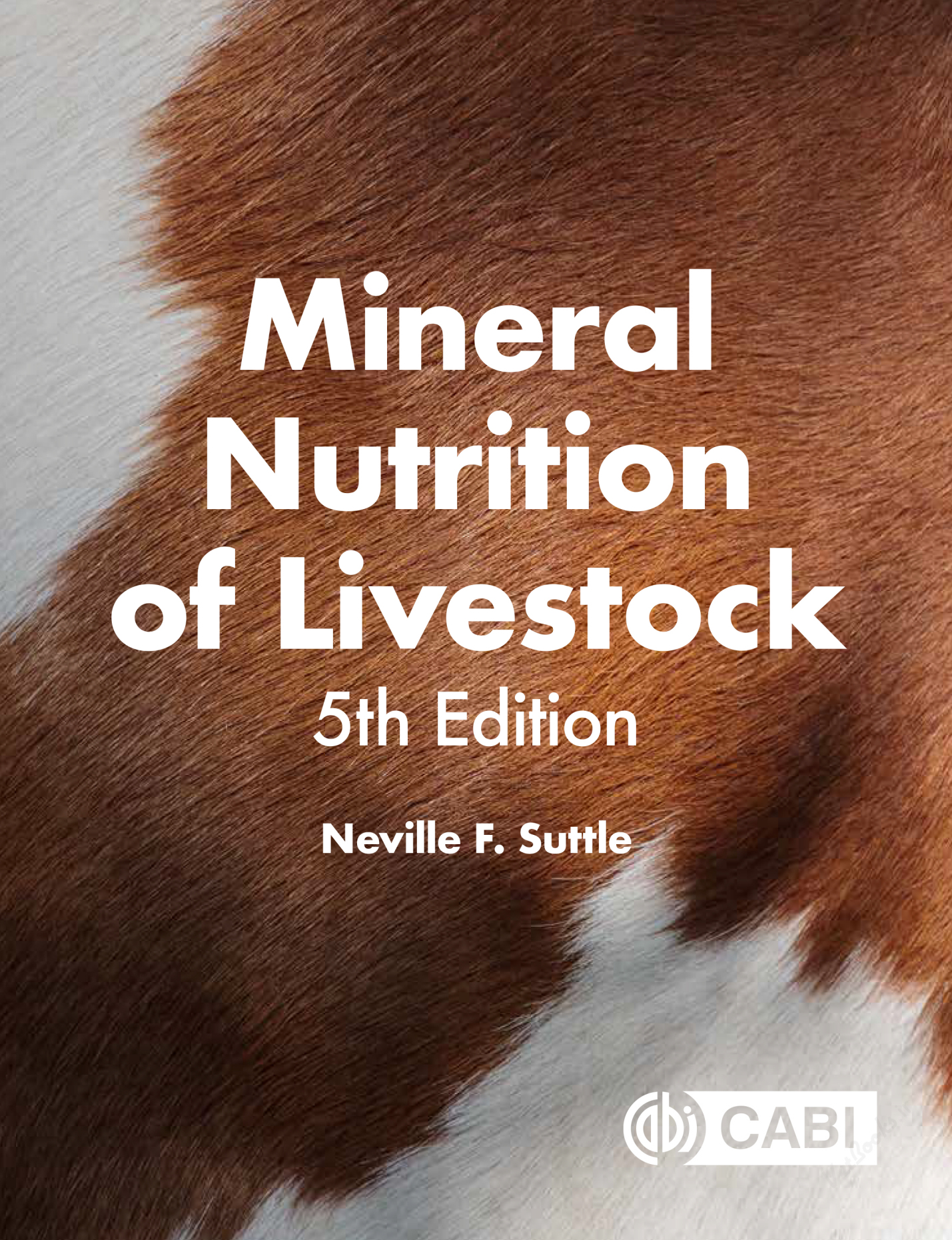
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5th Edition
Neville F. Suttle
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Preface

Catching up with publications on the mineral nutrition of livestock from the last decade has proved to be quite a challenge, though an excellent way of coping with Covid-induced confinement. The exercise has taken me a year longer than anticipated and has tested patience at the CABI headquarters in Wallingford, UK. The publication rate had soared due to a manifold increase in the number of journals, publishing on matters relating to nutrition, and the immediacy of online publications. Unfortunately, quality has declined and radical improvements in understanding mineral nutrition have been few and far between. It is all too easy to conduct copycat studies, just changing species, breed or gene product, and let the computer crunch the numbers, without stopping to ask the question, 'What is the animal trying to tell me?'. For this reason alone, the book begins, as before, with the basics of mineral nutrition before grappling with the plethora of new works. Straight away, there is an example of a new technique, thoughtlessly replicated, that has augmented the literature, without adding proportionately to knowledge: the use of comparative slaughter to define net mineral requirements for maintenance and growth.

There was a danger of cramming so much new information into an old framework that clarity would be lost and boredom generated. Two wise men of mineral nutrition, Dave Masters (CSIRO Agriculture and Food, Wembley, WA) and Andrew Sykes (Christchurch, NZ), fired early warning shots across my bow on macro mineral nutrition and I owe them a great debt for doing so. The result was a reordering that put animals before diets and relegated old or equivocal data to endnotes. On trace elements, I am greatly indebted to one of the inquisitive young bucks entering the field and moving mineral nutrition forward – Daniel Brugger (with Institute of Animal Nutrition and Dietetics, Vetsuisse-Faculty, University of Zurich, Switzerland). He straightened me out on Mn, Se and Zn: he was strong where I was outside my comfort zone, on genomics.

The hundreds of genes that are tweaked to cope with fluctuations in mineral supply will continue to swell the flow of publications, telling us 'how' but rarely 'why' animals respond as they do, 'how much' dietary mineral is enough and whether changes are cause or effect. I have had the temerity to question aspects of current use of gene expression to define requirements and evaluate sources of Fe, Mn and Zn, encouraged by Daniel. Suppliers of inappropriately named 'organic' minerals sources have exploited what might be called the 'mineraleome' for all its worth, assuming every difference in gene expression between mineral sources indicates superior value for the organic source. My view remains that the pursuit of hyper-availability through chelation continues to waste research resources and farmers' money. Far better to wage war on mineral antagonists. Advances made in the efficient use of macro and trace elements through the use of feed additives, like organic acids, phytases and other enzymes, represent some of the successes of the last decade. Efficient use of dietary minerals protects the environment by reducing mineral footprints and conserving scarce resources like phosphorus. Concerns to protect the environment increasingly constrain the mineral nutrition of livestock and could eventually compromise animal welfare. Such issues might arise if ever a sixth edition should come into being and my sympathies will be with whoever takes on that challenge!

1 The Requirement for Minerals

Early Discoveries

All animal and plant tissues contain widely varying amounts and proportions of mineral elements, which largely remain as oxides, carbonates, phosphates and sulfates in the ash after burning off organic matter. In the 17th century, a Swedish chemist, Gahn, found calcium phosphate to be the major component of bone ash. In 1875, Sir Humphrey Davy identified the element potassium in the residues of incinerated wood and gave it the name 'pot ash'. The earliest hint of nutritional significance for such apparently inert substances came when canaries on a seed diet were shown to require a supplement of 'calcareous earth' to remain healthy and produce eggs (Fordyce, 1791). Calcium supplements were eventually used for the prevention of rickets, a childhood disorder of bone development that had plagued people for centuries. The discovery that iron was a component of blood led Blaud (1832) to link blood iron content with 'chlorosis' (anaemia) in people. In livestock, Boussingault (1847) showed that cattle had a dietary need for common salt, and Babcock (1905) induced calcium deficiency in dairy cows by feeding diets low in calcium. A craving of sick cattle and sheep for the bones of dead animals on the South African veldt led to the identification of phosphorus deficiency in cattle (Theiler, 1912). Chatin (1850–1854) linked environmental iodine deficiency to the incidence of endemic goitre in man and animals and, early in the next century, the iodine-rich molecule thyroxine was isolated from thyroid tissue (Harington, 1926). By the 1930s, agricultural chemists at Wisconsin University were revealing effects of vitamin D deficiency on concentrations of calcium and phosphate in the blood of laboratory rats (Elvejhem and Kline (1933). There was clearly more to minerals than met the eye.

Essentiality

Elvejhem's group at Wisconsin were extending their studies to trace elements, using specially purified diets lacking copper, manganese and zinc, all of which proved to be essential for health in the 1930s (Underwood, 1977). These discoveries provided explanations for problems of ill-health, affecting grazing livestock on many continents. Deficiencies of copper and cobalt were reported in North America, Australia and Europe. Further studies with rats, maintained in plastic isolators to exclude atmospheric contamination, extended the list of essential minerals to include selenium (Smith and Schwarz, 1967), an element previously renowned for its toxicity to livestock. By 1981, 22 mineral elements were considered essential for animal life: seven major or macronutrient minerals – calcium, phosphorus, potassium, sodium, chlorine, magnesium and sulfur – and 15 trace or micronutrient mineral elements – iron, iodine, zinc, copper, manganese, cobalt, molybdenum, selenium, chromium, tin, vanadium, fluorine, silicon, nickel and arsenic (Underwood, 1981). Subsequently, dietary supplements of aluminium, boron, cadmium, lithium, lead and rubidium were shown to improve growth or health in rats, goats, pigs or poultry reared in highly specialized conditions, without either inducing specific clinical abnormalities or specific metabolic dysfunctions (see Chapters 17 and 18). The essentiality of these elements and some previously labelled 'newer essential elements' (notably fluorine, nickel, tin and vanadium) (Underwood and Suttle, 1999) must be re-examined in the light of hormesis, a phenomenon whereby animals respond positively when exposed to small doses of functionless agents that are toxic at higher doses (Calabrese and Baldwin, 1988); ubiquitous lead, nickel and vanadium may be such agents (pp. 498, 468 and 472, respectively). All animal tissues contain a further

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20–30 mineral elements, mostly in small and variable concentrations that probably arise from contact with a chemically diverse environment, and that may have hormetic attributes.

Complexity

By the end of the 20th century, molecular biology had permeated studies of mineral metabolism and function. Complex mechanisms by which minerals were safely transported across cell membranes and incorporated into functional intracellular molecules were being clarified (O'Dell and Sunde, 1997). For potassium alone, ten different membrane transport mechanisms had been identified. Genes controlling the synthesis of key metalloproteins such as metallothionein, selenoenzymes such as glutathione peroxidases and superoxide dismutases (SODs) were isolated. Zinc deficiency was found to alter expression of genes controlling synthesis of molecules that did not contain zinc: among them was uroguanylin, which is linked to satiety, the feeling of 'fullness' (Blanchard *et al*., 2001). New functions were revealed, such as the role of zinc in alkylation reactions and some, such as activation of methionine synthetase by copper, suggested that copper might be able to compensate for lack of the previously acknowledged activator, cobalt. The new millennium has seen an explosion of activity in the molecular field and a new focus: signalling mechanisms by which intracellular needs for cell survival are communicated and orchestrated. Calcium, superoxide ions and selenocysteine each play pivotal roles, that of selenocysteine being said to 'alter our understanding of the genetic code' (Hatfield and Gladyshev, 2002). Recent advances in understanding the molecular basis of mineral metabolism have been gathered in a single publication (Collins, 2016). Changes in mineral intake and form can trigger numerous molecular changes. Supra-supplementation with selenium yeast, a selenium protein or selenite induced different changes in immunological and other markers in heat-stressed broiler chicks (Liao *et al*., 2012). Zinc, chelated to glycine, evokes responses in mRNA in intestinal tissue that differ from those induced by inorganic zinc (100 mg kg^{-1}DM; Jaroscz *et al*., 2017). Such changes have yet to be associated with improved function (e.g. growth, disease resistance, longevity) and may not need to influence ration formulation. Pragmatism is called for when raising dietary Zn within a commonly encountered range (from 28 to 95mg kg^{-1}DM) can induce changes in 150 genes (p. 437).

Functions

Minerals perform four broad types of function in animals:

1. *Structural*: minerals can form structural components of body organs and tissues, exemplified by minerals such as calcium, phosphorus and magnesium; silicon in bones and teeth; and phosphorus and sulfur in muscle proteins. Minerals such as zinc and phosphorus can also contribute structural stability to the molecules and membranes of which they are a part.
2. *Physiological*: minerals occur in body fluids and tissues as electrolytes concerned with the maintenance of osmotic pressure, acid–base balance, membrane permeability and transmission of nerve impulses. Sodium, potassium, chlorine, calcium and magnesium in the blood, cerebrospinal fluid and gastric juice provide examples of such functions.
3. *Catalytic*: minerals can act as catalysts in enzyme and endocrine systems, as integral and specific components of the structure of metalloenzymes and hormones or as activators (coenzymes) within those systems. The number and variety of metalloenzymes and coenzymes identified has continued to increase since the late 1990s. Activities may be anabolic or catabolic, life-enhancing (oxidant) or life-protecting (antioxidant).
4. *Regulatory*: minerals regulate cell replication and differentiation; for example, calcium ions influence signal transduction and selenocysteine influences gene transcription, leading to its nomination as 'the 21st amino acid' (Hatfield and Gladyshev, 2002). The pivotal metabolic role of thyroxine has been attributed to the influence of triiodothyronine on gene transcription (Bassett *et al*., 2003). Table 1.1 indicates the breadth and functional importance of metalloenzymes and metalloproteins.

Multiplicity of Function

Different functions can be performed simultaneously by the same element, not only in animals but also in plants on which animals depend (e.g. glutathione peroxidase) and pathogens, both microbes and parasites, that infect them (e.g. MnSOD and CuZnSOD). Preoccupation with structural functions of calcium and phosphorus initially drew attention away from their manifold activities in soft tissues. These include: maintenance of calcium ion concentrations in ECF for orderly transmission

Table 1.1. Some important metalloenzymes and metalloproteins in livestock.

Metal	Metalloenzyme or metalloprotein	Function
	Hepcidin	Iron-regulating hormone
Fe	Succinate dehydrogenase	Oxidation of carbohydrates
	Haemoglobin	Oxygen transport in blood
	Catalase	Protection against hydrogen peroxide
Cu	Cytochrome oxidase	Terminal oxidase
	Lysyl oxidase	Lysine oxidation
	Hephaestin	Iron absorption
	Caeruloplasmin	Copper transport
	Superoxide dismutases (3*)	Dismutation of superoxide radical, O_2^-
Mn	Pyruvate carboxylase	Pyruvate metabolism
	Superoxide dismutase (MnSOD)	Antioxidant by removing O_2^-
	Glycosyl aminotransferases	Proteoglycan synthesis
Se	Glutathione peroxidases (8)	Removal of H_2O_2 and hydroperoxides
	Type 1 and 2 deiodinases	Conversion of tetra- to tri-iodothyronine
	Selenocysteine	Selenium transport and synthesis of selenoenzymes
	Thioredoxin reductase	Formation of disulphide bonds
Zn	Carbonic anhydrase	Formation of carbon dioxide
	Alkaline phosphatases (2)	Hydrolysis of phosphate esters
	Phospholipase A_2	Hydrolysis of phosphatidylcholine

*numbers in parentheses indicate multiple, isomeric forms of the same enzyme

of nerve impulses and in ICF for cell signalling; intracellular energy exchanges, through the making and breaking of high-energy phosphate bonds. Transmission of the genetic code by translation and transcription requires phosphorus as a constituent of key regulatory proteins and nucleic acids. Copper is an essential constituent of many cuproenzymes and cuproproteins with functions as diverse as electron transfer, iron absorption and antioxidant defence (Table 1.1). In metalloenzymes, a fixed number of metal atoms are firmly attached to a protein molecule and cannot be removed without loss of enzyme activity. In CuZnSOD, the two metals present serve different purposes, copper changing valency to dismute the free radical, superoxide, while zinc stabilizes the molecule. Manganese changes valency to serve a similar function to copper in MnSOD.

Multiplicity of Form and Location

A SOD family protects animals from oxidative stress in different locations: in the cell cytosol as SOD1, a dimeric aggregation of two molecules; in mitochondria as dimeric SOD2 and tetrameric MnSOD and extracellularly as tetrameric SOD3. Gene deletion studies in mice show that SOD1 and SOD2 are essential for survival but SOD3 is not. Two families of zinc transporters or ZIP proteins, 24 in all, have different roles, carrying zinc either into the cell cytosol or into organelles (p. 417). The precise number and/or position of atoms in regulatory proteins or peptides can determine function. Thyroxine contains four atoms of iodine, two attached to an outer and two to an inner tyrosine ring: removal of an outer atom creates physiologically active triiodothyronine, while removal of an inner atom creates an inactive analogue. Deiodination is accomplished by a family of three selenium-dependent deiodinases, synthesized from selenocysteine and operating at different sites (Beckett and Arthur, 1994). Cobalt is a unique element in that its functional significance stems from presence at the core of a single large molecule, vitamin B_{12}, with two different functions, determined by a side chain (p. 229).

Mineral Absorption

Minerals follow labyrinthine pathways through the animal once ingested and Fig. 1.1 illustrates the principal routes and intersections. The first intersection is that between digesta and apical membranes of cells forming the gut mucosa, wherever absorption begins. Prior digestive processes may either enhance mineral absorption, by releasing minerals bound to organic dietary constituents, or constrain absorption by allowing minerals to become bound to products of digestion. However, minerals are not broken down into metabolizable forms (i.e. 'digested') in the way that organic dietary components are: minerals excreted in faeces can be just as 'digestible' as those that were consumed. Absorbtion occurs by either active or passive processes: the

former consume energy and are often mineral dependent, relying on sodium pumps powered by adenosine triphosphatase (Goff, 2018). Passive mechanisms include diffusion, down either an electrochemical or concentration gradient, and solvent drag (Goff, 2018). Passive mechanisms are more influential for major than for trace elements, at high rather than low mineral intakes, and affected by the integrity of tight junctions between enterocytes (Buckley and Turner, 2018). Absorption of many trace elements is carefully regulated but some compete for a shared regulator. Iron and manganese compete for a divalent metal transporter (p. 355) and are delivered to a shared protective binding protein, transferrin, within the gut mucosa. Elements that share transport mechanisms compete with each other for absorption. Occasionally, minerals can be absorbed as complexes (e.g. selenium and manganese bound to methionine).

Mineral Uptake by Tissues

Minerals are usually transported from the serosal side of the gut mucosa and thence to the liver in free or bound forms via the portal blood stream, but trace elements can get 'stuck' in the mucosa. From the liver, minerals are transported by the peripheral bloodstream to be taken up by different organs and tissues at rates determined by local transporter mechanisms in cell membranes and organelles (Fig. 1.1b). A detailed insight is given later into the intricacies of such movements for zinc (Fig. 16.1). Mineral turnover rates vary from tissue to tissue but are generally high in intestinal mucosa and liver, intermediate in other soft tissues and slow in bone. Turnover rates and fluxes between compartments are influenced by physiological (e.g. lactation) and nutritional (deficient or overloaded) states. Mineral turnover is measurable from rates of change in specific radioactivity in selected pools after a single intravenous radioisotope dose. Compartmental analysis, using computer programs such as SAAM[27], has clarified changes in Ca accretion in and resorption from the skeleton during pregnancy and lactation in sheep (Braithwaite, 1983), Ca and P metabolism in growing lambs (Dias *et al*., 2006) and P metabolism in growing pigs (Dias *et al.,* 2010). Minerals with interrelated pathways, such as Ca and P, can be tracked simultaneously (Fernandez, 1995). Fig. 1.1a is a macromodel showing the fluxes of Ca at a single moment in time in lactating ewes. Similar intracellular fluxes and sequential events take place on a much smaller, nano-scale between organelles (Fig. 1.1b). Whereas the same transporter for Ca, calmodulin, can promote uptake from digesta as well as movement within cells, intracellular movements of trace elements such as copper are facilitated by an array of specific transporters at each interface.

Net Requirements for Maintenance

Before ingested minerals can begin to perform their proscribed functions, the amounts lost insidiously during the processes of surviving, working and producing must be replaced. Minerals leave the transport pool by secretion in sweat and digestive juices, by excretion in urine and by exhalation. Those secreted into the gut prior to sites of absorption may be partially reabsorbed (Fig. 1.1a). Together, these losses constitute the maintenance requirement (*M*) and consist mostly of minerals unavoidably excreted in faeces as sloughed mucosal cells, microbial residues and unabsorbed digestive secretions, the faecal endogenous loss (*FEL*). When minerals are supplied and absorbed in excess of need, either mineral secretion into the gut increases (e.g. for P in ruminants and for Cu and Mn in all species) or reabsorption of secreted minerals from the gut decreases (e.g. Ca, ruminants; Fe, all species) to void the excesses. Maintenance requirements are neither easily defined nor measured. If defined as the mineral lost when none is consumed, i.e. minimum endogenous loss (^{m}FEL), estimates can be obtained from intercepts of linear regressions of faecal and urinary excretion against mineral intake. Thus, ^{m}FEL of 12 mg P kg^{-1} liveweight (LW) were estimated (ARC, 1980) for sheep and cattle but subsequently considered to be gross underestimates (AFRC, 1991).

Estimating maintenance needs on mineral-free diets

Using semi-purified, mineral-free basal diets, ^{m}FEL of Ca and P ($^{m}FEL_{Ca,P}$) were determined in pigs (Fan *et al*., 2001; Petersen and Stein, 2006) and poultry (Shastak and Rodehutscord, 2013). Such diets present a number of problems: since animals cannot survive for long without Ca and P, balance periods must be short; initially, the diet was also protein-free (Fan *et al*., 2001), compounding the malnutrition problem; faecal output from highly

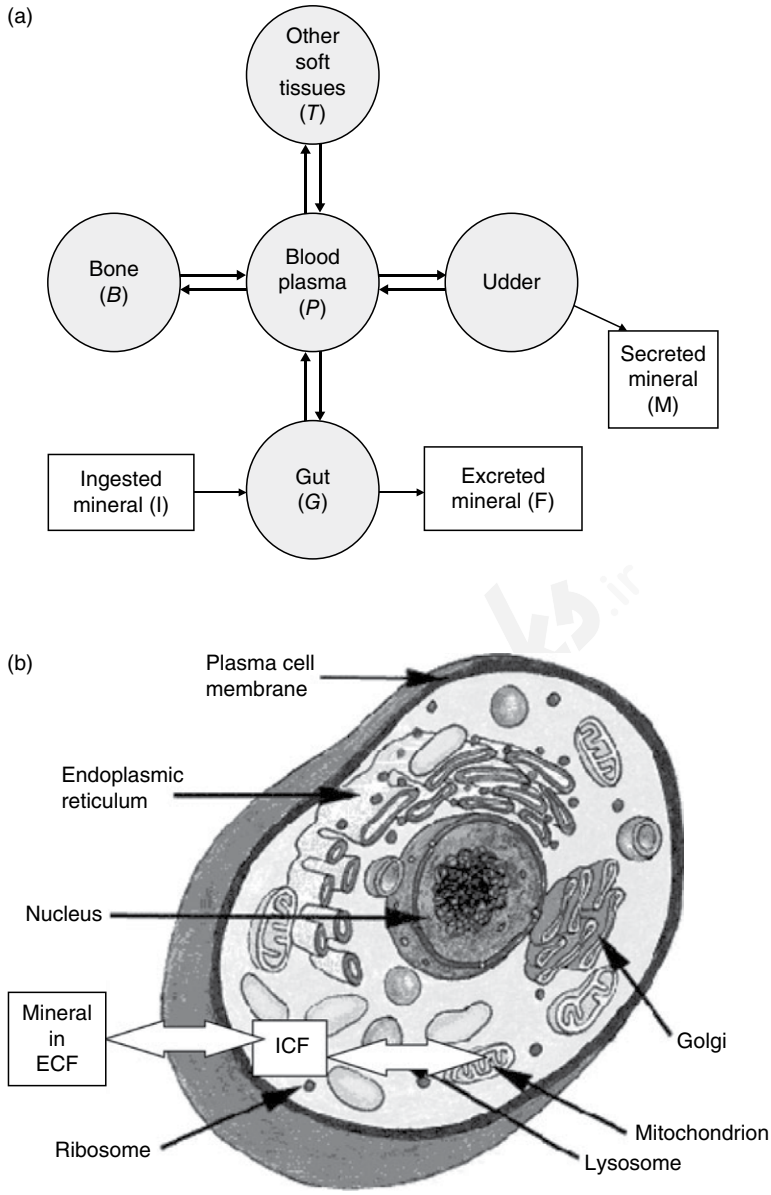

Fig. 1.1. (a) Two-way flows of ingested mineral (I) between body pools (circles) can be calculated by labelling mineral in gut (G) and/or plasma pools (P) with isotopes, giving the amounts of mineral absorbed and partitioned. In one such study (Braithwaite, 1983), the major daily calcium flows (in grams) in early lactation were: I, 5.2; absorption (*GP*), 2.5; secretion into gut (*PG*), 1.4; *PB,* 1.0; *BP,* 3.1; with faecal excetion (F) at 4.1, most of the calcium secreted in milk (M, 3.0) came from bone rather than diet. (b) Whole-body fluxes are the outcome of innumerable flows of mineral into, within and from each cell, with extracellular fluid (ECF) the provider and intracellular fluid (ICF) the distributor acting in similar ways to the diet and plasma in Fig. 1.1a and the organelles (e.g. mitochondria) participating in two-way flows with mineral in ICF.

digestible diets was extremely low and growth often poor. The method was improved by including gelatin as a Ca- and P-free protein source and substituting test feeds for gelatin. Standardized protocols have been recommended to minimize errors (WPSA, 2013) but intercepts can still have large errors and fail to attain statistical significance (e.g. $^{m}FEL_{P}$; Anwar *et al*., 2015). Furthermore, results can reflect choices made by experimenters rather than animal attributes (Perryman *et al*., 2017). Meta-regression analysis of data on faecal Ca excretion in pigs, which took out five important sources of variation, gave a mean $^{m}FEL_{Ca}$ with 95% confidence limits of 114–364mg Ca kg^{-1}DMI (Misiura *et al*., 2018). In conducting meta-analyses, whatever the species, experiments should be selected along lines suggested by WPSA (2013) to increase precision.

Estimating maintenance needs with mineral-rich diets

Simultaneous estimates of ^{m}FEL for several macrominerals were obtained by regression of either faecal excretion or carcase content of mineral against mineral intake in ruminants grown to different liveweights on mineral-rich diets (Fernandes *et al*., 2012). The technique has been widely copied (e.g. Ji *et al*., 2015). In some experiments, food allowances were restricted to halt growth in some groups, while in others, low-quality roughage was fed, with and without different amounts of concentrate. Substantial extrapolation from excessive to zero mineral intakes often generated ^{m}FEL that differed between studies (Ji *et al*., 2015) and from conventional estimates. Because mineral intake is confounded with food intake, ^{m}FEL is predicted for animals consuming no food, i.e. fed below maintenance. The method has been used to estimate ^{m}FEL for trace minerals (Cu, Fe, Mn, Zn) (Zhang *et al*., 2015), but again, given inconsistent values (Zhang *et al*., 2018), with differences often attributed by default to variation between breeds (Suttle, 2017).

A new approach for measuring maintenance needs

Ideally, endogenous losses should be measured at the minimum mineral intake needed to sustain a given level of production, as illustrated in Fig. 1.2 and following the long-standing principle for measuring energy requirements for maintenance (Corbett and Ball, 2002). The 'x' intercept values generated from the relationship between apparent absorption and intakes of mineral around the required amount indicate obligatory loss (^{o}FEL). Measurement of ^{o}FEL can be achieved by adding a given mineral in small increments to more conventional diets of marginal rather than neglible mineral content, while meeting all other WPSA (2013) conditions and avoiding pitfalls associated with semi-purified diets. Maintenance requirements in ruminants are more closely related to food intake (or metabolic body weight, $LW^{0.75}$) than body weight *per se* and should be expressed on a kg^{-1} dry matter (DM) intake basis, but a different scheme may be needed for non-ruminants (Lee *et al*., 2018). High food intakes increase 'wear and tear' on the mucosal lining as well as secretion of minerals into the gut, producing a 'productivity increment' in maintenance requirements (Milligan and Summers, 1986). Lactating animals eat more food per unit of body weight than non-lactating animals and probably have relatively high maintenance requirements.

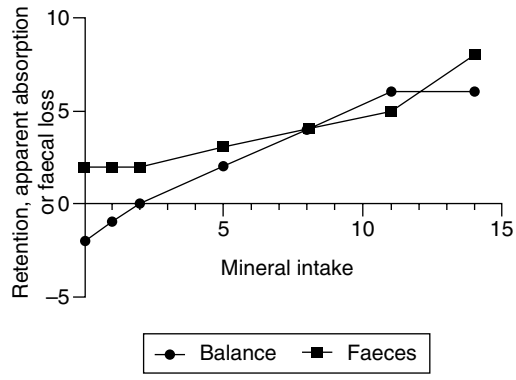

Fig. 1.2. A hypothetical model for determining gross mineral needs for maintenance as intercepts on the 'x' axis of non-linear relationships between either faecal excretion or carcase retention (determined by balance or comparative slaughter methods) and mineral intake: the common practice of extrapolating inappropriate linear relationships to zero intake (i.e. 'y' intercepts) and estimating 'minimum mineral loss' adds error without defining the mineral input needed to avoid negative balance.

Net Requirements for Work

With grazing stock, movement may raise maintenance needs for energy by 10–20% above those of confined animals at rest. With working animals, such as draft horses and bullocks, maintenance needs for energy increase several fold. The extra food supplied to working animals will usually supply sufficient additional minerals, even in cows that are increasingly used for draft purposes. Exceptions occur during hard physical work, especially in hot conditions, when losses of sodium, potassium and chlorine in sweat greatly increase. No other increased requirements have been reported for macrominerals with physical work. However, increased consumption of oxygen during exercise may increase requirements for trace elements such as selenium that are involved in antioxidant defence (Avellini *et al*., 1995). The role of minerals in antioxidant defence (Table 1.1) has featured prominently in proceedings of the International Society of Antioxidants (ISANH).

Net Requirements for Reproduction

Net requirements for reproduction are usually equated to the mineral content of the fetus plus products of conception (i.e. placenta, uterus and fetal fluids) and therefore increase exponentially to reach a peak in late gestation (Fig. 1.3). For the twin-bearing ewe, the calcium requirement in late gestation actually exceeds that of lactation, leading to important contrasts in the period of vulnerability to calcium deprivation when compared with the dairy cow (p. 66). There is also a small additional requirement for growth of mammary tissue and accumulation of colostrum prior to parturition. For those minerals where the generosity of maternal nutrition determines size of the fetal reserve (e.g. copper and selenium) (Langlands *et al*., 1984), it may be prudent but not essential to allow for maximal fetal retention.

Particular problems arise in defining and meeting requirements for reproduction, including lactation. The mother invariably gives priority to offspring and daily deficits between need and dietary provision are met by drawing on maternal mineral reserves. Requirements do not need to be met continuously from the diet throughout gestation or lactation. A classic experiment was performed by Kornegay *et al*. (1973) in which sows were fed constant daily amounts of calcium and phosphorus (10.3 g and 11.0 g day^{-1}, respectively) throughout five reproductive cycles. The allowance was grossly inadequate for lactation, but sufficient to allow some recovery of reserves between lactations. No harm was done to offspring and even the fifth generation remained

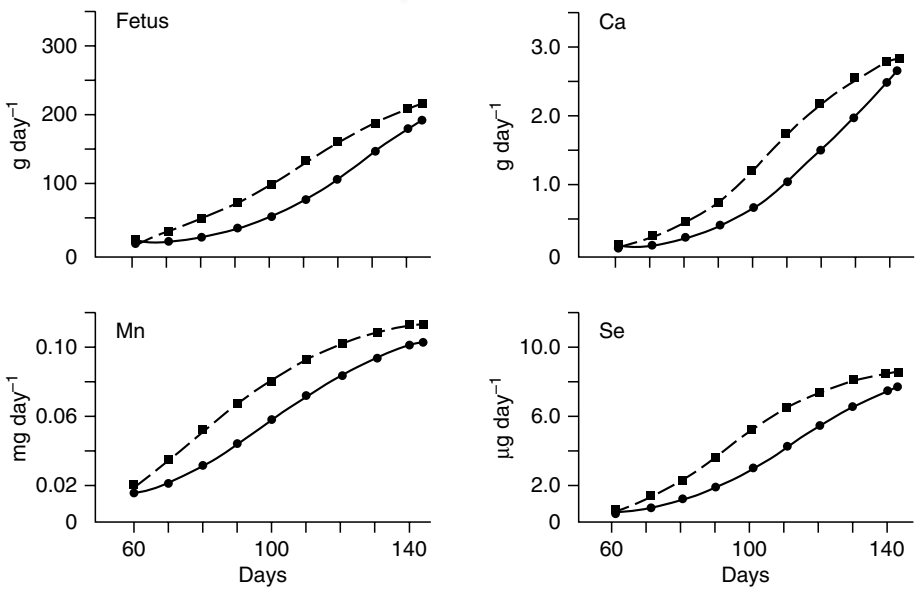

Fig. 1.3. Patterns of fetal accumulation of calcium, manganese and selenium in single- (•) and twin-bearing ewes (▪) during gestation reflect fetal weight gain (Grace *et al*., 1986).

biochemically normal. The only production trait to suffer was sow longevity, which was chiefly reduced by the gradual development of lameness and other leg abnormalities. Longevity is an important trait in economic terms. Lengthy experiments are required to define mineral requirements for lifetime production of breeding females and have yet to be carried out for any species (including the sow) for most minerals. When a first pregnancy precedes attainment of mature weight, requirements for both growth and pregnancy must be met in the short term, otherwise dam or offspring may suffer. When a cow has to conceive at peak lactation, there may be no reserves to draw on and fertility may suffer if peak daily mineral requirements are not met.

Net Requirements for Production

Net requirements for production (*P*) have always been equated to the mineral content of each unit of production, such as weight gain (*WG*), milk yield (*Y*) or fleece growth (*F*), and assumed to remain constant (ARC, 1980; NRC, 2012). Provided mineral composition of the product is not influenced by plane of mineral nutrition or age, the assumption is safe and generally holds true for milk yield. However, animals may adjust to suboptimal mineral intake by reducing mineral concentrations in tissues and products, such as eggs. Shell strength of eggs and tensile strength of wool fibres may be reduced in order to conserve Ca and Cu, respectively, for more essential functions. In such situations, net requirements must be sufficient to optimize quality as well as quantity of product. Milk is exceptional in that normal mineral concentrations are usually maintained during deficiencies, with priority being given to the suckling at the expense of the mother. Where provision of excess mineral increases mineral concentrations in milk (e.g. iodine), it is unnecessary to replace all secreted mineral. By analogy with maintenance requirements, only obligatory mineral secretion in milk needs to be budgeted for.

Mineral Needs for Growth

Net mineral requirements for growth (*G*) are traditionally described by mineral density (e.g. g Ca kg^{-1} WG), which changes throughout development. In early life, the skeleton grows rapidly in size and mineral density may decline until weaning, after which dietary mineral:energy ratios increase. Bones are far richer in Ca and P than soft tissues and respective requirements for growth diminish slightly as bone makes progressively smaller contributions to successive increments in WG (AFRC, 1991). However, macro- and trace elements can be stored in tissues when consumed in excess of need and mobilized when supplies are inadequate. Such storage is not obligatory but is a useful adaptation to counter fluctuations in dietary supply. The Brazilian model for determining *M* (Fernandes *et al.*, 2012, p. 6) was also used to estimate *G* in early-weaned goat kids from allometric equations relating carcase mineral content to body weight, allegedly following a precedent (ARC, 1980). In fact, ARC (1980) tested but rejected the allometric approach, as did their successors (AFRC, 1991). Relationships between carcase mineral content and body weight in the short-term goat studies were linear not logarithmic and differed from one to another (Fig. 1.4). Differences in allometrically determined *G* were often attributed by default to effects of genotype (e.g. Ji *et al.*, 2015) but are probably artefacts of experiments in which food and mineral intakes, the latter often excessive, were confounded and animals varied in rate of transition from milk to solid diets.

- Mineral density can reflect facultative as well as obligatory mineral retention.

There is rarely a need to define net mineral requirements for individual breeds. The exceptions are Ca and P requirements for heavy- and light-boned breeds and S for fine- and coarse-wooled sheep and goats. Although total mineral content of joints and remaining carcase of weaned pigs from 'low-lean' and 'high lean' lines differed, mineral density in the whole carcase did not (Wiseman and Mahan, 2010).

Differences between species in mineral needs for growth

Growth requirements for Ca and P do differ greatly between species, foals having far greater needs than lambs (Table 1.2) due to both a higher proportion and mineral density of bone in the equine skeleton. There are also species differences in growth requirements for trace elements. Copper, iron and zinc requirements are lower for broilers and turkeys than for weaned calves and lambs because respective

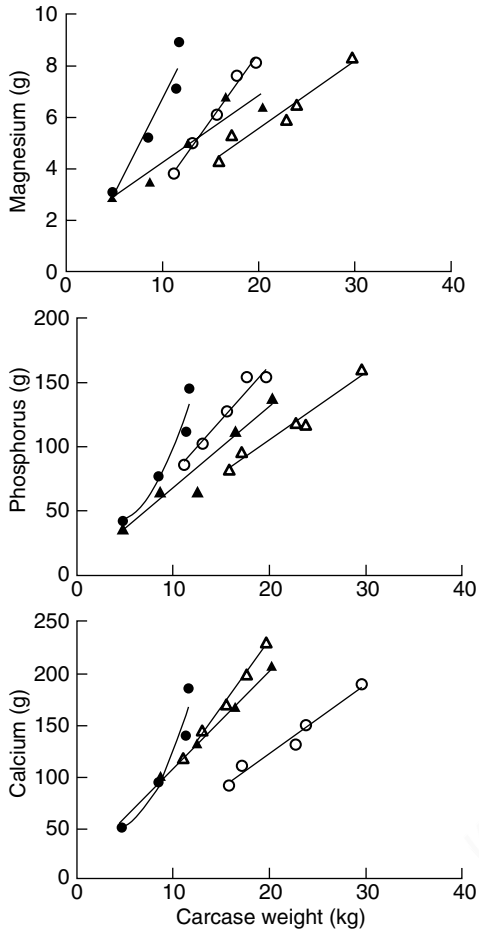

Fig. 1.4. Relationships between carcase weight and content of three minerals in groups of goat kids, reared differently in separate studies, are mostly linear but differ widely in slope and, sometimes, intercept. Groups were reared either naturally (Araujo *et al*., 2010) or artificially, in cages, on cow's milk, and weaned either early (Gomes *et al*., 2011; Teixeira *et al*., 2015) or late (Fernandes *et al*., 2012) onto *ad lib* or restricted amounts of mineral-rich, commercial rations.

concentrations in white meat are less than a quarter of those in red meat (Gerber *et al*., 2009). Species differ in propensity to store copper in the liver (rank: poultry < horse, deer, goat < pig < cattle < sheep) but requirements for extra-hepatic accretion are relatively constant at 3mg kg^{-1}WG, as are those for zinc, including the liver (23mg Zn kg^{-1}WG).

Gross Requirements

Net mineral requirements underestimate gross dietary needs for minerals because ingested minerals are incompletely absorbed and retained, the degree of underestimate being *inversely* related to the efficiency with which a given mineral is absorbed from a given diet. For some minerals (e.g. sodium and potassium), absorption is virtually complete under all circumstances, but for others (e.g. copper and manganese), most of the ingested mineral can remain unabsorbed. For such elements, a range of gross requirements (*GR*) will usually be appropriate, inversely related to the extent to which 'conditioning' or antagonistic factors limit mineral absorption from a particular grazing or ration (Fig. 1.5). By the same reasoning, there must be a range of maximum 'tolerable' dietary levels for a given mineral, which are *directly* related to antagonist potency (Fig. 1.5). The chemical form in which a mineral is present in the diet can determine the efficiency with which it is absorbed. Although cereals are rich in phosphorus, the mineral is largely present as phytate, which is not only poorly absorbed by pigs and poultry but also varies in availability with the type of phytate present. There are two principal methods for estimating the needs of livestock for minerals: factorial models and dietary trials.

Factorial models

Factorial models traditionally summated all components of net requirement and divided the total by the fraction of ingested mineral likely to be absorbed. Thus, the calcium need of a lactating ewe was given by:

$$GR_{Ca} - (M_{Ca} + P_{Ca})/A_{Ca} = (M_{Ca} + L_{Ca} + F_{Ca})/A_{Ca}$$

where *GR* is the gross requirement, A_{Ca} is the absorption coefficient for calcium and *M*, *P*, *L* and *F* are net requirements of calcium for maintenance, production, lactation and fleece growth, respectively. Choice of value for *A*, present to allow for inefficient use of ingested mineral, can greatly influence predicted requirements. Absorption coefficients are, in turn, dependent on error-prone corrections made for endogenous loss (p. 6). Minerals like calcium are often absorbed from or secreted into the gut according to need, while phosphorus is secreted and excreted according to need. For such

Table 1.2. Net mineral requirements for production are determined by mineral concentrations in the product: these data for calcium in carcases of different species indicate that net calcium requirement is about 70% greater for growth of foals than lambs.

	Ca concentration (g kg^{-1} empty body weight)				
	Sheep	Goats[d]	Cattle[e]	Deer[c]	Horses[c]
Newborn	10.1[a]	10.7	12.2	18.4	18.2
Weanling	9.6[b]	11.4	15.1	14.9	17.1
Young adult	10.5[c]	12.8	11.6	15.0	16.7

[a]Field and Suttle (1967); [b]Field *et al*. (1975); [c]Grace *et al*. (2008); [d]from references in Fig. 1.3; [e]data from AFRC, 1991)

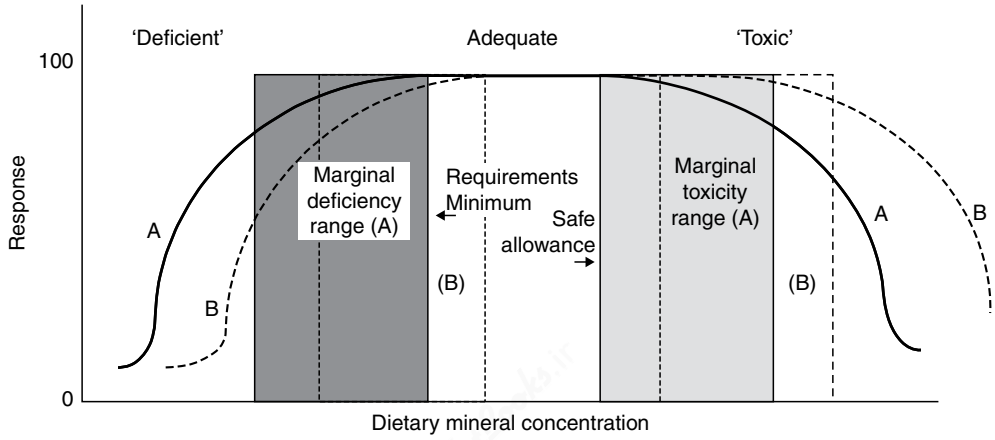

Fig. 1.5. The dose–response relationship between mineral supply and animal production, showing marginal bands between the central adequate band and either inadequate or toxic dietary concentrations: all bands move right as availability of mineral declines; thus A represents the more and B the lesser absorbable of two mineral sources. 'Requirements' are variously set within the central band, ranging from minimum requirements to safe allowances, depending on the caution of formulators in allowing for differences in variables such as availability.

minerals, *A* can easily become an animal rather than a dietary attribute, as explained later (p. 354). NRC (2012) estimates of phosphorus requirements for pigs were cast in terms of 'digestible phosphorus' to allow for the impact of phytate in different feeds. Factorial models have again been widely used to generate tabulated mineral requirements in this edition because requirements can be predicted for a wide range of production circumstances (Suttle, 1985; White, 1996). Where possible:

- maximal values for fractional absorbability (*A*), rather than an average, observed fractional absorption (*A*), have been derived from the literature;
- values for ***M*** are for obligatory (^{o}*FEL*), not minimum (^{m}*FEL*) losses (Fig. 1.2).

Incomplete or partial models are tried for important but problematic elements such as calcium (p. 79) and phosphorus (p. 141), where ***A*** and ^{o}*FEL* are ill-defined, indirectly allowing ***M*** to vary by using apparent absorbability (***AA***) as the denominator; thus:

$$GR = \mathbf{P/AA}$$

AA of a mineral supplement can be calculated from the increment in faecal mineral excretion, divided by the increase in mineral intake, the 'method of additions'.

Dietary trials

Mineral requirements can be estimated by feeding groups a range of mineral inputs above and below the minimum requirement and measuring responses in a relevant variable such as growth rate or blood composition. However, five or more different

mineral input levels may be needed to precisely define the optimum requirement, and the result can still depend on the statistical model used to describe the response (Remmenga *et al*., 1997). If purified ingredients are used to obtain sufficiently low mineral inputs, the diet may then lack naturally occurring antagonists and underestimate gross requirements on natural diets (Suttle, 1983; White, 1996). Another difficulty is that it is impractical to allow for requirements that vary with time. Where the requirement for production is high relative to that for maintenance, as it is for iron and zinc in growing livestock, requirements will fall with time when expressed as a proportion of a steadily increasing food intake if the growth rate remains constant (Suttle, 1985). The dietary approach gave much higher zinc requirements for turkey poults than chicks on the same semi-purified diet (25 versus 18mg Zn kg^{-1}DM) (Dewar and Downie, 1984). This difference may be partly attributable to higher food conversion efficiency of the poult. Dietary estimates of requirement are needed to validate factorially derived requirements.

Criteria of 'Requirement'

Criteria of adequacy determine estimated mineral requirements because mineral-dependent metabolic processes vary in sensitivity to deprivation. For instance, the processes of pigmentation and keratinization of wool are the first and sometimes only processes to be affected by low copper status in sheep and copper requirements are higher if wool growth, rather than growth rate or blood haemoglobin, is used to assess adequacy. Minimum zinc requirements for spermatogenesis and testicular development in young male sheep are higher than for body growth (Underwood and Somers, 1969). and requirements are therefore lower for meat production than breeding purposes. Optimum mineral intakes are often lower when defined by production traits rather than by biochemical traits. For example, optimum dietary zinc intakes for chicks were 18, 24 and 27mg kg^{-1}DM, respectively, for growth, plasma zinc and tibia zinc (Dewar and Downie (1984). Adequacy of phosphorus nutrition can be assessed on the basis of growth, bone dimensions, bone composition or bone histology, but bone ash and growth plate hypertrophy are the most sensitive indicators of need in turkey poults (Fig. 1.6) (Qian *et al*., 1996). Criteria of adequacy may be chosen for environmental rather than pathological reasons. Providing sufficient phosphorus and calcium for maximal bone mineralization simultaneously increases phosphorus excretion, with adverse consequences for the environment. To minimize phosphorus footprints (p. 529) when needs are high, young chicks have been fed less calcium than they immediately need for full bone mineralization: compensatory mineralization of bone occurred later in life without loss of productivity (p. 83).

Disagreement on Requirements

National authorities recommended mineral requirements to ensure that the production of native livestock is not impaired by dietary mineral imbalances but agreement was rare, whether poultry (Blair *et al*., 1983), cattle (AFRC, 1991) or sheep (White, 1996) were being prescribed for. When NRC (2012) revised standards for Ca and P in pigs, this did not stop workers in The Netherlands from revising their standards (Bikker and Blok, 2017). Recommendations continue to differ through use of different criteria of adequacy, different components in factorial models and use of different safety factors to give 'safe allowances' (Fig. 1.5) that leave no individual at risk.

- Whatever the origin, tables of requirements should not be regarded as absolutes.

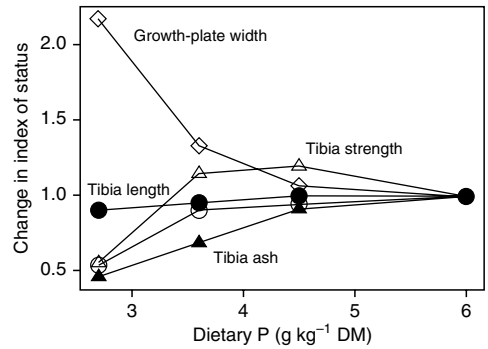

Fig. 1.6. Measures of bone quality in turkey poults vary in sensitivity to dietary phosphorus supply and assessments of need vary accordingly. Sensitivity of growth rate is shown by values relative to those at maximal phosphorus intake in open circles (data from Qian *et al*., 1996).

The proliferation of such tables often exaggerates uncertainties on the precise mineral needs of livestock in specific local contexts. Marginal bands or different categories will be attached to most of the mineral requirements presented in this book, rather than a single misleading average value. Average requirements can be useful for a preliminary assessment of the adequacy of mineral supplies from forages for grazing livestock, but minimum requirements are preferable for diagnosing deficiencies because of individual variations in absorption, which also affect tolerance (Suttle, 1991). Complete avoidance of mineral deprivation may not be compatible with avoidance of toxicity, for example in providing copper and phosphorus to breeds of sheep that are vulnerable to copper poisoning (see Chapter 11) and urinary calculi (see Chapter 6), respectively. There is rarely sufficient knowledge for precise safety factors to be calculated and their deployment is seldom acknowledged. The ARC (1980) used a safety factor to allow for a low A_{Mg} in some pastures and the AFRC (1991) used one to allow for high FEL_p on dry roughages. Feed compounders and farmers generally apply their own safety factors, regardless of the machinations of national authorities, and it is common for farm rations to provide far more than the already generous amounts recommended by some authorities (see Chapter 20).

Expression of Mineral Requirements

Mineral requirements can be expressed in several ways: in amounts per day or per unit of product, such as milk, eggs or weight gain; in proportions, such as percentage, parts per million (ppm), mass per mass (e.g. mg kg^{-1}); or moles (sometimes micro- or millimoles) per kg DM of the whole diet. Of the various options, required daily amounts are the most precise but they can vary with food intake and quality of diet. Expression as dietary concentrations has the merits of relative constancy and simplicity and is acceptable as long as the diet is palatable and food intake is not constrained. Daily P requirements of hens increase with onset of egg production but remain a constant proportion of the diet, whereas the required calcium concentration increases almost tenfold. Required mineral concentrations are affected by the efficiency with which organic constituents in the diet are utilized. Broiler chicks require diets that are richer in minerals than weaned pigs consuming similar diets because chicks convert food to body weight more efficiently. Although high-yielding dairy cows require more dietary Ca and P than low-yielding cows, the dietary concentrations required increase far less because feed intakes rise as milk yield increases (AFRC, 1991). Whether expressed as amounts or concentrations, precision can be greatly enhanced by allowing for factors that limit absorption of a given mineral, as advocated for copper in ruminants in previous editions. Similar allowances have been advocated for phosphorus in pigs (NRC, 2012) and poultry (WPSA, 2013) but, arguably, require more accurate values for available P in phytate-rich feeds (p. 138).

References

AFRC (1991) Technical Committee on Responses to Nutrients. Report No. 6: A reappraisal of the calcium and phosphorus requirements of sheep and cattle. *Nutrition Abstracts and Reviews* 61, 573–612.

Anwar, M.N., Ravindran V., Morel, P.C.H., Ravindran, G. and Cowiesom, A.J. (2015) Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens. *Animal Feed Science and Technology* 206, 100–107.

Araujo, M.J., de Madeiros, A,N., Teixera, I.A.M.A., Costa, R.J., Marques, C.A.T., Resende, K.T. and de Melo, G.M.P. (2010) Mineral requirements for growth of Moxoto goat in the semi-arid region of Brazil. *Small Ruminant Research* 93, 1–9.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

Avellini, L., Silvestrelli, M. and Gaiti, A. (1995) Training-induced modifications in some biochemical defences against free radicals in equine erythrocytes. *Veterinary Research Communications* 19, 179–184.

Babcock, S.M. (1905) The addition of salt to the ration of dairy cows. In: *University of Wisconsin Experiment Station, 22nd Annual Report*.

Bassett, J.H.D., Harvey, C.B. and Williams, G.R. (2003) Mechanisms of thyroid hormone receptor-specific nuclear and extra-nuclear actions. *Molecular and Cellular Endocrinology* 213, 1–11.

Beckett, G.J. and Arthur, J. (1994) The iodothyronine deiodinases and 5′ deiodination. *Baillieres Clinical Endocrinology and Metabolism* 8, 285–304.

Bikker, P. and Blok, M.C. (2017) Phosphorus and calcium requirements of growing pigs and sows. CVB Documentation Report no. 59. Wageningen, The Netherlands.

Blair, R., Daghir, N.J., Morimoto, H., Peter, V. and Taylor, T.G. (1983) International nutrition standards

for poultry. *Nutrition Abstracts and Reviews, Series B* 53, 673–703.

Blanchard, R.K., Moore, J.M., Green, C.L. and Cousins, R.J. (2001) Modulation of intestinal gene expression by dietary zinc status: effectiveness of cDNA arrays for expression profiling of a single nutrient deficiency. *Proceedings of the National Academy of Sciences* 98, 13507–13513.

Blaud, P. (1832) Sur les maladies chlorotiques et sur un mode de traitement specifique dans ses affections. *Revue Médicale Française Étrangère* 45, 341–367.

Boussingault, J.B.(1847) *Comptes Rendus des Séances de l'Académie des Sciences* 25, 729. Cited by McCollum, E.V. (1957) *A History of Nutrition*. Houghton Mifflin, Boston, Massachusetts.

Braithwaite, G.D. (1983) Calcium and phosphorus requirements of the ewe during pregnancy and lactation. 1. Calcium. *Journal of Agricultural Science, Cambridge* 50, 711–722.

Buckley, A. and Turner, J.R. (2018) Cell biology of tight junction barrier regulation and mucosal disease. *Cold Spring Harbor Perspectives in Biology* 10(1): a029314. doi: 10.1101/cshperspect.a029314. PMID: 28507021; PMCID: PMC5749156.

Calabrese, E.J. and Baldwin, L.A. (1998) Hormesis as a biological hypothesis. *Environmental Health Perspectives* 106 (Suppl. 1), 357–362.

Chatin, A. (1850–1854) Recherche de l'iode dans l'air, les eaux, le sol et les produits alimentoures des Alpes de la France. *Comptes Rendus des Séances de l'Académie des Sciences*, 30–39.

Collins, J. (2016) *Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals*. Elsevier.

Corbett, J.L. and Ball, A.J. (2002) Nutrition for maintenance. Chapter 7 in: Freer, M. and Dove, H. *Sheep Nutrition*. CAB International, Wallingford, UK, pp. 143–164.

Dewar, W.A. and Downie, J.N. (1984) The zinc requirements of broiler chicks and turkey poults fed on purified diets. *British Journal of Nutrition* 51, 467–477.

Dias, R.S., Kebreab, E., Vitti, D.M.S.S., Roque, A.P., Bueno, I.C.S. and France, J. (2006) A revised model for studying phosphorus and calcium kinetics in growing sheep. *Journal of Animal Science* 84, 2787–2794.

Dias, R.S., Lopez, S., Moreira, J., Schulin-Zeuthen, M., Vitti, D., Kebreab, E. and France, J. (2010) Application of a kinetic model to describe phosphorus metabolism in pigs fed a diet with a microbial phytase. *Journal of Agricultural Science* 148, 277–286.

Elvejhem, C.A. and Kline, B.E. (1933) Calcium and phosphorus studies in chicks. *Journal of Biological Chem*istry 103, 733–744.

Fan, M.Z., Archbold, T., Sauer, W.C., Lackeyram, D., Rideout, T. *et al*. (2001) Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. *Journal of Nutrition* 131, 2388–2396.

Fernandes. M.H.M.R., Resende, K.T., Tedeschi, L.O., Teixeira, I.A.M.A., Fernandes, Jr, J.S. (2012) Macromineral requirements for the maintenance and growth of Boer crossbred kids. *Journal of Animal Science* 90, 4458–4466.

Fernandez, J. (1995) Calcium and phosphorus metabolism in growing pigs. II. Simultaneous radio-calcium and radio-phosphorus kinetics. *Livestock Production Science* 41, 243–254.

Field, A.C. and Suttle, N.F. (1967) Retention of calcium, phosphorus, magnesium, sodium and potassium by the developing sheep foetus. *Journal of Agricultural Science, Cambridge* 69, 417–423.

Field, A.C., Suttle, N.F. and Nisbet, D.I. (1975) Effects of diets low in calcium and phosphorus on the development of growing lambs. *Journal of Agricultural Science, Cambridge* 85, 435–442.

Fordyce, G. (1791) *A Treatise on the Digestion of Food*, 2nd edn. London, J. Johnson; cited by McCollum, E.V. (1957) *A History of Nutrition*. Houghton Mifflin, Boston, Massachusetts.

Gerber, N., Brogioli, R., Hattendof, B., Scheeder, M.R.L., Wenk, C. and Gunther, D. (2009) Variability in selected trace elements of different meat cuts determined by ICP-MS and DRC-ICPMS. *Animal* 3, 166–172.

Goff, J.P. (2018) *Invited review:* Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *Journal of Dairy Science* 101, 2763–2813.

Gomes, R.A., Oliveira-Pascoa, D., Teixera, I.A.M.A., de Madeiros, A.N., Resende, K.T., Yanez, E.A. and Ferreira, A.C.D. **(**2011) Macromineral requirements for growing Saanen goat kids. *Small Ruminant Research* 99, 160–165.

Grace, N.D., Watkinson, J.H. and Martinson, P. (1986) Accumulation of minerals by the foetus(es) and conceptus of single- and twin-bearing ewes. *New Zealand Journal of Agricultural Research* 29, 207–222.

Grace, N.D., Castillo-Alcala, F. and Wilson, P.R. (2008) Amounts and distribution of mineral elements associated with live-weight gains of grazing red deer (*Cervus elaphus*). *New Zealand Journal of Agricultural Research* 51, 439–449.

Harington, C.R. (1926) Chemistry of thyroxine. *Biochemistry Journal* 20, 300–313.

Hatfield, D.L. and Gladyshev, V.N. (2002) How selenium has changed our understanding of the genetic code. *Molecular and Cellular Biology* 22, 3565–3576.

Jarosz, L., Marek, A., Gradzki, Z., Kwiecien, M., Zylinska, B and Kaczmarek, B. (2017) Effect of feed supplementation with zinc glycine chelate and zinc sulfate on cytokine and immunoglobulin gene expression profiles in chicken intestinal tissue. *Poultry Science* 96, 4224–4235.

Ji, S.-K., Gui-shan Xu, G.-S., Diao, Q.-Y., Deng, K.-D., Jiang, C.-J., Tu, Y. and Nai-feng Zhang, N.-F. (2015) Macromineral requirements of Dorper×thin-tailed Han crossbred female lambs. *Journal of Integrative Agriculture* 14, 1617–1626.

Kornegay, E.T., Thomas, H.R. and Meacham, T.N. (1973) Evaluation of dietary calcium and phosphorus for reproducing sows housed in total confinement on concrete or in dirt lots. *Journal of Animal Science* 37, 493–500.

Langlands, J.P., Bowles, J.E., Donald, G.E. and Smith, A.J. (1984) Deposition of copper, manganese, selenium and zinc in 'Merino' sheep. *Australian Journal of Agricultural Research* 35, 701–707.

Lee, S.S., Casas, G.A. and Stein, H.H. (2018) The level of feed intake does not influence digestibility of calcium and phosphorus in diets fed to gestating sows, but gestating sows have reduced digestibility of calcium and phosphorus compared with growing gilts. *Canadian Journal of Animal Science* 98, 589–592.

Liao, X., Lu, L., Li, S., Liu, S., Zhang, L., Wang, G., Li, A. and Luo, X. (2012) Effects of selenium source and level on growth performance, tissue selenium concentrations, antioxidation, and immune functions of heat-stressed broilers. *Biological Trace Element Research* 150, 158–165.

Milligan, L.P. and Summers, M. (1986) The biological basis of maintenance and its relevance to assessing responses to nutrients. *Proceedings of the Nutrition Society* 45, 185–193.

Misiura, M.M., Filipe, J.A.N., Walk, C.L. and Kyriazakis, I. (2018) Do not neglect calcium: a systemic review and meta-analysis (meta-regression) of its digestibility and utilisation by growing and finishing pigs. *British Journal of Nutrition* 119, 1207–1219.

NRC (2012) *Nutrient Requirements of Swine*. 11th edn. NRC Press, Washington, DC.

O'Dell, B.L. and Sunde, R.A. (1997) *Introduction to Handbook of Nutritionally Essential Minerals*. Marcel Dekker Inc., New York, pp. 8–11.

Perryman, K.R., Massey-O'Neill, H.V., Bedford, M.R. and Dozier, W.A. (2017) Methodology affects measures of phosphorus availability in growing broilers. 1. Effects of calcium feeding strategy and dietary adaptation period length on true ileal phosphorus digestibility and predicted endogenous phosphorus losses. *Poultry Science* 96, *611–621*.

Petersen, G.I. and Stein, H.H. (2006) Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. *Journal of Animal Science* 84, 2126–2132.

Qian, H., Kornegay, E.T. and Veit, H.P. (1996) Effects of supplemental phytase and phosphorus on histological, mechanical and chemical traits of tibia and performance of turkeys fed on soybean meal-based, semi-purified diets high in phytate phosphorus. *British Journal of Nutrition* 76, 263–272.

Remmenga, M.D., Milliken, G.A., Kratzer, D., Schwenke, J.R. and Rolka, H.R. (1997) Estimating the maximum effective dose in a quantitative dose–response experiment. *Journal of Animal Science* 75, 2174–2183.

Shastak,Y. and Rodehutschord, M. (2013) Determination and estimation of phosphorus availability in growing poultry and their historical development. *World Poultry Science Journal* 69, 569–586.

Smith, J.C. and Schwarz, K. (1967) A controlled environment system for new trace element deficiencies. *Journal of Nutrition* 93, 182–188.

Suttle, N.F. (1983) Assessing the mineral and trace element status of feeds. In: Robards, G.E. and Packham, R.G. (eds) *Proceedings of the Second Symposium of the International Network of Feed Information Centres, Brisbane*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 211–237.

Suttle, N.F. (1985) Estimation of requirements by factorial analysis: potential and limitations. In: Mills, C.F., Bremner, I. and Chesters, J.K. (eds) *Proceedings of the Fifth International Symposium on Trace Elements in Man and Animals*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 881–883.

Suttle, N.F. (1991) Mineral supplementation of low quality roughages. In: *Proceedings of Symposium on Isotope and Related Techniques in Animal Production and Health*. International Atomic Energy Agency, Vienna.

Suttle, N.F. (2017) Challenges in predicting needs for mineral supplementation in grazing animals. Chapter 15 in: Singh, B.R., McLoughlin, M.J. and Brevik, E.C. (eds) *The Nexus of Soils, Plants and Animals and Human Health*. Catena Schwezerbart, Stuttgart, pp. 105–110.

Teixeira, I.A.M.A., Hunter, C.I., Pereira Filho, J.M., Sobrinho, A.G. and Resende, K.T. **(**2015) Macromineral requirements for the maintenance and growth of F1 Boer × Saanen kids. *Journal of Animal Science*, 93, 2349–2356.

Theiler, A. (1912) Facts and theories about styfziekte and lamziekte. In: *Second Report of the Directorate of Veterinary Research*. Onderstepoort Veterinary Institute, Pretoria, South Africa, pp. 7–78.

Underwood, E.J. (1977) *Trace Elements in Human and Animal Nutrition*, 4th edn. Academic Press, London.

Underwood, E.J. (1981) *The Mineral Nutrition of Livestock*, 2nd edn. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Underwood, E.J. and Somers, M. (1969) Studies of zinc nutrition in sheep. I. The relation of zinc to growth, testicular development and spermatogenesis in young rams. *Australian Journal of Agricultural Research* 20, 889–897.

Underwood, E.J. and Suttle, N.F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

White, C.L. (1996) Understanding the mineral requirements of sheep. In: Masters, D.G. and White, C.L. (eds) *Detection and Treatment of Mineral Nutrition Problems in Grazing Sheep*. ACIAR Monograph No. 37, Canberra, pp. 15–29.

Wiseman, T.G. and Mahan, D.C. (2010) Partition of minerals in body components from a high- and low-lean genetic line of barrows and gilts from 20 to 125 kilograms of body weight. *Journal of Animal Science* 88, 3337–3350.

WPSA (Working Group No. 2: Nutrition of the European Federation of Branches of World Poultry Science Association) (2013) Determination of phosphorus availability in poultry. *World's Poultry Science Journal* 69, 687–698.

Zhang, H., Nie, H.T., Wang, Q., Wang, Z.Y, Zhang, Y.L, Guo, R.H. and Wang, F. (2015) Trace element concentrations and distributions in the main body tissues and the net requirements for maintenance and growth of Dorper × Hu lambs. *Journal of Animal Science* 93, 2471–2481.

Zhang, H., Nie, H.T., Wang, Z. and Wang, F. (2018) The net iron, manganese, copper and zinc requirements for maintenance and growth of Dorper × Hu lambs. *Italian Journal of Animal Science.* Available at: https://doi.org/10.1080/1828051X.2018.1431964 (accessed 23 April 2021).

2 Natural Sources of Minerals

Livestock can satisfy most of their needs for minerals from those naturally present (inherent) in feeds and forages but are still rarely allowed to do so by 'fail-safe' commercial practices that outsource responsibility to suppliers of mineral supplements. This chapter describes the mineral composition of natural feeds and forages and capacity to meet the mineral needs of animals. Plants and animals have different needs: plants do not need iodine (I) or cobalt (Co) at all and need less magnesium (Mg) than livestock at times. By contrast, plants need more potassium (K) and manganese (Mn) than animals: applications of K and Mn in fertilizers can increase crop and pasture yields on soils where grazing stock remained healthy. Mineral concentrations in plants are principally influenced by soil environment, plant genotype, climate and stage of maturity. The importance of a given factor varies between minerals and is influenced by interactions between factors and with aspects of crop or pasture husbandry, including use of fertilizers, soil amendments and irrigation.

Soil Environment

Mineral concentrations in plants generally reflect the availability of soil minerals at the plant root surface but plants, like animals, can sometimes adapt to low availability by reducing mineral concentrations in tissues. as well as restricting growth.

Geographical associations

The primary reason for mineral deficiencies and excesses of phosphorus (P), sodium, (Na), Co and selenium (Se) in grazing animals is that soils in certain regions are inherently low or high in the respective plant-available mineral: the soil effect is particularly strong for Se.[i] Occurrence of mineral imbalances in livestock can occasionally be predicted by soil-mapping techniques (Plant *et al*., 1996; White and Zasoski, 1999). Areas of molybdenum (Mo)-induced, copper (Cu) deficiency in cattle in England were predicted by reconnaissance of stream sediment Mo (Thornton and Alloway, 1974). Soil zinc (Zn) maps that indicate widespread risks of Zn deficiency in crops (Alloway, 2008) are of no use in animals and the potential of soil mapping has yet to be realized in the livestock production context (Fordyce *et al*., 1996). Multivariate analysis of comprehensively mapped data for soil mineral status in the UK (McGrath and Loveland, 1992; Patterson *et al*., 2011) and Europe (De Vos *et al*., 2006) may facilitate prediction of mineral status in livestock (Suttle, 2000; Suttle, 2017). Prediction of macromineral composition of the leaf canopy by remote sensing accounted for 88% of variation in herbage P in alpine grassland (Gao *et al*., 2019) but was less successful for tropical grassland, where prediction was most accurate for herbage Mg (47%; Mutanga *et al*., 2005).

Local soil factors

Geographical relationships can be disrupted by local influences, such as that of soil pH. Effects of soil pH on trace element concentrations in a grass sward are illustrated in Fig. 2.1 and they are even more striking in legumes. Molybdenum uptake increases as soil pH rises and pasture Mo can rise sufficiently on alkaline or calcareous soils (clays, shales and limestones) to induce Cu-responsive disorders in livestock (p. 261). By contrast, Mo uptake can be sufficiently poor on acidic soils to cause Mo deficiency in legumes. Absorption of Co and Mn by plants is favoured by low soil pH (Underwood and Suttle, 1999). Pasture Co, Mo and Mn greatly increase when soils become waterlogged (Burridge *et al*., 1983). Soil samples are treated with chemical extractants to measure 'plant-available' mineral in the hope of adjusting for influences of factors such as

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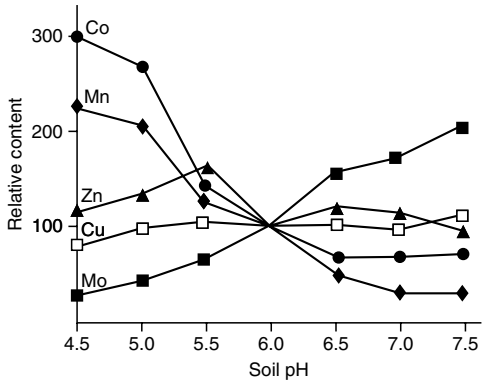

Fig. 2.1. Effects of soil pH on trace mineral concentrations in a ryegrass sward in Aberdeenshire (from COSAC, 1982): concentrations of cobalt and manganese decrease markedly as soil pH increases, while those of molybdenum increase and those of copper and zinc show little change. Values at pH 6.0 were (mg kg^{-1}DM): Co 0.03, Cu 3.1, Mn 51.0, Mo 2.7 and Zn 23 and given a relative value of 1.0.

soil pH, but methods often lack accuracy (Silanpaa 1982; Jumba *et al*., 1996). Prediction of herbage Co can be improved by multiple regression (Suttle *et al*., 2003) but more sophisticated simulations of the root micro-environment are needed (Suttle, 2017).

Plant Genotype

Pasture and forage species

Leguminous species are generally much richer in macro-elements than grasses growing in comparable conditions, whether temperate or tropical. Temperate and tropical legumes contained 14.2 and 10.1g Ca kg^{-1}DM, respectively, against 3.7 and 3.8g kg^{-1}DM in the corresponding grasses (Minson, 1990). Many tropical legumes were exceedingly low in Na, with half containing less than 4g Na kg^{-1}DM. Mixed swards of *Lolium perenne* and *Trifolium repens* contain more Ca, Mg and K than pure swards of *L. perenne*, but Na, S and P were similar with no nitrogen applied (Hopkins *et al*., 1994). Leguminous species are generally richer in trace elements than gramineous species in temperate climates, as are mixed as opposed to grass swards (Burridge *et al*., 1983; Hopkins *et al*., 1994): differences are narrowed when soils lack available elements and reversed for Cu in tropical climates (Minson, 1990). On Mo-rich 'muck' soils, red clover can be ten times richer in Mo than grasses (Kincaid *et al*., 1986).

Grass species also vary widely in mineral concentrations.[ii] While some variation in Co and Mn may be due to soil contamination, this in turn may reflect genetic characteristics, such as growth habit (upright or prostrate). Pasture species are either natrophilic, with Na-rich shoots and leaves but Na-poor roots, or natrophobic, with an opposite distribution (Smith *et al*., 1978). Natrophobic species such as red clover and timothy, growing inland, could supply insufficient Na to grazing livestock. In New Zealand, short-rotation (H1) hybrid ryegrass contained 90% less I than perennial ryegrass (*L. perenne*), far outweighing the influence of soil I. Botanical composition was far more important than soil composition in determining mineral concentrations of tropical grasses in western Kenya, where an introduced species (kikuyu, *Pennisetum clandestinum*) was richer in most macro- and trace elements than native species (Fig. 2.2). In Florida, Se was twice as high in Bermuda grass as in Bahia grass and Mg 60% higher in sorghum than corn silage (Kappel *et al*., 1983). Leafy brassicas are naturally rich in Se. Herbs are generally richer in minerals than cultivated plants (Wilman and Derrick, 1994; Harrington *et al*., 2006). In Mediterranean climates, increasing use is made of cereals, grazed in the vegetative state by sheep, to sustain productivity, but imbalanced mineral concentrations may frustrate that ambition (Masters *et al*., 2019).

Grains and seeds

Genetic differences in mineral composition of leafy plants are not generally mirrored in their seeds but leguminous seeds and oilseeds are usually richer in most minerals than those of grasses and cereals. Cereals are inherently low in Ca and Na. Cereal species differ fivefold in Mn, typical values being: wheat and oats, 35–40, barley and sorghum, 14–16 and maize 5–8mg Mn kg^{-1}DM (maize is also low in Co): variation amongst lupin seeds is even greater and not confined to Mn.[iii] With the foregoing exceptions, seeds and grains are generally more uniform in mineral composition than other feeds and forages, before processing, and uniformly high in P. Shed seeds of subterranean clover can provide an unintended source of supplementary P and can avert deficiency in sheep and cattle in New South Wales.

Browse species

Browse species are important sources of minerals in Mediterranean-type environments, particularly to smallholders and are likely to play an increasing role

in large-scale intensive intercropping systems, but their mineral composition has only recently come under scrutiny. A study of nine browse species in Tanzania has revealed wide variations in both macro- and trace mineral concentrations (Table 2.1) (Mtui *et al*., 2008). *Balanites aegyptiaca* has an exceptionally high Ca:P ratio of 35.6 and a low Cu(mg):S(g) ratio of 0.66, which indicates risks of P and Cu insufficiency, respectively, if the species is heavily relied upon. Seven Mediterranean browse species also had high Ca:P ratios but lower P concentrations (0.6–1.0g kg^{-1}DM), both goats and sheep preferring a species (*Erica multiflora*) with the lowest Ca:P ratio and goats consuming more than sheep (Rogosic *et al*., 2006). Data for several salt-tolerant species in Australia show generally high concentrations of all major cations, not only Na, but low Cu and Zn concentrations (Revell *et al*., 2013).

Interactions between Soil and Plant Genotype

Effects of plant genotype on mineral composition can be magnified on mineral-enriched soils. In saline soils, saltbushes (*Atriplex* species) and bluebushes (*Kochia* species) typically contain 80–140g NaCl kg^{-1}DM compared to about 1g NaCl kg^{-1}DM in most pasture species. Similarly, certain species of *Astragalus* growing on seleniferous soils contain >5000mg Se kg^{-1}DM compared to <20mg Se kg^{-1} DM in common herbage species on the same soils. The role of 'Se accumulator' plants in causing selenosis in grazing livestock is considered

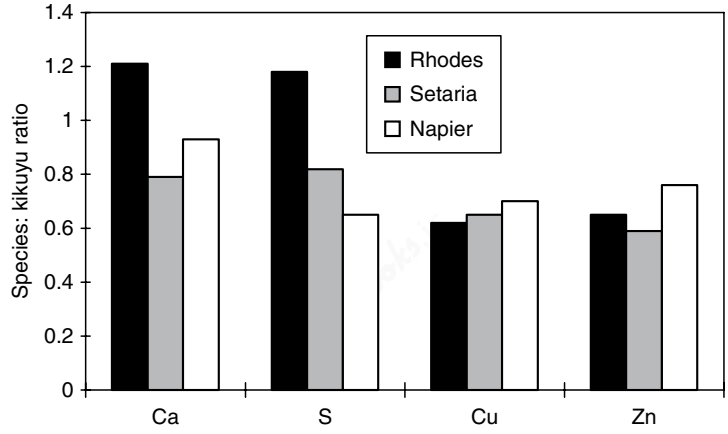

Fig. 2.2. Species differences in macro- and trace mineral composition amongst four grasses sampled during the dry season in western Kenya. Values of three species are shown as fractions of values for the fourth species, kikuyu, which were 1.4 and 1.7g kg^{-1}DM for calcium and sulfur, respectively, and 6.0 and 30.1mg kg^{-1}DM for copper and zinc, respectively (Jumba *et al*., 1996).

Table 2.1. Mineral concentrations in the leaves of nine browse species from Tanzania indicate some exotic mineral profiles that could cause mineral imbalances in browsing livestock (Mtui *et al*., 2008).

	(g kg^{-1}DM)				(mg kg^{-1}DM)			
Species	Ca	P	Mg	S	Zn	Cu	Mn	Se
Acacia nilotica	9.5	1.2	1.0	1.3	18.9	13.8	32.8	0.19
Acacia tortilis	14.7	2.2	3.0	1.8	29.0	2.5	52.7	0.20
Balanites aegyptiaca	35.6	1.0	6.6	6.2	12.5	4.4	42.6	0.77
Cryptolepis africana	15.1	2.3	3.4	1.7	17.5	5.4	33.6	0.05
Ficus sycomorus	18.2	3.8	5.7	2.1	29.1	10.0	29.9	0.07
Lantana camara	10.2	2.5	3.1	1.4	26.4	9.6	190.3	0.02
Lawsonia inermis	11.2	4.3	3.0	2.2	47.5	9.8	72.8	0.17
Senna siamea	7.5	1.9	2.0	1.9	2.3	2.9	23.4	0.07
Trichilia emetia	13.3	2.5	3.1	1.6	41.2	5.5	21.4	0.14

later (p. 397). Strontium-accumulating species have also been reported (Underwood and Suttle, 1999).

Intervention with Fertilizers

Phosphorus

Most soils in the tropics supply insufficient P for maximum crop and pasture growth and yields can be doubled by applying P in fertilizers at 20–50 kg ha^{-1}, depending on site, (Jones, 1990), but insufficiently for optimum animal performance. Additonal superphosphate can significantly improve weight gains in sheep and cattle, wool yields and lamb and calf crops in Australia by improving palatability and digestibility of herbage (Winks, 1990) but positive responses were not always obtained. On a mixed tropical pasture, P-fertilizer suppressed the more nutritious legume component (Coates *et al*., 1990). The opposite effect was found on temperate Australian pasture, where subterranean clover content increased when 20 kg P ha^{-1} was applied, allowing stocking rates to be doubled (Fig. 2.3). Improved pasture growth required careful adjustment of stocking type as well as stocking rate to obtain maximum responses in animal production: in the early stages of the lengthy experiment, use of castrate male sheep resulted in a mismatch between pasture growth and animal need (Saul *et al*., 1999). Wheat, barley and oat grains grown on soils low in available P generally contain 50–60% less P than those on more fertile soils but grain P can be raised from 2–3 to 4–5g kg^{-1}DM by superphosphate applications, while increasing grain yields.

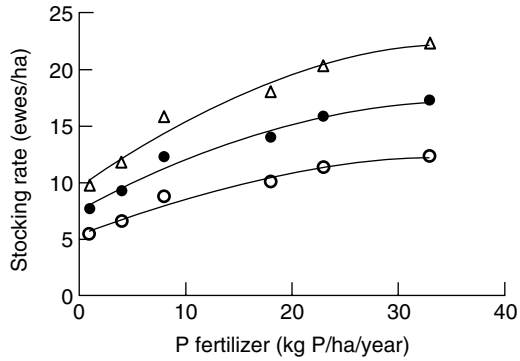

Fig. 2.3. Stocking rate (SR) of breeding ewes had to be increased to make the most of increased herbage mass that became available when phosphorus fertilizers were applied at increasing rates to a pasture containing subterranean clover in South Australia: weaned lamb weight was around 21 kg, whether SR was low (open circle), medium (solid circle) or high (triangle) (data from Saul *et al*., 1999).

Trace elements and sulfur

In some coastal areas in Australia, small applications of Mo to deficient soils markedly increase legume yields, herbage Mo and protein concentrations. Increases in Mo are of no value unless Cu intakes are high: then Cu retention is decreased and risk of chronic copper poisoning reduced. Zinc and selenium concentrations in grains and pastures reflect the soil status of those minerals and fertilizer usage. Zinc in wheat grain from Zn-deficient soils in Western Australia was increased from 16 to 35mg Zn kg^{-1}DM by applying ZnO at 0.6kg ha^{-1} (Underwood, 1977). Selenium fertilizers increased pasture Se from 0.03 to 0.06mg kg^{-1}DM and improved sheep production in Western Australia (p. 394). In seleniferous areas in India, sugarcane top Se was reduced from 15.2 to 5.1mg kg^{-1}DM by applying gypsum at 1t ha^{-1} (Dhillon and Dhillon, 1991). Much smaller applications of gypsum are used to raise pasture S (p. 218). Sulfur in superphosphate fertilizers can also raise pasture S.

Lime, nitrogenous and potassic fertilizers

Improvement of permanent pasture by liming, reseeding and applying nitrogenous (N) fertilizer does not increase risk of mineral deficiencies in grazing livestock, contrary to popular belief.[iv] Experiments on improved hill pastures in Scotland between 1985 and 1987 failed to show improvements in growth prior to weaning in lambs supplemented orally with Cu and parenterally with Co (as vitamin B_{12}) and Se in successive years (Suttle, 2017). However, N and K are applied to increase forage and crop yields, increasing the rate at which minerals are 'exported' in crop and animal products and risk of mineral deficiency may increase in the long term. Heavy applications of fertilizer N can depress legume growth, thereby reducing mineral content of a mixed sward. Applications of fertilizer K can raise herbage yields and herbage K while decreasing herbage Mg and Na: in cereals grazed in the vegetative state, the net effect may be to induce deficiencies in sheep (Masters *et al*., 2019).

Maturity and Season

Plants mature partly in response to factors inherent in their genetic make-up and partly in response to external factors, either natural (e.g. season, climate)

or man-made (e.g. provision of irrigation, shelter), and there are associated changes in mineral composition. Crops and forage P declines markedly with advancing maturity, although the decline is less in legumes than in grasses (Coates *et al*., 1990): concentrations of other elements rarely decline to the same extent. Pasture Fe and Co decline rapidly in spring but can increase massively in autumn, due to soil contamination (e.g. Sprinkle *et al*., 2018). Seasonal changes often reflect increases in proportions of stem to leaf and old to new leaves, stems and old leaves generally having the lower mineral concentrations (Burridge *et al*., 1983; Minson, 1990). However, Ca remains stable while Si increases as plants mature because straw contains more Ca and Si than grain. Sugarcane tops contain more Se than stems (5.7–9.5 and 1.8–2.1mg kg^{-1}DM, respectively) and both values decline with maturity (Dhillon and Dhillon, 1991). Seasonal fluctuations persisted in Bahia grass kept in an immature state by rotational grazing and clipping (Kappel *et al*., 1983). In Florida, concentrations of K, P and Mg were, respectively, 110, 60 and 75% higher in wet- than in dry-season forages (Kiatoko *et al*., 1982) but in Idaho, P and K in rangeland pasture were lower in autumn than in summer, whether or not pasture was irrigated (Sprinkle *et al*., 2018). Wheat grain Se was negatively correlated with rainfall (White *et al*., 1981) and tendencies for forage Cu to increase and Se to decrease with increasing altitude in the dry season (Jumba *et al*., 1996) may have reflected covariation in rainfall. Minerals are lost with the shedding of seed and the remaining stem or straw is low in most minerals. Furthermore, standing straw is subject to leaching of some macro-elements, P and K in Australian barley straw falling from 0.7 to 0.4 g and from 9 to 0.9g K kg^{-1}DM, respectively, in five months following harvest. In the USA, Cu, Zn and Mn showed no such decline (Southern, 1979).

Soil Ingestion

Soil and dust that contaminate herbage can contribute significantly to mineral intakes, especially in grazing livestock when either stocking rates are high or pasture availability is low. Soil intake can rise to 163g d^{-1} in sheep in arid conditions (Vaithiyanathan and Singh, 1994) and constitute 10–25% of total DM intake in outwintered sheep and cattle in temperate zones (Healy *et al*., 1974; Thornton, 1974). With elements such as Co, I and Se, which are more abundant in soils than in plants, soil ingestion can benefit livestock (Grace *et al*., 1996; Grace, 2006). By contrast, the Cu antagonists Fe, Mo and Zn ingested in soils may exacerbate hypocuprosis in cattle and 'swayback' (copper deficiency) in sheep (Suttle *et al*., 1984). Soil ingestion can provide an important route for toxic elements such as Pb (Hill *et al*., 1998), Cd (Bramley, 1990) and F (Grace *et al*., 2003a, b) to accumulate in tissues of grazing livestock. Soil contamination is a common cause of anomalously high Co, Fe and Mn concentrations in silages and conservation at low pH increases extractability of soil Fe (Hansen and Spears, 2009). Release of trace elements during digestion is hard to predict and influenced by soil particle size, mineral composition and dwell time at different sites in the gut (Suttle, 2017).

Atmospheric and Industrial Inputs

Significant amounts of minerals can enter ecosystems from the atmosphere. A New Zealand study found that inputs of S in rainfall varied from 0.5 to 15.0kg ha^{-1}, decreasing exponentially with distance from the coast (Ledgard and Upsdell, 1991). Similar gradients exist for I and Na. Inputs of S from industrial sources declined in many countries under the influence of stricter controls of pollution. In Scandinavia, the decreasing acidity of rain increased soil pH, with consequent increases in plant Mo towards the end of the last century. Contamination with F-containing dusts and fumes from industrial sources and dispersal of wastes from mining activities posed further hazards to livestock in some areas (see Chapter 18). Removal of Pb from petrol in Finland in 1993 was associated with a reduction in liver and kidney Pb in moose, probably because less atmospheric Pb became trapped in peat (p. 499)

Mineral Concentrations in Feeds

Analytical and experimental precision

Feed analyses have improved in accuracy, thanks to widespread application of quality control measures and approved standard feed materials (e.g. from the National Bureau of Standards, USA). Errors may still arise from contamination during collection and processing of samples for analysis. Milling can contaminate samples with Fe, Co, Mn and nickel but contamination can be reduced by using mills with agate-tipped blades or coffee grinders instead of laboratory mills with steel screens. When dietary mineral composition is manipulated by adding

supplements, intended mineral concentrations may not be attained and should always be checked before an experiment is started to correct mistakes: undetected, such errors can profoundly affect conclusions drawn.

Crops and forages

Given the many factors that can influence mineral composition of a particular crop or forage, derivation of representative values is difficult and of questionable use, as illustrated in Table 2.2: historic NRC (1982) values needed revision (Berger, 1995). Coefficients of variation for alfalfa hay from Alberta ranged from 23.8% for P to 107.4% for Mn for over 220 samples (Suleiman *et al*., 1997). New, unqualified mean values from NRC (2012) do not fix the problem and some qualified values from elsewhere (e.g. 9 ± 6mg Cu kg^{-1}DM for barley (INRA, 2004) do not inspire confidence. Agreement tends to be close at low levels of mineral supplementation but large discrepancies between calculated and analysed Ca can be found, probably caused by mixing errors and supplements of abnormal Ca content (e.g. Dersjant-Li *et al*., 2018).

A study of the impact of hurricane Irene on mineral composition of corn silage in north-eastern states in the USA highlighted variation in forage composition. Corn silage from unflooded fields deviated considerably from 'book' values, old and newer (Table 2.2). Some silages from flooded fields (not tabulated) had high Fe and Mn values, indicating soil contamination. Many US states have their own sets of data, containing abnormally distributed trace element values (e.g. Dairy One, Ithaca, NY).

- Skewed population distributions described by median and range rather than mean values would show closer agreement between states and countries.
- Adequacy of mineral provision from compound rations can rarely be predicted with sufficient accuracy from tables of mineral composition for principal feed constituents.

Crop by-products

Crop residues such as oilseed cakes, by-products of industrial fermentation, leaves and straws are being increasingly used as sources of energy, protein and fibre in livestock diets, often enriching the whole diet with many minerals. However, crop by-products can

Table 2.2. Old and newer data for mean (standard deviation) mineral concentrations in three major foodstuffs from different countries; wide variation within and between sources suggest unreliability of mineral composition of rations derived from tables rather than by local analysis of feed samples.

			Macro-element (g kg^{-1}DM)						
Feed	IFN	Source	Ca	P	K	Mg	Cu	Mn	Zn
Barley	4–07–939	CAN	0.6 (0.2)	3.7 (0.6)	5.4 (1.8)	1.5 (0.2)	5.4 (2.4)	18.9 (6.7)	42.3 (10.5)
	4–00–549	USA	0.7 (0.02)	3.8 (0.07)	6.3 (0.11)	1.4 (0.02)	7.0 (2.0)	22.0 (6.0)	38.0 (7.0)
	4–00–549	UK	0.9 (0.6)	4.0 (0.5)	5.0 (0.7)	1.2 (0.2)	4.2 (1.5)	18.5 (3.6)	32.5 (8.5)
		NRC[1]	0.5	3.7	4.7	1.5	9	18.0	17
		NRC[2]	0.6	3.5	3.8	1.4	–	–	–
		INRA	0.8 (0.7)	3.9 (0.3)	5.6 (0.7)	1.3 (0.2)	9.0 (6.0)	17 (6)	35 (9)
Lucerne hay	1–00–059	CAN	17.9 (5.3)	2.1 (0.5)	17.5 (4.9)	3.1 (0.9)	6.1 (2.5)	43.7 (41.8)	25.4 (11.7)
	1–00–059	USA	13.1 (3.3)	3.0 (0.6)	26.3 (4.4)	2.7 (0.8)	4.4 (6.3)	22.7 (13.9)	13.4 (17.5)
	1–00–078	UK	15.6 (1.8)	3.1 (0.7)	27.3 (5.0)	1.7 (0.3)	–	–	–
		NRC[1]	14.2	2.2	25.4	3.3	11.1	31.0	24.8
Maize silage	3–00–216	CAN	2.6 (1.2)	2.3 (0.5)	11.2 (2.6)	2.5 (0.9)	4.4 (2.2)	37.8 (18.8)	34.0 (13.1)
	3–28–250	USA	2.5 (0.14)	2.3 (0.6)	10.8 (3.3)	1.8 (0.4)	3.0 (2.7)	17.1 (20.3)	12.2 (18.8)
	3–02–822	UK	4.3 (2.0)	2.6 (1.2)	12.3 (4.1)	2.2 (0.7)	5.2 (0.75)	14.6 (7.2)	45.3 (13.9)
		NRC[1]	2.3	2.2	9.9	1.9	10.1	30.0	21.0
		NES	1.8 [1.3]	2.3 [1.8]	8.8 [11.3]	1.6 [1.1]	6 [5]	30 [10]	24 [19]

IFN, International Food Number; CAN, data from Alberta (Suleiman *et al*., 1997); USA, pooled data from the states of New York, Indiana, Idaho and Arizona (Berger, 1995); UK, data from the Ministry of Agriculture, Fisheries and Food (Givens *et al*., 1990); NRC[1], US-Canadian Feed Composition Tables (1982); NRC[2]; data from ingredient library (NRC, 2012); INRA, European data gathered by Sauvant *et al*. (2004); NES, samples from eight farms in unflooded areas of north-eastern States of USA in 2011; value in square brackets are the extreme values, lowest or highest (Kung *et al*., 2015).

vary more widely in mineral composition than the whole crop (Arosemena *et al*., 1995). The effect of industrial process on mineral content is illustrated in Table 2.3. Dual-purpose crops, grown for fuel as well as feeds, will continue to increase supplies of crop by-products such as dried distillers grain solubles (DDGS), providing valuable sources of highly available P (Widmer *et al*., 2007). Mineral concentrations in DDGS are generally threefold higher than those in the original grains but vary in Ca, Na and S, due to additions during processing (Liu, 2011). High and variable Cu values in distillery by-products from the UK arise from widespread use of copper stills for the distillation process. Differences between countries for soybean meal (SBM) were small because USA is the major UK supplier but can be much wider (Ibáñez *et al*., 2020). For a given by-product, only P is sufficiently uniform to predict contributions to the total ration. Most brans are good sources of P but beet pulp, citrus pulp and sorghum hulls are not. In tropical countries, by-products of the sugarcane industry such as bagasse and molasses sustain successful beef and milk production enterprises. Any antagonist content (e.g. goitrogens in rapeseed meals; phytate in cereals and protein concentrates) must be defined and overcome.

Minerals in Drinking Water

Drinking water is not normally a major source of minerals, although there are exceptions (Shirley, 1978; Linn, 2010). Water from deep aquifers can contain 750mg S l^{-1}, adding nearly 4g S kg^{-1}DM to the diet as sulfates. This is far more than any nutritional requirement and sufficient to induce copper insufficiency (Smart *et al*., 1986) and sulfur toxicity when consumed in conjunction with S-rich DDGS (NRC, 2017). Iodine concentrations in drinking water can be affected by local geology, barely detectable in east Denmark but as high as 139μg l^{-1} in islands off the west coast where groundwater filters through marine sediments, rich in I (p. 533). In most natural fluorosis areas, such as those in the semi-arid interior of Argentina, water supplies rather than feeds cause fluorosis (p. 493). Drinking water can be so saline that it provides far more Na and Cl than livestock can tolerate (p. 197). Some 'hard' waters are rich in Ca (120 mg l^{-1}) and Mg (80 mg l^{-1}) but trace element concentrations are usually low (<1 mg l^{-1}), except for Fe, which can reach 20 mg l^{-1} and induce copper insufficiency (p. 272). Individual daily water consumption can be as variable as mineral composition, varying fourfold in grazing beef cattle (Wright *et al*., 1978). Equations for predicting water intake from metabolic body weight are available but differ between temperate and tropical breeds and climates (Zanetti *et al*., 2017a). Meaningful 'average' mineral intakes from drinking water are therefore hard to calculate but a useful attempt has been made for temperate conditions (Linn, 2010).

Minerals in Milk

The most important animal source of minerals for species allowed to suckle their young is milk. The length of time that suckled offspring remain with their mothers varies widely according to species and management practice, but would commonly be 21 days for piglets, 120 days for lambs, 150 days for kids, 180 days for foals and 300 days for beef

Table 2.3. Mean mineral composition of crop by-products can vary with source and industrial process (with standard deviation).

			(g kg^{-1}DM)		(mg kg^{-1}DM)	
Crop by-product	IFN	Source	Ca	P	Cu	Zn
Brewers' grains	5–02–141	USA[1] (maize)	3.3 (1.2)	5.9 (0.8)	11.2 (5.0)	97.0 (16.0)
	5–00–517	USA[1] (barley)	3.5 (1.4)	5.1 (1.0)	18.7 (9.2)	72.8 (12.5)
Distillers' dried grains	5–28–236	USA[1] (maize)	2.9 (1.5)	8.3 (1.7)	9.7 (9.0)	73.5 (47.0)
		USA[2]	0.4 (0.25)	8.9 (1.1)	4.1 (1.3)	114 (85.7)
	5–12–185	UK (barley)	1.7 (0.32)	9.6 (0.80)	40.5 (16.8)	55.4 (4.7)
Soybean meal	5–04–612	USA[1]	4.1 (2.9)	7.2 (2.8)	17.7 (7.0)	69.5 (141)
		USA[2]	5.3 (1.5)	8.0 (2.3)	16.7 (3.8)	60.5 (5.5)
	5–04–604	UK	3.9 (1.6)	7.4 (0.44)	15.8 (2.6)	49.0 (9.9)

IFN, International Food Number; USA, data pooled from four states (Berger, 1995); UK data collected by ADAS in England (Givens *et al.*, 1990); USA[2] is data from (Kerr *et al*., 2008).

calves. In all species, milk composition varies with parity, stage of lactation, nutrition and presence of disease but the major influence is that of genotype.

Differences between species and breeds

Huge biodiversity in the mineral composition of milk has been noted (Medhammar *et al*., 2012) and is confirmed in Table 2.4, which presents average macro-mineral concentrations for main milks of 15 species, ranked in decreasing order of milk protein concentration. It is immediately obvious that milk Ca and P rank in a similar order to milk protein. For milk Ca, the linear relationship across species, omitting the outlying yak, was

Milk Ca (g/kg) = 0.4 (± 0.15) + 0.027 (± 0.0028) protein (g/kg) (r^2 = 0.88).

The corresponding relationship of milk P with milk protein was equally strong and the average Ca:P ratio 1.13; relationships with milk fat were weaker (adjusted r^2 < 0.7)

- Biodiversity in milk Ca and P reflects the capacity of milk of a given species to promote growth in the suckling by virtue of its metabolisable protein and energy densities.

Table 2.4. Mean macro mineral and protein concentrations (g kg^{-1}) in main milks of domesticated animals

Species	Protein	Ca	P	Mg	K	Na
Moose[1]	105	2.8	2.8	0.23	1.11	0.78
Reindeer[1]	104	3.2	2.7	0.19	1.56	0.48
Red deer[2]	71	2.73	1.91	0.18	1.26	0.41
Sow[3]	58	1.97	1.51	0.11	0.78	0.34
Ewe[4]	55	1.94	1.59	0.18	1.38	0.44
Yak[1]	52	1.29	1.06	0.1	0.95	0.16
Llama[1]	49	1.95	1.85	0.15	1.12	0.7
Buffalo[1]	40	1.91	1.91	0.12	1.12	0.47
Camel (B)[1]	39	1.94	1.54	0.08	1.86	0.66
Goat[5]	35	1.34	1.2	0.2	1.81	0.41
Cow[5]	32	1.2	1.0	0.12	1.52	0.58
Dromedary[1]	31	1.14	0.86	0.13	1.51	0.66
Mare[1]	20	0.95	0.58	0.07	0.51	0.16
Donkey[2]	13	0.54	0.43	0.06	1.1	0.12
Human[5]	12	0.33	0.43	0.04	0.55	0.15

Data sources
[1] Medhammar et al (2012);
[2] Malacarne et al., (2015);
[3] Csapo et al., 1996);
[4] USDA (2009);
[5] Park (2010).
B = Bacterian

- The relationship for Mg (r^2 = 0.58) implies that Mg also plays an important role in bone growth.
- Artificial rearing of a species on cow's milk changes Ca, P and Mg: protein ratios compared with natural suckling, reducing capacity for mineralising the skeleton in piglets, lambs and kids but increasing that capacity in foals.
- All milks from domesticated species appear to provide sufficient minerals for human infants.
- Cows' milk is inferior to most milks for correcting malnutrition in adults.

Milk K and Na also vary widely between species but are unrelated to milk protein and may reflect adaptation to extreme in fluctuations in dietary supply of K and Na in natural environments. Biodiversity in trace- as well as macro-mineral composition has been reported (Chen *et al*., 2020) but is unrelated to milk protein. Tabulation of trace element composition would be unhelpful because values can reflect dietary intake (e.g of Se and I) and contamination can lead to wide variation in values (e.g. of Fe and Mn; Chen *et al*., 2020; Mederhammer *et al*., 2012). Milk is particularly rich in Zn, concentrations varying from 2.2 in the donkey (Malacarne *et al*., 2015) to 6.5mg l^{-1} in the sow (Csapo *et al*., 1996).

Breed differences in mineral composition were evident in mare, buffalo, camel (Medhammar *et al*., 2012) and sow (Csapo *et al*., 1996) milks but were relatively small. Cattle breeds producing milk with relatively high fat and protein concentration, such as the Jersey and Guernsey, produced milk with 1.3g Ca kg^{-1} compared with 1.13g Ca kg^{-1} in the British Friesian (ARC, 1980). An equation relating milk Ca to milk fat was commended for predicting milk Ca for a given breed but milk protein would probably be a better predictor. Milk from Holstein-Friesians is also lower in P and Mg than that from Jerseys (Manuelian *et al*., 2018). Ash content of milk (mostly K, Ca and P) tends to decline with lactation length as does relative growth rate of suckled offspring, so that mineral concentrations in milk tend to match need within breeds and species.

Effects of lactation and parity

First milk (colostrum) is usually richer in minerals than later milk, as shown for bovine colostrum in Table 2.5 but far less so than in fat (Hadjipanayiotou, 1995). By the fourth calving, concentrations of Ca, P, Mg and Zn in colostrum

fell to 72–83% of values for the first calving but there was no effect of parity on milk composition after four days of lactation (Kume and Tanabe, 1993). Peak concentrations of Ca and P in mare's milk, 1.25 g and 0.90 g l^{-1}, respectively, were not reached until the seventh day of lactation (Grace *et al*. 1999).

Effects of diet and season

Effects of diet on milk composition vary greatly between minerals (Kirchgessner *et al*., 1967). Lack of Ca, P, Na and Fe are reflected in diminished milk yield of constant mineral composition. By contrast, lack of dietary Cu and I lower milk Cu and I and lack of Co decreases milk vitamin B_{12}. Milk B_{12} can be boosted by dietary Co supplements in ruminants but not non-ruminants (p. 241). Milk I and Mo can be increased by raising respective mineral intakes but those of Cu, Mn and Zn cannot. Apparent effects of season on milk minerals differed between sheep and goats and overall mineral status was deemed inadequate for both species in Pakistan (Khan *et al*., 2006) but season was confounded with dietary change, lactating animals grazing pastures with free access to a mineral mix during spring and summer but housed during winter. Mineral status in milk did not correlate with that of the diet. Access to browse in the semi-arid conditions may have resulted in species differences in mineral intake, given the abnormal mineral content of some browse species (Table 2.1). Milk Ca, Cu and Zn were far below values in Table 2.5, those for milk Cu lower in summer than winter. Dietary effects of Se vary with source: inorganic Se has a limited effect on milk Se but Se in selenomethionine or brewer's yeast is preferentially partitioned to milk, giving large increases in milk Se (Givens *et al*., 2004). Some potentially toxic elements in the mother's diet can be passed to offspring via milk (e.g. Pb), but maternal F exposure has little effect on milk F.

Table 2.5. Mean colostrum yield and mineral concentrations in colostrum of 21 cows (kg day-1) shortly after parturition (Kume and Tanabe, 1993). Comparative values for mare's milk at 24 h and 72 h are given in parentheses (Grace *et al*., 1999).

	Time after parturition (h)				
	0	12	24	72	SD
Colostrum	11.7	–	14.9	21.6	2.4
Ca (g l^{-1})	2.09	1.68	1.43 (0.69)	1.25 (1.16)	0.19
P (g l^{-1})	1.75	1.43	1.25 (0.71)	1.01 (0.89)	0.17
Mg (g l^{-1})	0.31	0.21	0.15 (0.30)	0.11 (0.10)	0.04
Na (g l^{-1})	0.69	0.64	0.58 (0.56)	0.53 (0.17)	0.008
K (g l^{-1})	1.48	1.49	1.58 (0.96)	1.50 (0.98)	0.12
Fe (mg l^{-1})	2.0	1.5	1.2 (0.79)	1.1 (0.34)	0.6
Zn (mg l^{-1})	17.2	10.3	6.4 (5.5)	5.2 (2.7)	2.5
Cu (mg l^{-1})	0.12	0.09	0.08 (0.76)	0.08 (0.37)	0.03
Mn (mg l^{-1})	0.06	0.04	0.03	0.02	0.02

SD, standard deviation.

Animal By-products

Bovine milk and by-products of butter and cheese manufacture – skimmed milk, buttermilk and whey, in either liquid or dried forms – are valuable mineral as well as protein supplements for pigs and poultry on grain-based diets, particularly for Ca (Table 2.6). Skimmed milk and buttermilk differ little in mineral composition from whole milk, since little mineral, other than I, is separated with the fat in cream and butter manufacture: they also vary little from source to source, except in iron content. Whey contains less Ca and P than separated milk or buttermilk, because much of the P and a proportion of Ca and other minerals in milk are removed with the curd in cheese manufacture but whey is a valuable source of many minerals. Meat and fish by-products from abattoirs and processing factories, given primarily to supply protein, also supply minerals, especially Ca, P (Table 2.6), Fe, Zn and Se. Commercial meat meals and fish meals, on the other hand, are usually rich in Ca, P, Mg and Zn, depending on the proportion of bone present. Ash content of meat and bone meals varies from 4% to 25% and consists mostly of Ca and P. A typical meat meal contains 6–8% Ca (and 3–4% P), about 100 times (10 times) more than the cereal grains they commonly supplement (Table 2.2). Meat and bone meals vary in particle size, which affects availability of Ca (p. 85) and P (p. 147) to poultry. Fishmeal contains appreciable amounts of NaCl as well as Ca and P. Use of animal by-products has been restricted in Europe for fear of transmitting harmful pathogens but recently eased for fish meals. Mineral content varies greatly between sources, due partly to differences in raw materials and processing

Table 2.6. Macromineral concentrations (g kg^{-1}DM) in animal by-products: data from an older (Givens *et al*., 1990, standard deviations in parentheses) and a newer source (italicised values, NRC, 2012).

	Ca	P	Mg	Na	K	S
Blood meal	0.6 (0.26)	1.5 (0.19)	0.2 (0.01)	3.6 (0.32)	1.9 (0.12)	8.4 (0.25)
	0.5	2.1	1.1	6.3	1.5	4.7
Butter milk	*14.4*	10.1	5.2	9.0	9.0	0.9
Feather meal	5.6 (0.07)	3.1 (0.06)	0.4 (0.04)	1.4 (0.01)	1.5 (0.01)	18 (0.6)
	4.1	2.8	2.0	3.4	1.9	13.9
Fish meal	56 (6.0)	38 (14.5)	2.3 (0.3)	11.2 (1.5)	10.2 (1.3)	5.0
	43	29	1.3	–	6.2	–
Meat and bone meal	90 (13)	43 (6.5)	2.2 (0.2)	8.0 (1.0)	5.2 (0.6)	9.2 (0.72)
	109	53	4.1	6.1	6.5	3.8
Whey	*6.2*	6.9	1.3	7.0	19.6	7.2

methods. Blood meal, liver meal and whale meal contain only 2–3% total ash and are low in Ca and P, but they can be valuable sources of Fe, Cu, Se and Zn.

Marine Sources of Minerals

Materials of marine origin are popular with organic farmers as sources of minerals, both as soil fertilizers and food supplements. Mineral composition of marine materials reflects that of seawater and the needs of marine biota rather than livestock. Brown seaweeds, for example, contain 14–35% ash, of which 2–3% is K, 0.9–4.0% Na and similar proportions of Ca, sulfate and chloride. Of the trace elements, I is most abundant, some species containing up to 11g kg^{-1}DM (Makkar *et al*., 2016). The prolific growth rate of seaweeds is being investigated as a cheap source of protein for livestock but will supply adventitious minerals, some, like Ca, in unique, soluble forms. Mass culture of marine algae is being investigated for similar purposes. Some fish species contain Ca in hyperabsorbable forms that may have uses in animal nutrition. Scallop fishing produces large volumes of shell waste that can be pulverized to form Ca supplements of equal biological value to limestone as can dead coral.

Forms of Minerals in Feeds

Minerals occur in plants and foods in diverse forms that vary in availability to livestock. Some Ca in soybeans is bound to proteins differing in molecular weight, those of low weight being preferentially absorbed but stabilities and availabilities of such proteins during digestion have yet to be studied. Phytate is an inositol hexaphosphate (IP6) and provides most of the organic P in commercial pig and poultry rations but forms unabsorbable complexes with added Ca and several trace elements, lowering mineral availability. The highly absorbable storage protein, phytoferritin, binds Fe and Mn as does less phytate in cereals. However, poultry absorb Fe well from wheat, in which it occurs as a well-absorbed mono-ferric phytate (Morris and Ellis, 1976). Enzymes such as cytochrome oxidase and glutathione peroxidase contribute Cu and Se, respectively, to fresh plant sources. Certain components of milk play a crucial part in neonatal development. For example, Fe is present, bound to lactoferrin, a form that can be used by animals but not by some potentially harmful gut pathogens. Milk also contains minerals as enzymes (e.g. Mo in xanthine oxidase) and hormones (triiodothyronine): the former is present in milk fat globule membranes and has antimicrobial and anti-viral properties; the latter facilitates early maturation of the intestinal mucosa in sucklings. Much of the Zn in milk is bound to casein and the precise binding may influence the ability of one species to absorb Zn from the milk of another. Milk also contains other mineral-binding components such as citrate.

Measuring the 'Mineral Value' of Feeds

The nutritional value of minerals in feeds (and supplements) depends on the proportions that can be utilized by the animal, a property termed 'bioavailability' (for reviews see Hazell, 1985 and

Ammerman *et al*., 1995). Bioavailability has four components:

- **accessibility** – potential access of dietary mineral to site(s) of absorption mucosa;
- **absorbability** – capacity of the gut mucosa to take up and transfer accessible minerals;
- **retainability** – potential retention of transferred minerals by the body;
- **functionality** – potential for retained minerals to enter functional forms in tissues.

The many methods for measuring bioavailability (Suttle, 1985; Ammerman *et al*., 1995) generally rank feeds satisfactorily but few yield maximal, absolute values that incorporate all four components of bioavailability. The animal's genotype, age, mineral status and needs can affect absorbability, retainability and functionality.

Measuring Accessibility

Measuring accessibility *in vitro* avoids animal influences but early methods generally lacked credibility. Reliance on simplistic attributes, such as solubility of plant mineral in water or unphysiological extractants (e.g. citric acid), produced results that correlated poorly with *in vivo* assessments of availability, but methods have improved. Solubility after sequential simulation of gastric and intestinal digestion was used to indicate accessibility of phytate P in corn and SBM to poultry (Walk *et al*., 2012). Under similar conditions, completeness of extrinsic labelling with an isotope (e.g. ^{65}Zn) can predict tracer accuracy *in vivo* (Schwarz *et al*., 1982). With a final dialysis stage added, interlaboratory collaboration yielded a standardized procedure for measuring what was termed the 'bioaccessibility' of Fe (Luten *et al*., 1996). Relative estimates approximating to 'absorbability' can be obtained by culturing mucosal cells in the dialysate (e.g. Caco 2 cells (p. 333). For ruminants, accessibility of minerals to rumen microflora is crucial and, for Co, measurable as rates of vitamin B_{12} synthesis in continuous cultures of rumen microbes. However, cellular uptake of mineral alone does not always equate to availability. Uptake of selenomethionine by cultured rumen microbes (Galbraith *et al*., 2016) or Caco 2 cells (Zeng *et al*., 2008) overestimates functionality (p. 28). Such *in vitro* techniques will be increasingly used but exclude interactions between feeds: results will always have to be validated *in vivo,* a much more difficult task.

Measuring Absorbability

Apparent absorption

Mineral values have been most commonly measured as differences between intake and faecal excretion of mineral in balance or 'digestibility' trials, i.e. the fractions 'apparently' absorbed (*AA)*. If a given mineral is maximally absorbed, usable for all functions dependent on that mineral and minimally excreted (i.e. not being supplied in excess of need) then

- *AA* coefficients can indicate absorbability, the potential mineral value of any source.

Unfortunately, minerals have usually been provided in excess of need, causing absorbability to be widely underestimated (e.g. Ca in alfalfa; p. 78). In poultry, AA_P gives no indication of absorbabilty of cereal P because excess absorbed P re-enters the gut in urine. By adding inert, unabsorbable markers such as chromic and titanium oxides to poultry diets, disappearance of mineral from the gut can be measured before digesta reach the cloaca: 'pre-caecal digestibility' (PCD), the equivalent of *AA*, is being widely measured. Such markers colour the faeces, allowing faecal collection to be synchronized with feeding period: their use in pigs has been reviewed (She *et al*., 2017) but outcomes are awkwardly described as 'apparent total tract digestibilities' (ATTD) for no good reason.

'Standardized digestibility'

Absorbability is invariably underestimated by *AA* because absorbed mineral renters the gut from endogenous sources and is partially lost in excreta (p.10). Standardized (S) values correct ATTD for minimum faecal endogenous loss (^{m}*FEL*: p.6) and STTD became the recommended method for describing availability of P in pigs (NRC, 2012) and poultry (WPSA, 2013), for which SPCD is the equivalent term. The original correction method (Fan *et al*., 2001) broke the golden rule for determining mineral requirements – that no other mineral or nutrient should be lacking – by using SBM and corn as sole incremental sources of Ca, P and protein in a basal diet that lacked all three nutrients. Gelatin has since been used as a Ca- and P-free protein source but does not eliminate problems of unstable mineral status and unrepresentative diet (p. 140). Nevertheless, STTD is being widely used to characterize Ca (Gonzales-Vega and Stein, 2014) and P sources for pigs (She *et al*., 2017).

Substantial variation in $^{m}FEL_{Ca}$ (Misiura *et al.*, 2018) and corrected digestibilities of P in pigs (p. 140) and poultry (p. 141) questioned the efficacy of 'standardization'. An international ring test, using a common SBM source and WPSA (2013) methodology, gave SPCD coefficients ranging from 0.09 to 0.56 in broiler chicks (Rodehutscord *et al.*, 2016); differences in pre-treatment diets and housing conditions were implicated. However, estimates of $STTD_p$ and $^{m}FEL_p$ in chicks remain method-dependent with set pre-treatment conditions (Perryman *et al.*, 2017). $^{m}FEL_p$ increased by more than 50% with DMI in pigs given diets containing lignocellulose (Bikker *et al.*, 2017).

- Current standardization methods do not improve upon AA_p in describing the value of P-rich feeds and blends.

Ligated intestinal loops

Superficially, complications of measuring mineral disappearance from the gut *in vivo* can be avoided by using simple 'hybrid' methods such as the ligated intestinal loop. Here, a known amount of mineral in buffered, 'physiological' saline is placed in a flushed-out segment of gut in an anaesthetized animal, and rate of mineral disappearance is equated to rate of absorption. Thus, absorbability of iron as $FeSO_4$, $Fe_2(SO_4)_3$ and an Fe:glycine chelate were compared in broiler chicks and the chelate allegedly shown to be of superior availability and less inductive of divalent metal-binding protein (Jia *et al.*, 2015). However, conditions in the gut lumen were far from 'physiological'. Injecting solutions containing 333 mg Fe l^{-1} might raise free Fe^{2+} and $^{3+}$ concentrations sufficiently to cause mucosal dysfunction. With 40 mg Zn l^{-1}, interactions of phytate with inorganic and organic sources differed (Yu *et al.*, 2010) but are of dubious nutritional significance because effects of digestion in the acid gizzard and stomach in a Ca-rich milieu were avoided. Use of the ligated loop to study effects of chelation on Mn absorption may have produced false concordance with dietary treatments (p. 361).

Estimating true absorption using isotopes

Radioactive isotopes have long been used to measure faecal endogenous losses (*FEL*) of mineral in balance studies and derive true absorption coefficients (*A*) from *AA* (p. 10), re-entry of absorbed mineral into the gut being measured by labelling the pool from which gut secretions are drawn.[v] Sustained faecal excretion of isotope after unabsorbed oral dose of isotope has passed through the animal is of entirely endogenous origin and amounts of stable mineral being lost can be calculated from post-absorptive rates of faecal excretion from a body pool of known specific radioactivity, using kinetic models (p. 4). Similarly, mineral excreted in urine must once have been absorbed and absorptive efficiencies can be calculated from kinetic models of whole-body retention after oral and parenteral administration of isotope in small animals (Fig. 2.4). 'Hybrid' techniques can combine parenteral isotope excretion with stable mineral retention. Thus, P absorption was measured in broiler chicks by measuring endogenous losses of P^{32} after intramuscular dosage and adding the fraction of P^{32} lost via urine and faeces to the fraction of dietary P retained in the carcase.

To give valid information on mineral absorption, an isotope must mimic physiological and biochemical behaviour of all forms of mineral as they flow from gut through body and are excreted (e.g. Buckley, 1988). As mineral accessibility of feed sources decreases (p. 26), orally administered tracer (usually given as soluble and highly accessible inorganic salts) becomes more likely to overestimate mineral value. Failure to recognize the distinctive metabolism

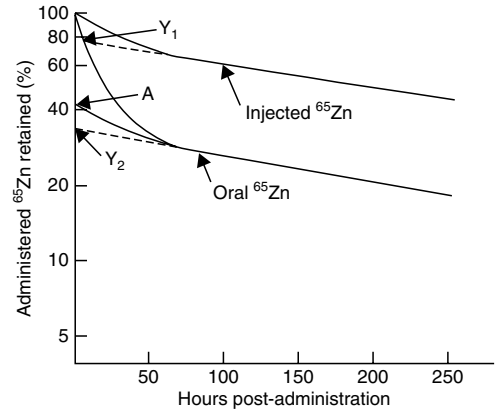

Fig. 2.4. Fractional absorption (*A*) of zinc in rats, calculated from a comparison of retention curves for Zn^{65} given in feed and by intramuscular injection: *y* intercept of the extrapolated retention curve for oral ^{65}Zn, Y_2, is simply divided by Y_1, corresponding intercept of the curve for injected ^{65}Zn. (From Heth and Hoekstra, 1965)

of different forms of Se in feeds may have given aberrant values for A_{Se} in hay, using selenite as tracer (Krishnamurti *et al*., 1997) (p. 391). When tracer is given parenterally, only accessible and absorbable mineral is 'traced' and errors are generally reduced, albeit incompletely with Mn (Davis *et al*., 1992). Such problems are more likely to arise with trace- than with macro-elements. Accuracy of tracing can be improved by growing plants in isotope-enriched (radioactive or stable) nutrient solutions ('intrinsic labelling') but the technique is laborious and costly to apply to large farm animals. Furthermore, results with laboratory animals were often similar to those obtained with extrinsic labelling (Johnson *et al*., 1991). The natural presence of stable mineral isotopes in feeds promised to simplify assessments of trace element availability (Lowe and Broome, 2003). Natural isotope ratios can be perturbed by dosing animals with the least-abundant isotope: absorption coefficients are calculated from the rates at which normal abundance ratios are restored (Fairweather-Tait *et al*., 1989). Stable isotope use avoids hazards and safety constraints associated with radioisotopes but requires expensive equipment[vi] and the amounts administered may disturb mineral metabolism.

Measuring retainability

Retainability is measurable as the fractional retention (R) of mineral in balance and carcase analysis studies when mineral intakes are low-to-marginal, but is often a small yet variable statistic that bears cumulative errors of estimating intake, faecal excretion and urinary excretion. When excretion or secretion of mineral is closely correlated to mineral intake (e.g. urine Mg or milk Mo and I): slope ratios indicate relative availabilities. If retention and *FEL* remain constant, each slope represents absorbability mineral source: thus, A_{Mg} has been assessed from rates of urinary Mg excretion (see Fig. 5.8). Relative retainability is also indicated by comparing relationships between mineral concentrations or contents of the whole body or a body part (e.g. liver, tibia, haemoglobin) and diet (Littel *et al*., 1995, 1997) but are more suited to Type 1 than Type 2 elements (see Fig. 3.2) and subject to dietary and husbandry influences (Fig. 1.4; Suttle, 2017). Curvilinearity can give false relative values unless comparisons are made across appropriate ranges of intake for compared sources (e.g. p. 356 and p. 424) and retention of one mineral can depend on retention of another (e.g Ca on P; Fig. 6.6). Relative biological values (RBV) cannot be converted to absolute values by meta-analysis (Shastak and and Rodehutscord, 2015). Absolute values for R can be obtained from carcase retention data by adjusting for ^{m}FEL, given by the intercepts of allometric regression against mineral intake (Zanetti *et al*., 2017b) but the method is prone to error (p. 9). If an organ can be biopsied, is responsible for a high proportion of total mineral accretion and of predictable weight, A can be calculated from changes in mineral concentration in the organ (e.g. liver Cu in sheep and cattle; ARC, 1980). Comparing responses in plasma Cu (i.e. partial retention) to oral and intravenous repletion and applying the comparative balance principle (p. 26) estimated A_{Cu} (Suttle, 1974): the method is applicable to other elements (Suttle, 2000).

- Absorbability is underestimated by R when: mineral intake exceeds need; mineral is lost by sweating (e.g. K and Na in tropical conditions); mineral is lost by exhalation (of Se and S) and urinary excretion (most Type 2 elements: Fig. 3.2).
- Availability is overestimated by R if a mineral either accumulates in poorly exchangeable forms (e.g. tissue calcification, urinary calculi, mucosal ferritin, Cu and Zn as metallothionein in kidney; selenomethionine in muscle protein) or is accompanied by absorbable agonists or antagonists (e.g. thiomolybdates (Fig 17.11)).
- Experiments to establish mineral value by carcase retention should be subject to rules like those laid down to measure $STTD_P$ for poultry (WPSA, 2013) (p.6).

Measuring functionality

The ability of a feed to ameliorate, prevent or potentiate clinical signs of mineral deprivation indicates functionality and is a composite measure of bioavailability, reflecting accessibility, absorbability, retainability and physiological potency (p. 26).[vii] Ranking in terms of functionality may not agree with rankings of availability by other methods. When natural feedstuffs are added to mineral-deficient diets as the major mineral source and non-specific functional indices, such as bone strength and growth rate are used, the tested feed may provide other essential nutrients or antagonists that affect performance (Suttle, 1983). A phytase-rich grain, like wheat or rye, will provide more absorbable P but also more residual *myo*-inositol (p. 535). Phytase also improves amino acid availability and improvements in growth and bone ash in phytase-supplemented broilers

reflect more than improvements in P availability (Perryman *et al*., 2017). Capacity to stimulate metal-responsive genes and synthesis of specific functional metalloproteins has been introduced for assessing Zn (p. 434) and Mn (p. 361) availabilities in pigs and broilers but raised new problems of interpretation.

Assessing mineral value in feed blends

Single foodstuffs rarely comprise the entire ration of livestock because individual feeds can rarely meet requirements for all nutrients. When ingredients are mixed to provide a balanced diet, interactions can occur that affect mineral value, which is not simply the arithmetic mean of values for constituents. Standardization of digestibility (p. 26) is alleged to improve the additivity of individual feed values for phosphorus value in blends for pigs but there are larger sources of error (p. 26). In striving for dietary balance with feed constituents of contrasting composition, resultant iso-energetic and isonitrogenous blends may have no practical significance. Some studies have been complicated unnecessarily by perceived needs to preserve arbitrary Ca:P ratios, the latter as phytate (*Py*P), non-*Py*P or digestible P (e.g. p. 139.)[viii] Availability of Cu in total mixed rations for ruminants is probably much higher than that suggested by availabilities for individual constituents (p. 276). Availabilities of minerals in supplements have been studied far more than those in feeds but can be dependent on those of the supplemented feed, as shown in Fig. 2.5. The diet based on soy bean hulls had a lower AA_{Ca} than one based on corn silage but $CaHPO_4$ was more available when added to the former ration. The same contrast was evident in a related study (Zanetti *et al*., 2017c) but may have been attributable to increased growth rate (and therefore demand for Ca) as $CaHPO_4$ alleviated a P deficiency. The sugarcane diet may also have lowered AA_{Ca} in $CaHPO_4$ (Fig. 2.5). Similar calculations from data for young, penned bulls, given or denied supplements of Ca-rich cassava leaves to improve performance on rice straw and para hay, low in Ca (3g Ca kg^{-1}DM; Sath *et al*., 2013), placed AA_{Ca} at 0.24 for cassava leaves compared with 0.18 in the dry roughages but growth was poor on diets also low in P (1.3g kg^{-1}DM). Low A_p in some corn-rich diets (Roque *et al*., 2007; Zanetti *et al*., 2017b, c) may be attributable to poor utilization of P in ground corn.

- Published estimates of 'mineral value' of supplements, feeds and blends are often characteristics of experiments rather than dietary components.

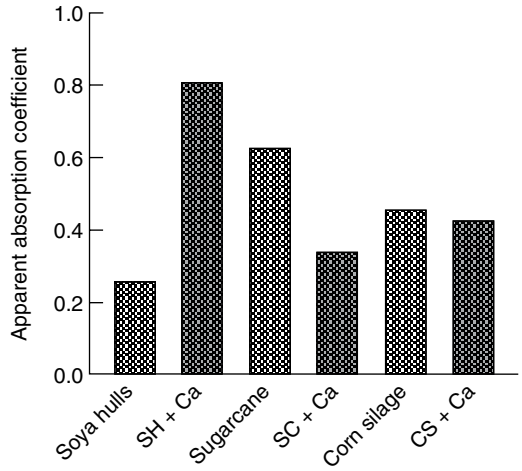

Fig. 2.5. Apparent availability (*AA*) of calcium (Ca) to Nellore bulls given one of three contrasting diets, rich in soybean hulls (SBH), sugarcane (SC) or corn silage (CS), all low in phosphorus (1.4–2g P kg^{-1}DM), with Ca as $CaHPO_4$ added to each: values in supplements were calculated as the reciprocal of increase in faecal Ca/Ca intake. (Data from Zanetti *et al*., 2017b)

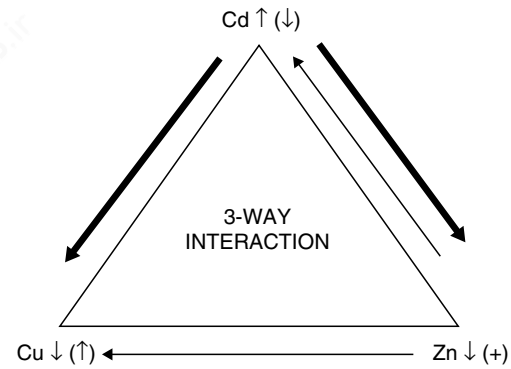

Fig. 2.6. Mutual antagonisms between copper, cadmium and zinc lead to complex three-way interactions. Raising the level of one interactant can lower the status of the other two, as shown for Cd (↑). However, inequalities in the strength of particular interactions, such as the strong antagonism of Cu by Cd, may mean that supplements of Zn (+) may raise (↑) Cu status by reducing (↓) the Cd–Cu interaction.

Interactions between Minerals

Interactions arise when minerals share metabolic pathways but evolution has probably minimized the impact of those that can be harmful. Uptake and export of Fe and Mn by and from intestinal mucosal cells share dependency on dependent DMT 1 and ferriportin: the

same pairing assists uptake by cells in other tissues. Similarities end there because Fe is a Type 1 mineral, absorbed according to need but avidly stored, whereas Mn is a Type 2 mineral, minimally absorbed, poorly stored and readily excreted. Absorption of Fe is regulated by body stores of ferritin (p. 329). Under most circumstances, differences in metabolism prevent interaction but dietary excess of one element can induce insufficiency of the other by competing for both carriers at many sites (e.g. between Fe and Mn; p. 354). Cu and Zn each have specific transporters but can both be taken up by DMT though not transported by ferroportin. Again, dietary imbalance can result in reciprocal deficiencies (Fig. 3.4) but the two antagonists are usually kept apart, Cu being a Type 1 and Zn a Type 2 mineral (Fig. 3.2). Thus,

- potential antagonists are normally and necessarily kept apart but if dietary imbalances are contrived, each can induce deprivation of the other.

The ability of moderately bound chelates of Cu, Fe, Mn and Zn to be absorbed intact independently of specific transporters and DMT1, and differentially induce ferroportin if proven might allow each chelate to induce genomic changes when compared with inorganic sources. Such changes have invariably been welcomed as a beneficial sign of hyperavailability but:

- absorbable chelates of Cu, Fe, Mn and Zn may, by subverting normal homeostatic mechanisms, induce genomic aberrations that do not reflect availabilities.

Interactions between minerals from feeds are a major cause of variation in availability and also influence potential toxicity. An interaction is demonstrated when responses to an element, added at two or more levels of another element, are dissimilar. An example of the interaction between Fe, Mo and Cu is given in Table 2.7. Large groups are often needed to show significant interactions because the interaction term has few degrees of freedom (df) in the analysis of variance (only 1 df in a 2×2 experiment). As a result:

- the frequency of physiological interactions between minerals is grossly underestimated.

Interactions between minerals can affect each component of bioavailability (p. 26) and two or more components may be affected simultaneously. The net effect is usually a change in retention and occasionally function throughout the body, but there may be only localized changes in tissue distribution in one organ (e.g. kidney), if the interactions occur post-absorptively. Interactions between minerals usually have negative effects, but they can be beneficial: small Cu supplements can enhance Fe utilization but large Cu supplements can increase Fe requirements.

Predicting outcomes of interactions

Strong interactions can usefully be predicted between: K and Mg (p. 140), Cu, Mo and S in ruminants (Fig. 11.10); between Zn, Ca and P (Fig. 16.8), phytate P and Zn absorption in non-ruminants. Outcomes are harder to predict when three mutually antagonistic elements are involved, such as Cu, Cd and Zn[ix] (Fig. 2.6). The clinical outcome of such three-way interactions will depend upon which element is the first to constrain

Table 2.7. Outcome of a 2×2 factorial experiment investigating separate and combined antagonisms of iron and molybdenum towards copper, using liver copper concentrations to assess copper status in cattle (Bremner *et al.*, 1987). Different totals on diagonals (italicized) indicate a strong interaction almost equal to apparent main effects, indicated by different row (for Mo) and column (for Fe) totals (bold): effects of molybdenum and iron must be qualified by reference to the accompanying antagonist.

		Fe treatment			
		0	+	**Mo totals**	
Mo treatment	0	1.18 (***0.52***)	0.35	**1.53**	
		(***1.32***)			
	+	0.17	0.14	**0.31**	
	Fe totals	**1.35**	**0.49**	**1.89**	**mmol Cu kg^{-1}DM**

performance. This, in turn, will be determined by the strength of separate antagonisms, which are each doubly dose-dependent. Thus, Cd is capable of inducing either Cu or Zn deprivation. Prediction of three-way interactions is also difficult if there is a shared potentiator. Thus, S (as sulfide) is necessary for both Fe- and Mo-mediated antagonism of Cu in ruminants. When Fe and Mo are added together in a diet marginal in Cu, there is no additive antagonism towards Cu (Table 2.7; Bremner *et al.*, 1987), possibly because Fe and Mo compete for sulfide. Researchers of interactions between Ca, phytate and inorganic P in broilers used 'nutritional geometry' to describe the complex matrix. The primary mechanisms whereby minerals interact to affect bioavailability are:

- formation of unabsorbable complexes in the gut (e.g. metal phytates);
- competition between cations for a divalent cation transporter (Fe and Mn');
- competition between anions for common absorption channel (SO_4^{2-} MoO_4^{2-}); and
- induction of non-specific metal-binding proteins (ferritin by Fe and metallothionein by Cu, Zn or Cd).

Experiments with elaborate treatment matrices, feasible in the small broiler chick, have been advocated to disentangle complex interactions between Ca, phytate P and non-phytate P but the pragmatic and environmentally more sensitive approach might be to minimize variability by phytase supplementation (p. 147)

Mineral Cycles, Losses and Legacies

Mineral status in farm ecosystems can change, due to substantial inputs of minerals, just described, withdrawals of minerals in harvested crops and/or livestock products and variable recycling via excreta. Fig. 2.7 illustrates effects of N fertilizer usage on the P cycle in a dairy enterprise on tropical grass pasture. Harmful effects of excessive inputs of P on watercourses were modelled (see Turner *et al.*, 2002) and the continuing need for remedial measures is discussed in the final chapter. However, around 80% of superphosphate, continuously applied at 1-33kg P ha^{-1} to grazed pasture for 20 years remained in the surface soil: only 6% was removed in animal products and a similar proportion was exported to other paddocks (McCaskill and Cayley, 2000). Sulfur applied in superphosphate disappeared into sub-surface soils, displacing soil K as it did so, but export via sheep totalled only 9%. A 'legacy' from continuous use of P fertilizers

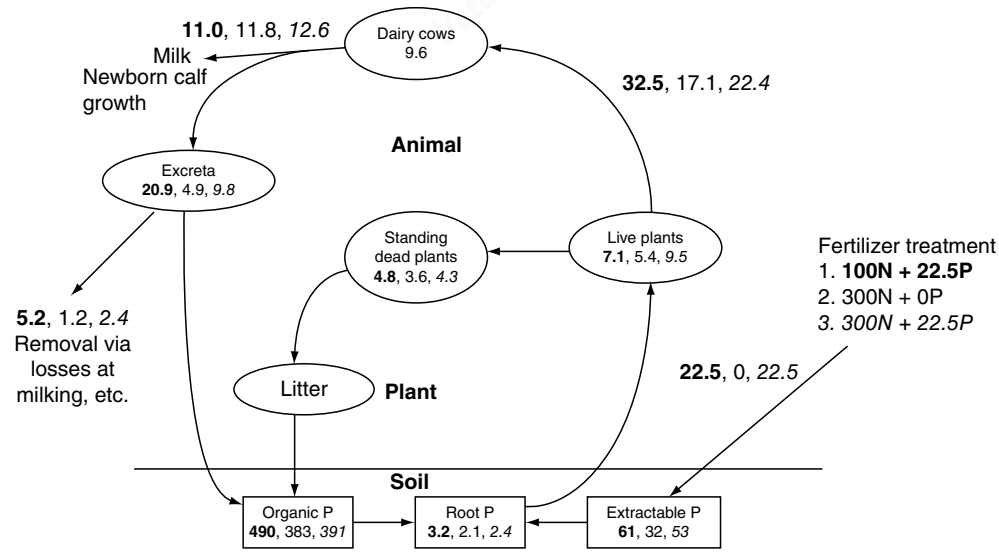

Fig. 2.7. Example of a mineral cycle, showing pool sizes and flows of phosphorus (kg P ha^{-1}) for a dairy herd grazing tropical pasture under three different fertilizer regimens (after Davison *et al.*, 1997): raising input of nitrogen (N) and P (treatment 3 versus 1) shifted route of recycling from excreta to plant litter, a less efficient process; application of P prevented a steady decline in extractable soil P (treatment 3 versus 2); treatment 3 increased milk yield by increasing herbage mass, not P status of the cow.

in Canada was sufficient to sustain wheat yields for 15 years after the last application. Movements of potentially toxic Cd from fertilizer inputs to food chain are described on p. 487. Net flows of utilizable mineral to grazing animals can vary widely from season to season and from year to year (Suttle, 2017). Where herbage barely meets animal requirements for minerals, atmospheric, climatic or seasonal influences may determine incidence and prevalence of deprivation.[x] The cyclical nature of mineral nutrition necessitates regular review of need for intervention.

Notes

[i] Median values for wheat grain Se were 0.80 and 0.05 mg kg^{-1} dry matter (DM) on soils high and low in Se, respectively, in USA (Scott and Thompson, 1971) and even lower (down to 0.005) in parts of New Zealand, Finland and Sweden. Sugarcane tops contained 6–14 times more Se in seleniferous than in non-seleniferous areas of India (Dhillon and Dhillon, 1991).

[ii] Total ash, 40–122, Ca, 0.9–5.5 and P 0.5–3.7 g kg^{-1}DM amongst 58 species in East Africa; Co, 0.05–0.14, Cu, 4.5–21.1 and Mn. 96–815 mg kg^{-1}DM amongst 17 species grown in Florida (Underwood and Suttle, 1999).

[iii] White lupins (*Lupinus albus*) contained potentially toxic levels of manganese (817–3397 mg kg^{-1}DM), 10–15 times as much as other species growing on the same sites in Western Australia (for citations, see Underwood and Suttle, 1999). *L. albus* seed was three times richer in cobalt than *L. augustifolius* but zinc and selenium concentrations were similar in the two species (29–30 mg Zn kg^{-1}DM and 57–89 µg Se kg^{-1}DM). Wheat grain harvested from the same sites contained 22 mg Zn and 23 µg Se kg^{-1}DM (White *et al*., 1981).

[iv] At 16 sites in England and Wales, herbage concentrations of Mg, Na and Zn rose, Ca, Mn, Mo and S fell, while K, Co and Cu were not consistently changed over 4 years (Hopkins *et al*., 1994). Risk of deficiency only increased where availability was likely to decrease (for Cu and Mg).

[v] Following a single parenteral injection of radioisotope (^{45}Ca), total collection of faeces and measurements of total excretion of both radioisotope and stable mineral, Comar (1956) calculated FEL_{Ca} in cattle by assuming that ratios of radioactive to stable Ca in gut secretions and plasma samples became the same, once equilibrium was reached (urine or saliva samples can also be used), thus:

$$\text{Absorption} = I - (F - FEL)$$

where, *I* is intake and *F* is faecal excretion of calcium. Giving radioisotopes orally *and* intravenously, either to the same animals after an appropriate clearance phase or simultaneously to two matched groups (Atkinson *et al*., 1993), absorption was given by:

$$\text{Absorption} = O - (Fo - Fi)$$

where *Fo* and *FI* denote faecal excretion of oral (O) and intravenous doses (I), respectively. This 'comparative balance' technique is useful for elements that may contaminate experimental environments, polluting both feed and faeces (e.g. iron, zinc and copper) and invalidating a conventional balance study. Where two isotopes of the same element are suitable (e.g. ^{55}Fe and ^{59}Fe), (Fairweather-Tait *et al*., 1989), comparative balance can be assessed after simultaneous oral and intravenous dosage: five elements were assessed simultaneously in piglets (Atkinson *et al*., 1993). Isotope ratio in milk after simultaneous oral administration of $Na^{131}I$ and $Na^{125}IO_3$ in lactating goats indicated that 0.86 less iodine was absorbed when given as iodate rather than iodide (Lengemann,1969).

[vi] An inductively coupled 'plasma' generator and mass spectrometer are required and stable isotope techniques have only been used to estimate Cu absorption in livestock (Buckley, 1988), to the knowledge of the author.

[vii] Relative availability of plant and animal feed sources of Se for chicks was assessed from their ability to prevent exudative diathesis and pancreatic atrophy (Cantor *et al*., 1975) and differed markedly between sources. In a chick growth assay using a Zn-deficient basal diet, Zn from wheat, fish meal and non-fat milk was 59%, 75% and 82%, respectively, as available as that in $ZnCO_3$ (Odell *et al*., 1972). Rates of recovery from or induction of anaemia have been measured as blood haemoglobin responses in Fe-depleted animals and compared with those to a standard source of high bioavailability ($FeSO_4$). The shear strength of bone can indicate relative P availability in pigs (Ketaren *et al*., 1993).

[viii] Blends of distillers' dried grains solubles (DDGS) with corn (48:50) had a mean (s.d) AA_P coefficient of 0.51 (± 0.025) and corn alone a lower value of 0.19 (Pedersen *et al*., 2007). Assuming that corn retained its 'phosphorus value' when mixed with DDGS, the latter's 'phosphorus value' was upgraded to 0.59. Lack of calcium in both feeds was corrected by adding limestone but twice as much was added to corn than to blends, giving dietary Ca of 8.4 and 3.9–5.4g kg^{-1}DM, respectively: the advantage for DDGS over corn would probably have been nearer twofold in blends that were equally low in Ca.

[ix] Since Cd and Zn each separately impaired Cu metabolism in sheep, addition of both elements was expected to severely compromise Cu status (Campbell and Mills, 1979). However, Zn protected lambs from the Cu-depleting effects of Cd and exceedingly high levels of dietary Zn were needed to exacerbate Cu depletion (Bremner and Campbell, 1980). When each element can negatively affect the other, a supplement of any one element may have a positive net effect either by countering an antagonism against itself or neutralizing the antagonistic properties of the second element upon the third.

[x] Risk of 'swayback' in newborn lambs, deprived of Cu *in utero*, was high after mild winters in the UK; decreased inputs of Cu from supplementary feeds and increased ingestion of antagonists in soil, not covered by snow, were implicated (Suttle *et al*., 1975).

References

Alloway, B.J. (2008) *Zinc in Soils and Crop Nutrition*. 2nd edn. IZA Publications, International Zinc Association, Brussels, pp. 1–16.

Ammerman, C.B., Baker, D.H. and Lewis, A.J. (1995) *Bioavailability of Nutrients for Animals*. Academic Press, New York.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

Arosemena, A., DePeters, E.J. and Fadel, J.G. (1995) Extent of variability in the nutrient composition within selected by-product feedstuffs. *Animal Feed Science and Technology* 54, 103–120.

Atkinson, S.A., Shah, J.K., Webber, C.E., Gibson, I.L. and Gibson, R.S. (1993) A multi-element tracer assessment of the fractional absorption of minerals from formula with additives of calcium, phosphorus, zinc, copper, and iron in young piglets. *Journal of Nutrition* 123, 1586–1593.

Berger, L.L. (1995) Why do we need a new NRC data base? *Animal Feed Science and Technology* 53, 99–107.

Bikker, P., van der Peet-Schwering, C.M.C., Gerrits, W.J.J., Sips. J.V., Walvoort, C. and van Laar, H. (2017) Endogenous phosphorus losses in growing-finishing pigs and sows. *Journal of Animal Science* 95, 1637–1643.

Bramley, R.G.V. (1990) Cadmium in New Zealand agriculture. *New Zealand Journal of Agricultural Research* 33, 505–519.

Bremner, I. and Campbell, T.K. (1980) The influence of dietary copper intake on the toxicity of cadmium. *Annals of the New York Academy of Sciences* 355, 319–332.

Bremner, I., Humphries, W.R., Phillippo, M., Walker, M.J. and Morrice, P.C. (1987) Iron-induced copper deficiency in calves: dose–response relationships and interactions with molybdenum and sulfur. *Animal Production* 45, 403–414.

Buckley, W.T. (1988) The use of stable isotopes in studies of mineral metabolism. *Proceedings of the Nutrition Society* 47, 407–416.

Burridge, J.C., Reith, J.W.S. and Berrow, M.L. (1983) Soil factors and treatments affecting trace elements in crops and herbage. In: Suttle, N.F., Gunn, R.G., Allen, W.M., Linklater, K.A. and Wiener, G. (eds) *Trace Elements in Animal Production and Veterinary Practice*. British Society of Animal Production Occasional Publication No. 7, Edinburgh, pp. 77–86.

Campbell, J.K. and Mills, C.F. (1979) The toxicity of zinc to pregnant sheep. *Environmental Research* 20, 1–13.

Cantor, A.H., Scott, M.L. and Noguchi, T. (1975) Biological availability of selenium in feedstuffs and selenium compounds for prevention of exudative diathesis in chicks. *Journal of Nutrition* 105, 96–105.

Coates, D.B., Kerridge, P.C., Miller, C.P. and Winter, W.H. (1990) Phosphorus and beef production in Australia. 7. The effect of phosphorus on the composition, yield and quality of legume based pasture and their relation to animal production. *Tropical Grasslands* 24, 209–220.

Comar, C.L. (1956) Radiocalcium studies in pregnancy. *Annals of the New York Academy of Sciences* 64, 281.

COSAC (1982) Trace elements in Scottish soils and their uptake by crops, especially herbage. In: *Trace Element Deficiency in Ruminants*. Report of a Scottish Agricultural Colleges (SAC) and Research Institutes (SARI) Study Group. SAC, Edinburgh, pp. 49–50.

Csapó, J., Martin, T.G., Csapó-Kiss, Z.S. and Házas, Z. (1996) Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days. *International Dairy Journal* 6, 881–902.

Davis, C.D., Wolf, T.L. and Greger, J.L. (1992) Varying levels of dietary manganese and iron effect absorption and gut endogenous losses of manganese by rats. *Journal of Nutrition* 122, 1300–1308.

Davison, T.M., Orr, W.N., Doogan, V. and Moody, P. (1997) Phosphorus fertilizer for nitrogen-fertilized dairy pastures. 2. Long-term effects on milk production and a model of phosphorus flow. *Journal of Agricultural Science, Cambridge* 129, 219–31.

De Vos, W., Travainen, T. and Reeder, S. (eds) (2006) *Geochemical Atlas of Europe. Part 2: Interpretation of Geochemical Maps, Additional Tables, Figures, Maps, and Related Publications*. Geological Survey of Finland.

Dersjant-Li, Y., Evans, C. and Kumar, A. (2018) Effect of phytase dose and reduction in dietary calcium on performance, nutrient digestibility, bone ash and mineralization in broilers fed corn-soybean meal-based diets with reduced nutrient density *Animal Feed Science and Technology* 242, 95–110.

Dhillon, K.S. and Dhillon, S.K. (1991) Accumulation of selenium in sugarcane (*Sacharum officinarum* Linn) in seleniferous areas of Punjab, India. *Environmental Geochemistry and Health* 13, 165–170.

Fairweather-Tait, S.J., Johnson, A., Eagles, J., Gurt, M.I., Ganatra, S. and Kennedy, H. (1989) A double label stable isotope method for measuring calcium absorption from foods. In: Southgate, D., Johnson, I. and Fenwick, G.R. (eds) *Nutrient Availability: Chemical and Biological Aspects*. Royal Society of Chemistry Special Publication No. 72, Cambridge, pp. 45–47.

Fan, M.Z., Archbold, T., Sauer, W.C., Lackeyram, D., Rideout, T., Gao, Y., de Lange, C.F.M. and Hacker, R.R. (2001) Novel methodology allows simultaneous measurement of the true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. *Journal of Nutrition* 131, 2388–2396.

Fordyce, F.M., Masara, D. and Appleton, J.D. (1996) Stream sediment, soil and forage chemistry as predictors of cattle mineral status in northeast Zimbabwe. In: Appleton, J.D., Fuge, R. and McCall, G.H.J. (eds) *Environmental Geochemistry and Health*. Geological Society Special Publication No. 113, London, pp. 23–37.

Galbraith, M.L., Vorachek, W.R., Estill, C.T., Whanger, P.D., Bobe, G., Davis, T.Z. and Hall, J.A. (2016) Rumen microorganisms decrease bioavailability of inorganic selenium supplements. *Biological Trace Element Research* 171, 338–343.

Gao, G., Meng, B., Liang,T., Feng, Q., Wu, C. *et al*. (2019) Modeling alpine grassland forage phosphorus based on hyperspectral remote sensing and a multi-factor machine learning algorithm in the east of Tibetan Plateau, China. *ISPRS Journal of Photogrammetry and Remote Sensing* 147, 104–117.

Givens, D.I., Moss, A.R., ADAS Feed Evaluation Unit, Stratford-on-Avon, UK, Ministry of Agriculture, Food & Fisheries and Food Standing Committee on Tables of Feed Composition (UK) (1990) *UK Tables of the Nutritive Value and Chemical Composition of Feedingstuffs*. Food and Agriculture Organization of the United Nations, Rome.

Givens, D.I., Allison, R., Cottrill, B. and Blake, J.S. (2004) Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. *Journal of the Science of Food and Agriculture* 84, 811–817.

Gonzales-Vega, J.C. and Stein, H.H. (2014) Invited review: calcium digestibility and metabolism in pigs. *Asian-Australasian Journal of Animal Sciences* 27, 1–9.

Grace, N.D. (2006) Effect of ingestion of soil on the iodine, copper, cobalt (vitamin B_{12}) and selenium status of grazing sheep. *New Zealand Veterinary Journal* 54, 44–46.

Grace, N.D., Rounce, J.R. and Lee, J. (1996) Effect of soil ingestion on the storage of Se, vitamin B_{12}, Cu, Cd, Fe, Mn and Zn in the liver of sheep. *New Zealand Journal of Agricultural Research* 39, 325–331.

Grace, N.D., Pearce, S.G., Firth, E.C. and Fennessy, P.F. (1999) Concentrations of macro- and trace elements in the milk of pasture-fed thoroughbred mares. *Australian Veterinary Journal* 77, 177–180.

Grace, N.D., Loganathan, P., Hedley, M.J. and Wallace, G.C. (2003a) Ingestion of soil fluorine: its impact on the fluorine metabolism and status of grazing young sheep. *New Zealand Journal of Agricultural Research* 46, 279–286.

Grace, N.D., Loganathan, P., Hedley, M.J. and Wallace, G.C. (2003b) Ingestion of soil fluorine: its impact on the fluorine metabolism of dairy cows. *New Zealand Journal of Agricultural Research* 48, 23–27.

Hadjipanayiotou, M. (1995) Composition of ewe, goat and cow milk and of colostrum from ewes and goats. *Small Ruminant Research* 18, 255–262.

Hansen, S.L. and Spears, J.W. (2009) Bioaccessibility of iron from soil is increased by silage fermentation. *Journal of Dairy Science* 92, *2896–2905*.

Harrington, K.C., Thatcher, A. and Kemp, P.D. (2006) Mineral composition and nutritive value of some common pasture weeds. *New Zealand Plant Protection* 59, 261–265.

Hazell, T. (1985) Minerals in foods: dietary sources, chemical forms, interactions, bioavailability. *World Review of Nutrition and Dietetics* 46, 1–123.

Healy, W.B., Rankin, P.C. and Watts, H.M. (1974) Effect of soil contamination on the element composition of herbage. *New Zealand Journal of Agricultural Research* 17, 59–61.

Heth, D.A. and Hoekstra, W.G. (1965) Zinc-65 absorption and turnover in rats. 1. A procedure to determine zinc-65 absorption and the antagonistic effect of calcium in a practical diet. *Journal of Nutrition* 85, 367–374.

Hill, J., Stark, B.A., Wilkinson, J.M., Curran, M.K., Lean, I.J., Hall, J.E. and Livesey, C.T. (1998) Accumulation of potentially toxic elements by sheep given diets containing soil and sewage sludge. 2. Effect of the ingestion of soils historically treated with sewage sludge. *Animal Science* 67, 87–96.

Hopkins, A., Adamson, A.H. and Bowling, P.J. (1994) Response of permanent and reseeded grassland to fertilizer nitrogen. 2. Effects on concentrations of Ca, Mg, Na, K, S, P, Mn, Zn, Cu, Co and Mo in herbage at a range of sites. *Grass and Forage Science* 49, 9–20

Ibáñez, M.A., de Blas, C., Cámara, L. and. Mateos, G.G. (2020) Chemical composition, protein quality and nutritive value of commercial soybean meals produced from beans from different countries: a meta-analytical study. *Animal Feed Science and Technology* 267, 114531,

INRA (2004) Tables of composition and nutritive value of feed materials. pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. (eds Sauvant, D., Perez, J.-M., Tran, G.). INRA Editions, Versailles, Paris.

Jia, Y.F, Jiang, M.M., Sun, J., Shi, R.B. and Liu, D.S. (2015) Studies on different iron source absorption by in situ ligated intestinal loops of broilers. *Biological Trace Element Research* 163, 154–161.

Johnson, P.E., Lykken, G.I. and Korynta, E.D. (1991) Absorption and biological half-life in humans of intrinsic and extrinsic ^{154}Mn tracers for foods of plant origin. *Journal of Nutrition* 121, 711–717.

Jones, R.J. (1990) Phosphorus and beef production in northern Australia. 1. Phosphorus and pasture productivity: a review. *Tropical Grasslands* 24, 131–139.

Jumba, I.O., Suttle, N.F., Hunter, E.A. and Wandiga, S.O. (1996) Effects of botanical composition, soil origin and composition on mineral concentrations in dry season pastures in Western Kenya. In: Appleton, J.D., Fuge, R. and McCall, G.J.H. (eds) *Environmental Geochemistry and Health*. Geological Society Special Publication No. 113, London, pp. 39–45.

Kappel, L.C., Morgan, E.B., Kilgore, L., Ingraham, R.H. and Babcock, D.K. (1983) Seasonal changes of mineral content in southern forages. *Journal of Dairy Science* 66, 1822–1828.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S.L., Bearson, B.L., Shurson G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Ketaren, P.P., Batterham, E.S. and Dettman, B. (1993) Phosphorus studies in pigs 2. Assessing phosphorus availability for pigs and rats. *British Journal of Nutrition* 70, 269–288.

Khan, Z.I., Ashraf, M., Hussain, A.M., McDowell, L.R. and Ashraf, M.Y. (2006) Concentrations of minerals in milk of sheep and goats grazing similar pastures in a semiarid region of Pakistan. *Small Ruminant Research* 65, 274–278.

Kiatoko, M., McDowell, L.R., Bertrand, J.E., Chapman, H.C., Pete, F.M., Martin, F.G. and Conrad, J.H. (1982) Evaluating the nutritional status of beef cattle herds from four soil order regions of Florida. I. Macroelements, protein, carotene, vitamins A and E, haemoglobin and haematocrits. *Journal of Animal Science* 55, 28–47.

Kincaid, R.L., Gay, C.C. and Krieger, R.I. (1986) Relationship of serum and plasma copper and caeruloplasmin concentrations of cattle and the effects of whole sample storage. *American Journal of Veterinary Research* 47, 1157–1159.

Kirchgessner, M., Friesecke, H. and Koch, G. (1967) *Nutrition and the Composition of Milk*. Crosby Lockward, London, pp. 209–238.

Krishnamurti, C.R., Ramberg, C.F., Shariff, M.A. and Boston, R.C. (1997) A compartmental model depicting short-term kinetic changes in selenium metabolism in ewes fed hay containing normal or inadequate levels of selenium. *Journal of Nutrition* 127, 95–102.

Kume, S.-I. and Tanabe, S. (1993) Effect of parity on colostral mineral concentrations of Holstein cows and value of colostrum as a mineral source for newborn calves. *Journal of Dairy Science* 76, 1654–1660.

Kung, L, Lim, J.M., Hudson, D.J., Smith, J.M. and Joerger, R.D. (2015) Chemical composition and nutritive value of corn silage harvested in the northeastern United States after tropical storm Irene. *Journal of Dairy Science* 98, 2055–2062.

Ledgard, S.F. and Upsdell, M.P. (1991) Sulfur inputs from rainfall throughout New Zealand. *New Zealand Journal of Agricultural Research* 34, 105–111.

Lengemann, F.W. (1969) Comparative metabolism of $Na_{125}IO_3$ and $Na_{131}I$ in lactating cows and goats. In: *Trace Mineral Studies with Isotopes in Domestic Animals*. International Atomic Energy Agency, Vienna, pp. 113–120.

Linn, J (2010) Impact of minerals in water on dairy cows. eXtension webinar, Univerity of Minnesota.

Littell, R.C., Lewis, A.J. and Henry, P.R. (1995) Statistical evaluation of bioavailability assays. In: *Bioavailability of Nutrients for Animals*. Academic Press, San Diego, California, pp. 5–34.

Littell, R.C., Lewis, A.J., Henry, P.R. and Ammerman, C.B. (1997) Estimation of relative bioavailability of nutrients. *Journal of Animal Science* 75, 2672–2683.

Liu, K (2011) Chemical composition of distillers grains: a review. *Journal of Agricultural and Food Chemistry* 59, 1508.

Lowe, N.M. and Broome, C.S. (2003) Evaluation of trace mineral status and bioavailability using stable isotopes (zinc, copper, selenium, molybdenum). In: Abrams, S.A. and Wong, W.W. (eds) *Stable Isotopes in Human Nutrition: Laboratory Methods and Research Applications*. CAB International, Wallingford, UK, pp. 61–84.

Luten, J., Crews, H., Flynn, A., Dael, P.V., Kastenmayer, P. and Hurrel, R. (1996) Inter-laboratory trial on the determination of the *in vitro* iron dialysability from food. *Journal of Science in Food and Agriculture* 72, 415–424.

Makkar, H.P.S., Tran, G., Heuze, V., Giger-Reverdin, S., Lesire, M., Labas, F. and Ankers, P. (2016) Seaweeds for livestock diets: a review. *Animal Feed Science and Technology* 212, *1–17*.

Malacarne, M., Franceschi, P., Formaggioni, P. Pisani, G.M., Petrera, F. et al. (2015) Minerals content and distribution in milk from red deer (Cervus elaphus), fallow deer (Dama dama) and roe deer (Capreolus capreolus). *Small Ruminant Research* 130, 208–215.

Manuelian, C.L., Penasa, M., Visentin, G. Zidi, A., Cassandro, M. and De Marchi, M. (2018) Mineral composition of cow milk from multibreed herds. *Animal Science Journal* 89, 1622–1627.

Masters, D.G., Norman, H.C. and Thomas, D.T. (2019) Minerals in pastures: Are we meeting the needs of livestock? *Crop & Pasture Science* 70(12).

McCaskill, M.R. and Cayley, J.W.D. (2000) Soil audit of a long-term phosphate experiment in south-western Victoria: total phosphorus, sulfur, nitrogen, and major cations. *Australian Journal of Agricultural Research* 51, 737–748.

McGrath, S. and Loveland, P.J. (1992) *The Soil Geochemical Atlas of England and Wales*. Blackie Academic and Professional, Glasgow, UK.

Medhammar, E., Wijesinha-Bettoni, R., Stadlmayr, B. Nilsson, E., Charrondiere, U.R. and Burlingame, B. (2012) Composition of milk from minor dairy animals and buffalo breeds: a biodiversity perspective. *Journal of Science in Food and Agriculture* 92, 445–74.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Misiura, M.M., Filipe, J.A.N., Walk, C.L. and Kyriazakis, I. (2018) Do not neglect calcium: a systemic review and meta-analysis (meta-regression) of its digestibility and utilisation by growing and finishing pigs. *British Journal of Nutrition* 119, 1207–1219.

Morin, D.E., Rowan, L.L., Hurley, W.L. and Braselton, W.E. (1995) Composition of milk from llamas in the United States. *Journal of Dairy Science* 78, 1713–1720.

Morris, E.R. and Ellis, R. (1976) Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. *Journal of Nutrition* 106, 753–760.

Mtui, D.J., Lekule, F.P., Shem, M.N., Hayashida, M. and Fujihara, T. (2008) Mineral concentrations in leaves of nine browse species collected from Mvomero, Morogo, Tanzania. *Journal of Food, Agriculture & Environment* 6, 226–230.

Mutanga, O., Skidmore, A.K. Kumar, L. and Ferwerda, J. (2005) Estimating tropical pasture quality at canopy level using band depth analysis with continuum removal in the visible domain. *International Journal of Remote Sensing* 26, 1093–1108.

NRC (2012) *Nutrient Requirements of Swine*, 11th edn. NRC Press, Washington, DC.

NRC (2016) *Nutrient Requirements of Beef Cattle*, 8th edn. NRC Press, Washington, DC.

Odell, B.L., Burpo, C.E. and Savage, J.E. (1972) Evaluation of zinc availability in foodstuffs of plant and animal origin. *Journal of Nutrition* 102, 653–660.

Park, Y. (2010) Goat Milk: Composition, Characteristics. In: Pond, W.G. and Bell, N. (eds) *Encyclopedia of Animal Science*, 2nd Edition. CRC Press, Baton Rouge, Florida.

Patterson, E., Campbell, C., Coull, M. and Shand, C. (2011) *Geochemical Atlas of Scottish Topsoils*. Macaulay Land Use Research Institute, Aberdeen, UK.

Pedersen, C., Boersma, M.G. and Stein, H.H. (2007) Digestibility of energy and phosphorus in ten samples of distillers dried grains with solubles fed to growing pigs. *Journal of Animal Science* 85, 1168–1176.

Perryman, K.R., Massey-O'Neill, H.V., Bedford, M.R. and Dozier, W.A. (2017) Methodology affects measures of phosphorus availability in growing broilers. 1. Effects of calcium feeding strategy and dietary adaptation period length on true ileal phosphorus digestibility and predicted endogenous phosphorus losses. *Poultry Science* 96, 611–621.

Plant, J.A., Baldock, J.W. and Smith, B.I. (1996) The role of geochemistry in environmental and epidemiological studies in developing countries: a review. In: Appleton, D., Fuge, R. and McCall, G.H.J. (eds) *Environmental Geochemistry and Health*. Geological Society Special Publication No. 113, London, pp. 7–22.

Revell, D.K., Norman, H.C., Vercoe, P.E., Phillips, N., Toovey, A., Bickell, S., Hulm, E., Hughes, S. and Emms, J. (2013) Australian perennial shrub species add value to the feed base of grazing livestock in low- to medium-rainfall zones. *Animal Production Science* 53, 1221–1230.

Rodehutscord, M., Adeola, O., Angel, R., Bikker, P., Delezie, E., Dozier III, W.A. and Witzig, M. (2016) Results of an international phosphorus digestibility ring test with broiler chickens. *Poultry Science* 96, 1679–1687.

Rogosic, J., Pfister, J.A., Provenza, F.D. and Grbesa, D. (2006) Sheep and goat preference for and nutritional value of Mediterranean maquis shrubs. *Small Ruminant Research* 64, 169–179.

Roque, A.P., Dias, R.S., Vitti, D.M.S.S., Bueno, I.C.S., Cunha, E.A., Santos, L.E. and Bueno, M.S. (2007) True digestibility of calcium from sources used in lamb finishing diets. *Small Ruminant Research* 71, 243–249.

Sath, K., Pauly, T. and Holtenius, K. (2013) Mineral status in cattle fed rice straw and Para Grass combined with different levels of protein derived from cassava foliage. *Asian-Australasian Journal of Animal Sciences* 26, 59-66.

Saul, G., Cayley, J. and McCaskill, M. (1999) *The Hamilton Long-term Phosphorus Experiment*. Agriculture Victoria, Hamilton, Australia.

Schwarz, R., Balko, A.Z. and Wien, E.M. (1982) An *in vitro* system for measuring intrinsic dietary mineral exchangeability: alternatives to intrinsic labelling. *Journal of Nutrition* 112, 497–504.

Scott, M.L. and Thompson, J.N. (1971) Selenium content of feedstuffs and effects of dietary selenium levels upon tissue selenium in chicks and poults. *Poultry Science* 50, 1742–1748.

Shastak, Y. and Rodehutscord, M. (2015) Recent developments in determination of available phosphorus in poultry. *Journal of Applied Poultry Research* 24, 283–292.

She, Y., Li, D. and Zhang, S. (2017) Methodological aspects of determining phosphorus digestibility in swine: a review. *Animal Nutrition* 3, 97–102.

Shirley, R.L. (1978) Water as a source of minerals. In: Conrad, J.H. and McDowell, L.R. (eds) *Latin American Symposium on Mineral Nutrition Research with Grazing Ruminants*. Animal Science Department, University of Florida, Gainsville, Florida, pp. 40–47.

Silanpaa, M. (1982) *Micronutrients and the Nutrient Status of Soils: A Global Study*. Food and Agriculture Organization, Rome.

Smart, M.E., Cohen, R., Christensen, D.A. and Williams, C.M. (1986) The effects of sulphate removal from the drinking water on the plasma and liver copper and zinc concentrations of beef cows and their calves. *Canadian Journal of Animal Science* 66, 669–680.

Smith, G.S., Middleton, K.R. and Edmonds, A.S. (1978) A classification of pasture and fodder plants according to their ability to translocate sodium from their roots into aerial parts. *New Zealand Journal of Experimental Agriculture* 6, 183–188.

Southern, P. (1979) The value of cereal crop residues. In: *Our Land* (W. Australia), p. 3.

Sprinkle, J.E., Baker, S.D., Church, J.A., Findlay, J.R., Graf, S.M. *et al*. (2018) Case study: regional assessment of mineral element concentrations in Idaho forage and range grasses. *The Professional Animal Scientist* 34, 494–504.

Suleiman, A., Okine, E. and Goonewardne, L.A. (1997) Relevance of National Research Council feed composition tables in Alberta. *Canadian Journal of Animal Science* 77, 197–203.

Suttle, N.F. (1974) A technique for measuring the biological availability of copper to sheep using hypocupraemic ewes. *British Journal of Nutrition* 32, 395–401.

Suttle, N.F. (1983) Assessing the mineral and trace element status of feeds. In: Robards, G.E. and Packham, R.G. (eds) *Proceedings of the Second Symposium of the International Network of Feed Information Centres, Brisbane*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 211–237.

Suttle, N.F. (1985) Estimation of requirements by factorial analysis: potential and limitations. In: Mills, C.F., Bremner, I. and Chesters, J.K. (eds) *Proceedings of the Fifth International Symposium on Trace Elements in Man and Animals*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 881–883.

Suttle, N.F. (2000) Minerals in livestock production. *Asian-Australasian Journal of Animal Science* 13, 1–9.

Suttle, N.F. (2017) Challenges in predicting needs for mineral supplementation in grazing animals. In: Singh, B.R., McLoughlin, M.J. and Brevik, E.C. (eds) *The Nexus of Soils, Plants and Animals and Human Health*. Catena Schwezerbart, Stuttgart, pp. 105–110.

Suttle, N.F., Alloway, B.J. and Thornton, I. (1975) An effect of soil ingestion on the utilisation of dietary copper by sheep. *Journal of Agricultural Science, Cambridge* 84, 249–254.

Suttle, N.F., Abrahams, P. and Thornton, I. (1984) The role of a soil × dietary sulfur interaction in the impairment of copper absorption by ingested soil in sheep. *Journal of Agricultural Science, Cambridge* 103, 81–86.

Suttle, N.F., Bell, J., Thornton, I. and Agyriaki, A. (2003) Predicting the risk of cobalt deprivation in grazing livestock from soil composition data. *Environmental Geochemistry and Health* 25, 33–39.

Thornton, I. (1974) Biogeochemical and soil ingestion studies in relation to the trace-element nutrition of livestock. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Trace Element Metabolism in Animals – 2*. University Park Press, Baltimore, Maryland, pp. 451–454.

Thornton, I. and Alloway, B.J. (1974) Geochemical aspects of the soil–plant–animal relationship in the development of trace element deficiency and excess. *Proceedings of the Nutrition Society* 33, 257–266.

Turner, B.L., Paphazy, M.J., Haygarth, P.M. and McKelvie, I.D. (2002) Inositol phosphates in the environment. *Philosophical Transactions of the Royal Society of London, B* 357, 449–469.

Underwood, E.J. (1977) *Trace Elements in Human and Animal Nutrition*, 4th edn. Academic Press, London.

Underwood, E.J. and Suttle, N.F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

USDA (2009) United States Dairy Association National Nutrient Database for Standard Reference. Available at: http://www.nal.usda.gov/fnic/foodcomp/search/

Vaithiyanathan, S. and Singh, M. (1994) Seasonal influences on soil ingestion by sheep in an arid region. *Small Ruminant Research* 14, 103–106.

Walk, C.L., Bedford, M.R. and McElroy, A.P. (2012) In vitro evaluation of limestone, dicalcium phosphate, and phytase on calcium and phosphorus solubility of corn and soybean meal. *Poultry Science* 91, 674–682.

White, C.L., Robson, A.D. and Fisher, H.M. (1981) Variation in nitrogen, sulfur, selenium, cobalt, manganese, copper and zinc contents of grain from wheat and two lupin species grown in a range of Mediterranean environments. *Australian Journal of Agricultural Research* 32, 47–59.

White, J.G. and Zasoski, R.J. (1999) Mapping soil micronutrients. *Field Crops Research* 60, 11–26.

Widmer, M.R., McGinnis, L.M. and Stein, H.H. (2007) Energy, phosphorus, and amino acid digestibility of high-protein distillers dried grains and corn germ fed to growing pigs. *Journal of Animal Science* 85, 2994–3003.

Wilman, D. and Derrick, R.W. (1994) Concentration and availability to sheep of N, P, K, Ca, Mg and Na in chickweed, dandelion, dock, ribwort and spurrey compared with perennial ryegrass. *Journal of Agricultural Science, Cambridge* 122, 217–223.

Winks, L. (1990) Phosphorus and beef production in northern Australia. 2. Responses to phosphorus by ruminants – a review. *Tropical Grasslands* 24, 140–158.

WPSA (2013) Working Group No 2 [Nutrition] of the European Federation of Branches of World Poultry Science Association: determination of phosphorus availability in poultry. *World's Poultry Science Journal* 69, 687–698.

Wright, D.E., Towers, N.R., Hamilton, P.B. and Sinclair, D.P. (1978) Intake of zinc sulphate in drinking water by grazing beef cattle. *New Zealand Journal of Agricultural Research* 21, 215–221.

Yu, Y., Lingeng, L., Wang, R.L., Lin, X., Luo, X.G. and Liu, B. (2010) Effects of zinc source and phytate on zinc absorption by in situ ligated intestinal loops of broilers. *Poultry Science* 89, 2157–2165.

Zanetti, D., Prados, L.F., Menezes, A.C.B., Pereira, J.M.V., Detmann, E., Engle., T.E. and Valadares Filho, S.C. (2017a) Water intake prediction for beef cattle in Brazil. *Journal of Animal Science* 95, 290.

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle, T.E., Silva, B.C. *et al.* (2017b) Estimating mineral requirements of Nellore beef cattle with or without inorganic mineral supplementation and the influence on mineral balance. *Journal of Animal Science* 95, 1696–1706.

Zanetti, D., Valadares Filho, S.C., Prados, L.F., Detmann, E., Pacheco, M.V.C., Rennó, L.N., Godoi, L.A. and Engle, T.E. (2017c) Impacts of reduction of phosphorus in finishing diets for Holstein-Zebu steers. *Livestock Science* 198, 45–51.

Zeng, H., Botnen, J.H. and Johnson, L.K. (2008) A selenium-deficient Caco-2 cell model for assessing differential incorporation of chemical or food selenium into glutathione peroxidase. *Biological Trace Element Research* 123, 98–108.

3 Mineral Status of Livestock

The previous chapter exposed continuing limitations of mineral composition of soils, plants and dietary components as indicators of mineral status in supported livestock. Mineral insufficiencies cannot be delineated with a set of pasture mineral analyses on the one hand and a set of mineral requirements on the other, although some still try to do so (Sprinkle *et al*., 2018; Khan *et al*., 2006). Relationships break down because many factors influence the flow of minerals from soils to active sites in animals (Fig. 3.1). Past attempts to simulate these influences at important interfaces between soil and plant, plant and animal, digesta and intestinal mucosa and between tissue cells were prone to error (Suttle, 2017).

Feed Intake

Mineral intakes are the product of feed intake and mineral concentrations. Voluntary feed intake is roughly proportional to metabolic body weight ($W^{0.75}$) but limited by physico-chemical factors that affect digestibility and increase gut fill, including water content and water-holding capacity in pigs (Ndou *et al*., 2013; Whittemore *et al*., 2003). Prediction of food intake and need for supplements are particularly challenging in grazing animals (Tedeschi *et al*., 2019). Individual dry-matter intake (DMI) can be measured in groups of animals by means of electronically operated gates, triggered by microchips, borne on collars. Measuring DMI at pasture requires the use of indigestible markers of the type used in studies of mineral absorption (p. 26). A novel approach is to administer indigestible marker in a rumen bolus, which releases a marker at a known rate, and measures marker concentrations in faeces. Thus DMI of 0.8 and 0.5 kg d^{-1}, respectively, were estimated for co-grazing alpaca and sheep, based on a predetermined release rate for Cr from a bolus containing chromium sesquioxide (Judson *et al*., 2018). Competition for limited trough space increases individual variation in food intake. In the following chapters, reliance is placed on mineral concentrations at assumed food intakes rather than daily amounts of mineral required to simplify the assessment of feedstuffs and expression of animal needs, but readers should recognize this limitation.

Selective feed consumption

Selective consumption of feeds by livestock can lead to differences in mineral consumption between what is presented and what is consumed. Mineral concentrations in plant species consumed while grazing can be important but are particularly hard to ascertain. When herbage is plentiful, livestock express preferences for different types and parts of plants that can vary widely in mineral concentration and are not simulated in hand-cut or -plucked samples. Such effects can be minimized by taking the mean of mineral concentrations of herbage samples before and after grazing off most of the herbage in strip, dartboard and paddock grazing systems (Suttle, 2017). Selected forage can be sampled via oesophageal fistulae but contamination of masticated food with saliva can complicate prediction of mineral intake, particularly that of Na (see Araujo *et al*., 2010). Selectivity greatly influences assessment of P intake (p. 149) and all mineral intakes where browse material is available (Fordyce *et al*., 1996). Experimenters may unwittingly deselect contaminating soil from herbage by washing samples, thereby removing soil minerals normally consumed and partially utilized. When housed livestock are offered mixtures of dietary ingredients differing in texture, moisture content and palatability, animals may consume a blend that differs from the one provided. Pelleting a heterogenous blend eliminates selection but increases rate of food intake and reduces time spent ruminating, both of which can influence metabolism, particularly of S, P and Na. Simply

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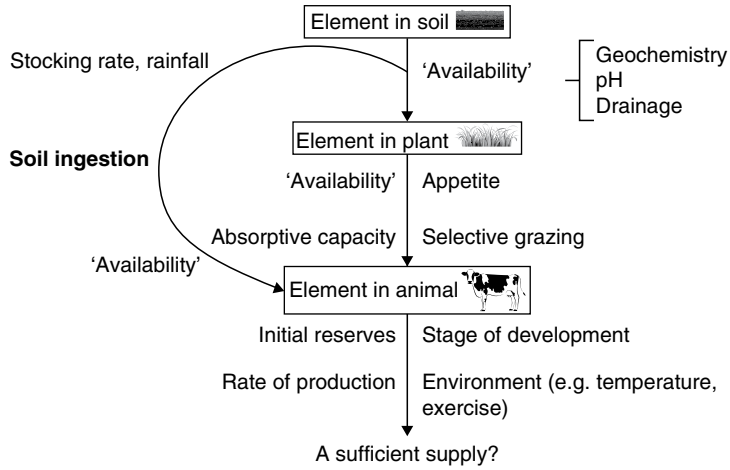

Fig. 3.1. Summary of the many and varied factors that can influence flows of mineral from soil to grazing animal and thus determine whether a given mineral supply will meet mineral requirement.

weighing feed refusals and relying on prior composite mineral analyses may give false estimates of mineral intake from loose mixtures of feeds. When offered limestone separately from the main ration, laying hens vary consumption of limestone according to Ca and P supplies from the ration (Barkley *et al*., 2004). Calcium appetite is expressed early in life and affects intake of food as well as mineral supplement (p. 84).

Clinical Signs of Abnormal Mineral Status

Weak relationships between mineral status in soils, plants and animals mean that direct indicators of mineral status are required. Unfortunately, the clinical and pathological abnormalities associated with lack of many minerals are indistinguishable from each other and from those other nutritional and infectious disorders (Phillippo, 1983). A scheme for differential diagnosis that eliminates non-mineral causes of disorder is given in Appendix 1. Diagnosis of mild insufficiency is particularly difficult and the most reliable criterion is still a positive response in health or productivity to a specific mineral supplement in a well-designed and well-executed trial (Phillippo, 1983; Chapter 19). Few pictures of 'deficient animals' are presented in this book because they can be misleading. Non-specificity arises because clinical abnormalities are final expressions of defective but different links along the same chain of metabolic events. Impaired reproduction caused by lack of Mn, Se and Zn reflects different primary effects on the reproductive process. The nature of clinical and pathological changes can vary greatly within species, depending on age at onset of mineral deprivation. Goitre (an enlarged thyroid gland) and congenital swayback in newborn lambs are well-known signs of lack of I and Cu, respectively, in late pregnancy but later deprivation may impair health in flocks or herds unaffected by these disorders.

Biochemical Indicators of Low Mineral Status

The non-specificity of clinical and pathological consequences of mineral deprivation shifts the focus to detecting biochemical changes in tissues and fluids of animals that accompany mineral insufficiency (Appendix 1, step 3c), valuable in the pre-clinical detection of mineral imbalances (Mills, 1987). Minerals (and other nutrients) have been divided into two categories, Type 1 and Type 2 based on capacity to store mineral when diets provide more mineral than animals require (Golden, 1996). Lack of Type 1 minerals is characterized by a prolonged initial depletion phase and terminal phase of specific clinical disorder (e.g. microcytic anaemia due to lack of Fe and swayback caused by lack of Cu). Type 2 elements are generally characterized by immediate entry into the deficiency phase and early, non-specific signs of terminal disorder. The sequence of events for Type 1 elements is

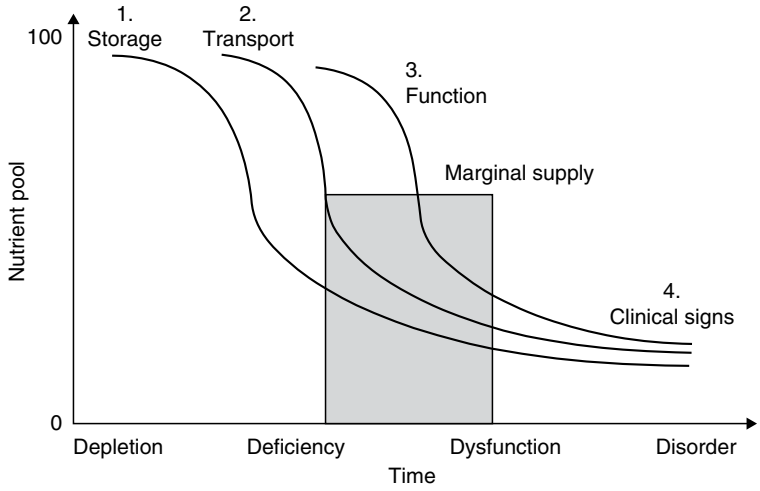

Fig. 3.2. The sequence of pathophysiological changes during deprivation differs between minerals (Golden, 1996). For Type 1 minerals (Ca, Co, Cu, Fe and I), there are generally four phases: depletion, deficiency, dysfunction and specific disorder (e.g anaemias). The sequence for Type 2 elements (K, Mg, Mn, Na, P, S and Se) usually lacks a depletion phase, because minimal stores are held, and ends with non-specific clinical disorders (anorexia and weight loss). For all elements, there is a marginal area (shaded) where stores and transport pools are almost exhausted and functions start to fail but animals appear healthy. Upper limit (100) on the 'y' axis represents maximum or normal pool size.

presented in Fig. 3.2 and can be divided into four phases:

- 'Depletion', during which storage pools of the mineral are reduced;
- 'Deficiency', during which transport pools of the mineral are reduced;
- 'Dysfunction', when mineral-dependent functions become rate-limited; and
- 'Disorder', during which clinical abnormalities become apparent to the naked eye.

Subdivision avoids the use of the same word, 'deficient', to describe diets low in minerals, healthy animals with subnormal mineral status and clinically sick animals. A diet is only 'mineral-deficient' if it is likely to be fed long enough to induce subnormal mineral status and – if fed – may not necessarily result in clinical disease. The classification is not absolute. Rapid increase in demand for Ca with onset of lactation can lead to acute hypocalcaemia and a specific clinical disease (milk fever), while large reserves remain in the skeleton; biochemical evidence of deficiency can occur before Fe reserves are exhausted (Chesters and Arthur, 1988); lack of Mn in the unborn can produce specific birth defects, while weaned animals take months to deplete (p. 350).

- Concepts of what constitutes a store may have to change.

For Cu, accumulation of CuZnSOD in the liver at luxus intakes contributes to body stores, and accumulation in red blood cells (rbc) may be similarly viewed. Large increases in MnSOD in the hearts of broiler chicks (p. 358) at intakes above requirement may constitute adaptation to oxidant stress.

- Tolerance of low dietary Mn and Zn intakes may be enhanced by unrecorded reductions in skin Mn and Zn.

The point of transition from deficiency to dysfunction and disorder varies with demand on the critical pathway. Low Se status may be tolerable until changes in diet and increased freedom of movement (i.e. turnout to spring pasture) present a twofold oxidative stress (Fig. 15.2); low I status may be tolerable until cold stress induces a thermogenic response (p. 302). Simultaneous lack of Se and I increases the likelihood that deprivation of either element will cause dysfunction (Arthur, 1992).

Biochemical markers are used as remote sensors of rate-limiting, intracellular events in vulnerable organs

Table 3.1. Sequential pathophysiological changes during development of six mineral-responsive disorders.

Mineral	Depletion	Deficiency	Dysfunction	Disorder
Ca	Young: bone ↓	Serum ↓	Chondrodystrophy	Rickets
	Old: bone ↓	Serum ↓	Irritability ↓	Recumbency
Mg	Young: bone ↓	Serum ↓	Irritability ↑	Convulsion
	Old: bone ?	Serum ↓	Irritability ↑	Convulsion
Cu	Liver ↓	Serum ↓	Disulfide bonds ↓	Wool crimp ↓
Co	Liver vitamin B_{12} ↓ Serum vitamin B_{12}↓	Serum vitamin B_{12} ↓	MMA ↑	Appetite ↓
I	T4 ↓ Thyroid colloid ↓ Thyroid iodine ↓	T3 ↓	BMR ↓ Thyroid hypertrophy	Goitre
Se	GPx in blood and liver ↓	Serum Se ↓	Serum creatine kinase ↑	Myopathy

BMR, basal metabolic rate; GPx, glutathione peroxidase; MMA, methylmalonic acid; T3, triiodothyronine; T4, thyroxine.

but, historically, few succeeded. Sample sites and analytes were selected for convenience rather than diagnostic merit, often indicating depletion and deficiency rather than dysfunction and disorder (Table 3.1). Subnormal analytical values in the diagnostic algorithm (step 3d in Appendix 1) are not synonymous with loss of health. Relationships between markers and dysfunction can clarify trace (Mills, 1987) and macro-element status (Manangi *et al*., 2018).

Criteria of physiochemical dysfunction

Alongside the 'snapshot' of macromineral metabolism presented in Fig. 1.1, a small-scale ('nano') model indicated the myriad balances struck in individual cells that make up the bigger picture, like 'pixels' in a screenshot. Similarly, the sequence shown in Fig. 3.2 has its 'nano' equivalent. The following sequence of intracellular events occurs in vulnerable blood cells during Zn deprivation:

- depletion of Zn stores in membranes;
- deficiency of alkaline phosphatase, protein and lipid in membranes;
- dysfunctional changes in intracellular Ca^{2+} ions and carbonic anhydrase activity;
- disorder, seen as periparturient bleeding, due to impaired platelet aggregation and increased erythrocyte fragility.

(O'Dell, 2000)

Biochemical markers of dysfunction are more useful than indices of depletion and deficiency because they change nearer to the onset of clinical disease. Increases in methylmalonic acid (MMA) indicate that MMA coenzyme A isomerase activity has become rate-limiting in cobalt (vitamin B_{12}) deficiency. Increases in creatine kinase indicate that muscle membrane integrity has been compromised by lack of Se. However, a rise in plasma MMA may precede loss of appetite (p. 241), an important clinical sign of lack of dietary Co. Animals have sophisticated, intracellular signalling systems for recognizing and correcting low mineral status. mRNA for transport storage proteins such as ferritin and cytosolic glutathione peroxidase are downregulated by lack of Fe and Se, respectively, while the mRNA for cholecystokinin is induced by lack of Zn. The use of cDNA arrays to unravel the intricacies of zinc homeostasis was suggested.

- Molecular markers involved in intracellular mineral trafficking, gene expression and 'crosstalk' between messengers indicate the priority that the animal is giving to a particular function for a limited mineral supply (e.g. Zn; p. 419).
- However, molecular markers can complicate rather than refine diagnosis and estimation of mineral needs by failure to correlate regulator with rate-limiting function of pigs and poultry (p. 361).

A specific term, *physiochemistry* is proposed to focus attention on this neglected area of mineral nutrition and veterinary diagnosis. Routine diagnosic use of cDNA and mRNA arrays is likely to remain prohibitively expensive.

Delineating marginal values

Throughout this book, biochemical criteria will be interpreted using a three-tier system (Table 3.2) with 'marginal bands' to separate normal values from those indicating dysfunction or disorder (Fig. 3.2), rather than a diagnostically unhelpful 'reference range' that abruptly separates the statistically normal from

Table 3.2. Interpretation of biochemical and other indices of Type 1 mineral deprivation (or excess) in livestock is enhanced by the use of a marginal band between values consistent with health and ill-health.

	Deprivation		Exposure	
Status	Phase[a]	Response to supplementation	Phase	Response to withdrawal/treatment
Normal	Depletion	None	Accretion	None
Marginal	Deficiency	Unlikely	Overflow	Unlikely
	Dysfunction	Possible	Dysfunction	Possible
Abnormal	Disorder	Probable	Disorder	Probable

[a]See Fig. 3.2 for definition and illustration of terminology: for Type 2 minerals, marginal bands are narrow and may not include a deficiency phase.

aberrant values without considering clinical condition. Individual variation within the flock or herd is also informative, high coefficients of variation indicating an abnormal population and possible dysfunction in some of its members. Detailed temporal studies of physiochemical changes during mineral imbalances are required to delineate marginal bands.[i] Critical values depend on index of adequacy. With optimal wool growth rather than weight gain as the goal, the lower limit of marginality for plasma Zn rose from 6.7 to 7.7 μmol l^{-1} (White *et al.*, 1994). Optimal blood or plasma Se for wool growth also differs from those for optimum LWG (p. 386). Use of single, uncorroborated threshold values has led to small ruminant populations in large areas being labelled 'mineral-deficient' and in need of supplementation (e.g lacking Se in Sweden) and many minerals in Punjab, Pakistan (Suttle, 2017).

- Genomic markers should be related to restriction of health and productivity and also interpreted using marginal bands.
- Confounded effects of physiological and developmental changes must be recognized.

The normal newborn animal often has 'abnormal' tissue or blood mineral composition when judged inappropriately by adult standards, as in the case of liver and plasma Cu, Se and I. Most blood mineral concentrations also change abruptly and briefly in the dam around parturition and sampling at this time is only diagnostically useful for acute disorders of Ca and Mg metabolism.

Choice of Sample

Surveys of soil/plant/animal relationships indicate that soil and plant samples rarely give information that would be sufficient for diagnostic purposes (Suttle, 2017). Blood, urine, saliva, integument and excreta samples have a common advantage of accessibility by simple, minimally invasive procedures but usefulness varies between minerals and sites. The most important pairings are summarized below.

Blood, plasma or serum?

Blood samples are usually allowed to clot after sampling and serum analysed to avoid the cost and possible analytical complications of adding anticoagulant, give a more stable, haemolysis-free, form for transportation: generally, serum gives similar values to plasma samples. However, values for plasma Cu are often higher than serum Cu, the difference rising as concentration rises in ruminants (Laven and Livesey, 2006).

- Among grazing species, only equines have been shown to be immune from loss of Cu to the blood clot and reliably monitored by serum Cu.

Results for plasma and serum generally reflect mineral status of the transport pool, and low values indicate onset of 'deficiency' (Fig. 3.2) but are susceptible to influences of non-nutritional, acute phenomena, such as parturition, infection and extreme change of temperature.

- Plasma and serum Cu, Fe and Zn change in the acute phase of microbial infection, Cu rising, the latter decreasing: anomalous ratios are better indices of infection than mineral status.

Mineral concentrations generally decline more slowly in whole blood than in plasma during deprivation, due to slower decline in red blood cells (rbc). Minerals such as Cu, Fe and Zn are incorporated as functional units in immature erythrocytes, prior to release into the bloodstream, and mineral content of younger erythrocytes reflects recent mineral nutriture. Once released, mineral status of

an rbc may remain unchanged throughout its life, despite marked fluctuations in mineral status of the diet. Thus:

- activities of rbc enzymes indicative of Cu and Se status (CuZnSOD and glutathione peroxidase, respectively) do not increase until about 3 weeks after a rise in Cu or Se provision;

then there are sufficient enriched young cells to increase mean values for the entire erythrocyte population. This may be an advantage if animals have been treated or have inadvertently gained access to minerals (e.g. during handling) prior to blood sampling, since rbc enzyme activities in subsequent blood samples would be unaffected: however, a recent downturn in mineral supply may be missed. In extremely deficient animals, rbc may be released with apo-enzyme, lacking functional mineral. Assay of enzyme protein by ELISA or extent of *in vitro* apo-enzyme reconstitution may offer new insights into the severity of depletion (e.g. of zinc) in such circumstances (p. 419).

- Where function is expressed by rbc, as in membrane stability and/or resistance to oxidative stress in Cu, P and Se deprivation, functional status may be indicated by *in vitro* haemolysis or presence of Heinz bodies in rbc but such tests are non-specific.
- Zn and alkaline phosphatase in erythrocyte membranes may reflect status better than plasma Zn (O'Dell, 2000) but carbonic anhydrase activity declines as rapidly as plasma Zn (p. 419).

Leucocytes and platelets have a half-life measured in days rather than weeks and give contemporary measures of Cu (Harvey *et al*., 2009) and Se (Reszka *et al*., 2012) status but have yet to be used in livestock.

Secreta and excreta

Saliva samples are easily procured, using a 'trombone slide' gag to open the mouth, and mineral composition is particularly sensitive to lack of dietary Na.

- As salivary Na declines, K rises and the Na:K ratio in parotid saliva can decrease tenfold or more in ruminants on diets low in Na (Morris, 1980).

Preparatory mouthwashes can reduce contamination from feed and digesta without disturbing the Na:K ratio. Analysis of Na:K in muzzle secretions can also indicate Na status (Kumar and Singh, 1981).

- Mineral excretion in urine generally correlates with mineral intake above requirement for Type 2 minerals (p. 40), low excretion indicating heightened risk of deprivation, while high concentrations indicate risk of toxicity.

Raised sulfur metabolites in urine can indicate S toxicity (p. 221) and raised urine F can indicate fluorine toxicity. However, high urinary excretion may reflect mobilization of mineral from the skeleton (e.g. P) and high urinary F reflects loss of skeletal F, accumulated during previous exposure. The influence of water intake, including that from lush pasture, must be corrected for by using mineral:creatinine ratios (e.g Masters *et al*., 2018). Mineral to alkane ratios in faeces of grazing animals can be applied to minimize such effects. Low faecal mineral concentrations indicate prolonged exposure to low mineral intakes (e.g. faecal P), particularly if corrected for extremes of digestibility by use of ratios to indigestible pasture components (e.g. alkanes).

Tissues and appendages

Liver and bone are accessible via simple techniques, such as aspiration biopsy and trephine, respectively. Subnormal liver Fe and Cu indicates 'depletion' of these elements but liver analyses are of little use for Type 2 elements (Fig. 3.2). Subnormal bone Ca and P can suggest respective deficiencies and high bone F indicates excessive F intakes. However, variation between bones, with age and modes of expression (fresh, dried, de-fatted or ashed bone basis) complicate interpretation (Table 6.3). With heterogeneous organs such as kidney, results can be affected by the area sampled: cortex Se is two- to fivefold higher than medulla Se) (Millar and Meads, 1988). Analysis of minerals in hair, hoof, fleece or feathers has a chequered diagnostic history (Combs, 1987) and relationships to other indices of status, both biochemical and clinical, can be poor. Exogenous and endogenous (e.g. sweat and suint) contamination, differences in moisture content and sampling interval are avoidable sources of variation. Sampling newly grown hair or fleece from a recently shaved site (e.g. of liver biopsy) can measure recent mineral status. Dividing hair, fleece and core samples of hoof into proximal, medial and distal portions can record past changes in mineral nutrition. Colour of sampled hair and wool is not correlated with mineral

concentration because minerals are not contained in pigments.

- The skin is a neglected sampling site, where reductions in concentration of abundant elements, like Zn and Mn, probably make a significant contribution in adapting to deprivation.

Products: milk and eggs

Mineral concentrations in milk rarely correlate with mineral status of diet or animal. Major exceptions are I, Mo and Se, for which samples of bulk milk collections can indicate seasonal changes in mineral status, with appropriate interpretation. With Mo, diagnostic significance is dependent on interactions with Cu and S in the gut, while marginal bands have yet to be defined for I. Milk Se is affected by Se source and may overestimate functional status (p. 381). Comparisons between 'organic' and conventionally managed dairy herds in Spain showed relatively low concentrations of Cu (0.041 and 0.067), I (0.078 and 0.265), Se (0.009 and 0.019) and Zn (3.3 and 3.9 mg l^{-1}), respectively. Differences were assumed to reflect, in part, reduced inputs of inorganic mineral supplements (Rey-Crespo *et al*., 2013). A survey of mineral composition of ewe and goat milk in Pakistan claimed that mineral deficiencies were prevalent (Khan *et al*., 2006) but outdated 'critical' values were used and some species differences were at variance with historic data (see Table 2.4): both Ca and Cu were particularly low in sheep in summer. Large species differences in milk Zn may require species-specific marginal bands for diagnostic purposes. Eggshell Ca and P quickly reflect poor dietary supplies of either Ca or P and are also affected by dietary Mn.

Functional and genomic indices of status

Serum assays for vitamin B_{12} rather than Co, for triiodothyronine rather than protein-bound I, for caeruloplasmin rather than Cu and for glutathione peroxidase rather than Se provided early functional indicators of status. However, caeruloplasmin assay did not improve the accuracy of assessing Cu status (Suttle, 2008). Advances in assay procedures continue to increase the range and sensitivity of diagnostic techniques, such as specific selenoproteins in platelets and leucocytes (Reszka *et al*., 2012). Superoxide dismutase assays in specific tissues are being used to define Mn (p. 358) requirements in pigs and poultry, but present difficulties of interpretation. Varied supply of mineral can induce genomic changes, as illustrated in Fig. 3.3 but interpretation again presents difficulties. Expression of the same enzyme in liver peaked around 67mg Fe kg^{-1}DM, while haemoglobin expression in bone marrow showed no sign of peaking with 147mg Fe kg^{-1}DM.

- Using heart COX as the arbiter of need for Fe in broilers is only justified if lack of cardiac COX presents the first constraint on health as dietary Fe decreases.

Copper uptake by the gut mucosa is mediated by an apically located CRT1 transporter and upregulated by lack of dietary Cu and the major zinc transporter, ZIP4, is upregulated by lack of dietary Zn (Myers, 2015): each might be used to indicate respective requirements. Expression of genomic markers varies with stage of development, species, duration of imbalance and tissue sampled and molecular criteria of inadequacy require careful interpretation.[ii] Until reliable diagnostic genomic markers of low mineral status are developed, conventional markers must be used.

Balance between Minerals

Balances between amounts of minerals absorbed from the diet and retained in the body can be

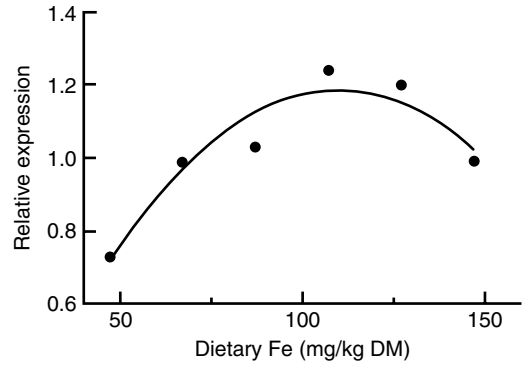

Fig. 3.3. The relationship between relative expression of mRNA for cytochrome oxidase (COX) in heart tissue and dietary iron in broilers indicated a requirement for 104–110mg Fe kg^{-1}DM to the authors (Liao *et al*., 2017).

important. Cations such as Na^+, K^+, Ca^{2+} and Mg^{2+} are alkali-producing, whereas anions such as Cl^- and SO_4^{2-} are acid-producing. Preservation of acid-base balance is important for all species, from poultry to dairy cows and cation–anion imbalance can result in clinical disorders (p. 177). Highly digestible energy- and protein-rich diets that sustain high levels of production may increase risk of mineral imbalance when compared with low-quality feeds and lower pH in gut and body (Goff, 2018). Each mineral involved in antioxidant defence (i.e. Cu, Mn, Se and Zn) is capable of inducing changes in activities of enzymes dependent on the other minerals, reflecting crosstalk between messengers to minimize peroxidation. Elements that share pathways of uptake and/or transportation to functional sites, like Fe, Mn, Cu, Zn and Cd, are potential antagonists but are protected from each other by an elaborate network, which can be summarized in a new concept, 'metal distancing', illustrated in Fig. 3.4.

Several elements can be taken through the apical membrane of the intestinal mucosa and from extracellular fluid into tissues by the divalent metal carrier protein, DMT1; other transport and storage proteins are shared by some elements. If dietary concentrations are pushed to extremes, one element can induce insufficiencies of another by competing for transporter. Potential antagonists are normally kept apart by a series of protective mechanisms, beginning in the gut mucosa, where Fe and Cd can be safely stored as ferritin and metallothionein, respectively. Cu and Zn have alternative, specific carriers, while Fe and Mn are passed to ferriportin, for which Fe has priority. These mechanisms, which are repeated at post-absorptive tissue sites, keep potential antagonists apart (Fig 3.4). Separation is reinforced by differences in propensity for storage, Fe and Cu being avidly stored, the former as haemosiderin, Mn and Zn being weakly stored (see Fig. 3.2). The possibility of storage of Mn and Zn as SOD is prompted by recent studies of responses to Mn- and Zn-chelates and follows the precedent set by Cu, for which hepatic CuZnSOD is clearly a safe storage form for Cu.

Self-correction of Mineral Imbalance

Contrary to popular belief, appetite for minerals, particularly trace elements, is not a reliable measure of animal need, and cannot be relied upon to correct dietary imbalances (Pamp *et al.*, 1976). Grazing sheep dosed orally with minerals to overcome deficiencies consumed as much mineral lick as undosed animals (Cunningham, 1949) and livestock may reject supplements containing minerals they need. Physiological and behavioural explanations probably lie in the absence of cognitive distress and/or

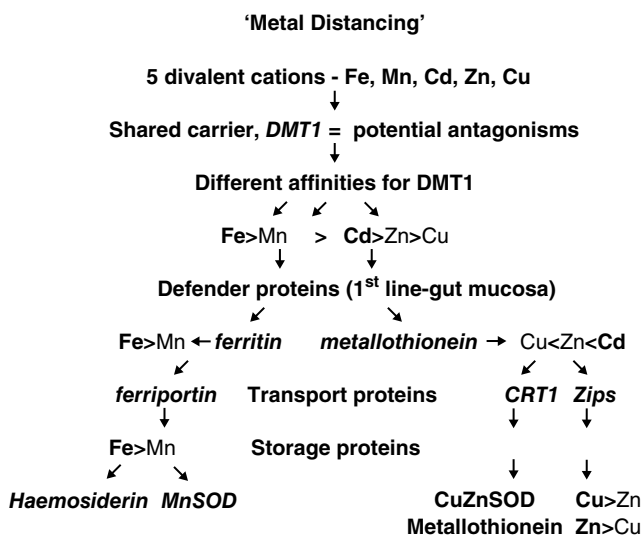

Fig. 3.4. Metal distancing: a concept advanced to indicate mechanisms by which potential competition between elements that share transport proteins is limited: transporters include divalent metal transporter 1 (DMT1) and copper transporter 1 (CRT1); bold symbols indicate elements with relatively strong affinity for a carrier.

inability to associate immediate alleviation of distress, caused by previous lack of mineral, with consumption of a beneficial mineral source. Voluntary consumption of mineral mixtures or 'licks' is determined more by palatability than physiological need.

Most examples of 'mineral wisdom' involve macro-elements. Pullets, approaching lay, self-select diets rich enough in Ca to meet the combined needs of bone deposition and shell calcification. Laying hens show preferences for Ca-rich diets on egg-laying days and a hen deprived of P will adapt to a decreased need for Ca. Calcium appetite develops during the first weeks of life in broiler chicks and may be exploitable commercially. Providing Ca supplements separately from an unsupplemented feed, low in Ca, can increase food intake (Wilkinson *et al*., 2013). Sodium-deficient ruminants develop an appetite for salt and will even consume sources to which they are normally averse, such as $NaHCO_3$. However, if salt is provided in a free-access mixture also rich in Mg, excessive consumption of Mg may cause diarrhoea (Suttle *et al*., 1996). When offered choices between feeds containing excess or normal concentrations of salt and high- or low-energy and protein concentrations, sheep select combinations that improve the quality rather than salt content of their diet (Thomas *et al*., 2006). The relish with which livestock consume mineral deposits around receding water sources may have unintended nutritional benefits. A rare example of apparently beneficial preference for a trace element was provided by the desire of pigs to lick Cu-containing rings supporting water pipes in their pens: copper supplements were subsequently found to stimulate growth. However, piglets showed no preference for a ration containing a pharmacological concentration of ZnO, where such a choice would have benefitted health and performance (Reynolds *et al*., 2010)

Mineral Supplementation

Ideally, mineral supplements should be used only when requirements cannot be met by the judicious combinations of natural feeds.[iii] If supplementation is necessary; the ideal procedure is influenced by climate, topography, conditions of husbandry and economics.

Intervention with fertilizers

Soil treatments are indicated when crop or pasture growth has been limited by mineral deprivation (Fig. 2.6) but contra-indicated where insufficiencies in animals are caused by poor availability to the plant. Acid soils, low in available P, may trap added P in unavailable forms while alkaline soils similarly affect added Co. Fertilization is often impracticable under extensive range conditions and adverse climatic conditions, such as dry and monsoon seasons, but aerial top-dressing remains the most practical method on many upland pastures. Application of sulfur fertilizers to seleniferous soils can prevent Se toxicity in grazing animals unless soils are already high in sulfate. Residual effects of all soil treatments vary widely between locations and determine the frequency of subsequent application.

Adding minerals to rations

Ensuring that the most vulnerable individuals get sufficient minerals without harming either the least vulnerable or the environment is more problematic than it might seem, even when adequate trough-time and trough-space is allowed. Choice of mineral source is determined by:

- purity and unit cost;
- stability in the feed bag and availability to the animal;
- particle size, which can affect ease and safety of mixing;
- freedom from harmful impurities, such as Cd and F;
- compatibility with other ingredients, including vitamins;
- risk of toxicity, often the converse of availability; and
- residual effects on products for human consumption and the environment.

With P supplements, the first four factors can each greatly influence choice of material, whereas with trace elements cost can be less important but availability and safety can be the major concerns. Inclusion of a less-soluble and reactive form of Cu in broiler rations, tribasic copper chloride, protected vitamin E from oxidation in the feed bag (Luo *et al*., 2005). Soluble (sulfate) sources reduced digestibility of neutral detergent fibre when compared with less-soluble hydroxide sources for heifers (Faulkner and Weiss, 2017) but source did not affect uterine health and milk yield (Yasui *et al*., 2014). Feeding Cu and Zn as hydroxychlorides rather than sulfates did not enhance pig performance unless 80 rather than 20mg Zn kg^{-1}DM was (unnecessarily) added (Van

Kuijk *et al*., 2019). Particle size is important to poultry because coarse particles can be trapped in the gizzard, enhancing bone and eggshell strength but interacting with phytase supplements and complicating balance studies (p. 76). In sparse grazing conditions, treatment of the water supply via a metering device may be practicable (e.g. Farmer *et al*., 1982) unless the supply is saline. Limitations remain because voluntary water intake is affected by weather and season and may be uncontrollable under range conditions.

Free-access supplementation

Minerals for pastured livestock are usually provided as loose mixtures or blocks, with common salt (NaCl) as the main ingredient (30–40%) and 'carrier' of less palatable minerals (McDowell *et al*., 1993). Mineral intake by cattle was higher from blocks (218 ± 38) than loose mixes (128 ± 15g d^{-1}) and intake of the latter halved on difficult terrain (Bailey and Welling, 2007). Blocks commonly contain molasses, whose sweetness can be used as a pasture management tool, luring livestock to relatively inaccessible pasture (Bailey *et al*., 2008; Aubel *et al*., 2011; Probo *et al*., 2013). However, individual variation in consumption can be marked (Bowman and Sowell, 1997). In one study, 19% of a ewe flock commonly consumed nothing, while others consumed feed block at 0.4–1.4 kg day^{-1} (Ducker *et al*., 1981). Consumption from protected, trailer-drawn blocks by a beef suckler herd, grazing Californian rangeland, ranged from 0 to 129; about a mean of 27g $day^{-1}cow^{-1}$(± calf) over consecutive seven-day periods (Fig. 3.5) (Morris *et al*., 1980). Seasonal variation in mineral consumption was less from granulated than block sources (Rocks *et al*., 1982). Mineral blocks can be used pharmacologically: molybdate-containing salt licks have prevented chronic copper poisoning in sheep and cattle in low Mo areas and the author knows one ingenious farmer who places copper oxide needles in holes drilled in a mineral releasing block. Providing a loose mineral mixture with distillery by-products, rather than salt, to cattle grazing Californian rangeland increased weight gain by 9% and increased profit margins (Forero *et al*., 2019).

Oral dosage

Pulse doses of minerals in solutions, suspensions and pastes allow all animals to receive known amounts of

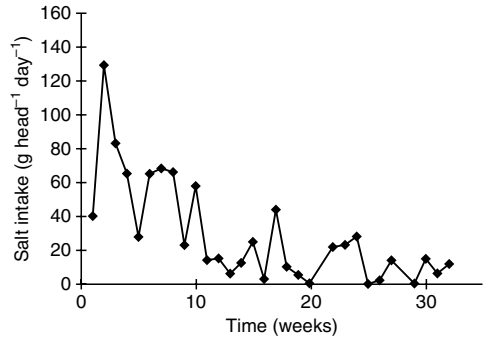

Fig. 3.5. Variation in average salt intake by a group of beef suckler cows given free access to salt from covered trailers on Californian rangeland. Observations began with calving in November. (Data from Morris *et al*., 1980)

mineral at known intervals but retention will be less efficient than with continuously administered mineral. Discontinuous supplementation carries high labour costs if animals have to be driven long distances, handled frequently and dosed only with one mineral. Oral dosing with Se salts had long residual effects and was combined with regular anthelmintic administration but reductions in anthelmintic use to reduce anthelmintic resistance has reduced such opportunities. Care must be taken when using the drench route to provide Co for ruminants. Although transient responses in plasma B_{12} were invariably attained when Co was administered in 10–20 ml of anthelmintic drench to ewes (p. 245), none were found when 5 ml drenches were used in lambs (Williams *et al*., 2017). Stimulation of the oesophageal groove may have caused most of the smaller doses to by-pass the rumen and be unusable for microbial synthesis of vitamin B_{12}.

Slow-release formulations

Some disadvantages of discontinuous supplementation can be overcome by using large doses in relatively inert, slowly solubilized forms, given either orally or by injection. Heavy pellets containing Co (p. 245) and Se (Miller *et al*., 1988; p. 395) and multi-element, soluble glass boluses (Zervas, 1988) are retained in the rumen and slowly release absorbable mineral.[iv] Administration of ZnO in a slow-release bolus worked equally well in alpaca and sheep, releasing 40 mg Zn d^{-1} for 60–70 days (Judson *et al*., 2018). Retention site is dependent on specific gravity and particle size, small, narrow rods of CuO becoming trapped in the abomasum

(p. 279) (Judson *et al*., 1991). Highly reactive and potentially toxic elements, such as Cu and Fe, can be safely administered by injection as slowly translocated organic complexes. Rate of translocation of complexed Cu affects efficacy (p. 279). A single intramuscular injection of Fe as a dextran complex, prevents anaemia in newborn piglets (p. 337). Co can also be given parenterally as vitamin B_{12} in a slowly released form (p. 245).

Inorganic versus chelated ('organic') sources

A steady stream of publications continues to sustain two popular misconceptions: that chelated ('organic') minerals are intrinsically more available and cause less environmental harm than inorganic sources, because inclusion rates can be lowered. Specific amino-acid complexes and non-specific mixtures of minerals with hydrolysed protein ('proteinates') have rarely improved performance or reduced excretion at equivalent intakes (Cu, p. 278; Mn, p. 360; Zn, p. 433). Chelation was originally claimed to provide elements in their absorbed forms, metal-peptides, and some manufacturers' literature still claims '100% availability' for chelated minerals. However, absorptive mechanisms differ between elements and are not peptide-dependent. Opportunities for inorganic minerals to form metal-peptides during digestion abound. Chelates differ in bonding strength and metabolism is non-linearly related to bonding strength in broilers: weak chelation does not change metabolism, intermediate bonding changes metabolism; strong bonding inactivates the bound element (e.g. Mn chelates, Lu *et al*., 2007; Zn chelates, Huang *et al*., 2013). To maintain hyperavailability, bonding must be strong enough to resist natural dietary antagonists and digestive processes yet weak enough to allow eventual access to specific, cellular uptake processes. An early study showed that many amino acid–trace mineral complexes were broken down by simulated stomach digestion (Fig. 3.6), a finding confirmed for Mn 'chelates' (Lu *et al*., 2007).

- Use of the duodenal loop technique to compare the absorbability of inorganic and organic trace element sources is unacceptable as a nutritional tool, since possible degradation in acidic stomach digesta is by-passed.

Manganese glycinate, a chelate with intermediate bonding strength, appears to be a sufficiently strong complex to resist digestion in lambs, is absorbed but poorly incorporated into mangano-enzymes in liver

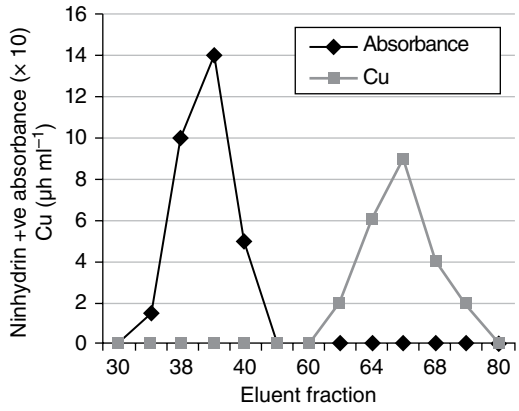

Fig. 3.6. The stability of trace element–amino acid complexes at pH conditions encountered during passage through the gut has been tested *in vitro*. The separate movements of the amino (ninhydrin-positive) and copper components of a copper–lysine complex after chromatography at a pH of 2 indicate probable breakdown of the complex in the stomach. Similar separation occurred for all 15 trace element–amino acid complexes tested, whether the pH was 2 or 5 (Brown and Zeringue, 1994), and explains the lack of efficacy of metal proteinates *in vivo*.

and accumulates in kidney cortex (Gresakova *et al*., 2016). Intermediate chelates are being widely studied and undoubtedly change molecular and genomic markers for metalloproteins in the intestinal mucosa and beyond: every difference is interpreted, without evidence, as beneficial and indicative of 'hyperavailability' but markers can be affected in different, dose-dependent manners. Fe glycinate may be absorbed intact and capable of inducing ferriportin but not DMT1 (p. 339), two carriers which usually work in concert to regulate absorption and tissue uptake according to need. Zn glycinate can increase expression of protective molecules, such as pancreatic metallothionein, superoxide dismutases and immunoglogobulins, that may reflect potential toxicity:

- Any capacity of intermediate chelates to be absorbed intact and subvert normal homeostatic mechanisms indicates potential danger rather than hyperavailability.
- Description of synthetic chelates that can subvert normal homeostatic processes as 'organic' is an oxymoron.
- Molecular markers cannot define availability of a source for all mineral-dependent processes.
- Tissue accretion is not synonymous with hyperavailability.

Chelation may be useful for one element, Cr, in stressed animals for reasons that are not fully understood (p. 455). Provision of Se as selenomethionine undoubtedly affects Se metabolism and some measures of Se status, without necessarily conferring any production benefit (p. 395).

- Evidence of consistent, high value for a specific organic mineral complex in a robust, production test of availability has yet to appear in a reputable, peer-reviewed scientific journal, with the possible exception of iron glycinate in non-ruminants.
- Rules for evaluating chelated mineral sources, similar to those advocated for sources of P (p. 142), are required and should be holistic and performance-related, rather than genomic.

Multi-element chelate supplementation in pigs and poultry

Inorganic minerals have often been partially replaced by chelated forms in multi-element supplementation experiments and benefit claimed if there is no loss of productivity and faecal mineral excretion (predictably) falls (e.g. Yuan *et al*., 2011; De Marco *et al*., 2017). Such claims cannot be justified in the absence of either unsupplemented groups or groups receiving less inorganic supplement. A linear relationship between dietary and faecal Zn in broiler chicks across sources belied a claim for environmental advantage (Yuan *et al*. 2011), just as it did in a similar study with Cu supplements for pigs (Fig. 20.1). When faecal excretion of four elements was compared between inorganic and proteinate mineral sources over similar ranges of dietary Cu, Fe, Mn and Zn, over three successive phases in pigs, only faecal Cu was lower for chelated minerals in the later growing and gilt-developer phases, the most important in terms of environmental impact (Creech *et al*., 2004). Lower faecal excretion in pigs given Cu, Mn and Zn as 2-hydroxy-4-methylthiobutanoic acid rather than inorganic forms indicated higher absorption from a high phytate but not low phytate diet (Liu *et al*., 2014) but reductions (10% for Zn, 20% for Cu, 9% for Mn and 22% for Fe, given as glycinate) did not indicate a step change in availability in favour of these newer chelates: performance was not recorded. Antagonism from phytate can be easily overcome by phytase, which has other benefits (p. 147).

Multi-element chelate supplementation in ruminants

Replacing 25% of inorganic trace minerals with amino acid chelates did not affect uterine health and performance in lactating cows (Yasui *et al*., 2014). Substituting organic for inorganic Cu, Mn, Co and Zn affected rumen fermentation but had no effect on respective proportions excreted in faeces by heifers (Pino and Heinrichs, 2016). Hyperavailability was claimed for four trace element chelates, one (Co) as a glucoheptonate, compared with inorganic counterparts in lactating cows, but the protocol precluded any conclusions of practical significance (Osorio *et al*., 2016).[v] Chelation of minerals may have indirect benefits in limiting the degradation of other organic constituents during pelleting and storage but these have nothing to do with mineral availability to animals.

- Responses to multi-element supplementation cannot be attributed to any one mineral and will not be discussed in later chapters, dealing with specific elements.

Over-correction of Mineral Deficits

Most farmers still overfeed or oversupply minerals, lured by the promise of a quick fix for chronic ill-health and under-performance. However, supplying more minerals than livestock need increases costs with no benefit and can be harmful.[vi] Each of the next 13 chapters ends with a cautionary section on toxicity of individual elements. Mineral supplementation strategies should be periodically questioned to avoid long-term mineral imbalances. Surplus minerals are largely excreted and some (notably P, Zn and Cu) are regarded as hazards to the environment (see Chapter 20). Copper and zinc have the potential to oxidize lipids and fat soluble vitamins, copper as $CuSO_4$ being able to reduce vitamin E concentrations in broiler feed in the bag, during storage (Luo *et al*., 2005). Profligate use of dietary mineral supplements has created problems that are receiving more attention than the deficiencies that prompted their use (Lopez-Alonso, 2012).

Multi-element injections

Many papers have explored the virtues of multi-mineral injections that provide Cu, Mn, Se and Zn in rapidly translocated inorganic forms to cattle on nominally adequate mineral intakes. The same

challenging contexts as those used to test chelates have been used: transition cows, transported cattle, intensively managed, infertile heifers in dairy herds with high incidences of udder and uterine infections. The pseudo-scientific justification for oversupplying minerals includes the hypothesis that at times of high oxidative stress, neither diet nor body stores can meet the acute need for any mineral with antioxidant properties. Injecting animals of normal mineral status with minerals may be harmful. Injections increased weight loss after transportation in normally fed calves (Genther-Schroeder and Hansen, 2015), reducing plasma Il-8, a marker of inflammation: transportation weight loss was unaffected in injected calves, previously depleted of trace elements (Genther and Hansen, 2014). In dairy herds, no benefits from multi-element injections were found in biochemical markers for energy status, oxidative stress, immune function (Bicalho *et al.*, 2014) or mastitis incidence in herds with high somatic cell counts in bulk milk samples (Ganda *et al.*, 2016). Multiple injections did not improve pregnancy rates in beef heifers (Stokes *et al.*, 2018). Evidence to support the hypothesis is not robust and claims are exaggerated. Fertility may be improved by injections that enhance regression of the corpus luteum, a major determinant of fertility in herds where oestrus was routinely synchronized (Wiltbank *et al.*, 2014). Immune responses may be improved by the adjuvant effect of injections that enhance the acute phase response. There is no evidence that incidence of targeted infections was reduced in feedlot cattle. It is unlikely that all four elements are required to elicit the adjuvant effect and

- the scientific approach should be to include minimum amounts of the effective element, possibly Mn, in vaccines and agents of sychronization.

No further coverage of a burgeoning literature on multi-mineral injections will be given since no conclusions can be drawn on the contributions of specific elements.

Biochemical Indicators of Mineral Excess

Chronic exposure to mineral excess generally leads to the sequence of biochemical changes illustrated in Fig. 3.7: the sequence for Type 1 minerals has four phases and is a mirror image of events during deprivation (Fig. 3.2; Table 3.2):

- 'Accretion', during which mineral accumulates at any storage sites;
- 'Excess', when mineral concentrations in *transport* pools rise;
- 'Dysfunction', when abnormal metabolites or constituents appear in blood and tissues or excreta;
- 'Disorder', when clinical abnormalities become visible.

For example, initial increases in liver Cu are followed, eventually, by marginal increases in plasma Cu (*overflow*) and major increases in serum aspartate aminotransferase (AAT, *dysfunction*): these precede the termimal haemolytic crisis (*disorder*) in chronic copper poisoning in sheep, with AAT indicative of damage to hepatocytes. Increases in AAT also occur during development of white muscle disease caused by lack of Se, when the same enzyme leaks from damaged myocytes in muscle. Assays of glutamate dehydrogenase (found only in liver) and creatine kinase (found only in muscle) are of greater value than AAT in distinguishing the underlying site of dysfunction in copper poisoning. Marginal bands must be used in conjunction with biochemical criteria of mineral excess as with those of inadequacy to allow for individual variability in tolerance and rate of exposure, the latter affecting transition between phases. The sequence in Fig. 3.7 is telescoped for Type 2 minerals and for Type 1 minerals during acute toxicity, such as that following injection of excess copper (Table 11.8).

Genomic and Proteomic Markers of Mineral Excess

Ultra-sensitive molecular markers for dysfunction are being deployed to detect sub-clinical mineral toxicity and protect livestock and environments (e.g. Cu; Huang *et al.*, 2015) but notes of caution must be struck. A small increase in dietary Zn (29–95mg kg^{-1} DM; well within a tolerable range) caused changes in 150 transcriptional genes after just 40 days supplementation (Elgendy *et al.*, 2017). Interpretation is as problematical with mineral excesses as with deficiencies. A key regulator of phosphorus metabolism in mammalian kidney, FGF23, was weakly expressed in chick embryo tibia but strongly expressed in liver of laying hens: excessive P intakes upregulated the marker in kidney and tibia but not liver (Wang *et al.*, 2018). Responses in mRNA for pancreatic MT to 30 mg and

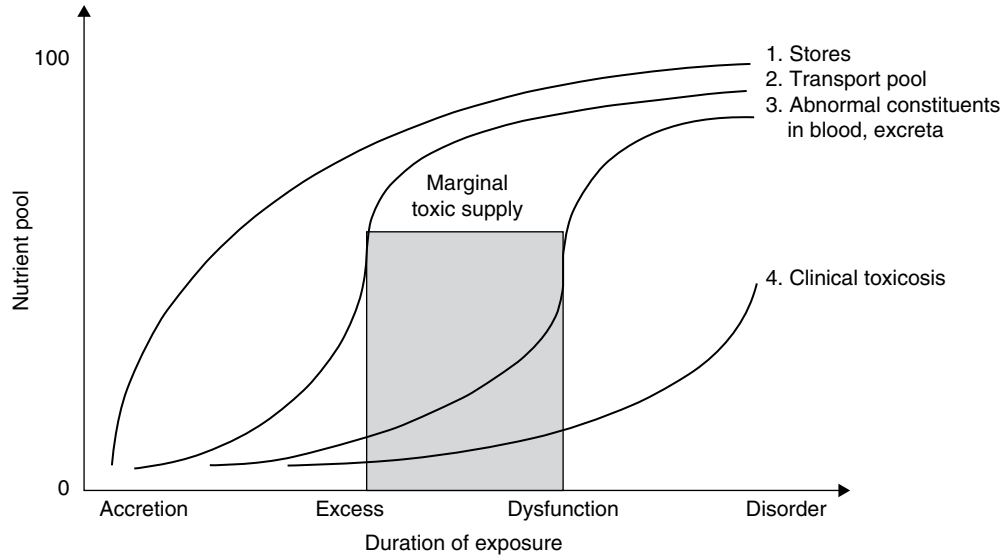

Fig. 3.7. Sequence of biochemical events during chronic exposure to excesses of Type 1 minerals: events can become telescoped during acute exposure (e.g. if mineral availability is high), extended in productive animals (mineral may be safely disposed of in products such as milk and eggs) but more or less toxic during challenge from pathogens (e.g. Fig. 11.16).

60mg Zn kg^{-1}DM were found in broiler chicks within six days and greater when chelated Zn rather than ZnO was the source: superior bioavailability was claimed (Huang *et al*., 2013). Metallothionein protects against trace element excesses and may indicate that chelated Zn by-passed normal transport mechanisms and posed a toxicity hazard.

- It is ironic that molecular studies of mineral excess are being more enthusiastically funded and pursued while those to determine mineral deficiency and minimum requirement are neglected.
- It is uncertainty about needs that causes minerals to be overfed and do harm!

Notes

[i] Zn and growth rate: a threshold value of 6.7 µmol Zn l^{-1}) – 95% of the critical value or asymptote – was defined, below which growth was likely to be impaired. A more pragmatic approach is to relate measures of mineral status to likelihood of livestock responding positively to mineral supplementation in field trials. Thus, lambs in New Zealand were considered likely to benefit from Co supplementation if plasma B_{12} fell below 300 pmol l^{-1} (Clark *et al*., 1985). A similar study (Grace and Knowles, 2002) suggested that lamb growth was unlikely to be retarded by lack of Se until blood Se fell below 130 nmol l^{-1}, far below the existing reference range limit of 500 nmol l^{-1}.

[ii] Addition of protein concentrates to grain mixtures raises concentrations of Ca, P, Zn, Cu and I. Antagonistic effects of phytate in plant protein sources on P, Ca and Zn can be minimized by including natural or synthetic phytases. Bran is freely available on many small, mixed farms and is an excellent source of P and Fe for ruminants (Suttle, 1991). However, reliance on natural mineral sources requires accurate information on mineral composition of local feeds, which is rarely available. Use of mineral-free, industrial nitrogen sources, such as urea, to provide protein and increase digestibility of low quality roughages to ruminants requires co-supplementation with P and S (Suttle, 1991).

[iii] In early Australian studies, lack of dietary Co was solved by use of heavy pellets that lodged in the reticulo-rumen and steadily released Co for many months but some were regurgitated and others became coated. Several trace elements can be administered simultaneously in the soluble glass bolus, which does not become coated. By ingenious capsule design, mineral sources of low density can be retained in the rumen (Cardinal, 1997). Minerals can also be given with vitamins when compressed into a polymer capsule that erodes slowly from its 'open' end (Allan *et al*., 1993).

[iv] Osorio *et al*. (2016) administered both sources as daily boluses in amounts likely to cause acute increases in respective rumen mineral concentrations (Zn, 1125, Mn, 975, Cu, 165 and Co, 15mg d^{-1}). Milk yields

followed DMI and were lower in cows given unchelated minerals, which probably inhibited fibre digestion. Low milk yields in cows given inorganic minerals were also attributed in part to poorer antioxidant status but of two markers of antioxidant status, one showed no effect of source and the other showed advantage for inorganic minerals. The test of cobalt availability used, liver Co, gives no indication of cobalt availability for vitamin B_{12} synthesis.

[v] Excess P and Mg can cause deaths from urinary calculi (Hay and Suttle, 1993), while an excess of Cu is quickly toxic to sheep. Iron is sometimes added to mineral mixtures in excess of need as Fe_2O_3 to colour the mix but to the possible detriment of Cu status. Excess provision of Fe may be partly responsible for the low availability of Mn in many pig rations (Finley *et al*., 1997). Proprietary, free-access mineral mixtures for livestock can provide minerals in excess of need (Suttle, 1983), concentrations driven upward by competition by the misconception amongst farmers that 'more' invariably means 'better'. Formulations can be targetted to meet local mineral requirements (Siromin®) (White *et al*., 1992).

[vi] Conception rate was apparently improved by giving heifers a 'cocktail' of elements (Cu, 50, Mn, 100, Se 25 and Zn, 100 mg) in a 5 ml, subcutaneous injection, 17 days prior to embryo transfer and synchronized oestrus (Sales *et al*., 2011). The pooled response was obtained across five farms in New York State, with animals of normal trace element status and without raising plasma concentrations of some elements. Random blind allocation of heifers to treatment on a 1:1 basis somehow yielded unequal groups of 279 and 216. Conception rate to first service remained poor in treated heifers (48%, Sales *et al*., 2011). Incidences of clinical mastitis and endometritis were reduced in injected groups in the same herds, oversupplied with dietary minerals, but incidences again remained high (18.7% and 27.6%, respectively): survivability was not affected (Machado *et al*., 2013). Incidence of calf diarrhoea in two of these herds was reportedly reduced and neutrophil activity raised by giving two injections (each providing 15 mg Cu, 10 mg Mn, 5 mg Se and 60 mg Zn) at 3 and 30 days of age; once more, incidence remained high amongst treated calves (41.6%) but overall mortality was low and unaffected by treatment (Texeira *et al*., 2014). Alleviation of oxidative stress was claimed in dairy cows, when combined injection of minerals with aflatoxin challenge did not upregulate GPX1 in liver (Pate and Carduso, 2018). Responses to aflatoxin challenge may have been atypical in cows with marginally toxic concentrations of liver Cu (>600mg kg DM^{-1}; see Table 11.8). Performance was not improved in calves weaned abruptly or in two stages, by injections given upon receipt at the feedlot (Rauch *et al*., 2019).

References

Allan, C.L., Hemingway, R.G. and Parkin, J.J. (1993) Improved reproductive performance in cattle dosed with trace element/vitamin boluses. *Veterinary Record* 132, 463–464.

Araujo, M.J., de Madeiros, A,N., Teixera, I.A.M.A., Costa, R.J., Marques, C.A.T., Resende, K.T. and de Melo, G.M.P. (2010) Mineral requirements for growth of Moxoto goat in the semi-arid region of Brazil. *Small Ruminant Research* 93, 1–9.

Arthur, J.R. (1992) Selenium metabolism and function. *Proceedings of the Nutrition Society of Australia* 17, 91–98.

Aubel, N.A., Jaeger J.R., Drouillard, J.S., Schlegel, M.D., Pacheco L.A. et al. (2011) Effects of mineral-supplement delivery system on frequency, duration, and timing of supplement use by beef cows grazing topographically rugged, native rangeland in the Kansas Flint Hills. *Journal of Animal Science* 89 3699–3706.

Bailey, D.W. and Welling, G.R. (2007) Evaluation of low-moisture blocks and conventional dry mixes for supplementing minerals and modifying cattle grazing patterns. *Rangeland Ecology and Management* 60, 54–64.

Bailey, D.W., VanWagoner, H.C., Weinmeister, R. and Jensen, D. (2008) Comparison of low-moisture blocks and salt for manipulating grazing patterns of beef cows. *Journal of Animal Science* 86, 1271–1277.

Barkley, G.R., Miller, H.M. and Forbes, J.M. (2004) The ability of laying hens to regulate phosphorus intake when offered two feeds containing different levels of phosphorus. *British Journal of Nutrition* 92, 233–240.

Bicalho, M.L., Lima, F.S., Ganda, E.K., Foditsch, C., Meira, E.B. Jr *et al*. (2014) Effect of trace mineral supplementation on selected minerals, energy metabolites, oxidative stress, and immune parameters and its association with uterine diseases in dairy cattle. *Journal of Dairy Science* 97, 4281–4295.

Bowman, J.G.P. and Sowell, B.F. (1997) Delivery method and supplement consumption by grazing ruminants: a review. *Journal of Animal Science* 75, 543–550.

Brown, T.F. and Zeringue, L.K. (1994) Laboratory evaluations of solubility and structural integrity of complexed and chelated trace mineral supplements. *Journal of Dairy Science* 77, 181–189.

Cardinal, J.R. (1997) Intrarumunal devices. *Advanced Drug Delivery Systems* 28, 303–322.

Chesters, J.P. and Arthur, J.R. (1988) Early biochemical defects caused by dietary trace element deficiencies. *Nutrition Research Reviews* 1, 39–56.

Clark, R.G., Wright, D.F. and Millar, P.R. (1985) A proposed new approach and protocol to defining mineral deficiencies using reference curves: cobalt deficiency in young sheep used as a model. *New Zealand Veterinary Journal* 33, 1–5.

Combs, D.P. (1987) Hair analysis as an indicator of the mineral status of livestock. *Journal of Animal Science* 65, 1753–1758.

Creech, B.L., Spears J.W., Flowers, W.L., Hill, G.M., Lloyd, K.E., Armstrong, T.A. and Engle, T.E. (2004) Effect of dietary trace mineral concentration and source (inorganic vs. chelated) on performance, mineral status, and fecal mineral excretion in pigs from weaning through finishing. *Journal of Animal Science* 62, 2140–2147.

Cunningham, I.J. (1949) Sheep dosed with minerals eat as much lick as undosed animals. *New Zealand Journal of Agriculture* 8, 583–586.

De Marco, M., Zoon, M.V., Margetyal, C., Pickart, C. and Ionescu, C. (2017) Dietary administration of glycine chelated trace elements can improve performance and slaughter yield in broilers and reduce mineral excretion. *Animal Feed Science and Technology* 232, 182–189.

Ducker, M.J., Kendall, P.T., Hemingway, R.G. and McClelland, T.H. (1981) An evaluation of feed blocks as a means of providing supplementary nutrients to ewes grazing upland/hill pastures. *Animal Production* 33, 51–58.

Elgendy, R., Plazzo, I.F., Castellani, F., Giantin, M., Grotta, L., Cerratini, I., Dacasto, M. and Martino, G. (2017) Transcriptome profiling and functional analysis of sheep fed with high zinc-supplemented diet: a nutrigenomic approach. *Animal Feed Science and Technology* 234, 195–204.

Farmer, P.E., Adams, T.E. and Humphries, W.R. (1982) Copper supplementation of drinking water for cattle grazing molybdenum-rich pastures. *Veterinary Record* 111, 193–195.

Faulkner, M.J. and Weiss, W.P. (2017) Effect of source of trace minerals in either forage- or by-product-based diets fed to dairy cows. 1. Production and macronutrient digestibility. *Journal of Dairy Science* 100, 5358–5367.

Finley, J.W., Caton, J.S., Zhou, Z. and Davison, K.L. (1997) A surgical model for determination of true absorption and biliary excretion of manganese in conscious swine fed commercial diets. *Journal of Nutrition* 127, 2334–2341.

Fordyce, F.M., Masara, D. and Appleton, J.D. (1996) Stream sediment, soil and forage chemistry as predictors of cattle mineral status in northeast Zimbabwe. In: Appleton, J.D., Fuge, R. and McCall, G.H.J. (eds) *Environmental Geochemistry and Health*. Geological Society Special Publication No. 113, London, pp. 23–37.

Forero, L.C., Davy, J.S., McMurry, B.E., and Oltjen, J.W. (2019) Monensin and mineral supplementation economically increase yearling cattle weight gain on California annual rangeland. *Translational Animal Science* 3, 737–741.

Ganda, E.K., Bisinotto, R.S., Vasquez, A.K., Teixeira, A.G.V., Machado, V.S. et al. (2016) Effects of injectable trace mineral supplementation in lactating dairy cows with elevated somatic cell counts. *Journal of Dairy Science* 99, 7319–7329.

Genther, O.N. and Hansen, S.L. (2014) Effect of dietary trace mineral supplementation and a multi-element trace mineral injection on shipping response and growth performance of beef cattle. *Journal of Animal Science* 92, 2522–2530.

Genther-Schroeder, O.N. and Hansen, S.L. (2015) Effect of a multielement trace mineral injection before transit stress on inflammatory response, growth performance, and carcass characteristics of beef steers. *Journal of Animal Science* 93, 1767–1779.

Goff, J.P. (2018) Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *Journal of Dairy Science* 101, 2763–2813.

Golden, M.H.N. (1996) Specific deficiencies versus growth failure: Type I and Type II nutrients. *Journal of Nutritional & Environmental Medicine* 6, 301–308.

Grace, G.D. and Knowles, S.O. (2002) A reference curve using blood selenium concentration to diagnose selenium deficiency and predict growth responses in lambs. *New Zealand Veterinary Journal* 50, 163–165.

Gresakova, L., Venglovska, K. and Cobanova, K. (2016) Dietary manganese source does not affect Mn, Zn and Cu tissue deposition and the activity of manganese-containing enzymes in lambs. *Journal of Trace Elements in Medicine and Biology* 38, 138–143.

Harvey, L.J., Ashton, K., Hooper, L., Casgrain, A. and Fairweather-Tait, S.J. (2009) Methods of assessment of copper status in humans: a systematic review. *American Journal of Clinical Nutrition* 89, 2009S–24S.

Hay, L. and Suttle, N.F. (1993) Urolithiasis. In: *Diseases of Sheep*, 2nd edn. Blackwell Scientific, London, pp. 250–253.

Huang, Y.L., Lu, L., Xie, J.J., Li, S.F., Li, X.L. *et al.* (2013) Relative bioavailabilities of organic zinc sources with different chelation strengths for broilers fed diets with low or high phytate content**.** *Animal Feed Science and Technology* 179, 144–148.

Huang, Y.L., Ashwell, M.S., Fry, R.S., Lloyd, K.E., Flowers, W.L. and Spears, J.W. (2015) Effect of dietary copper amount and source on copper metabolism and oxidative stress of weanling pigs in short-term feeding. *Journal of Animal Science* 93 2948–2955.

Judson, G.J., Babidge, P.J., Brown, T.H., Frensham, A.B., Langlands, J.P. and Donald, G.E. (1991) Copper oxide particles as an oral supplement: the retention of wire particles in the alimentary tract of sheep. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals, Dubrovnik*. IMI, Zagreb, pp. 15-10–15-11.

Judson, G.J, MacGregor, B.A., Ellis, K.J. and Howse, A.M. (2018) Evaluation of controlled-release devices

for providing chromium sesquioxide and zinc in Huacaya alpacas at pasture. *Australian Veterinary Journal* 96, 458–463.

Khan, Z.I., Ashraf, M., Hussain, A., McDowell, L.R., and Ashraf, M.Y. (2006) Concentrations of minerals in milk of sheep and goats grazing similar pastures in a semiarid region of Pakistan. *Small Ruminant Research* 65, 274–278.

Kumar, S. and Singh, S.P. (1981) Muzzle secretion electrolytes as a possible indicator of sodium status in buffalo (*Bubalus bubalis*) calves: effects of sodium depletion and aldosterone administration. *Australian Journal of Biological Sciences* 34, 561–568.

Laven, R.A. and Livesey, C.T. (2006) An evaluation of the effect of clotting and processing on the recovery of copper from bovine blood. *Veterinary Journal* 171, 295–300.

Liao, X., Ma, C., Lu, L., Zhang, L. and Luo, X. (2017) Determination of dietary iron requirements by full expression of iron-containing cytochrome c oxidase in the heart of broilers from 22 to 42 d of age. *British Journal of Nutrition*, 118, 493–499.

Liu, Y., Ma, Y.L., Zhao, J.M., Vazquez-Aaon, M. and Stein, H.H. (2014) Digestibility and retention of zinc, copper, manganese, iron, calcium and phosphorus in pigs fed diets containing inorganic and organic minerals. *Journal of Animal Science* 92, 3407–3415.

Lopez-Alonso, M. (2012) Trace minerals and livestock: not too much not too little. *ISRN Veterinary Science* 18, 704825. http://dx.doi.org/10.5402/2012/704825.

Lu, L., Luo, X.G., Ji, C., Liu, B. and Yu, S.X. (2007) Effect of manganese supplementation on carcass traits, meat quality and lipid oxidation in broilers. *Journal of Animal Science* 85, 812–822.

Luo, X.G., Ji, F., Lin, Y.X., Steward, F.A., Lu, L., Liu, B. and Yu, S.X. (2005) Effects of dietary supplementation with copper sulphate or tribasic copper chloride on broiler performance, relative copper bioavailability and oxidation stability of vitamin E in feed. *Poultry Science* 84, 888–889.

Machado, V.S., Bicalho, M.L.S., Pereira R.V., Caixeta, L.S., Knauer, W.A., Oikonomou, G., Gilbert, R.O. and Bicalho, R.C. (2013) Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows. *Veterinary Journal* 197, 451–456.

Manangi, M.K., Maharjan, P. and Coon, C.N. (2018) Effect of different concentrations of dietary P and Ca on plasma inorganic P and urinary P excretion using noncolostomized and colostomized broilers. *Poultry Science* 97, 522–530.

Masters, D.G., Hancock, S., Refshauge, G., Robertson, S., Bhanugopan, M., Friend, M. and Thompson, A.N. (2018) Mineral status of reproducing ewes grazing vegetative cereal crops. *Animal Production Science* 58, 2049–2060.

McDowell, L.R., Conrad, J.H. and Hembry, F.G. (1993) *Minerals for Grazing Ruminants in Tropical Regions*, 2nd edn. Animal Science Department, University of Florida, Gainsville, Florida.

Millar, P.R. and Meads, W.J. (1988) Selenium levels in the blood, liver, kidney and muscle of sheep after the administration of iron/selenium pellets or soluble glass boluses. *New Zealand Veterinary Journal* 36, 8–10.

Millar, P.R., Meads, W.J., Albyt, A.T., Scahill, B.G. and Sheppard, A.D. (1988) The retention and efficacy of soluble glass boluses for providing selenium, cobalt and copper to sheep. *New Zealand Veterinary Journal* 36, 11–14.

Mills, C.F. (1987) Biochemical and physiological indicators of mineral status in animals: copper, cobalt and zinc. *Journal of Animal Science* 65, 1702–1711.

Morris, J.G. (1980) Assessment of the sodium requirements of grazing beef cattle: a review. *Journal of Animal Science* 50, 145–152.

Morris, J.G., Dalmas, R.E. and Hall, J.L. (1980) Salt (sodium) supplementation of range beef cows in California. *Journal of Animal Science* 51, 722–731.

Myers, S.A. (2015) Zinc transporters and zinc signaling: new insights into their role in type 2 diabetes. *International Journal of Endocrinology* 2015, Article ID 167503.

Ndou, S.P., Gous, R.M. and Chimonyo, M. (2013) Prediction of scaled feed intake in weaner pigs using physico-chemical properties of fibrous feeds. *British Journal of Nutr*ition 110, 774–780.

O'Dell, B.L. (2000) Role of zinc in plasma membrane function. *Journal of Nutrition* 130, 1432S–1436S.

Osorio, J.S., Trevisi, E., Li, C., Drackley, J.K., Socha, M.T. and Loor J.J. (2016) Supplementing Zn, Mn, and Cu from amino acid complexes and Co from cobalt glucoheptonate during the peripartal period benefits post-partal cow performance and blood neutrophil function. *Journal of Dairy Science* 99, 1868–1883.

Pamp, D.E., Goodrich, R.D. and Meiske, J.C. (1976) A review of the practice of feeding minerals free choice. *World Review of Animal Production* 12, 13–17.

Pate, R.T. and Carduso, F.C. (2018) Injectable trace minerals (selenium, copper, zinc, and manganese) alleviate inflammation and oxidative stress during an aflatoxin challenge in lactating multiparous Holstein cows. *Journal of Dairy Science* 101, 8532–8543.

Phillippo, M. (1983) The role of dose–response trials in predicting trace element disorders. In: Suttle, N.F., Gunn, R.G., Allen, W.M., Linklater, K.A. and Wiener, G. (eds) *Trace Elements in Animal Production and Veterinary Practice*. British Society of Animal Production Special Publication No. 7, Edinburgh, pp. 51–60.

Pino, F. and Heinrichs, A.J. (2016) Effect of trace minerals and starch on digestibility and rumen fermentation in diets for dairy heifers. *Journal of Dairy Science* 99, 2797–2810.

Probo, M., Massolo, A., Lonati, M., Bailey, D., Gorlier, A., Maurino, L. and Lombardi, G. (2013) Use of mineral mix supplements to modify the grazing patterns by cattle for the restoration of sub-alpine and alpine shrub-encroached grasslands. *Rangeland Journal* 35, 85–93.

Rauch, J.C., Stokes, R.S. and Shike, D.W. (2019) Evaluation of two-stage weaning and trace mineral injection on receiving cattle growth performance and behaviour. *Translational Animal Science* 3, 155–163.

Reszka, E., Jablonska, E., Gromadzinska, J. and Wasowicz, W. (2012) Relevance of selenoprotein transcripts for selenium status in humans. *Genes and Nutrition* 7, 127–137.

Rey-Crespo, F., Miranda, M. and Lopes-Alonso, M. (2013) Essential trace and toxic element concentrations in organic and conventional milk in NW Spain. *Food and Chemical Toxicology* 55, 513–518.

Reynolds, F., Forbes, J. and Miller, H.M. (2010) Does the newly weaned piglet select a zinc oxide supplemented feed, when given the choice? *Animal* 4, 1359–1567.

Rocks, R.L., Wheeler, J.L. and Hedges, D.A. (1982) Labelled waters of crystallization in gypsum to measure the intake of loose and compressed mineral supplements. *Australian Journal of Experimental Agriculture and Animal Husbandry* 22, 35–42.

Sales, J.N.S., Peveira, R.V.V., Bicalho, R.C. and Baruselli, P.S. (2011) Effect of injectable copper, selenium, zinc and manganese on the pregnancy rate of crossbred heifers (Bos indicus~Bos taurus) synchronized for timed embryo transfer. *Livestock Science* 142, 59–62.

Sprinkle, J.E., Baker, S.D., Church, J.A., Findlay, J.R., Graf, S.M. et al. (2018) Case study: regional assessment of mineral element concentrations in Idaho forage and range grasses, *The Professional Animal Scientist* 34, 494–504.

Stokes, R.S., Volk, M.J., Ireland, F.A., Gunn, P.J. and Shike, D.W. (2018) Effect of repeated trace mineral injections on beef heifer development and reproductive performance. *Journal of Animal Science* 96, 3943–3954.

Suttle, N.F. (1983) Meeting the mineral requirements of sheep. In: Haresign, E. (ed.) *Sheep Production*. Butterworths, London, pp. 167–183.

Suttle, N.F. (1991) Mineral supplementation of low quality roughages. In: *Proceedings of Symposium on Isotope and Related Techniques in Animal Production and Health*. International Atomic Energy Agency, Vienna, pp. 101–104.

Suttle, N.F. (2008) Relationships between the concentrations of tri-chloroacetic acid-soluble copper and caeruloplasmin in the serum of cattle from areas with different soil concentrations of molybdenum. *Veterinary Record* 162, 237–240.

Suttle, N.F. (2017) Challenges in predicting the need to supplement pastured livestock with minerals. In: Singh, B.-R., McLaughlin, J. and Brevik, E. (eds) *The Nexus between Soils, Plants, Animals and Human Health*. Schweizerbart Science Publishers, Stuttgart, Germany.

Suttle, N.F., Brebner, J., McLean, K. and Hoeggel, F.U. (1996) Failure of mineral supplementation to avert apparent sodium deficiency in lambs with abomasal parasitism. *Animal Science* 63, 103–109.

Tedeschi, L.O., Molle, G., Menendez, H.M., Cannas, A. and Fonseca, M.A. (2019) The assessment of supplementation requirements of grazing ruminants using nutrition models. *Translational Animal Science* 3, 811–828.

Teixeira, R.M.X.S., Lima, F.S., Bicalho, M.L.S., Kussler, A., Lima., S.F., Philippe, M.J. and Bicalho, R.C. (2014) Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on immunity, health, and growth of dairy calves. *Journal of Dairy Science* 97, 4216–4226.

Thomas, D.T., Rintoul, A.J. and Masters, D.G. (2006) Sheep select combinations of high and low sodium chloride, energy and protein feed that improve their diet. *Applied Animal Behaviour Science* 105, 140–153.

Van Kuijk, S.A., Jacobs, M., Smits, C.H.M. and Han, Y. (2019) The effect of hydroxychloride trace minerals on the growth performance and carcass quality of grower/finisher pigs: a meta-analysis. *Journal of Animal Science* 97, 4619–4624.

Wang, R.M., Zhao, J.P., Wang, X.J., Jiao, H.C., Wu, J.M. and Lin, H. (2018) Fibroblast growth factor 23 mRNA expression profile in chickens and its response to dietary phosphorus. *Poultry Science* 97, 2258–2266.

White, C.L., Masters, D.G., Peter, D.W., Purser, D.B., Roe, S.P. and Barnes, M.J. (1992) A multi-element supplement for grazing sheep. 1. Intake, mineral status and production responses. *Australian Journal of Agricultural Research* 43, 795–808.

White, C.L., Martin, G.B., Hynd, P.T. and Chapman, R.E. (1994) The effect of zinc deficiency on wool growth and skin and wool histology of male Merino lambs. *British Journal of Nutrition* 71, 425–435.

Whittemore, E., Emmans, G., and Kyriazakis, I. (2003) The problem of predicting food intake during the period of adaptation to a new food: a model. *British Journal of Nutrition* 89, 383–399.

Wilkinson, S.J., Selle, P.H., Bedford M.R. and Cowieson A.J. (2013) Separate feeding of calcium improves performance and ileal nutrient digestibility in broiler chicks. *Animal Production Science* 54, 172–178.

Williams, J.R., Williams, N.E. and Kendall, N.R. (2017) The efficacy of supplying supplemental cobalt, selenium and vitamin B_{12} via the oral drench route in sheep. *Livestock Science* 200, 80–84.

Wiltbank, M., Souza, A., Carvalho, P., Cunha, A., Giordano, J., Fricke, P., Baez, J.M. and Diskin, M. (2014) Physiological and practical effects of progesterone on reproduction in dairy cattle. *Animal* 8, 70–81.

Yasui, T., Ryan, C.M., Gilbert, R.O., Perryman, K.R. and Overton, T.R. (2014) Effects of hydroxy trace minerals on oxidative metabolism, cytological endometritis and performance of transition dairy cows. *Journal of Dairy Science* 97, 3728–3738.

Yuan, J., Xu, Z., Huang, C., Zhou, S. and Guo, Y. (2011) Effect of dietary Mintrex-Zn/Mn on performance, gene expression of Zn transfer proteins, activities of Zn/Mn related enzymes and fecal mineral excretion in broiler chickens. *Animal Feed Science and Technology* 168, 72–79.

Zervas, G.P. (1988) Use of soluble glass boluses containing Cu, Co and Se in the prevention of trace-element deficiencies in goats. *Journal of Agricultural Science* 110, 155–158.

4 Calcium

Introduction

A need for calcium (Ca) for growth and milk production in dairy cows was one of the first consequences of mineral deprivation to be demonstrated (p. 1) but egg production in hens (Buckner *et al*., 1930; Deobald *et al*., 1936) and pullets (Common, 1938) and bone development in chicks (Elvejhem and Kline, 1933) provided more fruitful and convenient experimental models for studying the essentiality of Ca. Egg production increased in direct proportion to dietary Ca over the range of 1.9–30.9g kg^{-1}, rising from 10% to 93% egg days (Gilbert, 1983). As commercial poultry production intensified, energy-rich, grain-based diets were increasingly fed to housed birds and incidences of bone and egg disorders multiplied. Unwittingly, birds were being fed diets naturally deficient in Ca and rich in a Ca-antagonist, phytate, while simultaneously deprived of UV irradiation and, thus, 'starved' of vitamin D_3, essential for efficient utilization of Ca. Selection for growth rate and egg production, traits with high Ca requirements, and use of antibiotics, hormones and additives that improved feed conversion efficiency (FCE), increased the Ca density required in feeds. In a large survey of UK broiler flocks in the new millennium, 27.6% of birds showed poor locomotion and 3.3% were almost unable to walk, despite culling policies supposed to remove affected individuals (Knowles *et al*., 2008) but lack of dietary Ca was not evidently responsible. A ban on battery cages in Europe, designed to improve animal welfare, has increased the incidence of keel bone fractures (KBF) (Regmi *et al*., 2016): provision of particulate $CaCO_3$ decreased KBF, although lack of Ca was not the primary cause (Fleming *et al*., 2006). In milk production, selection of animals for high yield and diets for high FCE has left the majority of cows at risk from sub-acute clinical hypocalcaemia (SACH) (p. 61),[i] shortly after calving, despite an absence of overt bone disorders. Acute disorder, in the form of milk fever, is far less prevalent and preventable but the measures required, massive oral dosage with Ca or acidifying the pre-partum diet, are extreme. Smarter remedies are needed to prevent SACH and KBF, requiring improved delivery of Ca from skeleton as well as diet. Spontaneous fractures of the humerus in heifers has emerged as a problem in New Zealand (Dittmer *et al*., 2016), while the aetiology is multi-factorial, exposure to acidogenic diets may increase vulnerability.

Functions of Calcium

Development of the skeleton

Calcium is the most abundant mineral in the body and 99% is found in the skeleton, protecting vital organs, while allowing growth and movement and providing a store of minerals for use in times of need. Bones grow in length and width in response to growth hormone and other factors, which modulate bone-forming (osteoblasts) and bone-resorbing (osteoclasts) cells (Loveridge, 1999).[ii] Mammalian young are born with poorly mineralized bones and, while suckled, do not receive enough Ca from energy-rich milk to increase bone density: bone Ca can be increased by adding Ca to milk for calves (AFRC, 1991). Early weaning increases accretion of Ca in bone in lambs and kids (p. 9) and bone density increases after weaning in grazing lambs (Field *et al*., 1975; Spence *et al*., 1985). Treadmill activity increased cortical bone thickness in confined lambs (Wallace *et al*., 2014). Increased physical activity and load-bearing may contribute to post-weaning increases in bone density in free-moving animals at pasture. Striking evidence of the impact of mechanical stress on skeletal development and strength is provided by poultry. Bone ash concentration decreases in caged birds (Regmi *et al*., 2016) and increases

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with access to a perch but alighting bears an increased risk of KBF (Sandilands *et al*., 2009). Bone Ca increases with dietary Ca, particularly in pigs (Fig. 4.1) and poultry (Fig. 6.3). Partnerships between hormones, endocrine (parathyroid hormone, PTH) and exocrine (e.g. calcitriol, 1,25-$(OH)_2D_3$), and Ca-binding cytokines (e.g. osteocalcin, OC, and osteopontin, OP) facilitate Ca accretion in bone in the young and regulate Ca fluxes to and from bone in the sexually mature animal.[iii]

The skeleton as a reservoir of calcium in ruminants

Contrasts in Ca metabolism between first-calving and multiparous cows (Fig. 4.2) indicate protection of the skeletal reserve until growth is complete. Priority is given to bone-forming (e.g. OC and OP) over bone-resorbing cytokines (RANKL and osteprotegerin, OPG) in first-calvers, allowing net bone accretion. Around parturition, up to one fifth of Ca in the skeleton is removed in well-nourished, confined multiparous ewes (Braithwaite, 1983) and dairy cows (Ramberg *et al*., 1984), sufficient to be detected by radiography in dairy goats (Liesegang, 2008). The losses are unaccompanied by hypocalcaemia (Braithwaite, 1983; Liesegang *et al*., 2000), refractory to Ca (AFRC, 1991; Kamiya *et al*., 2005) and protein (Chrisp *et al*., 1989b) supplementation and partly obligatory. In minimally restricted, newly calved, multiparous cows, eventual decreases in bone-specific alkaline phosphatase (BSAP) (Sato *et al*., 2013) and deoxypryidine nucleotide (DPN) in serum, markers for bone resorption, suggest that obligatory loss of Ca normally lasts <21 days and is attenuated by Ca supplementation (Taylor *et al*., 2009; Sato *et al*., 2013; Gaignon *et al*., 2019).

Biochemical responses around parturition differ between goats, sheep and cows: only cows show a sharp decline in plasma Ca but both goats and cows show a rise in plasma calcitriol and decrease in ratio of bone formation to bone resorption. Ewes show

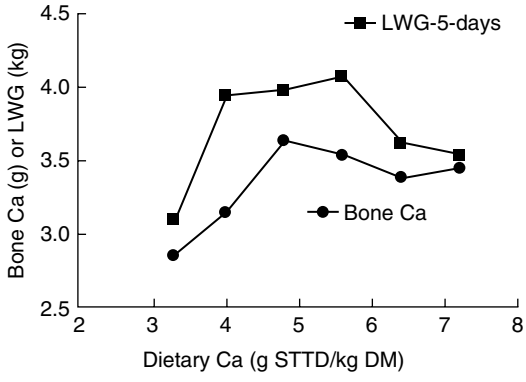

Fig. 4.1. More dietary calcium is required to maximize bone Ca than to maximize liveweight gain (LWG) in starter pigs. (Data from Gonzales-Vega *et al*., 2016: STTD is standardized total tract digestible Ca)

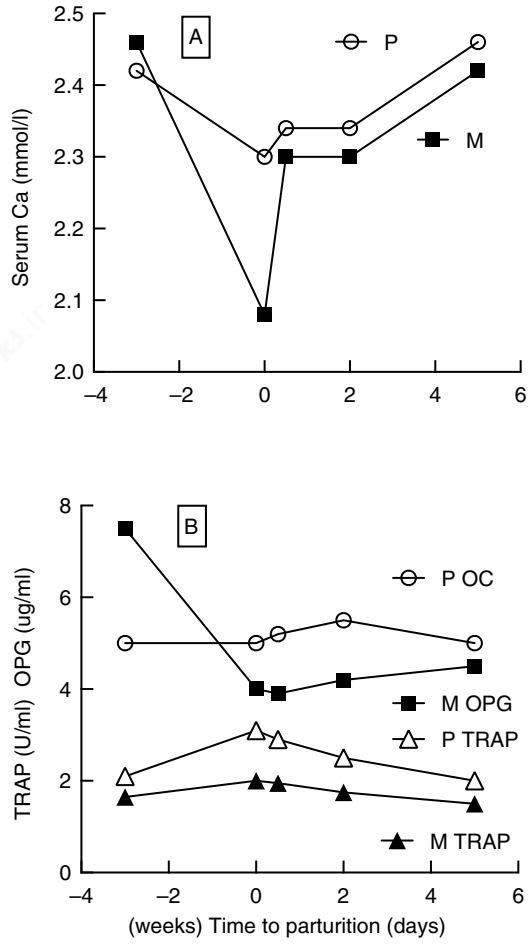

Fig. 4.2. Serum concentrations of calcium (A) and two markers indicating bone resorption (B) (osteoprotegerin, OPG) and tartrate-resistant acid phosphatase 5b (TRAP) in primiparous (P) and multiparous (M) cows before and after calving (data from Hatate *et al*., 2018).

pre-parturient adaptations to increased demands for Ca and are an unsuitable model for the dairy cow in some respects. Other components of the hormonal partnership need not concern us here[iv] except to note that husbandry practices can influence the skeletal reserve. Induction of synchronized oestrus (in which oestrogen displaces progesterone as the dominant hormone), 11 weeks after calving, was associated with decreased Ca retention (Taylor *et al*., 2009). Use of dairy ewes as models for dairy cows may also be inappropriate because ewes remain anoestrus while lactating.

Dietary protein and the skeletal reserve

The skeleton is influenced by non-mineral nutrition, particularly the supply of dietary protein, which affects both the synthesis and mineralization of bone matrix. Restriction of dietary N to 9g kg^{-1} DM reduced accretion of Ca in young goats and moderated responses to lack of Ca through changes in serum insulin-like growth factor (IGF 1) rather than PTH (Elfers *et al*., 2016): impaired hydroxlation of calcidiol by the kidney was implicated (Wilkens *et al*., 2016, 2019).[v] The Ca-depleting effect of nematode infection in naïve lambs (p. 68) may be secondary to a need to redistribute body protein for immunogenic purposes (Coop and Sykes, 2002). Seasonal bone resorption in outwintered ewes (Field *et al*., 1968) was partly attributable to protein depletion.

The calcium reservoir in avian species

Avian adults are uniquely susceptible to lack of dietary Ca while in lay, during which up to 30 times the total-body Ca is deposited in a skeleton-like shell around each egg laid (Gilbert, 1983). In a 24-hour cycle, OP in the ovum membrane is impregnated with 2g Ca, as $CaCO_3$, during passage down the oviduct. Calcium ebbs and flows from the skeleton to complement supply from the gut, under modulation by the same hormones and cytokines that regulate lactation in cows (Whitehead, 2004). Bone morphology changes with onset of sexual maturity. Release of oestrogen halts cancellous and structural bone formation and new medullary bone is woven onto the surface of existing cortical bone and bone marrow to provide a labile Ca reserve that maintains plasma Ca during shell formation (Whitehead, 2004). There is a diurnal peak in calcification overnight and provision of 24 h lighting stimulates Ca deposition and increases shell strength. Mineralization of the skeleton is influenced by genotype, housing and dietary Ca supply (Fleming *et al*., 2006). Aviary and 'furnished' cage housing systems that encourage activity increase weight and ash concentration of medullary pneumonic ('light-for-flight') and radius bones in pullets (Neijat *et al*., 2019).

Non-skeletal functions of calcium

Exchanges of Ca between the skeleton and the small but life-sustaining proportion (1%) of body Ca that lies outside it support Ca homeostasis in an 'elegant compromise' (Bain and Watkins, 1993). Non-skeletal Ca exists as free ions, bound to serum proteins and as complexes with organic and inorganic acids, and is essential for survival. Ionized Ca^{2+}, 50–60% of total plasma Ca, is essential for nerve conduction, muscle contraction and cell signalling (Carafoli, 1991), roles mediated via ubiquitous calcium sensors (Ray, 2015; Ellinger, 2016). The intricate network of processes that controls cellular Ca^{2+} bears comparison with the cytokine cascade of the immune response and is shared only by Zn and Se, amongst other minerals. Exchanges of Ca^{2+} within and between cells (Breitwieser, 2008) are modulated by vitamin D_3 metabolites and calcium-binding proteins (CaBP), which trigger inflammatory and immune responses. Calcium can activate or stabilize some enzymes and is required for normal blood clotting (Hurwitz, 1996). Calcium facilitates the conversion of prothrombin to thrombin, which reacts with fibrinogen to form the blood clot, fibrin. Failure of Ca homeostasis is life-threatening but effects vary with suddenness of onset.

Clinical Consequences of Acute Lack of Calcium

Milk fever in dairy cows

Milk fever was once estimated to cost the UK dairy industry £10 million annually (Allen and Sansom, 1985) and has been widely reviewed (Thilsing-Hansen *et al*., 2002; Goff, 2007; DeGaris and Lean, 2008). The disorder is caused by an abrupt increase in demand for Ca with onset of lactation in cows unlikely to have eaten for several hours. Plasma Ca begins to decline several hours before calving

(Megahed *et al*., 2018) and is subnormal in all cows at calving.[vi] Milk fever was a common reason for veterinary call-outs, affecting 3–10% of dairy cows in Europe (Heringstad *et al*., 2005). Incidence in a large grazing herd in New Zealand declined from 13.1% between 1970 and 1979 to 4.5% and 3.2% in successive decades, due largely to cross-breeding the Jersey with the Friesian (Roche and Berry, 2006), which is 2.25 times less susceptible to milk fever (Lean *et al*., 2006). Incidents are repeatable, previously affected cows being 2.2 times more likely to succumb in a current lactation than hitherto unaffected cows (Roche and Berry, 2006). Symptoms develop within 48 hours of calving in three stages: listlessness, coma and sternal recumbency[vii] but concomitant hypomagnesaemia occasionally confounds the presenting signs. Introduction of routine Mg supplementation in New Zealand in 1979 may have contributed to the subsequent decline in incidence (Roche and Berry, 2006). Diagnosis is presumptive, relying on clinical assessment and 'blind' response to intravenous infusion of calcium borogluconate (CaBG), with Mg, which revives about 40% of affected cows. Diagnosis is confirmed if pre-treatment plasma samples contained <1.7mmol Ca l^{-1} or low values are found in vitreous humor, taken *post mortem*: a further 40% will respond to re-treatments, need for which increases with delay in first treatment and severity of initial hypocalcaemia[viii]; those still recumbent ('downer' cows) are prone to abomasal displacement. Cows that recover are prone to limb injuries, mastitis and ketosis: milk yield can drop by 1–2 kg day^{-1} for weeks (Rajala-Schulz *et al*., 1999; Santos *et al*., 2019). Susceptibility is age-dependent and increases markedly between the second and fourth calvings (Fig. 4.3).

In New Zealand, several risk factors were identified: days of high evaporation, high rainfall and chilly nights; need for assistance at calving and extreme body-condition scores (Roche and Berry, 2006). Liver fatness may also predispose cows to milk fever, since affected cows have anomalous serum lipid profiles (Oikawa and Kato, 2002). Pastures with a high cation-anion difference (DCAD), due largely to high pasture K, were also associated with heightened risk. However, milk fever is generally less common in grazing than housed herds despite a much higher DCAD (Pehrson *et al*., 1999; McNeill *et al*., 2002; Roche *et al*., 2003a,b). Incidence of milk fever in Costa Rica was low (1.5%) and typical of countries with pastoral husbandry systems: there, additional risk factors included length of preceding dry period and previous milk yield (Saborio-Montero *et al*., 2017), which may partly explain the lower incidence compared to housed herds. The disease was more prevalent in a high-altitude, low-temperature zone than in low-altitude, wet forest conditions and incidence was higher in winter than in summer. The role of DCAD as opposed to effects of separate ions may have been exaggerated.[ix]

Subclinical Hypocalcaemia in Dairy Cows

Subclinical hypocalcaemia (SCH) has been widely studied and reviewed (Seifi and Kia, 2017) since the

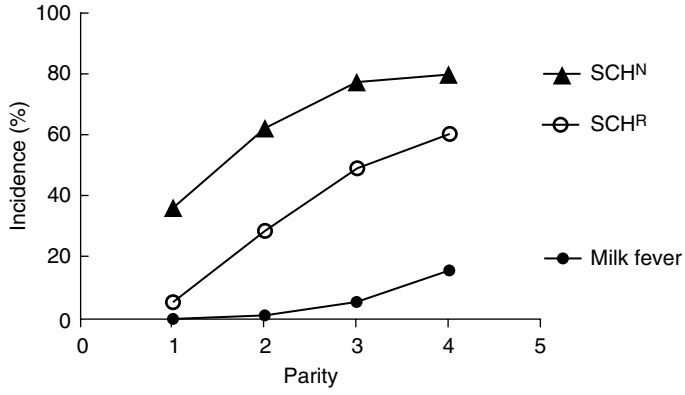

Fig. 4.3. Effect of parity on incidences of milk fever and subclinical hypocalcaemia (SCH) in German dairy herds. (Data from Venjakob *et al*., 2018, who used plasma Ca ≤ 2.0 (SCH^R) and < 2.2 mmol l^{-1} (SCH^N) to define hypocalcaemia)

last edition: it is a biochemically defined population that contains a subset of individuals likely to develop sub-acute, clinical diseases, which are partially prevented by Ca supplementation: the disorders include delayed conception, metritis, displaced abomasum, retained foetal membranes (RFM) and ketosis, all of which can have other causes. To define incidence and emphasize clinical significance, the subset of sub-acute disorders will be given a separate synonym, SACH, and discussed separately. SCH is defined loosely by plasma Ca thresholds, below which risk of associated disorders increases and was initially set at ≤2.0 mmol Ca l^{-1}, recorded within the 48 hours of calving (SCH^R) (Reinhart *et al.*, 2011). Refined statistical techniques suggested that the threshold was ≤ 2.2 mmol Ca l^{-1} in two large herds (Neves et al., 2017, 2018) and higher thresholds became used (SCH^N; e.g. 2.15mmol l^{-1}, Martinez *et al.*, 2018).[x] A higher threshold raises SCH incidence more in younger than older cows (Fig. 4.3) but primiparous cows remain less susceptible to SCH than multiparous cows, as with milk fever (Fig. 4.3). An SCH^N population remains loosely defined because plasma Ca changes rapidly within 48 hours, declining and then often returning to normal within two days, but sometimes lasting 3–5 days. At some point,

- the majority of multiparous cows in housed herds in the USA (above references) and Germany (Venjakob *et al.*, 2018) fell into the SCH category.

Occurrence of SCH, like that of milk fever may be lower but more prolonged in pastured than in housed herds. In a grazing herd in Argentina, average SCH^R incidence on the day of calving was only 27%, much higher in summer than in autumn but higher (37%) seven days after calving (Umana Sedó *et al.*, 2018).

Sub-Acute Clinical Hypocalcaemia (SACH)

Incidences of Ca-responsive diseases and subsequent culling rate are correlated with plasma Ca after calving (Tsiamidis *et al.*, 2016; Seifi and Kia, 2017; Venjakob *et al.*, 2018) but Ca supplementation and acidification rarely, if ever, reduce incidences of all or eliminate any constituent disease on a given farm (Cai *et al.*, 2018; Umana Sedo *et al.*, 2018). Like milk fever, diagnosis of SACH must be retrospective and rely on response to treatment. A single Ca bolus at calving improved milk yield and conception rate (Martinez *et al.*, 2016; Jahani-Moghadam *et al.*, 2018) and reduced incidence of constituent diseases in multiparous cows (Leno *et al.*, 2018). Published responses to Ca supplementation and risk factors are inconsistent partly because treatments vary in efficacy and partly because large group sizes are needed to attain significance, particularly where incidences are low (Venjakob *et al.*, 2018). However, relationships between disorder and plasma Ca are probably causative rather than associative. Resumption of rumen contractions and eating after calving follows recovery of plasma Ca (Wynn *et al.*, 2015). Muscle tone is adversely affected by lack of Ca and impaired rate of uterine involution has been implicated in RFM (Heppelmann *et al.*, 2015). Lack of Ca compromises intracellular signalling and coordination of the cytokine cascade: both are integral to immune defence and aspects of cellular immunity.[xi] Calcium and energy metabolism are interrelated and abnormal metabolite concentrations are raised in hypocalcaemic cows (Reinhart *et al.*, 2011; Leno *et al.*, 2018; Martinez *et al.*, 2018; Rodrigues *et al.*, 2020; Santos *et al.*, 2019): serum insulin and IGF-1 are reduced (Rodrigues *et al.*, 2020) and serum cortisol raised (Venjakob *et al.*, 2019). Time taken to resume oestrus cycling (Caixieta *et al.*, 2017) and conceive again (Venjakob *et al.*, 2018) in cows was correlated with plasma Ca after calving.

- SACH is endemic in housed herds in the USA and in Germany, affected about 25% of multiparous cows.
- If response to lowered DCAD is taken to indicate involvement of Ca, SACH is responsible for about two thirds of post-parturient diseases (from Santos *et al.*, 2019).
- SACH probably costs national dairy industries far more than milk fever.

Physiochemical changes due to acute and sub-acute lack of calcium

The physiochemical changes that accompany milk fever indicate attempted adaptation to a systemic lack of Ca: there are sharp increases in plasma PTH and slower increases in calcitriol (Fig. 4.4), which mobilize Ca from bone. Markers of bone matrix resorption, such as plasma hydoxyproline (Goff *et al.*, 2004), type 1 collagen

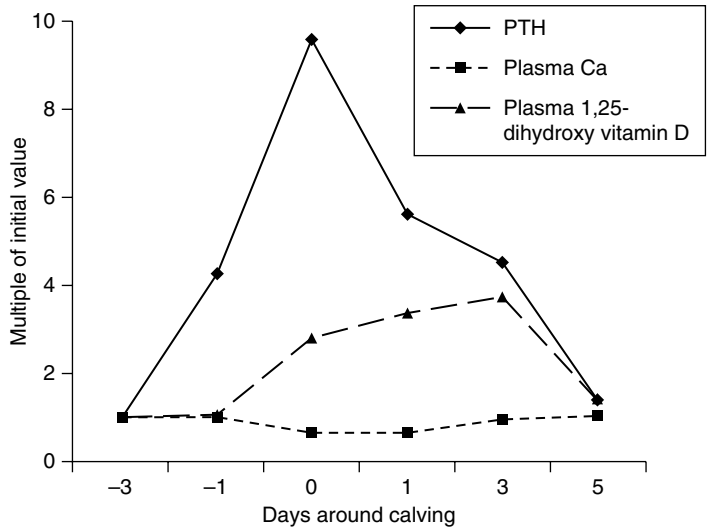

Fig. 4.4. Precipitous increases in demand for calcium at calving prompt an early rise in plasma parathyroid hormone (PTH) and slower rise in calcitriol (1,25-[OH]2D) in an attempt to curtail life-threatening reductions in plasma Ca (values are given relative to those 3 days before calving, approximately 60 pg ml^{-1} for PTH; 30 pg ml^{-1} for calcitriol and 2.4 mmol l–1 for plasma Ca (data for non-relapsing cows; Goff *et al*., 1989b).

metabolites and urinary excretion of DPN (Liesegang *et al*., 1998) all increase: these and other changes are generally evident but less extreme in healthy, primeparous cows (Fig 4.2). Failure of homeostatic mechanisms and target-organ insensitivity to PTH and calcitriol quickly causes neurological dysfunction due to reductions in extracellular Ca^{2+} concentrations (Goff *et al*., 1989b).

In SACH, biochemical changes in plasma are qualitatively similar but generally intermediate in severity between those seen in milk fever and healthy cows: the rate-limiting factor is probably intracellular Ca concentration (^{i}Ca) at key sites and times, such as uterine muscle during involution. To the author's knowledge, low ^{i}Ca concentrations have only recently been measured.[xii] The 'dynamic' of changes in plasma Ca up to 3 days into lactation has been stressed as a causal factor of endometritis (Venjakob *et al*., 2019) but the dynamic of ^{i}Ca may be more influential. Low plasma Ca^{2+} was associated with slowness to conceive but not endometritis (Mahen *et al*., 2018).

Prevention of Milk Fever and SACH in Housed Herds

Prevention of SCH and SACH would obviously prevent milk fever but treatments that prevent milk fever cannot be relied upon to prevent SACH. Prevention of SACH can improve profitability by $3–8 per calving for each of four sources of loss, low milk yield, lameness, metritis and RFM (McArt and Oertzel, 2015). Daily loss of milk yield was 2.19 kg in multiparous cows in early lactation in Germany (Venjakob *et al*., 2018) and 27% of a herd can have multiple disorders (Martinez *et al*., 2018).

Bolus dosage

Large Ca supplements are required to offset large acute Ca deficits. Initially, 50–75g Ca were given at calving as $CaCl_2$ by bolus or drench (Goff and Horst, 1993, 1994). Current products use gels containing less astringent Ca salts, like the formate and propionate, to avoid reductions in appetite, acidosis and abomasal inflammation (e.g. Leno *et al*., 2017). Maladministration of a large bolus may still damage the throat and cause aspiration pneumonia. Properly administered, two boluses given within 24 hours of calving raised plasma Ca 24 hours later and improved subsequent conception rate, without preventing SCH (Jahani-Moghadam *et al*., 2018). However, combining bolus dosage with

Ca injection and dietary supplementation with anions did not prevent 45% of multiparous cows in a housed Brazilian herd from developing SCH in autumn.

Dietary acidification in housed herds

Acidic diets are now routinely fed to pre-partum cows, an approach suggested by observations that milk fever rarely occurred in cows given silage preserved with acids in Scandinavia (Ender *et al.*, 1971). Anions vary in acidifying capacity, with HCl > NH_4Cl > $CaCl_2$> $CaSO_4$ > $MgSO_4$ > elemental S (Goff *et al.*, 2004) but use of calcium salts raises dietary Ca. Forage type *per se* does not affect urine pH. Acidification increases sensitivity of bone and kidney to PTH and increases Ca resorption (Phillippo *et al.*, 1994; Goff and Horst, 1997; Goff *et al.*, 2014) but may also increase Ca absorption from the rumen, independently of the calcitriol pathway (Wilkens *et al.*, 2016). Lowered plasma calcitriol and other D_3 metabolites may be a secondary consequence of increases in plasma Ca and Ca^{2+} (Rodney *et al.*, 2018; Martinez *et al.*, 2018). The target DCAD is generally -100 me kg^{-1} DM and reaching it raised plasma Ca to 2.04 ± 0.05 mmol l^{-1} at calving but an optimum DCAD could not be established (Santos *et al.*, 2019). Acid-base status of the cow can easily be monitored via pH in spot samples of urine, obtained by perineal stimulation and measured *in situ* by dipstick (Afsahi *et al.*, 2020) or pH paper (Constable *et al.*, 2019): target pH is < 7.5 (Goff and Korszewski, 2017) and is closely correlated with DCAD (Fig 4.5). Proneness to hypocalcaemia was weakly correlated with urine pH in commercial herds in Eastern Australia (McNeill *et al.*, 2002). Relationships between DCAD and acidity of rumen, plasma and urine are considered further on. Uncertainty surrounding correlations between DCAD and disease incidence[xii] will be partly attributable to confounded effects of the separate ions. The majority of treated multiparous cows still develop SCH, albeit of decreased severity (Weich *et al.*, 2013; Amanlou *et al.*, 2016; Leno *et al.*, 2017; Martinez *et al.*, 2018; Rodney *et al.*, 2018).[xiii] Incidence of RFM and morbidity can improve without improvements in metritis incidence and conception rate (Martinez *et al.*, 2018). However, meta-analysis showed significant reductions in incidences of RFM and metritis when DCAD was lowered in both first-calvers and multiparous cows (Fig. 4.6): no milk fever occurred in first-calvers but incidence was reduced from 11.7 to 2.8% in mulitiparous cows. Acidification improved milk yield by 1.1 kg in multiparous cows but decreased yield by 2.1 kg d^{-1} in nulliparous cows (Santos *et al.*, 2019). However,

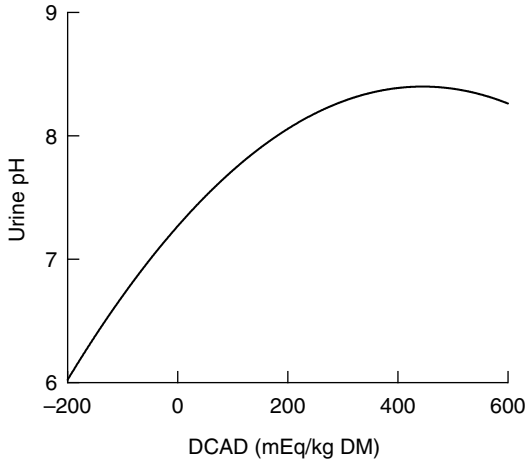

Fig. 4.5. Relationships between dietary cation-anion difference (DCAD) with urine pH from a meta analysis of 35 experiments in which DCAD was varied (Santos *et al.*, 2019).

- acidification prevented SACH more in nulliparous than multiparous cows; and
- prevented 2.5 times more SCH than milk fever incidents in multiparous cows.

Involvement of Ca is not proven by response to acidification but in an individual experiment, incidence of metritis was correlated with plasma Ca (Venjakob *et al.*, 2019). Acidogenic diets are abruptly withdrawn at calving for fear that subsequent food intake and milk yield may be reduced (Charbonneau *et al.*, 2006; Martinez *et al.*, 2018; Bani Hassan *et al.*, 2018).

- Herd health might be improved by consistently keeping plasma Ca >2.15 mmol l^{-1}.

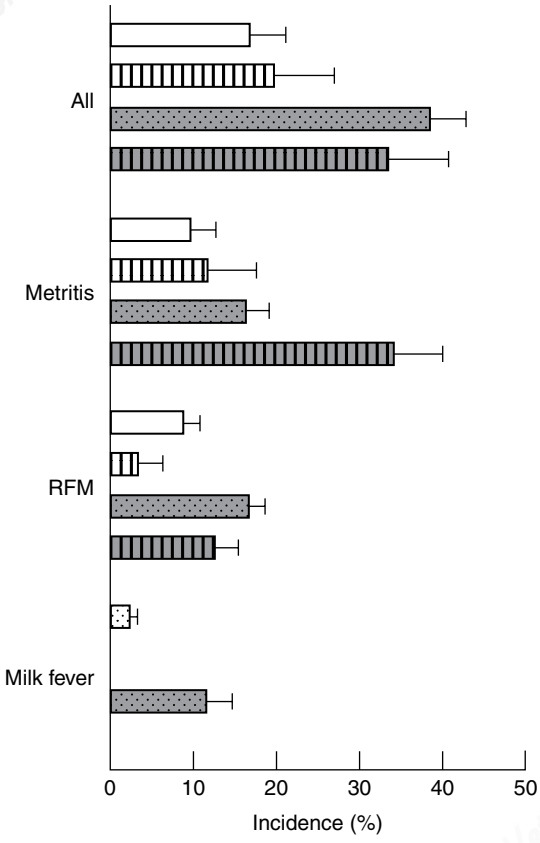

Fig. 4.6. Predicted effects of reducing dietary cation difference from +200 (patterned) to -100 m.e. $kg^{-1}DM$ (plain) in multiparous (unfilled) and nulliparous (filled columns) cows on incidence of disorders attributable to subacute clinical hypocalcaemia. (Data from Santos *et al.*, 2019)

- Abrupt increase in DCAD at calving may curtail efficacy, with plasma PTH yet to peak.
- Gradual reductions of less acidic supplements after parturition may improve efficacy.

Dietary acidification in grazing herds

Large anion inputs are needed to offset high K in many pastures grazed by spring calving herds (Pehrson *et al.*, 1999) but are achievable by mixing anionic salts with supplementary food: withdrawal after 21 days was recommended to maintain milk yield (Roche *et al.*, 2003a, b). A survey of grass-fed herds in Victoria suggested that SCH is alleviated by relatively small changes in DCAD (from -34 to +25 m.e $kg^{-1}DM$; Bani Hassan *et al.*, 2018). Alternatively, fertilizers can be used to raise pasture Cl (Perhson *et al.*, 1999). In pot trials, responses in alfalfa to NH_4Cl and $CaCl_2$ at rates of up to 112 kg Cl ha^{-1} followed the law of diminishing returns and effects on DCAD were relatively small, despite raising alfalfa Cl from 5.2 to 8.7g $kg^{-1}DM$ (Goff *et al.*, 2007). In field trials, application of 150kg Cl ha^{-1} to timothy pasture lowered DCAD in harvested hays. When fed to cattle, low-DCAD hays (10 me $kg^{-1}DM$) significantly reduced urine pH and increased Ca mobilization from the skeleton by EDTA infusion but efficacy in preventing milk fever was only implied (Heron *et al.*, 2009). In practice,

- spring calvers are most in need of supplementation to lower DCAD.
- pasture K should be the minimum needed to maximize pasture yield (p.178).

Inorganic acidic salts have no nutritive value and may be replaceable with nutritious supplements that lower DCAD. Combinations of fermented cereal by-products and anionic salts are widely used. Simulation of the traditional practice of 'steaming up' (ramping up concentrate feeding in the weeks before calving) improved the ability of cows to recover from an artificial hypocalcaemic challenge (Mellau *et al.*, 2004). Adding wheat grain rather than anions to transition diets also lowers urine pH and raises plasma Ca after calving (Nikkhah *et al.*, 2011).

Other preventive measures

Parenteral injections of vitamin D3, its analogue, $1\alpha OHD_3$ and PTH all provided partial protection against milk fever but are impractical (Goff *et al.*, 1989a). Subcutaneous injection of calcitriol within 6 hours of calving alleviated hypocalcaemia for five days in cows on an acidogenic diet and improved neutrophil function (Vieira-Neto *et al.*, 2017). Injections of calcidiol raised urine Ca in steers as much as acidifying the diet (p. 177). One or two subcutaneous injections of calcium BG (250 or 500 ml, given prophylactically either side of calving) increased food intake, reduced risk of endometritis and somatic cell counts (Amanlou *et al.*, 2016). Administration of such large injection volumes may be stressful and increase incidence and severity of SCH after 48 h, due to induced hypoparathyroidism (Goff *et al.*,

2014). Increasing dietary Ca prior to calving is counterproductive, down-regulating PTH synthesis (Ender *et al*., 1956; Goff and Horst, 1997; Lean *et al*., 2006) unless >20g Ca kg^{-1} DM is fed: negative responses then may be partly attributable to decreased appetite, milk production and demand for Ca. Feeding diets 'low' in Ca before calving to prime homeostatic pathways reduced incidence but effectiveness may have been partially attributable to concurrent decreases in dietary K (Goff and Horst, 1997). Diets with < 4.6 g Ca kg^{-1}DM may be required (Goff and Korszewski, 2017) but are easily formulated if whole crop silage is available (Table 2.2). Dietary supplementation with calcidiol rather than vitamin D_3 did not raise plasma Ca after calving in cows on either low or high DCAD diets but no cows receiving calcidiol developed RFM (Rodney *et al*., 2018).

Prevention by selection and culling

Heritabilities for milk fever within breeds can be as low as 2.6% in the Holstein–Friesian (Pryce *et al*., 1997) but increased from 9% to 13% in Norwegian Red dairy cattle between the first and third lactations (Heringstad *et al*., 2005). It is not possible to reduce herd incidence by sire selection within breeds when preventive methods are routinely used. When no preventive methods were used, incidence of milk fever in nine Greek herds was only 3.5% but SCH had a moderate heritability (H^2, 0.13–0.25; Tsiamadis *et al*., 2016). Repeatability is higher than heritability and culling cows that had milk fever will reduce subsequent herd incidence but tend to remove the highest yielders (Pryce *et al*., 1997; Rajala-Schulz *et al*., 1999; Saborio-Monteiro *et al*., 2017). Crossing susceptible breeds, such as Jerseys and Guernseys, with a less susceptible breed will reduce milk fever (Roche and Berry, 2006; Saborio-Monteiro *et al*., 2017).

- Reliance on preventive treatments keeps vulnerable individuals in the herd, as noted for hypomagnesaemic tetany (p. 117), whereas selection against SCH may gradually reduce incidences of milk fever and SACH acceptably, provided selection indices are weighted for milk yield.

Prevention by improving bone density

Since K-rich diets increase susceptibility to milk fever by increasing Ca retention during compensated alkalosis, it was hypothesized that K supplementation in late lactation and the early dry period might limit hypocalcaemia at parturition but it did not do so (Bhanugopan *et al*., 2010). In that study, cows normally kept at pasture were confined in a bare paddock and walked a short distance to individual stalls for feeding. Regular exercise alone may have increased bone mineral density. The principle may work for cows that are normally housed, particularly those confined in tie stalls, at increased risk of milk fever and fed acidic, transition diets, which leach Ca from the skeleton, lactation after lactation.

- The protective merits of exercise, combined with osteogenic diets, prior to transition should be explored as a physiological alternative to pharmacological dosage with Ca and anions in housed herds.

'Hypocalcaemia' in other species

Lactating females of other species, including the nanny, sow and mare, occasionally develop Hypocalcaemia, the clinical equivalent of milk fever. The clinical condition develops around parturition and is characterized by restlessness, apparent blindness and rumen stasis. Over half affected ewes become sternally recumbent, occasionally tetanic, and die (Mosdol and Wange, 1981). Symptomatically and aetiologically, Hypocalcaemia resembles pregnancy toxaemia (caused by energy deficiency) in being most likely to occur in females carrying more than one foetus, exposed to a change in or shortage of feed and stressed by transportation (Hughes and Kershaw, 1958) and/or adverse weather. In a large UK ewe flock, 43% of lambing ewes presenting with ataxia or recumbency were not hypocalcaemic (Cockcroft and Whitely, 1999):

- loss of appetite, caused initially by lack of Ca may cause Hypocalcaemia to develop into pregnancy toxaemia but the latter may cause hypocalcaemia.

Hypomagnesaemia often accompanies hypocalcaemia and presenting signs can include convulsions. Females in their first pregnancy are rarely affected (e.g. Mosdol and Wange, 1981), as with milk fever. Diagnosis is also retrospective and confirmed by response to scaled down doses of CaBG: 50 ml of a 20%w/v solution is appropriate for ewes and nannies. Hypocalcaemia is caused by acute failure of Ca supply to meet increasing demand for Ca but

peak demand (and onset) is pre- rather than post-parturient in multiple pregnancies for non-dairy breeds of sheep and goats. Low plasma OC prior to parturition indicates suppression of osteoblast activity and bone growth in the ewe to 'spare' Ca (Farrugia *et al*., 1989). Healthy, twin-bearing dairy goats show similar peri-parturient changes in plasma hormones and cytokines to cows (Wilkens *et al*., 2014): multiple caprine pregnancy demands roughly half as much Ca as the equivalent ovine pregnancy but milk yield is much higher in dairy breeds. Excess dietary P (Jonson *et al*., 1971, 1973) and K (p. 176) may increase susceptibility. Raising dietary K to 31g kg^{-1}DM decreased Ca absorption from the rumen and abomasum and reduced the renal tubular reabsorption of Ca in sheep (Bhanugopan *et al*., 2014) but the clinical significance of such imbalances are unclear.[xiv] Injection of cortisol-inducing vaccines in late pregnancy may be a risk factor and vaccination should be initiated as early as possible before lambing (Suttle and Wadsworth, 1991). Hypocalcaemia can occur after drought when sudden luxuriant growth of grass replaces emergency supplements of grain, low in Ca (Larsen *et al*., 1986).

Treatment and prevention of Hypocalcaemia

Some flock and herd owners inject CaBG prophylactically, dispersing 50-100ml between five sites to minimize tissue damage, but the procedure lacks experimental validation. Use of acid-preserved silage, $1\alpha OHD_3$ injections and oral supplementation with $CaCl_2$ and/or calcium propionate have been suggested, the latter offering cover against any accompanying pregnancy toxaemia (Leonhard-Marek *et al*., 2007). The same treatments are indicated for transport tetany, which can also affect cattle transported to feedlots (p. 103). Sustained protection requires adequate synthesis of calcitriol, which may be in short supply in outdoor flocks in early spring (Smith and Wright, 1981) and housed dairy flocks: both may require supplementation with vitamin D_3. Raising Cl and lowering K in pasture should benefit small ruminants.

Sub-acute hypocalcaemia in other species

The existence of a sub-acute form of the disease, equivalent to SACH in dairy cows, is not recognized but may occur in dairy goats, given the similarities in similar biochemical changes around parturition to those seen in cows (Wilkens *et al*., 2014). Hypocalcaemia and hypomagnesaemia may contribute to high lamb mortality (20-30%) in parts of Australia where lambing flocks graze cereals in the vegetative state (Friend *et al*., 2020), dietary Ca is often marginal (<4g kg^{-1}DM), K high (>30) and Na low (< 0.9g kg^{-1}DM).[xv] Mineral supplementation with Ca, Mg and Na did not raise serum Ca or improve lamb survival but lamb marking weight improved (McGrath *et al*., 2014): nine individual ewes had low serum Ca.

Consequences of Chronic Lack of Calcium

Growing ruminants and herbivores

Young horses developed osteofibrosis and lost weight when fed on cereals (Groenewald, 1937). Severe stunting and deaths occurred in lambs and young weaned sheep fed a wheat-based diet without additional Ca (Franklin *et al*., 1948) but grazing livestock rarely lacked Ca (McDonald, 1968). Lambs grazing cereals in the vegetative state are at risk (Masters *et al*., 2018). Osteodystrophic diseases and low productivity were reported in cattle consuming fast-growing grasses with <2g Ca kg^{-1}DM in tropical parts of India, in the Philippines and Guyana on acid, sandy and peaty soils (Underwood and Suttle, 1999). Ability to absorb and use dietary Ca depends on vitamin D_3 status and grazing livestock are dependent on UV irradiation of the skin to convert dietary precursors such as 7-dehydrocholesterol to D_3. Where animals are housed during winter or graze in areas of high latitude, low UV irradiation can cause clinical abnormalities at relatively high Ca intakes. Plasma calcidiol shows a seasonal decline in sheep, outwintered at northerly latitude (Fig. 4.7) and may have contributed to development of rickets in young sheep in central Scotland (Bonniwell *et al*., 1988) and newborn lambs in Oregon (Van Saun, 2004). Indigenous alpaca and llama normally synthesize sufficient vitamin D_3, despite bearing thick fleeces, because UV exposure is intense at high altitude and low latitude. When farmed in alien environments at low altitude and high latitude, vitamin D supplements are essential to avoid Ca insufficiency (Judson and Feakes, 1999).

In experimental Ca depletion, plasma Ca began a steady decline after a month in weaned lambs,

coinciding with loss of appetite (Fig. 4.8). Plasma Ca decreased less if the diet was also low in P and plasma P tended to rise on the low Ca diet (Fig. 6.2), reflecting simultaneous release of both elements from resorbed bone in meeting a single deficit. The accompanying changes in Ca and P in the skeleton are shown in Table 4.1. Skeletal size and weight increased substantially, without much increase in bone Ca, on the low-Ca diet. Remodelling allowed the initial mineral reserve to be redistributed in a larger bone volume and growth to take place without loss of function.

- No clinical bone or teeth deformities were seen.
- The contrast with pigs and chicks was probably due to a later onset of deprivation and slower relative growth rate.
- Changes in the Ca:Mg in bone may distinguish Ca from P insufficiencies in lambs and other species.

Lack of either Ca or P caused only small deviations in average Ca:P ratio of 2.1:1 but Ca:Mg was markedly reduced by lack of Ca, and tended to rise with lack of P (Table 4.1).

Lambs infected experimentally with larvae of nematodes that parasitize the gastrointestinal tract show species- and dose-dependent demineralization of the skeleton of a severity that matched anything achieved with Ca- (or P-) deficient diets (Fig. 4.9) (Coop and Sykes, 2002). Since natural nematode infections are commonplace in young grazing livestock, they may exacerbate dietary Ca (and P) insufficiency in the field. Demineralization associated with abomasal parasitism (by *Ostertagia circumcincta*) occurred with normal plasma Ca (and P) and normal absorption of the two minerals and was not attributable to loss appetite. Parasitism of the small intestine lowered plasma P (p,pp) and caused secondary loss of Ca from the skeleton. Systemic effects of gut parasites on bone demineralization may be triggered by similar factors to those that mobilize bone matrix during early

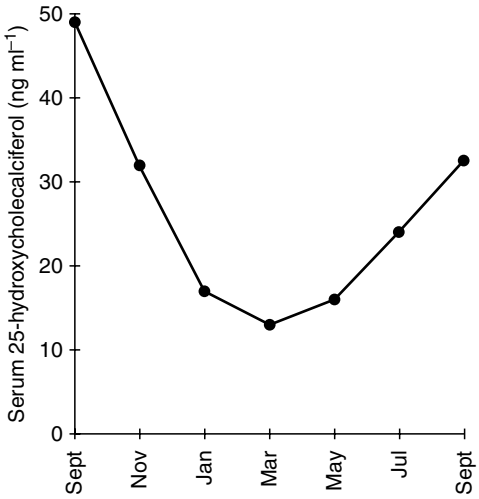

Fig. 4.7. Concentrations of vitamin D metabolite, 25-hydroxycholecalciferol, in the serum of pregnant ewes outwintered in Scotland, decline markedly due to lack of solar irradiation. (Data from Smith and Wright, 1981)

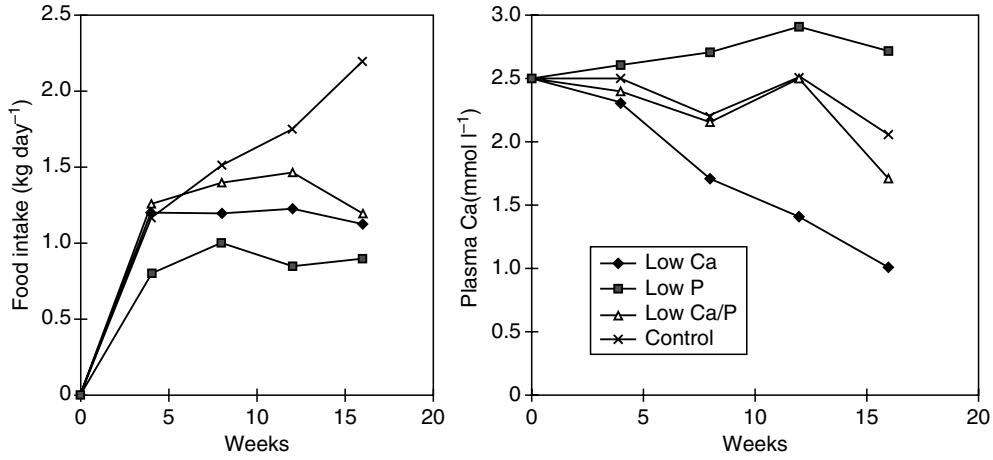

Fig. 4.8. Effects of single or combined deficiencies of calcium and phosphorus on food intake and plasma Ca in lambs: lack of Ca had lesser effects when P was also lacking. (From Field *et al*., 1975)

Table 4.1. Effects of feeding diets low in calcium and/or phosphorus for 18 weeks on the growth and mineral content of the lamb skeleton. (From Field *et al*., 1975)

	Changes in fat-free skeleton				Total mineral ratios	
Diet	Weight (kg)	Ca (g)	P (g)	Mg (g)	Ca:P	Ca:Mg
Control	+1.44	+197	+67	4.7	2.14	40.5
Low Ca	+1.00	+15	−18	2.4	2.13	25.7
Low P	+0.43	+57	−10	0.4	2.44	49.0
Low Ca/P	+0.9	+35	−14	0.8	2.30	39.4

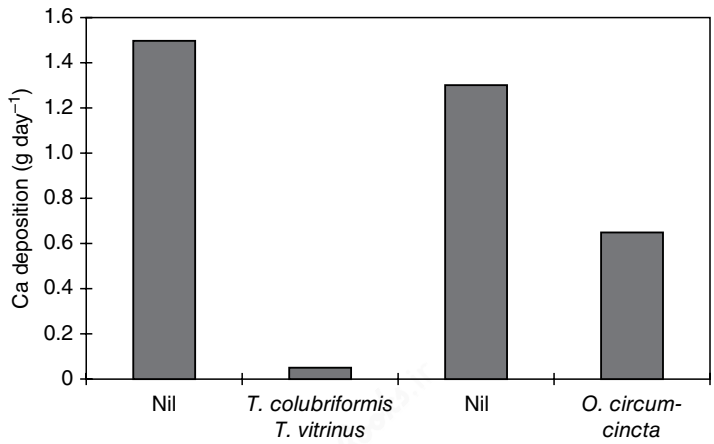

Fig. 4.9. Deposition of calcium in the skeleton of lambs is greatly reduced by parasitic infections of the abomasum (by *Ostertagia circumcincta*) or small intestine (by *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis*): trickle infections of nematode larvae were used and controls were pair-fed. (From Sykes, 1987; Nil = non-infected)

lactation to furnish amino acids for milk protein synthesis. Parasites of the liver have far lesser effects on bone mineralization. It would be surprising if debilitating infections of the gut by other pathogens (e.g. *Coccidia*) or in other hosts (calves, kids, young deer, piglets and chicks) did not sometimes cause demineralization of the skeleton.

Adult ruminants and herbivores

Lack of Ca in the adult causes osteomalacia, in which excessive mobilization of minerals leaves a surfeit of matrix and osteoporosis: bones contain less of a normally mineralized matrix. A combination of the two, matrix osteoporosis is caused by lack of dietary protein and not confined to the young (Sykes and Field, 1972; Sykes and Geenty, 1986). Once the epiphyseal plate has 'closed', bone changes are confined to shafts, where cortical bone thickness decreases, and in ends of bones, increased osteoclastic activity may be seen. Net withdrawal of minerals varies from bone to bone: those low in ash ('spongy'), such as rib, vertebra, sternum and the cancellous end of long bones, are affected first; those high in ash ('compact'), such as the shafts of long bones and small bones of the extremities, are affected last. A similar ranking of bones applies in young animals (Fig. 4.10c). Caudal vertebrae and metacarpal are not demineralized in early bovine lactation (Keene *et al*., 2004) and Ca:P ratios show little change in any bone. Ruminants and herbivores, fed largely on grain during droughts or in experiments, will develop osteoporosis unless supplemented (Larsen *et al*., 1986). An increased incidence of humerus fractures in heifers about to calve or recently calved at pasture caused concern in New Zealand (Dittmer *et al*., 2016). The osteoporotic humerus collapses suddenly as the heifer rises or is mounted. The cause is unknown but

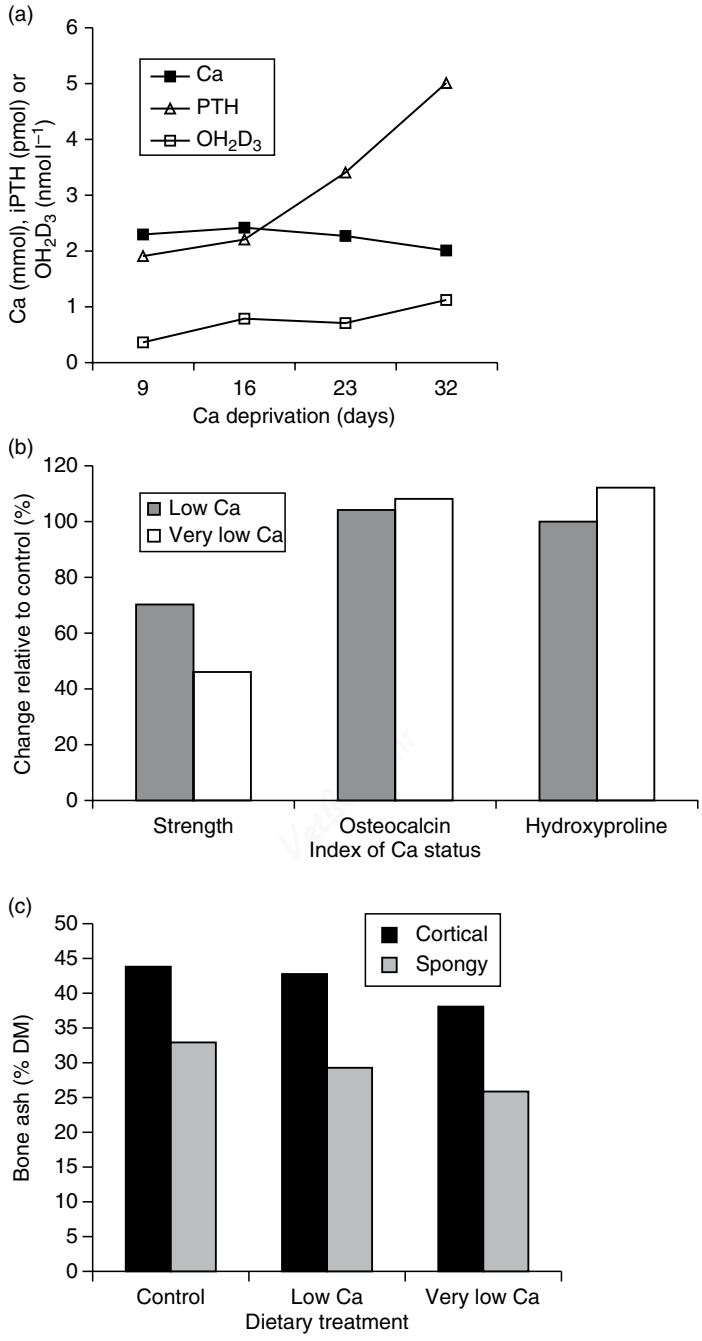

Fig. 4.10. Sequential (a) and terminal (b and c) changes in biochemical or physical measures of calcium status in weanling pigs (from Eklou-Kalonji *et al*., 1999). Diets containing 9.0 (control), 3.0 (low) or 1.1g Ca (very low) kg^{-1}DM were fed for 32 days. In (a), hormonal control (increases in parathyroid hormone (PTH) and calcitriol OH_2D_3) delays the fall in plasma Ca; in (b), plasma markers indicate increased bone growth (osteocalcin) but resorptive (hydroxyproline) activities probably reduce cortical bone strength (failure load of metatarsal); in (c), effects of Ca deprivation on ash content are greater in spongy than in cortical bone.

probably multifactorial in origin, earlier protein-calorie malnutrition, low P intake from fodder beet and Cu insufficiency being implicated in some but not all cases. Exposure to acidogenic diets intended to prevent milk fever in older cows may exacerbate the problem. Some lactating ewes given a cereal grain ration showed a <50% decline in serum Ca within a few weeks, whereas pregnant ewes, lambs and weaners were less affected (Franklin *et al.*, 1948).

Growing pigs

The outcome of an early study of severe Ca deprivation in baby pigs is summarized in Table 4.2. Unsupplemented animals developed a 'peg-leg' gait, enlarged and painful joints, bent and twisted long bones, arched backs and posterior paralysis. The probable sequence of events was shown in a later study (Fig. 4.10). The fall in plasma Ca is barely perceptible, plasma PTH and calcitriol slowly increase over a period of weeks: slight but significant increases in plasma OC and hydroxyproline indicate increased bone turnover when dietary Ca is lacking.

Table 4.2. Effects of dietary calcium concentrations on selected performance, skeletal and blood indices of calcium status in the baby pig given a synthetic milk diet from 0 to 6 weeks of age (Trial 2 in Miller *et al.*, 1962).

	Dietary Ca (g kg^{-1}DM)			
Criterion	4	6	8	10
Weight gain (g day^{-1})	170	210	180	200
Dry matter intake (g)	230	240	230	250
Feed gain (g: g^{-1})	1.33	1.16	1.31	1.27
Serum P (mmol l^{-1})	3.29	3.54	3.43	3.52
Ca (mmol l^{-1})	2.60	2.60	2.72	3.00
AP[c] (U l^{-1})	9.90	8.58	8.50	9.75
Humerus ash (%)[a]	42.2	43.3	46.1	47.1
Femur specific gravity	1.14	1.15	1.16	1.18
Femur weight (g)[b]	47.9	–	47.2	–
Eighth rib weight (g)[b]	3.62	–	3.44	–
Femur breaking load (kg)	57.7	65.9	65.9	84.5

[a]Dry fat-free basis
[b]Trial 1 data
[c]alkaline phosphatase

Biochemical, histological, radiological and physical measures of bone quality generally correlate well with each other in young pigs (Eklou-Kalonji *et al.*, 1999), as in other species, young and old (e.g. cows).

Growing chicks

Up to 25% of bone minerals was lost in Ca-deprived chicks, chiefly from the axial skeleton, and bones fractured (Common, 1938). Effects may be less pronounced in the much-studied tibia. Reducing dietary Ca from 10.4 to 4.4g kg^{-1}DM reduced tibia ash in 16-day-old broiler chicks from 39.6% to 35.1% (Driver *et al.*, 2005b). Long bones become deformed, with the ends enlarged; rib costochondral junctions are also enlarged. Histologically, uniform widening of epiphyseal-diaphyseal cartilages and excessive accumulation of uncalcified matrix (osteoid) were evident, a combination classified as 'rickets' and manifested in all livestock species. Rickets was exacerbated in broiler chicks by excess dietary P and also induced by lack of dietary P (Driver *et al.*, 2005b) but the two causes can be distinguished by histology (p. 130). Tibial dyschondroplasia (TD) can also be induced by low dietary Ca (Driver *et al.*, 2005b) but distinguished from rickets by plugs of avascular cartilage beneath the growth plate (Edwards and Veltmann, 1983). Older turkey poults are particularly vulnerable to TD but the disease has other more common causes. Modern hybrids may be unable to produce enough calcitriol at the growth plate in the fastest growing bones and lack of Ca exposes this anomaly (Whitehead, 1995). However, short periods of Ca depletion can be tolerated. A 44% reduction in dietary Ca (and P) for ten days in the middle of a 37-day study did not affect overall performance of broiler chicks, having reduced bone breaking strength, bone and toe ash by <13 % (Valable *et al.*, 2018).

Changes in adult birds

Although other factors are involved, lack of Ca can contribute to: cracked eggshells (Roland, 1986), cage layer fatigue (Whitehead, 1995) and bone fractures during transportation, slaughter (Wilson, 1991) and production. Incidence of KBF increased in Europe when hens could no longer be legally confined in battery cages: free-moving birds were

more exposed to physical injury to bones, still weak and osteoporotic (Fleming *et al.*, 2006). Transitional Ca nutrition and husbandry around onset of lay is crucial. If low-Ca diets are fed, pullets over-consume food in an effort to meet a burgeoning need for Ca, increasing risk of fatty liver haemorrhagic syndrome (Roland, 1986). In sexually mature birds, bone strength is overestimated by biochemical indicators of bone density: medullary bone lacks the strength of structural bone (Whitehead, 2004). To promote Ca deposition, serum Ca is normally higher and more variable in laying hens than in non-laying hens or chickens (Table 4.3), due to the presence of a Ca-phospholipoprotein, vitellogenin, a precursor of egg-yolk proteins (Hurwitz, 1996). During shell calcification, serum Ca declines, regardless of dietary Ca. Both egg and eggshell as well as the skeleton are denuded of Ca. Egg yield, egg weight, hatchability and eggshell thickness are all reduced by lack of Ca, with shell strength the most sensitive index of dysfunction (Hamilton and Cipera, 1981). Poor shell quality remains a major source of loss to the poultry industry: most hens end lay with osteoporosis and 'cage layer fatigue' (Whitehead, 1995).

Diagnosis of Calcium Imbalance

Unusually amongst minerals, abnormal values for Ca in the bloodstream indicate probable dysfunction because homeostatic mechanisms normally keep plasma Ca within narrow limits. Marginal bands for plasma Ca in different classes of livestock are given in Table 4.3. By contrast, assessments of bone quality alone can never provide a specific diagnosis because of the many factors that influence bone development. Guidelines for assessing bone quality are given in Table 6.4 because they are more widely used in diagnosing insufficiency of P: chronic Ca insufficiency is confirmed when bone abnormalities are combined with low serum Ca. Assessing the role of Ca in weakening eggshells and skeleton is complicated by diurnal fluctuations in serum Ca. Supporting evidence of inadequate Ca intake or form (too fine) can be adduced and diagnoses confirmed by responses to particulate Ca supplements. Failure to improve shell thickness does not rule out improvement in the Ca status of the skeleton (Fleming *et al.*, 2006) and normal serum Ca does not rule out historic Ca insufficiency.

Natural Sources of Calcium

Forages

Forages, particularly leguminous species, generally provide sufficient Ca for grazing livestock. Global average values for temperate and tropical legumes were 14.2 and 10.1g Ca kg^{-1}DM: corresponding values for grasses were 3.7 and 3.8g Ca kg^{-1}DM (Minson, 1990). In the UK, swards contained, on average, 5.4±1.7(s.d.)g Ca kg^{-1}DM (MAFF, 1990). Cultivar differences can be marked, but maturity and seasonal changes in legume contribution have greater influences. In New Zealand, pasture Ca increased from 4.4 to 5.7g kg^{-1}DM between spring and summer due to a seasonal increase in clover content (Stevenson *et al.*, 2003). The leaf generally contains twice as much Ca as the stem and pasture Ca is increased by applying fertilizer N but decreases with advancing maturity. Selectively grazed herbage is likely to contain more Ca than hand-plucked samples. Herbage Ca increases when pasture growth is slowed by flooding and seasonal reductions in soil temperature. Soil Ca

Table 4.3. Marginal bands[a] for mean total serum calcium in assessing low and high calcium status in various classes of livestock.

	Mean total serum Ca (mmol l^{-1})							
					Periparturient			Laying
	Lamb/Kid	Growing Pig	Chick[b]	Foal	Cow	Ewe	Goat	Hen
Low	1.8–2.0	1.7–2.0	2.0–2.5	2.2–2.5	2.0–2.2	1.5–2.2	2.0–2.2	3.8–5.0
High[c]	>3.2							>6.0

[a]Values within bands indicate probable dysfunction in some individuals, single values, a need for wider sampling.
[b]Turkeys have slightly higher ranges than those quoted, which are for fowl.
[c]Hypercalcaemia bears risk of tissue damage, particularly to the kidneys

(Jumba *et al*., 1995) and applications of lime, limestone or $CaCl_2$ have surprisingly little effect on forage Ca (e.g. Goff *et al*., 2007). Grass conserved as silage contains slightly more Ca than the more mature hay (6.4 versus 5.6g Ca kg^{-1}DM), while alfalfa silage is much richer and more variable (13.5–24.0g Ca kg^{-1}DM) (MAFF, 1990). Maize silages are generally low (1.4–3.0) (see Table 2.2 and McNeil *et al*., 2002), root crops and straws marginal (3–4g) and leafy brassicas rich in Ca (10–20g Ca kg^{-1}DM).

Concentrates

Cereals are low in Ca, with maize, wheat, oats and barley typically containing only 0.2, 0.6 and 0.6–0.9g kg^{-1}DM, respectively. By-products of cereal fermentation are often richer in Ca than the original grains (see Table 2.3). Although richer in Ca than cereals, vegetable protein sources usually contained no more than 2–4g Ca kg^{-1}DM (MAFF, 1990; NRC, 2012). Rapeseed meal, with 8.4g Ca kg^{-1}DM, is exceptional and, like sugarbeet pulp (6.0–7.5g Ca kg^{-1}DM), an excellent supplement for P-rich, grain-based diets for all livestock. Fish meal and meat + bone meals are rich sources, with 50–100g Ca kg^{-1}DM commonplace, but is concentration commensurate with feed value?

Control of Calcium Absorption

The availability of Ca in feeds cannot be assessed without reference to dietary Ca concentration at the growth stage in species that absorb Ca according to need.

Growing birds

In poultry, Ca is primarily absorbed by an active, saturable process in the small intestinal mucosa. On low Ca intakes, a decrease in plasma Ca^{2+} stimulates secretion of PTH, whereby vitamin D_3 is converted by hydroxylations in liver and kidney to a physiologically active form, calcitriol (1,25dihydroxycholeciferol). Calcitriol opens Ca^{2+} channels and increases vitamin D receptor (VDR) expression in the apical membrane, allowing CaBP to convey Ca across the mucosa for extrusion at the basal membrane. The role of one CaBP, calbindin, in facilitating absorption according to need in broilers is illustrated in Fig. 4.11. Ca-transporter genes are also upregulated when dietary Ca (and P) is lacking (Rouseau *et al*., 2016). Increases

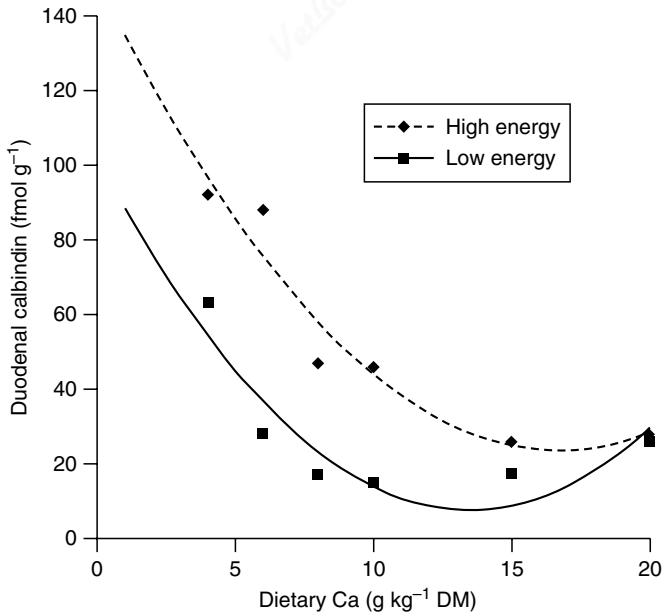

Fig. 4.11. Calcium is absorbed according to need by broiler chicks: as dietary calcium concentrations decrease, synthesis of a potentiator of absorption (calbindin) increases; if high-energy diets are fed, demand for calcium rises and broiler chicks synthesize yet more duodenal calbindin. (From Hurwitz *et al*., 1995)

in dietary Ca decreased fractional retention, an indicator of absorptive efficiency, (p. 28), in newly hatched broiler chicks but there were interactions with phytase and vitamin D supplements in a diet lacking Pi and vitamin D_3 (Fig. 4.12): similar results were reported by Driver *et al.* (2005b). With low dietary Ca, added phytase and D_3, nearly 70% of ingested Ca was retainable (and therefore absorbable) from the blend of $CaCO_3$, corn and SBM. Others have shown that AA_{Ca} (see p. 9 for notation) decreases as dietary Ca increases (Tamin *et al.*, 2004; Yan *et al.*, 2005; Olukosi and Fru-Nji, 2014) but increases between the first and second weeks of life (Fig. 4.13); subsequent decreases may reflect a growing excess of Ca provision over need.[xvi]

Growing pigs

In pigs, AA_{Ca} remains high and constant (0.60) over a wide range in dietary Ca (Stein *et al.*, 2011; Gonzales-Vega and Stein, 2014; Misiura *et al.*, 2018). Apparent failure to adjust A_{Ca} may arise from a reciprocal link between active absorption in the proximal small intestine and passive absorption in distal regions. As dietary Ca increases, passive absorbtion increases, driven by electrochemical gradients (Pérez *et al.*, 2008). In the pig, paracellular absorption is regulated by genes that control tight junctions in the intestinal mucosa, where regulatory proteins like occludin and claudin are synthesized: these genes are also upregulated by Ca deprivation in weanling pigs, from duodenum to ileum, and affected by dietary P (Fig. 4.14) (Lagos *et al.*, 2019a). Active absorption is regulated according to need (Fig. 4.14B) but reductions in active absorption will increase Ca concentrations in ileal digesta and increase passive A_{Ca}, unless there is proportionate distal downregulation.

- Pigs may not sense that demand for Ca is satisfied until bone Ca is very high.
- A_{Ca} is not a feed attribute in diets contrived to provide insufficient P.

Ruminants

It has become apparent during the last decade that control of Ca absorption (A_{Ca}, p. 9) is more complex than was once thought: control mechanisms differ between ruminants and non-ruminants, between adult cattle, sheep and goats (Wilkens *et al.*, 2011, 2012, 2014, 2016) and between lactating and non-lactating cows (Hyde *et al.*, 2019). Adult ruminants can actively absorb Ca from the rumen by mechanisms that are neither calcitriol- nor calbindin-dependent, sheep being less adaptive to lack of dietary Ca than goats (Wilkens *et al.*, 2011; Hyde *et al.*, 2019). Using absorption of Sr as a marker for

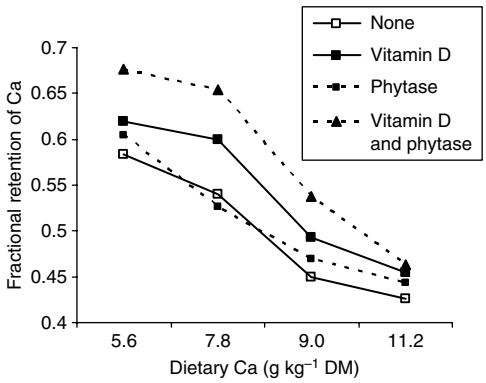

Fig. 4.12. Fractional retention of dietary calcium in broiler chicks in the first 21 days of life declined when calcium supply exceeded need but increased when deficiencies of available phosphorus and vitamin D_3 were lifted: supplementation with vitamin D_3 and phytase improved growth and feed conversion efficiency and allowed calcium value of sources (mostly $CaCO_3$) to be fully expressed. (From Qian *et al.*, 1997, using data from groups given 900 FU phytase and 660 µg D_3 kg^{-1}DM)

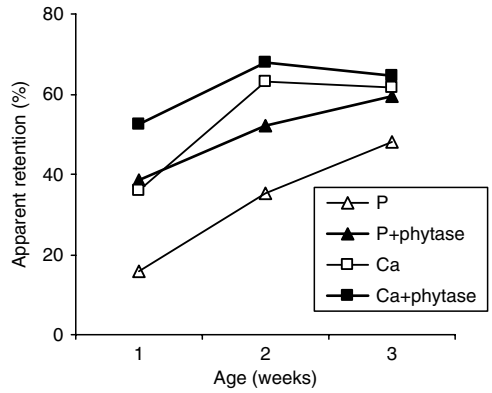

Fig. 4.13. Effect of age on apparent (ileal) absorption of calcium (AA_{Ca}) and phosphorus (AA_P) in broiler chicks: AA_{Ca} is low during the first three weeks of life but increased by a phytase supplement, which also increases AA_P, suggesting that the newly hatched chick has a low capacity to degrade phytate. (Data from Olukosi *et al.*, 2007)

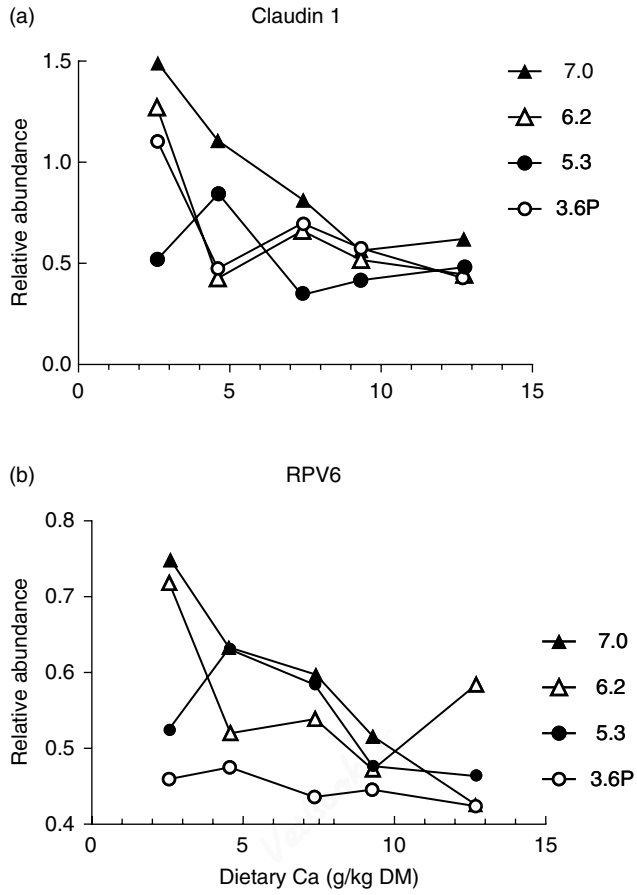

Fig. 4.14. Effects of dietary calcium and phosphorus concentrations on molecules regulating passive (a) and active (b) absorption of calcium in the duodenal (a) and ileal (b) mucosa of weanling pigs (from Lagos *et al*., 2019a): decreased expression of transient receptor vanilloid type 6 (RVP6) (b) and claudin (a) shows that both processes are downregulated as dietary Ca increases; P- deprivation also downregulates RVP6, probably by restricting deposition of Ca in bone, creating a physiological excess of Ca and raising plasma Ca^{2+}; simultaneous deprivation of Ca and P may aid adaptation by restoring equilibrium between Ca and P supplies.

A_{Ca}, passive absorption from the rumen was higher in lactating cows than in non-pregnant, non-lactating heifers (Hyde *et al*., 2019). Nevertheless, historic balance studies indicated that growing lambs absorbed Ca according to need (Fig. 4.15). The hypothesis is confirmed in Fig. 4.16, which links two subsequent studies with lambs and goat kids, neither of which recognized the influence of the chosen dietary treatments. A conclusion that A_{Ca} was not affected by Ca intake in kids (Dorigan *et al*., 2018) was only true within the low narrow range of dietary Ca employed. The average availability attributed to a $CaHPO_4$ supplement for lambs, 0.4 (Dias *et al*., 2013) was similar to that reported previously (Roque *et al*., 2007) but an underestimate, being measured at twice the required intake (6 and not 3g Ca kg^{-1}DM.

- Given the differences in Ca metabolism between heifers, continuing to grow, and multiparous cows (Fig. 4.2), it is possible that Ca metabolism in growing and adult ruminants differs.

Influence of net calcium flow to or from the skeleton

In adult ruminants, net flow of Ca from the skeleton often reduces the perceived need for absorbed

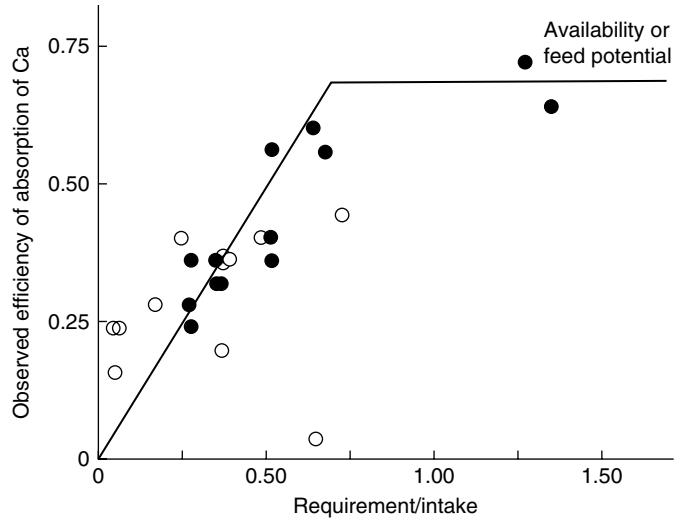

Fig. 4.15. Evidence that calcium is absorbed according to need in growing lambs: pooled data for efficiency of calcium absorption plotted against adequacy of dietary supply (ratio of net Ca requirement, R, to calcium intake, I) show a linear rise to a plateau that represents full potential of the source. (From AFRC, 1991; ● = nutritionally balanced diets; ○ = nutritionally imbalanced diets)

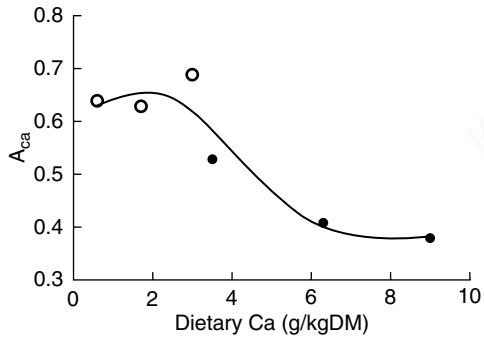

Fig. 4.16. Relationship between efficiency of calcium absorption (A_{Ca}) and dietary calcium concentration in young lambs and goats: A_{Ca} was uniformly high in goat kids given ground corn cob and grain, very low in Ca, with and without small limestone supplements (open symbols: Dorigan *et al*., 2018), suggesting that requirements were never exceeded; A_{Ca} decreased when $CaHPO_4$ was added to a hay-based diet for lambs because requirement was exceeded (solid symbols, Dias *et al*., 2013); linking the data with a smoothed spline (Lowess) plot gives a mirror image of Fig. 4.15.

Ca (Chrisp *et al*., 1989b; AFRC, 1991; Goff, 2018) and helps to explain low A_{Ca} from forages fed to lactating ewes (0.19; Chrisp *et al*., 1989b) and cows (0.25; Martz *et al*., 1999) in negative Ca balance and with high needs for Ca. Antagonism from high oxalate concentrations in alfalfa hay was implicated in the cows, which were confined in metabolism crates and losing 26–36g Ca d^{-1} after 60–90 days of lactation on a diet theoretically adequate in Ca (5.1g Ca kg^{-1}DM). Minimally confined but otherwise similar cows on a similar Ca intake approached positive Ca balance (Gaignon *et al*., 2019). The confined ewes were losing 2–4g Ca d^{-1} for the first six weeks of lactation, whereas ewes at pasture were estimated to have lost 0.8–1.4g Ca d^{-1} by comparative slaughter, equivalent to about 60 and 20mg Ca kg^{-1}LW, respectively, over similar periods (Sykes and Geenty, 1986).

- Could confinement of ruminants for experimental purposes underestimate A_{Ca} by reducing bone deposition and/or increasing bone resorption?

Carcase Ca, almost entirely contributed by bone, decreased at a rate exceeding that expected from allometric reduction in bone growth, with approach to maturity, in penned lambs (Fig. 4.17a) and calves (Fig. 4.17b) given adequate dietary Ca. Confined, weaned lambs remained in negative Ca balance, losing about 0.6g Ca d^{-1}, despite being fed twice their maintenance needs for energy as hay containing ample Ca (6.4g kg^{-1} DM; Chrisp *et al*., 1989a). By contrast, carcase Ca increased as

body weight increased in minimally restricted, weaned lambs (Bellof *et al*., 2006) and goat kids (Araujo *et al*., 2010). In feedlot lambs allowed to graze for 3, 6 or 9 h d^{-1} and given Ca-rich concentrate at 1.2, 0.84 and 0.48% LW, respectively, overnight (Assis *et al*., 2018), carcases were estimated to contain 9.9, 12.7 and 13.0 g Ca kg^{-1}EBW (empty body weight), respectively. Since concentrate supplementation *per se* has little effect on carcase Ca (Araujo *et al*., 2010), Ca retention may have increased with time spent grazing. Similar associations between freedom of movement and bone mineralization are found in poultry. Exercise increases bone density in hens (Casey-Trott *et al*., 2017) and pullets (Neijat *et al*., 2019). Low, even negative, Ca 'digestibilities' in confined, pregnant sows, not evident in confined, growing gilts on the same food intake (Lee *et al*., 2018), contrast with consistently high AA_{Ca} in growing pigs (Misiura *et al*., 2018). Further studies in sows on corn-based, 'Ca-free' diets, with and without phytase supplements, confirmed that $^{m}FEL_{Ca}$ (see p.4 et seq for notation) was fivefold higher during early pregnancy than growth but only three times higher by late pregnancy (Fig. 4.19): such changes could only be attributable to differences in mobilization of Ca from bone.

- Was bone resorption induced by confinement of heavy, newly pregnant sows in metabolism crates for 12–13 days, a similar phenomenon to that proposed for confined ruminants (p. 74)?
- Had sows adapted to confinement by late pregnancy or did Ca retention by fetuses reduce FEL_{Ca}?

Correction for endogenous loss in pigs and poultry

Authorities that opted for $STTD_P$ as the best way to describe the P value of feeds (NRC, 2012 and WPSA, 2013; p. 26) seem likely to lead their respective industries along a path that evaluates Ca in terms

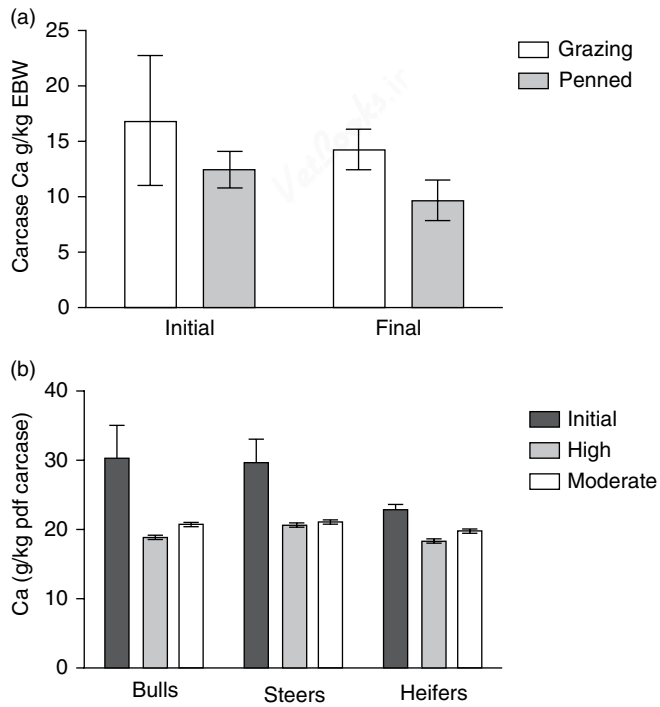

Fig. 4.17. Circumstantial evidence that confinement reduces calcium (Ca) accretion in bone in ruminants. (a) Mean carcase Ca decreased during experiments with penned but not grazing lambs lasting 6 weeks (n = 6 for each: references in Table 6 of AFRC, 1991) plus Thompson *et al*. (1988) and Costa e Siva *et al*. (2015); (b) Confined growing cattle, on moderate or high planes of energy nutrition showed gender-dependent loss of Ca after 12 weeks (Chizzoti *et al*., 2009).

of an $STTD_{Ca:P}$ ratio, building error upon error. Errors introduced in correcting *AA* for minimum (^{m}) FEL_{Ca} in non-ruminants were discussed earlier[xvii] and arise from experimenters' choices of basal diets, treatments and protocols, each of which can affect $^{m}FEL_{Ca}$.[xviii] Phytase supplementation lowered $^{m}FEL_{Ca}$ in sows on phytate-free diets (Fig. 4.19), possibly through an 'extra-phosphoric' stimulation of bone accretion, but may have different effects in commercial, phytate-rich diets. Whatever the Ca source or additive, comparisons of small, graded increments of sources and linear regression are recommended but may still not constitute a perfect design.[xix] The chief sources of error are curvilinear responses, groups losing weight and having no net growth requirement for Ca and, in poultry, retention of coarser feed and supplement particles in the gizzard.[xx]

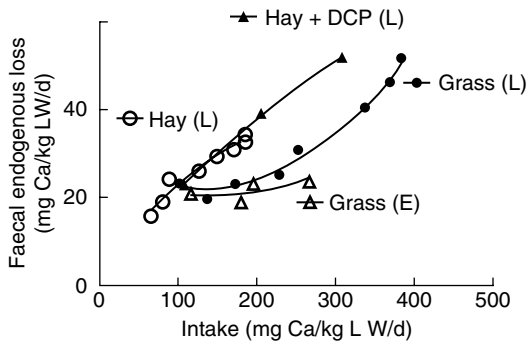

Fig. 4.18. Relationships between faecal endogenous loss and intake of calcium in sheep on forage diets of different types: relationships in lambs (L) given hay were similar and linear, whether offered as different amounts of the same hay (Chrisp *et al*., 1989a) or hay with dicalcium phosphate (DCP) added (Dias *et al*., 2013) but differed from those pertaining to fresh or defrozen forages consumed by lactating ewes (E) and lambs (Chrisp *et al*., 1989b).

- Effects of protocols far exceed those of standardization for $^{m}FEL_{Ca}$, which only marginally increases PCD_{Ca} (e.g. from 0.40 to 0.42 in chicks; David *et al*., 2019).
- Corrections of AA_{Ca} values for FEL_{Ca}, whether minimal or obligatory, are prone to error and best avoided in all species in estimating requirements.

Correction for endogenous loss in ruminants

Endogenous losses of Ca via faeces *(FEL_{Ca})* are appreciable but were thought to be determined by and predictable from dry matter intake (DMI) (AFRC, 1991). A comparison of new and old data in Fig. 4.18 suggests that FEL_{Ca} is affected by dietary Ca, diet type and complex interactions between confinement, bone accretion, physiological status and dietary protein.[xxi] Obligatory endogenous

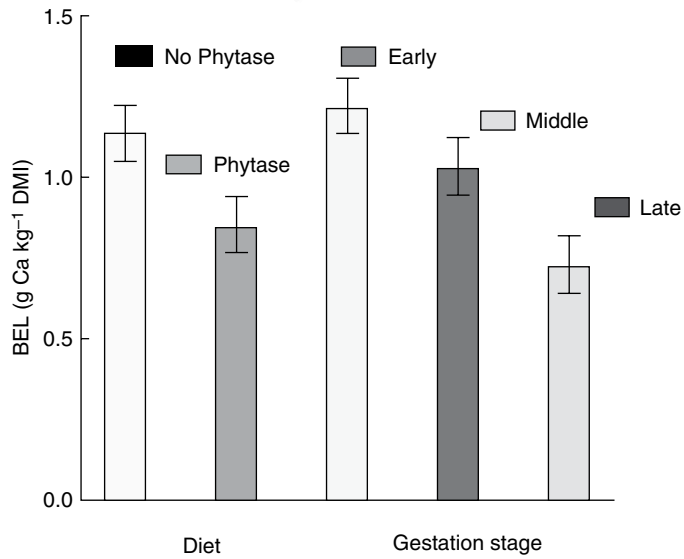

Fig. 4.19. Basal endogenous losses (BEL) in sows given corn-based, 'calcium-free' diets, with or without phytase supplements in early, mid- and late pregnancy: losses fell by 40% as pregnancy progressed and were reduced by 25% by phytase (Lee *et al*., 2019).

losses in urine are so small that retention of Ca can be used as a surrogate measure of AA_{Ca} (e.g. cattle: Zanetti *et al*., 2017a, b), unless the diet is acidogenic.[xxii] However, confinement may increase FEL_{Ca} as well as decrease $A_{Ca,}$ resulting in the underestimation of Ca value of a source.

Availability in Feeds and Supplements

Suggested rules for measurement

The following rules are suggested for measuring availability as a feed or supplement attribute in the light of the foregoing discussion of Ca absorption.

- *Golden rule:* Dietary Ca should be close to the minimum requirement.
- *Rule 2:* No other dietary factor, such as lack of P, S or protein, should constrain performance, bone accretion or mineralization.
- *Rule 3:* Experimental artefacts that induce bone resorption, such as confinement (p.74) and synchronization of oestrus (p. 59) should be avoided.
- *Rule 4:* Obligatory physiological drivers of net Ca accretion in and resorption from the skeleton, such as gestation, lactation and onset of lay, must be considered.
- *Rule 5:* Performance should be recorded to gauge need for Ca.
- *Rule 6:* While effects of feed type and dietary Ca on FEL_{Ca} are uncertain, AA_{Ca} should be used to indicate Ca value of feeds.

Calcium Absorbability in Feeds

Milk and milk replacers

Although milk and milk replacers derived from bovine milk are rich in Ca (Table 2.5), availability is high. In weanling pigs, AA_{Ca} of whey and skimmed milk powders were 0.84 and 0.86, respectively, when added to corn-based diets (She *et al*., 2016). In calves, high A_{Ca} of 0.83 (Challa and Braithwaite, 1989) and AA_{Ca} of 0.95 (Yuangklang *et al*., 2004) were reported. Calcium was marginally less absorbable from milk replacers based on soy protein (AA_{Ca} 0.87), and may be attributable to formation of insoluble Ca-phytates. Doubling dietary Ca from 6.4 to 11.5g kg^{-1}DM only decreased AA_{Ca} to 0.75 (Yuangklang *et al*., 2004) but may have increased bone Ca (p. 57).

Feeds for ruminants

Old and new values for $AA_{Ca,}$ and $A_{Ca,}$ are summarized in Table 4.4 but rarely approach the absorbable threshold of 0.68, indicated by AFRC (1991) (Fig. 4.14). However, maximal A_{Ca} within a comparison of grass species was found in lambs freely grazing the most nutritious grass species, on which growth was fastest (Thompson *et al*., 1988). When cows were fed a diet with more Ca than they required, AACa was 10 and 20% lower for high and low. Low AA_{Ca} is associated with unbalanced diets, confinement in metabolism crates and lactation, with two exceptions.[xxiii] There was no evidence for consistently higher $AA_{Ca,}$ of inorganic than inherent feed Ca in Zanetti *et al*. (2017a, b). Forage species high in oxalate, such as alfalfa, do not have consistently low AA_{Ca} values, probably because oxalate is partially broken down in the rumen (Duncan *et al*., 1997). Alfalfa had a similar A_{Ca} to inorganic sources when a new kinetic model was applied to the data of Roque *et al*., (2007) (Dias *et al*., 2006). Oxalate is unlikely to reduce post-ruminal absorption, given the high AA_{Ca} from alfalfa meal for non-ruminants (Soares, 1995). Napier grass had a low AA_{Ca} but supplementary DCP decreased faecal and urinary Ca, suggesting that lack of P rather than Ca was limiting milk yield (Rahman *et al*., 2014). Low A_{Ca} of 0.24–0.47 in green-feed oats and ryegrass/white clover given to stags with high Ca requirements for antler growth (Muir *et al*., 1987) may have been attributable to seasonal increases in bone resorption related to season or confinement.

Feeds for horses

Horses were thought to be particularly vulnerable to the Ca-depleting effects of oxalate-rich roughages but similar high AA_{Ca} coefficients (0.80 and 0.76) were reported for ponies given alfalfa meals, either low or high in oxalate, given with oats (1:2) in diets low in Ca (1.5g kg^{-1}DM) (Hintz *et al*., 1984). In horses with high BEL of 31mg kg^{-1}LW, AA_{Ca} was 0.75 with a mix of alfalfa, grass and concentrates but lower from concentrates (Pagan, 2001). Phytate may limit A_{Ca} in horses and *in vitro* studies suggested that the horse had distinctive mechanisms of Ca absorption (Wilkens *et al*., 2016). In weanling Thoroughbred foals, AA_{Ca} from grass containing 3.5g Ca kg^{-1} DM was lower (0.60) and did not decrease when supplements tripled Ca intake (Grace *et al*., 2002a). The equine body is relatively rich in Ca (Table 1.2), probably reflecting a

Table 4.4. Apparent availability coefficients for calcium (AA_{Ca}) in forages and forage-based diets and concentrates in studies with ruminants, with two exceptions: values are low when dietary Ca is high, dietary P is low or animals are confined. (Bold type indicates true availability values).

Main feed (fraction)	Other components	Ca	P	AA_{Ca}	Anomaly
Perennial Ryegrass[1] (1.0)	None	5.1	2.9	**0.48**	None
Timothy (1.0)	None	4.2	2.9	**0.30**	None
Tall Fescue (1.0)	None	4.8	2.6	**0.24**	Poor growth
Cocksfoot (1.0)	None	4.3	3.2	**0.31**	None
Tropical grass/hays[2]	None	BM		0.57	High oxalate
		BM		0.64	None
Timothy hay[3] (0.64)	RB, SBM	4.9	4.5	0.57	Confined/goats
Alfalfa hay, immature	RB, SBM	7.8	3.2	0.57	Confined/goats
Alfalfa hay, mature (0.72)	RB, SBM	6.2	3.7	0.54	Confined/goats
Sugarcane[4] (0.4)	GC, 0.30; SBH, 0.21	2.0	1.5	0.26	None
(0.4)	GC, 0.48	1.3	1.8	0.63	None
(0.6)	GC, 0.13; SBH, 0.14	3.8	1.0	0.47	None
(0.4)	GC, 0.27; SBH, 0.28	3.7	1.4	0.54	None
Corn silage (0.58)	GC, 0.27; SBH, 0.28	1.5	2.0	0.46	None
Corn silage[5] (0.42)	A, 0.24; HG, 0.29	5.1	2.1	0.22	Confined/lact.
Alfalfa hay (0.30)	HG, 0.34; CC, 0.22	5.2	1.5	0.16	Confined/lact.
Cereal silages[6]				0.24	
Alfalfa silage		c.10		0.34	Very high Ca
Grass/legume[7] (1.0)	None (Df)	12.1	6.0	**0.17**	Very high Ca
Grass/legume hay (1.0)	None	6.4	3.0	**0.19**	High Ca
Grass/legume[8] (1.0)	None	5.5	3.0	**0.19**	Confined/lact.
Green oats/grass (1.0)	None	3.8	3.0	**0.17**	Confined/lact.
Para grass[9]	RS	3.0	1.4	0.28	Low P; low S
Cassava foliage	PG; RS	6.4	1.4	0.24	Low P; low S
Napier grass[10]		5.6		0.26	Low P?
Citrus pulp[11] (0.35)	SC, 0.14; GC, 0.39	5.8		0.21	Confined
Alfalfa hay (0.45)	SC, 0.14; GC, 0.40	5.6		0.12	Confined
Corn silage[12] (0.4)	SBM, 0.2; HMC, 0.15; SBH, 0.12	5.2*	3.4	0.32	Confined
		(1.4)			
Barley[13]	SBM	1.8	4.2	**0.85**	Pelleted diet
Barley[14] (0.6)	Straw, LS	3.5	2.8	0.40	Pelleted diet
Corn silage[15] (0.7)	EC, 0.15; pSBM, 0.1	6.0	3.9	0.37	Tie stall
	LS, DCP	8.3	4.1	0.31	
Ground corn cob (0.58)	Corn (0.4)	0.6	2.5	0.65	Confined kids

[1–15]References: [1]Thompson *et al*., 1988; [2]Blaney *et al*., 1982; [3]Fredeen, 1989; [4]Zanetti *et al*., 2017b, 2019; [5]Martz *et al*., 1999; [6]Khorasani *et al*., 1997; [7]Chrisp *et al*., 1989a; [8]Chrisp *et al*., 1989b; [9]Sath *et al*., 2013; [10]Rahman *et al*., 2014; [11]Roque *et al*, 2007; [12]Taylor *et al*., 2009); [13]Field *et al*., 1985; [14]Freeden and Van Kessel, 1990; [15]Gaignon *et al*., 2019; [16]Dorrigan *et al*., 2018 RB, rolled barley; pSBM, protected soybean meal; EC, energy concentrate; GC, ground corn; SBH, soybean hulls; CS, corn silage; A, alfalfa; HG, hominy grits; RS, rice straw; PG, para grass; LS, limestone; BM, dietary Ca < maintenance: AACa 15% lower with Ca > maintenance; Df, defrozen grass.

relatively high proportion of bone. The weanling foal may resemble the young pig and deposit supplementary Ca in bone, rather than downregulating A_{Ca}. Relatively high AA_{Ca} of 0.54 was reported recently for Ca-supplemented hay (4.5g Ca kg^{-1}DM) given to mature Thoroughbreds (Pagan *et al*., 2020). High AA_{Ca} (0.63 ± 0.04) was also reported for unsupplemented roughage but values were lower from a mixture of roughage and oats (0.49 ± 0.04; Lof, 2010). A higher AA_{Ca} of 0.75 was found in lactating Thoroughbred mares given similar pasture to that fed to foals (Grace *et al*., 2002b), probably reflecting the higher Ca needs associated with lactation.

Feeds and Supplements for Poultry

Little was reported on A_{Ca} in feeds for poultry until recently because research focused on values for

inorganic supplements, necessarily added in large amounts to rations to complement Ca-poor cereals. Perspectives are changing (Selle *et al*., 2009; Li *et al*., 2017). The first PCD_{Ca} values (p. 26) for vegetable protein sources have been published (Table 4.5) and, with the exception of sunflower meal, are not sufficiently low to raise Ca requirements. Inclusion of rapeseed hulls with phytase improved PCD_{Ca} in broilers, possibly through an effect of fibre on gizzard development (Bournazel *et al*., 2018b). Unsaturated fat supplements lowered Ca retention from limestone in newly hatched broilers by forming indigestible calcium soaps (Atteh and Leeson, 1983) but was without effect in hens, given far more Ca (Atteh and Leeson, 1985).

Feeds and Supplements for Pigs

Variation in A_{Ca} in feeds, blends and supplements is less than in poultry but determined by the same factors, involving phytate (Selle *et al*., 2009). Linear regression meta-analysis revealed the following competing linear effects, accounting for 90% of variation in AA_{Ca} (g d^{-1}) (Misiura *et al*., 2018):

- Ca intake: 0.60 ± 0.029
- phytate P intake: -0.25 ± 0.057
- phytase (100 FU d^{-1}): 0.17 ± 0.031
- dietary Ca × phytase: -0.69 ± 0.101
- non-phytate P (n*Py*P) intake: 0.1 ± 0.052
- phytate × phytase: 1.21± 0.312

Thus benefits of phytase increase as phytate P increases but decrease as dietary Ca increases. Dietary Ca concentration had no independent influence on AA_{Ca} and a possible reason was given earlier. The positive effect of n*Py*P is unexpected since Pi supplements usually lower AA_{Ca} by inhibiting phytate degradation but may be explained by inclusion of results for groups given artificial, P-deficient diets, which retarded growth but lacked phytate. Meta-analysis does not entirely remove the influence of protocol. Other effects were related to dietary phytate content. A recent study of phytate digestibility (AA_P) in different cereal species and genotypes, with or without added phytase (McGhee and Stein, 2019), also measured AA_{Ca} and Table 4.5 includes a summary of results: AA_{Ca} and AA_P were highly and positively correlated, with

$$A_{Ca}\ (\text{coefficient}) = 0.59 \pm 0.023 + 0.38 \pm 0.051 AA_P\ (12\text{df})\ \text{adjusted}\ r^2 = 0.81$$

Table 4.5. Apparent availability coefficients of calcium (AA_{Ca}) for feeds and supplements included in cereal-based diets, with (italics) or without supplementary phytase in starter pigs.

Source	AA_{Ca}	Reference
Soybean meal (SBM)	0.54	Zhang *et al*. (2016)
Sunflower meal	0.25	Zhang *et al*. (2016)
Rapeseed meal (RSM)	0.46	Zhang *et al*. (2016)
RSM + SBM	0.50 *(0.51)*	Bournazel *et al*. (2018a)
Dehulled RSM + SBM	0.50 *(0.53)*	Bournazel *et al*. (2018a)
Meat and bone meal[a]	0.53–0.81	Sulabo and Stein (2013)
Limestone	0.65 (0.73)	Gonzales-Vega *et al*. (2015a, b)
Limestone with rye hybrids	0.71–0.76 (*0.75–0.85*)	McGhee and Stein (2019)
barley	0.72 (*0.85*)	
wheat	0.74 (*0.83*)	
corn	0.63 (*0.78*)	
sorghum	0.65 (*0.75*)	
Barley + RSM + Limestone	0.61 (*0.69*)	Sauer *et al*. (2003)
Barley + SBM + Limestone	0.55 (*0.72*)	Sauer *et al*. (2003)
Barley + SBM + Limestone	0.70 (*0.80*[c])	(Fig. 6.8)
Corn + SBM + limestone	0.64 (*0.74*[d])	Almeida *et al*. (2010)
Corn + SBM + limestone		Arredondo *et al*. (2019)
Dicalcium phosphate[b]	0.85	Almeida *et al*. (2010)
Fishmeal	0.73 (*0.84*)	Gonzales-Vega *et al*. (2015b)

[a]Only data for semi-purified diets available
[b]Calculated from increments in Ca intake and faeces with addition of DCP to negative control diet
[c]Phytate reduced by plant breeding, not phytase supplementation.
[d]Value with 500FU phytase: larger additions increased AA_{Ca} to 0.82

Higher AA_{Ca} in rye, wheat and barley than in corn and sorghum was probably attributable to lower phytase activity in corn and sorghum. Feeds, blends and supplements can be adequately characterized by AA_{Ca} as in Table 4.5[xxiv]. Distinction between pre-caecal and total tract absorption, net or apparent, is not required because post-ileal absorption of Ca is negligible (Zhang *et al*., 2016). Vegetable protein sources contain Ca of relatively low availability, even when phytase is added and requirements would be high on diets containing sunflower meal, if low availability was transferable to added Ca supplements.

Calcium Requirements

The principal factors affecting mineral requirements were described earlier (p.6 *et seq*.) and Ca was used to illustrate factorial modelling of composite needs (p. 9). All model components should be derived from studies following the rules on p. 77.

Sheep and cattle

Although AFRC (1991) factorial model uses maintenance needs that may have been wrongly based on DMI (p. 77), the strong correlation between DMI and LW means that provision for DMI also scales for LW, equating to ^{o}FEL of 19mg Ca kg^{-1}LW when DMI is 3% of LW. Net requirement for growth was based on the proportion of fully mineralized bone kg^{-1} LWG rather than carcase Ca, because the latter can vary (p. 9; Fig. 1.4) and poorly mineralized bones can fracture. The AFRC model used an A_{Ca} coefficient, 0.68, which is much higher than the 0.30 used by NRC (2001) but equivalent to AA_{Ca} values for feeds used in balanced diets (Table 4.4) and is retained for estimating the requirements for sheep and cattle in Tables 4.6 and 4.7, respectively. The requirement for young lambs on low-quality diets, 3.6g Ca kg^{-1}DM, is close to that for maximal A_{Ca} in Fig. 4.15. The estimates for cattle are supported by experiments with finishing steers (Zanetti *et al*., 2017a,b; Zanetti *et al*., 2019) and dairy cows (Taylor *et al*., 2009; Moreira *et al*., 2009). In the latter study with unconfined cows in early lactation, reducing dietary Ca from 6.3 to 4.6g kg^{-1}DM, with dietary P at 3.7 kg^{-1}DM, positive Ca balance was almost attained. Reducing dietary Ca in pre-partum diets from 7.0 to 4.6 g kg^{-1}DM was insufficient to trigger a response in PTH (Goff and Koszewski, 2017). With cows confined in crates and oestrus supressed in early lactation (Taylor *et al*., 2009), reducing dietary Ca decreased calcium retention but did not increase AA_{Ca}.

Note that:

- For lambs and calves, requirements decrease with age but increase with growth rate;
- For ewes, requirements rise rapidly in late pregnancy to equal those for lactation;
- For cows, requirements increase slowly during pregnancy and abruptly at calving;

Table 4.6. Dietary calcium requirements for housed sheep (AFRC, 1991).

			Diet Quality[a]			
			Low		High	
	Live-weight (kg)	Production level/stage	DMI (kg d^{-1})	Ca (g kg^{-1}DM)	DMI (kg d^{-1})	Ca (g kg^{-1} DM)
Lamb	20	100g d^{-1}	0.67	3.7	0.40	5.7
		200g d^{-1}	–	U	0.57	7.0
	40	100g d^{-1}	1.11	2.4	0.66	3.4
		200g d^{-1}	1.77	2.6	0.93	4.0
Ewe carrying twins[b]	75	13 weeks	1.28	2.0	0.85	2.6[c]
		17 weeks	1.68	2.9	1.13	3.9
		Term	2.37	3.2	1.62	4.3
Lactating (twins)	75	2–3kg milk^{-1}	2.8–3.7 2.3–3.2	2.8 (m)[d] 3.1 (bm)	1.8–2.4 (m) 1.5–2.1 (bm)	3.8 (m) 4.3 (bm)

[a]Low, poorly digestible diet, 'q' value 0.5; High, highly digestible diet, 'q' value of 0.7; U, unattainable performance.
[b]The requirements for small ewes carrying single lambs are similar, assuming they will eat proportionately less DM.
[c]Sufficient for a dry ewe.
[d]Requirements are influenced by the ability of the diet to meet energy needs and prevent loss of body weight (m); diets that allow loss of body weight at 0.1 kg day^{-1} (bm) are associated with higher requirements.

Table 4.7. Dietary calcium requirements of housed cattle on diets of low or high quality (AFRC, 1991).

	Liveweight (kg)	Production level/stage	Diet Quality		Gross need	
			Low	High	Low	High
			(kg DMI d^{-1})		(g Ca kg^{-1}DM)	
Growing cattle	100	0.5kg d^{-1}	2.8	5.2	1.7	8.0
		1.0kg d^{-1}	4.5	6.3	2.4	10.8
	300	0.5kg d^{-1}	5.7	3.0	3.4	4.4
		1.0kg d^{-1}	8.3	3.5	4.7	5.5
	500	0.5 kg d^{-1}	10.9	2.6	6.1	3.6
		1.0 kg d^{-1}	11.6	2.8	6.5	4.3
Pregnant	calf	23 weeks	6.3	2.1	4.0	2.7
	birth	31 weeks	7.2	2.3	4.7	3.0
	weight	39 weeks	9.1	2.7	6.1	3.5
	40kg	Term	11.2	2.8	7.5	3.6
Lactating	600	10kg d^{-1}	9.9	3.3 (bm)	NA	NA
		20kg d^{-1}	NA	NA	11.4	4.6 (m)
			NA	NA	10.1	5.1 (bm)
		30kg d^{-1}	NA	NA	15.3	4.8 (m)
			NA	NA	13.8	5.2 (bm)
		40kg d^{-1}	NA	NA	20.0	4.9 (m)
			NA	NA	18.4	5.3 (bm)

Low and high quality diets have ratios of metabolizable to gross energy of 0.5 and 0.7, respectively; m, fed to maintain body weight; bm, fed below maintenance.
*estimate added but based on AFRC (1991) assumptions

- When cows 'milk off their backs' (i.e. lose body weight to sustain production), requirements as concentration increase (by 8.0% in the example given).
- No figure need be met on a day-to-day basis but plentiful vitamin D_3 is required to utilize dietary Ca during prolonged housing.
- Induction of net bone resorption during confinement may underestimate dietary needs for free-moving sheep and cattle (p.71).

Sheep performance is unlikely to suffer if the diet provides an average of 3g Ca kg^{-1} DM throughout the year. Because there is less time for cows to replenish Ca reserves between lactations, average annual dietary Ca should be about 25% higher than for ewes and 15% higher in late pregnancy for primiparous heifers, still growing, than for older cows.

- Requirements of sheep and cattle grazing succulent green pasture would be lower than those in Tables 4.6 and 4.7 if maintenance needs were only half those of animals on dry, mixed diets, as indicated for sheep in Fig. 4.18.

Horses

Factorial estimates of requirements of grazing foals (Grace *et al.*, 1999a) and lactating mares (Grace *et al.*, 1999b) in New Zealand were based on data for mineral compositions of the carcase (Table 1.2) and milk (Table 2.4), similar FEL_{Ca} to those for sheep (16mg kg^{-1}LW; ARC, 1980), a milk yield of 20kg day^{-1} and A_{Ca} coefficients of 0.7 and 0.5 from grass in weaned thoroughbred foals and lactating mares, respectively; dietary needs were 4.6 and 4.9g Ca kg^{-1}DM but may be overestimates. Young thoroughbreds on grass containing 3.5g Ca kg^{-1}DM showed no benefit from Ca supplementation (Grace *et al.*, 2003) and a similar pasture was considered adequate for mares (Grace *et al.*, 2002b). There is no evidence that mares absorb Ca less efficiently than weaned foals. In reviewing the mineral requirements of horses NRC (2007a) noted that requirements were probably increased by regular exercise and lowered by inactivity and raised their previous estimates in the light of high reported FEL_{Ca} (Pagan, 2001). A later regression of faecal Ca against Ca intake placed $^{m}FEL_{Ca}$ at 12mg kg^{-1}

LW, similar to that of lambs (p.ca) and found no justification for increased requirements (Lof, 2010).

Pigs

Requirements of growing pigs were overestimated in early empirical feeding experiments and by a factorial model that assumed A_{Ca} to decline from 0.67 to 0.47 as pigs grew (ARC, 1981). Aspirational requirements, assuming a uniformly high AA_{Ca} for phytase-supplemented diets based on corn or other cereals (Table 4.7), are given in Table 4.8: they are lower than NRC (2012) but largely untested. However, reducing dietary Ca to 3.9g kg^{-1}DM and withdrawing a Pi supplement for finisher pigs (leaving 2.9g P kg^{-1}DM) did not reduce performance (other than reducing FCE), plasma Pi or bone P (Schlegel and Gutzwiller, 2020). Increasing dietary Ca from 4.0 to 7.5g (with 3.5g P) kg^{-1}DM produced only small improvements in starter pig performance in corn/SBM diets without added phytase (Lagos *et al.*, 2019b). Requirements for optimal growth are lower than those for maximal bone mineralization (Fig. 4.1): the net requirement for growth probably allowed for generous mineralization and constitutes a safety factor, since bone mineralization can be reduced without harm to the pig. An $STTD_{Ca}$:P ratio approach has been proposed to accommodate variation in A_{Ca} (Stein *et al.*, 2016; Lagos *et al.*, 2019b). In view of the uncertainties discussed earlier (p.76),

- precise Ca (and P) nutrition is more likely to be achieved by using phytase with minimal added Ca and Pi than by reliance on unrepresentative NRC (2012) values for $STTD_P$ and intra-experimental $STTD_{Ca}$ values for feeds and supplements.

Poultry: meat production

Estimated Ca requirements of growing birds have declined, driven by a need to maximize utilization of abundant phytate P (Bradbury *et al.*, 2014 and 2016; Li *et al.*, 2017; Dersjant-Li *et al.*, 2020). Early-life conditioning by feeding diets low in Ca and P may allow further reductions in Ca provision (Ashwell and Angel, 2010) but the approach requires validation (Valable *et al.*, 2018) and may not be needed in phytase-supplemented diets. Response surface analysis indicated that near optimum performance and tibia quality was obtained from 0–21 days with 6.5g Ca (with 3.9g non-phytate P) kg^{-1}DM (Fallah *et al.*, 2020), in line with previous studies (Henry and Pesti, 2002; Driver *et al.*, 2005b) (Fig. 4.20). Requirements are higher for maximum bone density than growth, as in the pig, but less dispensable. However, grower chicks can compensate for low dietary Ca immediately after hatching by increasing AA_{Ca} (Yan *et al.*, 2005; Rousseau *et al.*, 2016) and re-mineralizing bone without cost in terms of subsequent performance (Valable *et al.*, 2018). The standards presented in Table 4.9 are paired with those for P (Table 6.7) and assume that phytase supplements will reduce starter requirement by 10%.

- Ideally, requirements should probably be expressed relative to energy density rather than DM (Fig. 4.11).

Poultry are unique in showing an appetite for supplementary Ca, which develops during the growth phase. Uptake of $CaCO_3$, given separately from the diet, increases as dietary Ca intake decreases. Separate feeding of $CaCO_3$ stimulates food intake as the chick tries to satisfy calcium appetite by consuming more low Ca diet (Abdollahi *et al.*, 2016). Separate feeding of $CaCO_3$ and low Ca diet may have environmental as well as commercial advantages, enhancing the utilization of phytate P.

Table 4.8. New factorial[a] estimates of minimum calcium requirements for growing pigs on diets based on corn (USA) or other cereals (Europe) and containing 1000 FU phytase (intrinsic or exoxogenous), formulated for use with corresponding requirements for phosphorus (p.pp) and to protect environments.

Liveweight (kg)	DMI (kg day^{-1})	LWG (kg day^{-1})	Net need[a] (g day^{-1})	Gross need Europe (g kg^{-1}DM)	USA
20	1.0	0.4	3.4	4.9	4.3
40	1.6	0.6	5.3	4.7	4.1
80	2.5	0.8	7.0	4.0	3.5
100	3.0	1.0	8.7	4.1	3.6

[a]Net growth requirement; 8g Ca kg LWG day^{-1} and 8mg Ca kg^{-1}LW (Bikker and Blok, 2017). Apparent absorption coefficients: 0.7 for corn/soybean meal diets (USA) and 0.8 for cereal/mixed protein supplements in Europe: maintenance need, 0.239 mg kg^{-1}DM (Misiura *et al.*, 2018).

Poultry: egg production

For pullets entering lay, provision should increase from 10 to 30g Ca kg^{-1}DM one week before the first egg is anticipated (Roland, 1986). Whatever the chosen combinations of animal, diet and environment, net reqirement is affected by stage of lay, as illustrated in Fig. 4.21. Roland (1986) proposed a gradual increase in dietary provision from 3.75 to 4.25g Ca kg^{-1}DM throughout lay and subsequent studies supported this (Fig. 4.22) (Scott *et al*., 2000). NRC (1994) allowed for effects of food intake (or energy density) over the range of 80–120g day^{-1} by recommending 3.25g Ca kg^{-1}DM, on average, for white hens and less for brown hens, which consume more food. Requirements increased from 3.8 to 4.5g Ca kg^{-1}DM as egg size increased from 50 to 60g (Simons, 1986). For pullets entering lay, intake should increase from 10 to 30g Ca kg^{-1}DM one week before the first egg is anticipated (Roland, 1986). Requirements for peak egg production must sometimes be met on an hourly basis to maintain shell strength, necessitating 24-hour lighting to increase feed and Ca intakes overnight. Laying hens voluntarily consume more Ca supplement on laying than on non-laying days and more of a low- than a high-Ca ration every day (Gilbert, 1983).

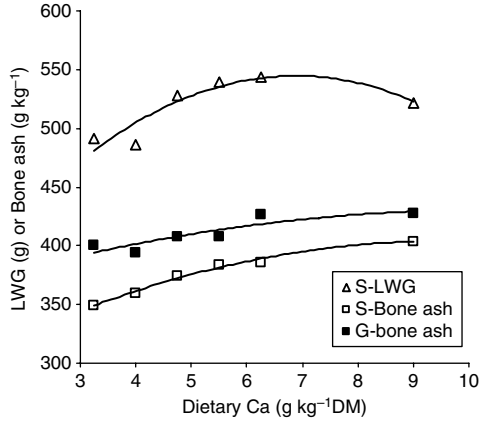

Fig. 4.20. Effects of dietary calcium on liveweight gain (LWG) and bone mineralization (tibia ash) in broiler chicks at starter (S, 0–16 day) and grower (G, 19–42 day) stages (from Driver *et al*., 2005a): 6.25g Ca kg^{-1}DM was optimal for growth in starters and bone mineralization in growers, supra-optimal for growth in growers (not shown) but suboptimal for bone mineralization in starters; a corn/soybean meal diet provided 4.5 g 'available' P kg^{-1}DM and food intake was 40 g and 113 g day^{-1} in starters and growers, respectively.

Prevention of Chronic Lack of Calcium

Poultry

Skeletal defects in early life can be irreversible, weak eggshells are irreparable and KBF painful, while reducing egg yield: preventive measures are essential but the scope for manipulation of dietary Ca without reducing P availability is small. Short-term lack of dietary Ca is tolerable in chicks (p. 83) and common Ca sources differ little in availability, exceptions being dolomitic limestone and soft-rock phosphate with low values of 0.65 and 0.70, respectively, relative to $CaHPO_4$ (Soares, 1995). Isolated claims of low AA_{Ca} in meat and bone meals may be anomalies of technique rather than supplement. Particle size is more important than source for broilers (Bradbury *et al*., 2016: Kim *et al*., 2018) and hens (Fleming *et al*., 2006).

Table 4.9. Suggested minimum dietary requirements for calcium (g kg^{-1}DM) for optimal growth in phytase-supplemented rations but without added inorganic P in broilers, indicated by recent research, compared with NRC (1994) standards and extrapolated to give needs for other birds.

	Broiler[a] stage (weeks)		
	Early (0–3)	Middle (3–6)	Late (6–8)
Broilers – new proposal	6.0	4.0	3.0
Broilers – NRC (1994)	10.0	9.0	8.0
Leghorn chick[b]	5.0	3.5	20.0[c]
Turkey poult[b]	7.0	5.0	4.0

[a] Corresponding growth stages are 0–6, 6–18 and >18 weeks for Leghorns and 0–4, 8–12 and 20–24 weeks for poults.
[b] Provisional recommendations, based on the degree to which requirements in broilers fell with concurrent reductions in dietary calcium and phosphorus.
[c] This value is high in preparation for lay.

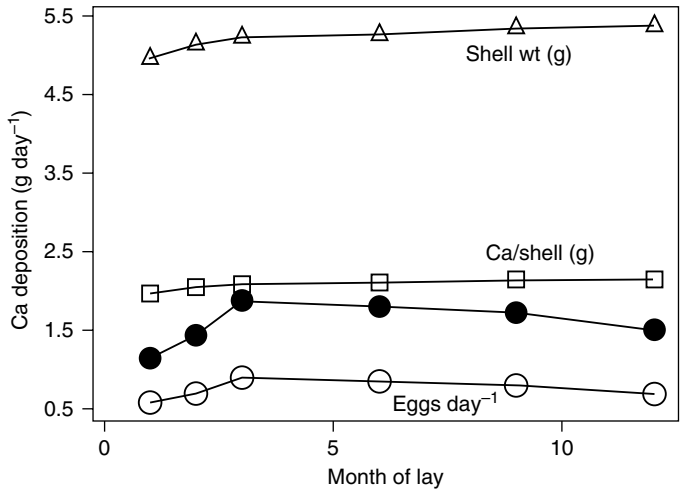

Fig. 4.21. Daily calcium (Ca) deposition in eggs by laying hens (solid circle) peaks after about three months of lay, due to increases in rate of egg production, amount of shell per egg and shell Ca. (Data from Roland, 1986)

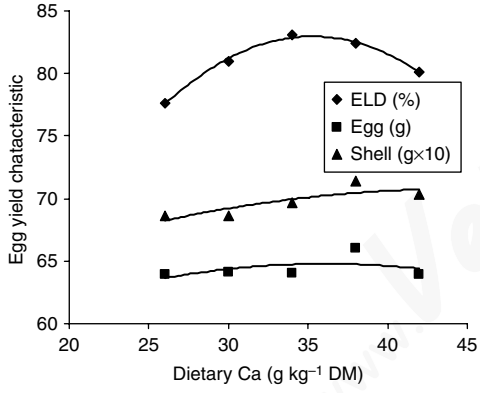

Fig. 4.22. Responses of Leghorn hens to increases in dietary calcium: egg-laying days (ELD) and both egg and shell weight were optimal with about 38g Ca kg^{-1}DM, but bone strength (not shown) increased linearly as dietary Ca increased (from Narvaez-Solarte *et al*., 2006; basal corn/soybean meal diet provided 3.5 g available P kg^{-1}DM).

Large particles remain in the gizzard longer and provide more retainable Ca than finely ground sources (Kim *et al*., 2018) and may achieve synchrony with diurnal fluctuations in shell calcification (Whitehead, 1995). Particulate limestone can improve bone quality and resistance to KBF but without improving egg production or shell strength (Fleming *et al*., 2006; Cudafar *et al*., 2011). Particle size may also influence P requirements

Table 4.10. Effects of dietary calcium and calcitriol ($1,25\text{-}(OH)_2D_3$) on incidence of tibial dyschondroplasia (TD) in broiler chicks (Whitehead, 1995).

		Calcitriol (µg kg^{-1})			
	Ca (g kg^{-1})	0	2.0	3.5	5.0
TD incidence (%)	7.5	50	15	5	0
	10	10	20[a]	5	5
	12.5	15	15	0	0

[a]Complex interactions between dietary Ca and $1,25\text{-}(OH_2)$ D occur: Ca uptake from diets low in Ca is enhanced by the vitamin; with high Ca and $1,25\text{-}(OH)_2D_3$, growth-inhibiting but TD-protective hypercalcaemia counters adverse effects on P status.

of broilers. Sources other than $CaCO_3$ generally offered no advantages.[xxv] Better control of TD was obtained by supplementation with calcitriol than by raising dietary Ca (Table 4.10) but non-dietary measures offer more protection against TD and KBF.

Heritabilities of other leg disorders, including TD, are sufficiently high for partial prevention to be achieved by genetic selection (Akbas *et al*., 2009). Selection for low or high bone strength[xxvi] produced a sixfold difference in bone fracture incidence after six generations from a smaller divergence (16–24%) in an index of bone quality of high heritability (0.4): particulate limestone improved bone quality in both lines (Bishop *et al*.,

2000). Comparisons between aviary and caged housing in the seventh generation showed benefits of freedom of movement by the end of lay in both selection lines (Fleming *et al*., 2006). Exercise increased bone density in pullets and residual effects were evidenced by decreased osteoporosis at the end of lay (Casey-Trott *et al*., 2017). Breeds differ in susceptibility to KBF, Plymouth Rock being relatively resistant (Regmi *et al*., 2016).

Pigs

The role of dietary Ca supplementation is simpler in one respect in pigs than in poultry, since particle size does not affect A_{Ca} or performance (Merriman and Stein, 2016) but is still a potential source of error in short-term availability tests (p. 76). Differences in availability between sources are similar to those in poultry (Soares, 1995). Calcium formate may improve gut health in early-weaned pigs but Ca is not the active ingredient. Confinement for farrowing may mobilize Ca from the skeleton (p. 76), aiding the litter but eventually requiring replacement, if lactation is not to be constrained (p. 8).

Ruminants

Additional Ca is rarely needed but routinely provided as $CaCO_3$ or DCP: the latter was of marginally superior availability to cattle (Soares, 1995) but not sufficiently to justify extra cost and waste of P. Lambs appeared to absorb two thirds less Ca from DCP than from $CaCO_3$ but of equal A_{Ca} to oyster meal (Roque *et al*., 2007). However, high Ca retention from DCP (around 2 g Ca d^{-1}) without weight gain suggesting retention of dense DCP particles, an anomaly previously thought to have lowered A_P from RP (p. 154). Acidotic salts, such as $CaCl_2$ and $CaSO_4$, are used to manipulate DCAD in late pregnancy for dairy cows to avoid milk fever (p. 63). Low and high DCAD mineral mixtures containing Ca were equally effective in raising Ca status of ewes in late pregnancy, grazing cereals in the vegetative state (Masters *et al*., 2018). When mixing Ca sources and other minerals and foodstuffs, even distribution is important. Inadequate mixing of limestone with cereals contributed to hypocalcaemia after drought feeding (Larsen *et al*., 1986) but can be avoided by using molasses to stick such supplements to grains: P-free Ca sources are ideal for grain-based diets. Calcium complexes with organic acids ('calcium soaps'), used as energy sources, do not appear to have been studied as inducers of calcium appetite or strengtheners of eggshells and may not replace particulate limestone.

Calcium toxicity

Calcium is not generally regarded as a toxic element because homeostatic mechanisms ensure that excess dietary Ca is extensively excreted in faeces. However, doubling dietary Ca for chicks to 20.5g kg^{-1}DM caused hypercalcaemia and growth retardation, with fast-growing strains more vulnerable than slow-growing strains (Hurwitz *et al*., 1995). Marginal bands for diagnosing hypercalcamia were given in Table 4.3. Adverse effects of excess dietary Ca are generally indirect and arise from impaired absorption of other elements; thus, deficiencies of P and Zn are readily induced in non-ruminants. Calcium soaps of fatty acids (CSFA) are used to increase the energy density of diets without depressing fibre digestibility in the rumen; significant improvements in milk yield can be obtained in dairy cows and ewes, but not without feeding gross excesses (about 10g Ca kg^{-1}DM) and sometimes matching excesses of P (6g kg^{-1}DM) (Underwood and Suttle, 1999): this excess dietary Ca in rations with added CSFA may reduce conception rate in cows. Hypercalcaemia can cause life-threatening tissue calcification but is usually a secondary consequence of phosphorus deprivation or over-exposure to vitamin D_3 (Payne and Manston, 1967) and its analogues (Whitehead, 1995), some of which occur naturally. Ingestion of $1\alpha OHD_3$ in *Solanum malacoxylon* can cause tissue calcification in cattle. The first sign of trouble is osteophagia and there may be no alternative but to temporarily destock pastures infested with the water-loving plant. Factors that safely maximize deposition of Ca in bone (heavily boned breeds; ample dietary P; alkaline mineral supplements) or lower Ca intake (grain feeding) may provide protection where *S. malacoxylon* is a problem. *Trisetum flavescens* also contains $1\alpha OHD_3$ and can cause calcification of the cardiovascular system and kidneys when fed as silage to cattle or sheep (Franz *et al*., 2007). Cattle were the more severely affected, moving unnaturally and exhibiting low body temperatures and erect hair: calcification of the aorta was detectable by ultrasonography. Exposed sheep had cardiac arrhythmia and kidney calcification. Horses are also vulnerable to calcinogenic plant species (NRC, 2007b).

Notes

[i] Although the abbreviation SCH stands for subclinical hypocalcaemia in common use, a case will be made for a stronger definition.

[ii] Bones grow in length as chondrocytes towards the end of regular columns in the growth (epiphyseal) plate become progressively hypertrophic and degenerate: peripherally, they concentrate Ca and P and exfoliate vesicles rich in amorphous $Ca_3(PO_4)_2$ (Wuthier, 1993). Thus, a Ca-rich milieu is provided to impregnate an organic osteoid template, laid down by osteoblasts (Donelly and Boskey, 2011). Crystalline bone mineral, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), eventually accumulates in a zone of provisional calsification in an apparently disorganized and largely inorganic matrix of trabecular bone. Hydroxyapatite can adsorb both cations (notably Mg^{2+} and Na^+) and anions (including PO_4^{2-} and CO_3^{2-}).

[iii] Genes for matrix proteins have vitamin D-responsive elements, which facilitate hormonal control of mineralization. Bones grow in width through inward deposition of compact, cortical bone in concentric shells by osteoblasts, behind a cutting cone of osteoclasts and beneath the surface of the bone shaft or periosteum. Receptors for $1,25\text{-}(OH)_2D_3$ on chondrocytes and osteoblasts facilitate Ca accretion, while receptors for PTH assist bone resorption. Osteoclasts respond to $1,25\text{-}(OH)_2D_3$ via CaBP, induced by osteoblasts (Norman and Horwitz, 1993; Donelly and Boskey, 2011; Alkukhun *et al*., 2017). Serum osteoprotegerin, a CaBP that preserves bone by regulating osteoclastogenesis, remains high in multiparous cows until parturition but then declines to allow osteoclastic bone resorption: primiparous cows, in which the skeleton is still growing, show no such change at calving (Fig. 4.2). Similarly, serum osteocalcin, a CaBP that promotes bone accretion, decreases sharply at parturition in multiparous cows (Rodney et al., 2019) but less so in first-calvers (Taylor *et al*., 2009). Serum osteocalcin is low at parturition but increases steadily for 20 weeks in minimally confined cows (Gaignon *et al*., 2019).

[iv] Mammary tissue can upregulate expression and release of a PTH-related protein (PTHrP) (Goff *et al*., 1991) that mimics PTH: plasma PTHrP increases after parturition (Filipović *et al*., 2008). Peripheral serotonin concentrations regulate PTHrP gene expression, mobilization of calcium from the skeleton and mammary epithelial cell calcium transport (Hernandez *et al*., 2012). Infusion of a serotonin precursor attenuated the fall in plasma Ca after parturition in cows and increased yield of colostrum (Hernandez-Castellano *et al*., 2017). Osteoclasts and osteoblasts both respond to oestrogens, which increase sensitivity to PTHrP (Goff, 2007) and modulate osteoptegrin (Hatate *et al*., 2018).

[v] In a further study, lack of N had a contrary effect to lack of Ca, the former downregulating, the latter upregulating 24-hydroxylase activity in the kidney, suppressing an adaptive increase in Ca absorption: both deficiencies increased the expression of bone resorbing factors by the kidney (Wilkens *et al*., 2019).

[vi] Hypocalcaemia can be episodic and recur at roughly nine-day intervals as cows repeatedly adjust to increasing demands until lactation peaks (Hove, 1986).

[vii] In the first stage, cows become listless, show muscular weakness, circulatory failure, muscular twitching, anorexia and rumen stasis (i.e. a parturient paresis); in the second, they become drowsy, have staring, dry eyes with dilated pupils and become sternally recumbent; finally, they become laterally recumbent and comatose. Blood flow to peripheral tissues decreases (Barzanji and Daniel, 1987), contributing to hypoxia (Barzanji and Daniel, 1988) and hypothermia (Fenwick, 1994). Displacement of the abomasum and myocardial lesions (Yamagishi *et al*., 1999) may limit responses to Ca alone.

[viii] Efficacy is not improved by extending the infusion period from the customary 15 min to 6 h (Braun *et al*., 2004). If a veterinary practitioner is not immediately available then the herdsman should intervene, warming the infusate before administration (Fenwick, 1994).

[ix] Incidence of milk fever was increased greatly but equally in vulnerable cows (Jerseys >4 years old) when dietary K and Na were raised from 10 to 30 g kg^{-1}DM by supplementation with either $KHCO_3$ or $NaHCO_3$ despite a large difference in DCAD (+500 or + 870me kg^{-1}DM) (Goff and Horst, 1997). Meta-analyses of 37 studies found that prediction of milk-fever incidence was poorer using the most popular DCAD equation ([Na + K] – [Cl + S]) (Model 1) than when K and S were included as independent variables, alongside Mg, Ca and P (Model 2) (Lean *et al*., 2006). A quadratic effect of dietary Ca was evident, with low risks of milk fever at low (<5g) and high (>20g Ca kg^{-1}DM) concentrations. Influences of Mg and P were also apparent: a 62% *decrease* in risk of milk fever was predicted as Mg increased from 3 to 4g kg^{-1}DM and an 18% *increase* as P increased by the same amount.

[x] Incidence of SCH^N in multiparous cows was 69.3% (Martinez *et al*., 2016) and 79.8% (Jahani-Moghadam *et al*., 2018). Mean (SE) plasma Ca was only 1.86 (0.08) mmol l^{-1}) in an untreated indicator group of 25 cows in one experiment (Leno *et al*., 2018).

[xi] The synthesis of proteins during the acute phase response to infection may be compromised (Feijo *et al*., 2017).

[xii] Polymorphonuclear lymphocytes have low intracellular Ca^{2+} and reduced *in vitro* killing capacity (Zhang *et al*., 2019).

[xiii] Although plasma Ca was normalized within 48h, 91% of a treated group on a diet high in Ca (11g kg^{-1}DM) developed SCH^N (Amanlou *et al*., 2016). With $MgCl_2$ as the major anion source, 61.5% of treated, multiparous cows developed SCH^R and herd health was not improved (Leno *et al*., 2017).

[xiv] Imbalanced intake of Ca, Mg, K, P, Na and K is common in ruminants grazing pastures and vegetative crops but it is unclear whether this affects incidence of clinical and subclinical disease (Masters, 2018)

[xv] High incidence in USA was attributable in part to widespread use of conserved alfalfa, rich in Ca and K, and excessive use of supplementary P (Goff and Horst, 2003). DCAD (or the separate ions) affects exchanges between skeletal and plasma Ca pools at calving.

[xvi] PCD_{Ca} decreased with age for two sources of monocalcium phosphate (MCP), which raised dietary Ca above need to 11g kg^{-1}DM: one source appeared to provide less absorbable Ca than $CaCO_3$ at 25 days (0.61 ± 0.022). Estimates of availability may have been affected by age but also by interactions between dietary Ca and phytate degradability, which changes with age.

[xvii] The inappropriateness of semi-purified diets for chicks (p. 6) was confirmed when AA_{Ca} increased from 0.36 to 0.44 ± 0.016 for four mineral sources when corn replaced corn starch as the major dietary constituent (David *et al*., 2019). Availability was maximal for limestone and minimal for meat and bone meal (MBM). ($PCAA_{Ca}$ was 0.49 and 0.30 ± 0.023, respectively) but the value for limestone increased to 0.65 ± 0.032 when the adaptation period was reduced from three days to one day.

[xviii] Poor piglet growth (e.g. Traylor *et al*., 2001) and weight loss in chicks (e.g. Anwar *et al*., 2015) in marker-to-marker studies may also have inflated FEL_{Ca}. Interactions between Ca source, phytate and non-phytate P (n*PP*) (Paiva *et al*., 2013; Bradbury *et al*., 2016) affect FEL_{Ca} in poultry. Effects of confinement in piglets and chicks may explain some of the variation in estimates of $^{m}FEL_{Ca}$ on Ca- and P-free diets.

[xix] Graded increments (20-80g kg^{-1}DM) of three meat and bone meals (MBM), added to a 'Ca-free' semi-purified diet, had PCD_{Ca} of 0.46-0.60 (Anwar *et al*., 2015): similar increments produced less variable values (0.54-0.61) for three limestones (Anwar *et al*., 2016). However, a follow-up comparison of inorganic (DCP, MCP), animal and vegetative sources, yielded equally low PCD_{Ca} (0.24-0.33), depending on method (Anwar *et al*., 2018).

[xx] Endogenous microbial phytases begin to degrade phytate in the gizzard, a process influenced by pH (Kim *et al*., 2018). Calcium sources differing in particle size and solubility influenced phytate disappearance in the gizzard and impacted PCD_{Ca}, which varied from 0.38 to 0.56 between supplementary limestones in one study (Kim *et al*., 2018).

[xxi] Reduced Ca accretion in bone by confined lambs on hay may have created a surplus of absorbed Ca, which was excreted via faeces. Early increases in calcium intake from protein-rich grass may have stimulated bone accretion in confined lambs, lowering FEL_{Ca}. Similarly, bone accretion may be stimulated and resorption attenuated in confined lactating ewes, in which milk secretion as well as endogenous loss provides an outlet for excess Ca, again lowering FEL_{Ca}.

[xxii] Urinary Ca generally remains low, regardless of dietary Ca, this despite the fact that renal tubular reabsorption is not controlled in sheep and goats (Herm *et al*., 2015) as it is in non-ruminants, by Ca^{2+} receptors such as TRVP6 (Alkukhun *et al*., 2017). However, urinary Ca rises significantly in dairy cows (Goff and Horst, 1998; Leno *et al*., 2017), steers (p. 177) and goats (Liesegang, 2008) given acidogenic diets: >4g Ca d^{-1} can be excreted in urine by acidotic cows (Megahed *et al*., 2018).

[xxiii] Firstly, high A_{Ca} were obtained for alfalfa in goats confined for 63 days, during which positive Ca balance was maintained during application of a Latin Square design (Freeden, 1989): non-lactating does may have adapted to confinement. Secondly, low A_{Ca} was recorded in cattle given a combination of sugar cane bagasse, ground corn and soybean hulls (Zanetti *et al*., 2017b, 2019).

[xxiv] Values obtained with semi-purified diets are of largely academic interest and disregarded because they cannot reflect the full effects of phytate and phytase. For example, fish meal added to a semi-purified diet had a far lower AA_{Ca} (0.51) and a smaller response to phytase (0.57) than values shown in Table 4.5 and AA_{Ca} was increased when fibre was added (Gonzales-Vega *et al*., 2015b).

[xxv] Monobasic calcium phosphate (CaH_2PO_4) is unsuitable for laying hens because it causes acidosis and poor shell quality. Soluble calcium citrate malate had similar value to $CaCO_3$ for broiler chicks (Henry and Pesti, 2002). However, calcium formate is advocated to promote gut health and reducing contamination of carcases and eggs with pathogenic bacteria.

[xxvi] Bone strength was measured as a score combining tibial and humeral strengths with radiographic density of the keel bone.

References

Abdollahi, M.R., Duangnumsawang,Y., Kwakkel, R.P., Steenfeldt, S. Bootwala, S.M. and Ravindran, V. (2016) Investigation of the interaction between separate calcium feeding and phytase supplementation on growth performance, calcium intake nutrient digestibility and energy utilisation in broiler starters. *Animal Feed Science and Technology* 219, 48–58.

AFRC (1991) Technical Committee on Responses to Nutrients Report No. 6. A reappraisal of the calcium and phosphorus requirements of sheep and cattle. *Nutrition Abstracts and Reviews* 61, 573–612.

Afsahi, A., Ahmadi-Hamedani, M. and Khodadi, M. (2020) Comparative evaluation of urinary dipstick and pH-meter for cattle urine pH measurement. *Heliyon* 6, e03316. Available at: https://doi.org/10.1016/j.heliyon.2020.e03316 (accessed 29 April 2021).

Akbaş, Y., Yalçın, S., Özkan, S., Kırkpınar, F., Takma, C., Gevrekçi, Y., Güler, H.C. and Türkmut, L.C. (2009) Heritability estimates of tibial dyschondroplasia,

valgus-varus, foot-pad dermatitis and hock burn in broiler. *Archive fur Geflügelkunde* 73 S, 1–6.

Alkukhun, A., Munoz-Abraham, A.S., Judeeba, S. and Geibel, J.B. (2017) Molecular aspects of the calcium-sensing receptor and calcium homeostasis. Chapter 2 in: Collins, J. (ed.) *Molecular, Genetic and Nutritional Aspects of Major and Trace Minerals.* Elsevier Inc., Amsterdam.

Almeida, F.N. and Stein, H.H. (2010) Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. *Journal of Animal Science* 88, 2968–2977.

Allen, W.M. and Sansom, B.F. (1985) Milk fever and calcium metabolism. *Journal of Veterinary Pharmacology and Therapeutics* 8, 19–21.

Amanlou, H., Akbari, A., Farsuni, N.E. and Silva-Del-Río, N. (2016) Effects of subcutaneous calcium administration at calving on mineral status, health, and production of Holstein cows. *Journal of Dairy Science* 99, 9199–9210.

Anwar, M.N., Ravindran, V., Morel, P.C.H., Ravindran, G. and Cowieson, A.J. (2015) Measurement of the true ileal calcium digestibility in meat and bone meal for broiler chickens. *Animal Feed Science and Technology* 206, 100–107.

Anwar, M.N., Ravindran, V., Morel, P.C.H., Ravindran, G. and Cowieson, A.J. (2016) Apparent ileal digestibility of calcium in limestone for broiler chickens. *Animal Feed Science and Technology* 213, 142–147.

Anwar, M.N., Ravindran, V., Morel, P.C.H., Ravindran, G. and Cowieson, A.J. (2018) Measurement of the true ileal calcium digestibility of some feed ingredients for broiler chickens. *Animal Feed Science and Technology* 237, 118–128.

Araujo, M.J., de Madeiros, A.N., Teixera, I.A.M.A., Costa, R.J., Marques, C.A.T., Resende, K.T. and de Melo, G.M.P. (2010) Mineral requirements for growth of Moxoto goat in the semi-arid region of Brazil. *Small Ruminant Research* 93, 1–9.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

ARC (1981) *The Nutrient Requirements of Pigs*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 215–248.

Arredondo, M.A., Casas, G.A. and. Stein, H.H. (2019) Increasing levels of microbial phytase increases the digestibility of energy and minerals in diets fed to pigs. *Animal Feed Science and Technology* 248, 27–36.

Ashwell, C.M. and Angel, R. (2010) Nutritional genomics: a practical approach by early life conditioning with dietary phosphorus. *Revista Brasileira de Zootecnia* 39 (Suppl. spe), 268–278.

Assis, D.S.M., Silva, A.M.A., Nascimento, N.J. Jr, Bezerra, L.R., Filho, J.M.P. et al. (2018) Dorper × Santa Ines F1 crossbred lambs under different grazing times and supplement levels in tropical regions: performance and macromineral requirements. *Italian Journal of Animal Science* 17, 931–940.

Atteh, J.O. and Leeson, S. (1983) Effects of dietary fatty acids and calcium levels on performance and mineral metabolism of broiler chickens. *Poultry Science* 62, 2412–2419.

Atteh, J.O. and Leeson, S. (1985) Responses of laying hens to dietary saturated and unsaturated fatty acids in the presence of varying dietary calcium levels. *Poultry Science* 62, 2412–2419.

Bain, S.D. and Watkins, B.A. (1993) Local modulation of skeletal growth and bone modelling in poultry. *Journal of Nutrition* 123, 317–322.

Bani Hassan, E., Nouri, M., Vogrin, S. and Pyman, M. (2018) Can neutral dietary cation-anion difference (DCAD) decrease occurrence of clinical periparturient hypocalcaemia in dairy cattle? *Australian Veterinary Journal* 96, 269–273.

Barzanji, A.A.H. and Daniel, R.C.W. (1987) Effect of hypocalcaemia on blood flow distribution in sheep. *Research in Veterinary Science* 42, 92–95.

Barzanji, A.A.H. and Daniel, R.C.W. (1988) The effects of hypocalcaemia on blood gas and acid–base parameters. *British Veterinary Journal* 144, 93–97.

Bellof, G., Most, E. and Pallauf, J. (2006) Concentrationn of Ca, P, Mg, Na and K in muscle, fat and bone tissue of lambs of the breed German Merino landsheep in the course of the growing period. *Journal of Animal Physiology and Animal Nutrition* 90, 385–393.

Bhanugopan, M.S., Fulkerson, W.J., Fraser D.R., Hyde, M. and McNeill, D.M. (2010) Carryover effects of potassium supplementation on calcium homeostasis in dairy cows at parturition. *Journal of Dairy Science* 93, 2119–2129.

Bhanugopan, M.S., Fulkerson, W.J., Hyde, M. and Fraser, D.R. (2014) Effect of dietary potassium supplementation on the calcium absorption capacity in the rumen and abomasum and fractional excretion of urinary minerals in sheep. *Animal Production Science* 55, 508–514.

Bikker, P. and Blok, M.C. (2017) Phosphorus and calcium requirements of growing pigs and sows. CVB Documentation Report no. 59. Wageningen, The Netherlands.

Bishop, S.C., Fleming, R.H., McCormack, H.A., Flock, D.K. and Whitehead, C.C. (2000) Inheritance of bone characteristics affecting osteoporosis in laying hens. *British Poultry Science* 41, 33–40.

Blaney, B.J., Gartner, R.J.W. and Head, T.A. (1982) The effects of oxalate in tropical grasses on calcium, phosphorus and magnesium availability to cattle. *Journal of Agricultural Science, Cambridge* 99, 533–539.

Bonniwell, M.A., Smith, B.S.W., Spence, J.A., Wright, H. and Ferguson, D.A.M. (1988) Rickets associated with vitamin D deficiency in young sheep. *Veterinary Record* 122, 386–388.

Bournazel, M., Lessire, M., Duclos, M., Magnin, M., Même, N. *et al.* (2018a) Effects of Rapeseed meal

fiber content on phosphorus and calcium digestibility in growing pigs fed diets without or with microbial phytase. *Animal* 12, 34–42.

Bournazel, M., Lessire, M., Klein, S., Même, N., Peyronnet, C., Quinsac, A., Duclos, M. and Narcy, A. (2018b) Phytase supplementation in diets rich in fiber from rapeseed enhances phosphorus and calcium digestibility but not retention in broiler chickens. *Poultry Science* 97, 1627–1640.

Bradbury, E.J., Wilkinson, S.J., Cronin, G.M., Thomson, P., Bedford, M. and Cowieson, A.J. (2014) Nutritional geometry of calcium and phosphorus nutrition in broiler chicks: growth performance, skeletal health and intake arrays. *Animal* 8, 1071–1079.

Bradbury, E.J., Wilkinson S.J., Cronin, G.M., Walk, C.L. and Cowieson, A.J. (2016) Effects of phytase, calcium source, calcium concentration and particle size on broiler performance, nutrient digestibility and skeletal integrity. *Animal Production Science* 58, 271–283.

Braithwaite, G.D. (1983) Calcium and phosphorus requirements of the ewe during pregnancy and lactation. 1. Calcium. *Journal of Agricultural Science, Cambridge* 50, 711–722.

Braun, U., Salis, F., Siegwart, N. and Hassig, M. (2004) Slow intravenous infusion of calcium in cows with parturient paresis. *Veterinary Record* 154, 336–338.

Breitwieser, G. (2008) Extracellular calcium as an integrator of tissue function. *International Journal of Biochemistry and Cell Biology* 40, 1467–1480.

Buckner, G.D., Martin, J.H. and Insko, W.M. Jr (1930) The blood calcium of laying hens varied by the calcium intake. *American Journal of Physiology* 94, 692–695.

Cai, C., Kong, Y., Wu, D. and Wang, J. (2018) Changes of macrominerals and calcitropic hormones in serum of periparturient dairy cows subject to subclinical hypocalcaemia. *Journal of Dairy Research* 85, 12–15.

Caixeta, L.S., Ospina, P.A., Capel, M.B. and Nydam, D.V. (2017) Association between subclinical hypocalcemia in the first 3 days of lactation and reproductive performance of dairy cows. *Theriogenology* 94, 1–7.

Carafoli, E. (1991) Calcium pump of the plasma membrane. *Physiological Reviews* 71, 129–149.

Casey-Trott, T.M., Korver, D.R., Guerin, M.T., Sandilands, V., Torrey, S. and Widowski, T.M. (2017) Opportunities for exercise during pullet rearing. Part II: Long-term effects on bone characteristics of adult laying hens at the end-of-lay. *Poultry Science* 96, 2518–2527.

Challa, J. and Braithwaite, G.D. (1989) Phosphorus and calcium metabolism in young calves with special emphasis on phosphorus homeostasis. 4. Studies on milk-fed calves given different amounts of phosphorus but a constant intake of calcium. *Journal of Agricultural Science, Cambridge* 113, 283–289.

Charbonneau, E., Pellerin, D. and Oetzel, G.R. (2006) Impact of lowering dietary cation–anion balance in non-lactating cows: a meta-analysis. *Journal of Dairy Science* 89, 537–548.

Chizzotti, M.L., Valadares Filho, S.-C., Tedeschi, L.O., Paulino, P.V.R., Paulino, M.F. et al. (2009) Net requirements of calcium, magnesium, sodium, phosphorus, and potassium for growth of Nellore × Red Angus bulls, steers, and heifers. *Livestock Science* 124, 242–247.

Chrisp, J.S., Sykes, A.R. and Grace, N.D. (1989a) Faecal endogenous loss of calcium in young sheep. *British Journal of Nutrition* 61, 59–65.

Chrisp, J.S., Sykes, A.R. and Grace, N.D. (1989b) Kinetic aspects of calcium metabolism in lactating sheep offered herbages with different calcium concentrations and the effect of protein supplementation. *British Journal of Nutrition* 61, 45–58.

Cockcroft, P.D. and Whiteley, P. (1999) Hypocalcaemia in 23 ataxic/recumbent ewes: clinical signs and likelihood ratios. *Veterinary Record* 144, 529–532.

Common, R.H. (1938) Observations on mineral metabolism in pullets 3. *Journal of Agricultural Science, Cambridge* 28, 347–366.

Constable, P.P., Megahed, A.A. and Hiew, M.W.H. (2019) Measurement of urine pH and net acid excretion and their association with urine calcium excretion in periparturient dairy cows. *Journal of Dairy Science* 102, 11370–11383.

Coop, R.L. and Sykes, A.R. (2002) Interactions between gastrointestinal parasites and nutrients. In: Freer, M. and Dove, H. (eds) *Sheep Nutrition*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 318–319.

Costa e Siva, L.F., Valdares Filho, S.C., Rotta, P.F., Marcandes, M.I., Zanetti, D., Gianbelli, M.P., Engle, T.E. and Paulino, M.F. (2015) Mineral requirements for beef cattle. Section 9 in *Nutrient Requirements of Zebu and Crossbred Cattle,* BR-Corte, Viscondo do Rio Branco, Brazil, pp. 213–249.

Cufadar, Y. Olgun, O. and Yildiz, A.Ö. (2011) The effect of dietary calcium concentration and particle size on performance, eggshell quality, bone mechanical properties and tibia mineral contents in moulted laying hens. *British Poultry Science* 52, 761–768.

David, L.S., Abdollahi, M.R., Ravindran, G., Walk, C.L. and Ravindran, V. (2019) Studies on the measurement of ileal calcium digestibility of calcium sources in broiler chickens. *Poultry Science* 98, 5582–5589.

DeGaris, P.J. and Lean, I.J. (2008) Milk fever in dairy cows: a review of pathophysiology and control principles. *The Veterinary Journal* 176, 58–69.

Deobald, H.J., Lease, E.J., Hart, E.B. and Halpin, J.G. (1936) Studies on the calcium metabolism of laying hens. *Poultry Science* 15, 179–185.

Dersjant-Li, Y. Archer, G., Stewert, A.M, Brown, A.A., Sobotik, E.B. et al. (2020) Functionality of a next generation biosynthetic bacterial 6-phytase in

enhancing phosphorus availability to broilers fed a corn-soybean meal-based diet. *Animal Feed Science and Technology* 264, 114481.

Dias, R.S., Kebreab, E., Vitti, D.M.S.S., Roque, A.P., Bueno, I.C.S. and France, J. (2006) A revised model for studying phosphorus and calcium kinetics in growing sheep. *Journal of Animal Science* 84, 2787–2794.

Dias, R.S., López, S., Patiño, R.M., Silva, T.S., Silva Filho, J.C., Vitti, D.M.S.S. *et al.* (2013) Calcium and phosphorus utilization in growing sheep supplemented with dicalcium phosphate. *Journal of Agricultural Science* 151, 424–433.

Dittmer, K.E., Hitchcock, B., McDougall, S. and Hunnam, J.C. (2016) Pathophysiology of humeral fractures in a sample of dairy heifers. *New Zealand Veterinary Journal* 64, 230–237.

Donnelly, E. and Boskey, A.L. (2011) Physical chemistry of mineralisation. In: Feldman, D., Pike, J.W. and Adams, J.S (eds) *Vitamin D*. Academic Press, New York.

Dorigan, C., Vargas, J., Härter, C., Resende, K., Vitti, D., Abdalla, A. and Teixeira, I. (2018) Dynamics of body calcium and net calcium requirements for maintenance of Saanen goats. *Journal of Agricultural Science* 156, 437–442.

Driver, J.P., Pesti, G.M., Bakali, R.I. and Edwards, H.M. Jr (2005a) Calcium requirements of the modern broiler chicken as influenced by dietary protein and age. *Poultry Science* 84, 1629–1639.

Driver, J.P., Pesti, G.M., Bakali, R.I. and Edwards, H.M. Jr (2005b) Effects of calcium and nonphytate phosphorus concentrations on phytase efficacy in broiler chicks. *Poultry Science* 84, 1406–1417.

Duncan, A.J., Frutos, P. and Young, S.A. (1997) Rates of oxalic acid degradation in the rumen of sheep and goats in response to different levels of oxalic acid administration. *Animal Science* 65, 451–455.

Edwards, H.M. and Veltmann, J.R. (1983) The role of calcium and phosphorus in the aetiology of tibial dyschondroplasia in young chicks. *Journal of Nutrition* 113, 1568–1575.

Eklou-Kalonji, E., Zerath, E.C., Lacroix, C.C., Holy, X., Denis, I. and Pointillart, A. (1999) Calcium-regulating hormones, bone mineral content, breaking load and trabecular remodeling are altered in growing pigs fed calcium-deficient diets. *Journal of Nutrition* 129, 188–193.

Elfers, K., Liesegang, A., Wilkens, M.R., Breves, G. and Muscher-Banse, A.R. (2016) Dietary nitrogen and calcium modulate bone metabolism in young goats. *Journal of Steroid Biochemistry and Molecular Biology* 164, 188–193.

Ellinger, I. (2016) The calcium-sensing receptor and the reproductive system. *Frontiers in Physiology* 7, 371.

Elvejhem, C.A. and Kline, B.E. (1933) Calcium and phosphorus studies in chicks. *Journal of Biological Chemistry* 103, 733–744.

Ender, F., Dishington, I.W. and Helgebostad, A. (1956) Parturient paresis and related forms of hypocalcemic disorders induced experimentally in dairy cows. *Nordisk Veterinaermedicin* 8, 507–513.

Ender, F., Dishington, I.W. and Helgebostad, A. (1971) Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic parturient paresis. *Zeitschrift fur Tierphysiologie Tierernahrung und Fultermittelkunde* 28, 233–256.

Fallah, H., Karimi, A., Sadeghi, G.H. and Behroozi-Khazaei, N. (2020) Modelling and optimizing of calcium and non-phytate phosphorus requirements of male broiler chickens from 1 to 21 days of age using response surface methodology. *Animal* 14, 1598–1609.

Farrugia, W., Fortune, C.L., Heath, J., Caple, I.W. and Wark, J.D. (1989) Osteocalcin as an index of osteoblast function during and after ovine pregnancy. *Endocrinology* 125, 1705–1710.

Feijo, J.O., Pereira, R.A., Montagner, P. Pino, F.A.P.D Schmitt, E. and Correa, M.N (2017) Dynamics of acute phase proteins in dairy cows with subclinical hypocalcemia. *Canadian Journal of Animal Science* 98, 1–8.

Fenwick, D.C. (1994) Limitations to the effectiveness of subcutaneous calcium solutions as a treatment for cows with milk fever. *Veterinary Record* 134, 446–448.

Field, A.C. and Suttle, N.F. (1967) Retention of calcium, phosphorus, magnesium, sodium and potassium by the developing sheep foetus. *Journal of Agricultural Science, Cambridge* 69, 417–423.

Field, A., Suttle, N. and Gunn, R. (1968) Seasonal changes in the composition and mineral content of the body of hill ewes. *Journal of Agricultural Science* 71, 303–310.

Field, A.C., Suttle, N.F. and Nisbet, D.I. (1975) Effects of diets low in calcium and phosphorus on the development of growing lambs. *Journal of Agricultural Science, Cambridge* 85, 435–442.

Field, A.C., Woolliams, J. and Dingwall, R.A. (1985) The effect of dietary intake of calcium and dry matter on the absorption and excretion of calcium and phosphorus by growing lambs. *Journal of Agricultural Science* 105, 247–253.

Filipović, N., Stojevic, Z., Zdelar-Tuk, M. and Kusec, V. (2008) Plasma parathyroid hormone-related peptide and bone metabolism in peri-parturient dairy cows. *Acta Veterinaria Hungarica* 56, 235–244.

Fleming, R.H., McCormack, H.A., McTeir, L. and Whitehead, C.C. (2006) Relationships between genetic, environmental and nutritional factors influencing osteoporosis in laying hens. *British Poultry Science* 47, 742–755.

Franklin, M.C., Reid, R.L. and Johnstone, I.L. (1948) *Studies on Dietary and Other Factors Affecting the Serum-calcium Levels of Sheep*. Bulletin, Council for Scientific and Industrial Research, Australia, No. 240.

Franz, S., Gasteiner, F., Schilcher, F. and Baumgartner, W. (2007) Use of ultrasonography for detecting calcification in cattle and sheep given calcinogenic *Trisetum flavescens* silage. *Veterinary Record* 161, 751–754.

Freeden, A.H. (1989) Effect of maturity of alfalfa (*Medicago sativa*) at harvest on calcium absorption in goats. *Canadian Journal of Animal Science* 69, 365–371.

Fredeen, A.H. and Van Kessel. J.S. (1990) Effect of sudden loss of calcium from the exchangeable calcium pool on bone calcium resorption in mature sheep. *Canadian Journal of Animal Science* 70, 887–894.

Friend, M.A., Bhanugopan, M.S., McGrath, S., Edwards, R., Hocking, J. *et al.* (2020) Do calcium and magnesium deficiencies in reproducing ewes contribute to high lamb mortality? *Animal Production Science* 60, 733–751.

Gaignon, P., Le Grand, K., Laza-Knoerr, A.L., Hurtaud, C. and Boudon, A. (2019) Effect of calcium intake and the dietary cation-anion difference during early lactation on the bone mobilization dynamics throughout lactation in dairy cows. *PLOS ONE* 14, e0218979.

Gilbert, A.B. (1983) Calcium and reproductive function in the hen. *Proceedings of the Nutrition Society* 42, 195–212.

Goff, J.P. (2007) The monitoring, prevention and treatment of milk fever and subclinical hypocalcaemia in dairy cows. *The Veterinary Journal* 176, 50–57.

Goff, J.P. (2018) *Invited review:* Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *Journal of Dairy Science* 101, 2763–2813.

Goff, J.P. and Horst, R.L. (1993) Oral administration of calcium salts for treatment of hypocalcaemia in cattle. *Journal of Dairy Science* 763, 101–108.

Goff, J.P. and Horst, R.L. (1994) Calcium salts for treating hypocalcaemia: carrier effects, acid–base balance and oral versus rectal administration. *Journal of Dairy Science* 77, 1451–1456.

Goff, J.P. and Horst, R.L. (1997) Effects of the addition of potassium or sodium, but not calcium, to prepartum rations on milk fever in dairy cows. *Journal of Dairy Science* 80, 176–186.

Goff, J.P. and Horst, R.L. (1998) Use of hydrochloric acid as a source of anions for prevention of milk fever. *Journal of Dairy Science* 81, 2874–2880.

Goff, J.P. and Horst, R.L (2003) Milk fever control in the United States. *Acta Veterinaria Scandinavica, Supplement* 97, 145–147.

Goff, J.P. and Koszewski, N.J. (2017) Comparison of 0.46% calcium diets with and without anion salts with 0.7% calcium anionic diet as a means to prevent periparturient hypocalcaemia. *Journal of Dairy Science* 101, 5033–5045.

Goff, J.P., Kerli, M.E. Jr and Horst, R.L. (1989a) Periparturient hypocalcaemia in cows: prevention using intramuscular parathyroid hormone. *Journal of Dairy Science* 72, 1182–1187.

Goff, J.P., Reinhardt, T.A. and Horst, R.L. (1989b) Recurring hypocalcaemia of bovine parturient paresis is associated with failure to produce 1,25 hydroxyvitamin D. *Endocrinology* 125, 49–53.

Goff, J.P., Reinhardt, T.A., Lee, S. and Hollis, B.W. (1991) Parathyroid hormone-related peptide content of bovine milk and calf blood assessed by radioimmunoassay and bioassay. *Endocrinology* 129, 2815–2819.

Goff, J.P., Riuz, R. and Horst, R.L. (2004) Relative acidifying activity of anionic salts commonly used to prevent milk fever. *Journal of Dairy Science* 87, 1245–1255.

Goff, J.P., Brummer, E.C., Henning, S.J., Doorenbos, R.K. and Horst, R.L. (2007) Effect of application of ammonium chloride and calcium chloride on alfalfa cation–anion content and yield. *Journal of Dairy Science* 90, 5159–5164.

Goff, J.P., Liesegang, A. and Horst, R.L. (2014) Diet-induced pseudohypoparathyroidism: a hypocalcaemia and milk fever risk factor. *Journal of Dairy Science* 97, 1520–1528.

Gonzáles-Vega, J.C. and Stein, H.H. (2014) Invited review – calcium digestibility and metabolism in pigs. *Asian Australasian Journal of Animal Science* 27, 1–9.

González-Vega, J.C., Walk, C.L. and Stein, H.H. (2015a) Effects of microbial phytase on apparent and standardised digestibilities of calcium in calcium supplements for pigs. *Journal of Animal Science* 93.

González-Vega, J.C., Walk, C.L. and Stein, H.H. (2015b) Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. *Journal of Animal Science* 93, 4808–4818.

González-Vega, J.C., Liu, Y., McCann, J.C., Walk, C.L., Loor. J.J. and Stein, H.H. (2016) Requirement for digestible calcium by eleven- to twenty-five-kilogram pigs as determined by growth performance, bone ash concentration, calcium and phosphorus balances, and expression of genes involved in transport of calcium in intestinal and kidney cells. *Journal of Animal Science* 94, 3321–3334.

Grace, N.D., Pearce, S.G., Firth, E.C. and Fennessy, P.F. (1999a) Content and distribution of macro- and trace-elements in the body of young pasture-fed horses. *Australian Veterinary Journal* 77, 172–176.

Grace, N.D., Pearce, S.G., Firth, E.C. and Fennessy, P.F. (1999b) Concentrations of macro- and trace-elements in the milk of pasture-fed Thoroughbred mares. *Australian Veterinary Journal* 77, 177–180.

Grace, N.D., Gee, E.K., Firth, E.C. and Shaw, H.L. (2002a) Determination of digestible energy intake, dry matter digestibility and mineral status of grazing New Zealand Thoroughbred yearlings. *New Zealand Veterinary Journal* 50, 182–185.

Grace, N.D., Shaw, H.L., Firth, E.C. and Gee, E.K. (2002b) Determination of digestible energy intake, and apparent absorption of macroelements of grazing, lactating Thoroughbred mares. *New Zealand Veterinary Journal* 50, 182–185.

Grace, N.D., Rogers, C.W., Firth, E.C., Faram, T.L. and Shaw, H.L. (2003) Digestible energy intake, dry matter digestibility and effect of calcium intake on bone parameters of Thoroughbred weanlings in New Zealand. *New Zealand Veterinary Journal* 51, 165–173.

Groenewald, J.W. (1937) Osteofibrosis in equines. *Onderstepoort Journal of Veterinary Science and Animal Industry* 9, 601–620.

Hamilton, R.M.G. and Cipera, J.D. (1981) Effects of dietary calcium levels during brooding, rearing and early egg laying period on feed intake, egg production and shell quality in white leghorn hens. *Poultry Science* 60, 349–357.

Hatate, K., Kawashima, C., Hanada, M., Kayano, M. and Yamagishi, N. (2018) Short communication: Serum osteoprotegerin concentrations in peripartu-rient dairy cows. *Journal of Dairy Science* 101, 6622–6626.

Henry, M.H. and Pesti, G.M. (2002) An investigation of calcium citrate-malate as a calcium source for broiler chicks. *Poultry Science* 81, 1149–1155.

Heppelmann, M., Krach, K., Krueger, L., Benz, P., Herzog, K., Piechotta, M., Hoedemaker, M. and Bollwein, H. (2015) The effect of metritis and subclinical hypocalcemia on uterine involution in dairy cows evaluated by sonomicrometry. *Journal of Reproduction and Development* 61, 565–569.

Heringstad, B., Chang, Y.M., Gianola, D. and Klemetsdal, G. (2005) Genetic analysis of clinical mastitis, milk fever, ketosis and retained placenta in three lactations of Norwegian Red cows. *Journal of Dairy Science* 88, 3273–3281.

Herm, G., Muscher-Banse, A.S., Breves, G., Schröder, B. and Wilkens, M.R. (2015) Renal mechanisms of calcium homeostasis in sheep and goats. *Journal of Animal Science* 93, 1608–1621.

Hernandez, L.L., Gregerson, K.A. and Horseman, N.D. (2012) Mammary gland serotonin regulates parathyroid hormone-related protein and other bone-related signals. *American Journal of Physiology, Endocrinology and Metabolism* 302, E1009–E1015.

Hernández-Castellano, H.E., Hernandez, L.L., Weaver, S. and Bruckmaier, R.M. (2017) Increased serum serotonin improves parturient calcium homeostasis in dairy cows. *Journal of Dairy Science* 100, 1580–1587.

Heron, V.S., Tremblay, G.F. and Oba, M. (2009) Timothy hays differing in dietary cation–anion difference affects the ability of dairy cows to maintain their calcium homeostasis. *Journal of Dairy Science* 92, 238–246.

Hintz, H.F., Schryver, H.F., Doty, J., Lakin, C. and Zimmerman, R.A. (1984) Oxalic acid content of alfalfa hays and its influence on the availability of calcium, phosphorus and magnesium to ponies. *Journal of Animal Science* 58, 939–942.

Hove, K. (1986) Cyclic changes in plasma calcium and the calcium homeostatic endocrine system of the postparturient dairy cow. *Journal of Dairy Science* 69, 2072–2082.

Hughes, L.E. and Kershaw, G.F. (1958) Metabolic disorders associated with movement of hill sheep. *Veterinary Record* 70, 77–78.

Hurwitz, S. (1996) Homeostatic control of plasma calcium concentration. *Critical Reviews in Biochemistry and Molecular Biology* 31, 41–100.

Hurwitz, S., Plavnik, I., Shapiro, A., Wax, E., Talpaz, H. and Bar, A. (1995) Calcium metabolism and requirements of chickens are affected by growth. *Journal of Nutrition* 125, 2679–2686.

Hyde, M.L., Wilkens, M.R. and Fraser, D,R. (2019) In vivo measurement of strontium absorption from the rumen of dairy cows as an index of calcium absorption capacity. *Journal of Dairy Science* 102, 5699–5705.

Jahani-Moghadam, M. Chashnidel, Y., Teimouri-Yansari, A., Mahjoubi, E. and Dirandeh, E. (2018) Effect of oral calcium bolus administration on milk production, concentrations of minerals and metabolites in serum, early-lactation health status, and reproductive performance of Holstein dairy cows. *New Zealand Veterinary Journal* 66, 132–137.

Jonson, G., Luthman, J., Mollerberg, L. and Persson, J. (1971) Hypocalcaemia in pregnant ewes. *Nordisk Veterinaermedicin* 23, 620–627.

Jonson, G., Luthman, J., Mollerberg, L. and Persson, J. (1973) Mineral feeding flocks of sheep with cases of clinical hypocalcaemia. *Nordisk Veterinaermedicin* 25, 97–103.

Judson, G.J. and Feakes, A. (1999) Vitamin D doses for alpaca (Lama pacos). *Australian Veterinary Journal 77, 310–315.*

Jumba, I.O., Suttle, N.F., Hunter, E.A. and Wandiga, S.O. (1995) Effects of soil origin and mineral composition and herbage species on the mineral composition of forages in the Mount Elgon region of Kenya. 1. Calcium, phosphorus, magnesium and sulfur. *Tropical Grasslands* 29, 40–46.

Kamiya, Y., Kamiya, M., Tanaka, M. and Shioya, S. (2005) Effects of calcium intake and parity on plasma minerals and bone turnover around parturition. *Animal Science Journal* 76, 325–330.

Keene, B.E., Knowlton, K.F., McGilliard, M.L., Lawrence, L.A., Nickols-Richardson, S.M. *et al.* (2004) Measures

of bone mineral content in mature dairy cows. *Journal of Dairy Science* 87, 3816–3825.

Khorasani, G.R., Janzen, R.A., McGill, W.B. and Kennelly, J.J. (1997) Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage or alfalfa silage. *Journal of Animal Science* 75, 239–248.

Kim, S.-W., Li, W., Angel, R. and Proszkowiec-Weglarz, M. (2018) Effects of limestone particle size and dietary Ca concentration on apparent P and Ca digestibility in the presence or absence of phytase. *Poultry Science* 97, 4306–4314.

Knowles, T.G., Kestin, S.C., Haslam, S.M., Brown, S.N., Green L.E., Butterworth, A., Pope, S.J., Pfeiffer, D. and Nicol, C.J. (2008) Leg disorders in broiler chickens: prevalence, risk factors and prevention. *PLOS ONE* 3, e1545.

Lagos, L.V., Lee, S.A., Fondevila, G., Walk, C.L., Murphy, M.R., Loor, J.J. and Stein, H.H. (2019a) Influence of the concentration of dietary digestible calcium on growth performance, bone mineralization, plasma calcium, and abundance of genes involved in intestinal absorption of calcium in pigs from 11 to 22 kg fed diets with different concentrations of digestible phosphorus. *Journal of Animal Science and Biotechnology* 10, 47.

Lagos, L.V., Walk, C.L. Murphy, M.R. and Stein, H.H. (2019b) Effects of dietary digestible calcium on growth performance and bone ash concentration in 50- to 85-kg growing pigs fed diets with different concentrations of digestible phosphorus. *Animal Feed Science and Technology* 247, 262–272.

Larsen, J.W.A., Constable, P.D. and Napthine, D.V. (1986) Hypocalcaemia in ewes after a drought. *Australian Veterinary Journal* 63, 25–26.

Lean, I.J., DeGaris, P.J., McNeill, D.M. and Block, E. (2006) Hypocalcaemia in dairy cows: meta-analysis and dietary cation–anion difference theory revisited. *Journal of Dairy Science* 89, 669–684.

Lee, S.A., Casas, G.A. and Stein H.H. (2018) The level of feed intake does not influence digestibility of calcium and phosphorus in diets fed to gestating sows, but gestating sows have reduced digestibility of calcium and phosphorus compared with growing gilts. *Canadian Journal of Animal Science* 98 589–592.

Lee, S.A., Lagos, L.V., Walk, C.L. and Stein, H.H. (2019) Basal endogenous loss, standardized total tract digestibility of calcium in calcium carbonate, and retention of calcium in gestating sows change during gestation, but microbial phytase reduces basal endogenous loss of calcium. *Journal of Animal Science* 97, 1712–1721.

Leno, B.M.R., Ryan, C.M., Stokol, T., Kirk, D., Zanzalari, K.P., Chapman, J.D. and Overton, T.R. (2017) Effects of prepartum dietary cation-anion difference on aspects of peripartum mineral and energy metabolism and performance of multiparous Holstein cows. *Journal of Dairy Science* 100, 4604–4622.

Leno, B.M.R., Neves, C., Louge, I.M., Curler, M.D., Thomas, M.J., Overton, T.R. and McArt, J.A.A. (2018) Differential effects of a single dose of oral calcium based on postpartum plasma calcium concentration in Holstein cows. *Journal of Dairy Science 101*, 3285–3302.

Leonhard-Marek, S., Becker, G., Breves, G. and Schroder, B. (2007) Chloride, gluconate, sulphate and short-chain fatty acids affect calcium flux rates across the sheep forestomach epithelium. *Journal of Dairy Science* 90, 1516–1526.

Li, W., Angel, R., Kim, S.-W., Jiménez-Moreno, E., Proszkowiec-Weglarz, M. and Plumstead, P.W. (2018) Impacts of age and calcium on phytase efficacy in broiler chickens. *Animal Feed Science and Technology* 238, 9–17.

Li, X., Zhang D. and Bryden, W.L. (2017) Measurement of the true ileal calcium digestibility of some feed ingredients for broiler chickens. *Animal Production Science* 57, 2304–2310.

Liesegang, A. (2008) Influence of anionic salts on bone metabolism in periparturient dairy goats and sheep. *Journal of Dairy Science* 91, 2449–2460.

Liesegang, A., Sassi, M.-L., Risteli, J., Eicher, R., Wanner, M. and Riond, J.-L. (1998) Comparison of bone resorption markers during hypocalcaemia in dairy cows. *Journal of Dairy Science* 81, 2614–2622.

Liesegang A., Eicher, R., Sassi, M.-L., Risteli J., Kraenzlin, M. and Riond, J.-L. (2000) Biochemical markers of bone formation and resorption around parturition and during lactation in dairy cows with high and low milk yields. *Journal of Dairy Science* 83, 1773–1781.

Lof, F. (2010) *Digestibility of Calcium and Phosphorus in Growing Horses*. Department of Animal Nutrition and Management, Swedish University of Agricultural Science, Uppsala, Sweden.

Loveridge, N. (1999) Bone: more than a stick. *Journal of Animal Science* 77 (Suppl. 2), 190–196.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Mahen, P.J., Williams, H.J., Smith, R.F. and Grove-White, D. (2018) Effect of blood ionised calcium concentration at calving on fertility outcomes in dairy cattle. *Veterinary Record.* doi:10.1136/vr.104932

Martinez, N., Sinedino L.D.P., Bisinotto, R.S., Daetz,†R., Risco, C.A., Galvão, K.N., Thatcher, W.W. and Santos, J.E.P. (2016) Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. *Journal of Dairy Science* 99, 8417–8430.

Martinez, N., Rodney, R.M., Block, E., Hernandez, L.L., Nelson, C.D., Lean, I.J and Santos, J.E.P. (2018) Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: health and reproductive responses. *Journal of Dairy Science*101, 2563–2578.

Martz, F.A., Belo, A.T., Weiss, M.F. and Belyca, R.L. (1999) True absorption of calcium and phosphorus from alfalfa and corn silage when fed to lactating cows. *Journal of Dairy Science* 82, 618–622.

Masters, D.G. (2018) Practical implications of mineral and vitamin imbalance in grazing sheep *Animal Production Science* 58, 1418–1450.

Masters, D.G., Hancock, S., Refshauge, G., Robertson, S.M., McGrath, S., Bhanugopan, M. and Thompson, A.N. (2018) Mineral supplements improve the calcium status of pregnant ewes grazing vegetative cereals. *Animal Production Science* 59, 1299–1309.

McArt, J.A.A. and Oertzel, G.R. (2015) A stochastic estimate of the economic impact of oral calcium supplementation in postparturient dairy cows. *Journal of Dairy Science* 98, 7408–7418.

McDonald, I.W. (1968) The nutrition of the grazing ruminant. *Nutrition Abstracts and Reviews* 38, 381–400.

McGhee, M.L. and Stein, H.H. (2019) Effects of microbial phytase on standardized total tract digestibility of phosphorus in hybrid rye, barley, wheat, corn, and sorghum fed to growing pigs. *Translational Animal Science* 3, 1238–1245.

McGrath, S.R., Bhanugopan, M.S., Dove, H., Clayton, E.H., Virgona, J.M. and Friend M.A. (2014) Mineral supplementation of lambing ewes grazing dual-purpose wheat. *Animal Production Science* 55, 526–534.

McNeill, D.M., Roche, J.R., McLachlan, B.P. and Stockdale, C.R. (2002) Nutritional strategies for the prevention of hypocalcaemia at calving for dairy cows in pasture-based systems. *Australian Journal of Agricultural Research* 53, 755–770.

Megahed, A.A., Hiew, M.W., El Badawy, S.A. and Constable, P.D. (2018) Plasma calcium concentrations are decreased at least 9 hours before parturition in multiparous Holstein-Friesian cattle in a herd fed an acidogenic diet during late gestation. *Journal of Dairy Science* 101,1365–1378.

Mellau, L.S.B., Jørgensen, R.J., Bartlett, P.C., Enemark, J.M.D. and Hansen, A.K. (2004) Effect of anionic salt and highly fermentable carbohydrate supplementations on urine pH and on experimentally induced hypocalcaemia in cows. *Acta Veterinaria Scandinavica* 45,139–147.

Merriman, L.A. and Stein, H.H. (2016) Particle size of calcium carbonate does not affect apparent and standardized total tract digestibility of calcium, retention of calcium, or growth performance of growing pigs. *Journal of Animal Science* 94, 3844–3850.

Miller, E.R., Ullrey, D.C., Zutaut, G.L., Baltzer, B.V., Schmidt, D.A., Hoefer, J.A. and Luecke, R.W. (1962) Calcium requirement of the baby pig. *Journal of Nutrition* 77, 7–17.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Misiura, M., Filipe, J., Walk, C. and Kyriazakis, I. (2018) Do not neglect calcium: a systematic review and meta-analysis (meta-regression) of its digestibility and utilisation in growing and finishing pigs. *British Journal of Nutrition* 119, 1207–1219.

Moreira, V.R., Zeringue, L.K., Williams, C.C., Leonardi, C. and McCormick, M.E. (2009) Influence of calcium and phosphorus feeding on markers of bone metabolism in transition cows. *Journal of Dairy Science* 92, 5189–5198.

Mosdol, G. and Waage, S. (1981) Hypocalcaemia in the ewe. *Nordisk Veterinaemedicin* 33, 310–326.

Muir, P.D., Sykes, A.R. and Barrell, G.K. (1987) Calcium metabolism in red deer (*Cervus elaphus*) offered herbages during antlerogenesis: kinetic and stable balance studies. *Journal of Agricultural Science, Cambridge* 109, 357–364.

Narvaez-Solarte, W., Rostagno, H.S., Soares, P.R., Uribe-Valasquez, L.L. and Silva, M.A. (2006) Nutritional requirement of calcium in white laying hens from 46–62 weeks of age. *International Journal of Poultry Science* 5, 181–184.

Neijat, M., Casey-Trott, T.M., Robinson, S., Widowski, T.M. and Kiarie, S. (2019) Effects of rearing and adult laying housing systems on medullary, pneumatic and radius bone attributes in 73-wk old Lohmann LSL lite hens. *Poultry Science* 98, 2840–2845,

Neves, R.C., Leno, B.M., Stokol, T., Overton, T.R. and McArt, J.A.A. (2017) Risk factors associated with postpartum subclinical hypocalcemia in dairy cows. *Journal of Dairy Science* 100, 3796–3804.

Neves, R.C., Leno, B.M., Bach, J.D. and McArt, J.A.A. (2018) Epidemiology of subclinical hypocalcemia in early-lactation Holstein dairy cows: the temporal associations of plasma calcium concentration in the first 4 days in milk with disease and milk production. *Journal of Dairy Science* 101, 9321–9331.

Nikkhah, A., Ehsanbakhsh, F., Zahmatkesh, D. and Amanlou, H. (2011) Prepartal wheat grain feeding improves energy and calcium status of periparturient Holstein heifers. *Animal* 5, 522–527.

Norman, A.W. and Horwitz, S. (1993) The role of vitamin D endocrine system in avian bone biology. *Journal of Nutrition* 123, 310–316.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2001) *Nutrient Requirements of Dairy Cows*, 7th edn. National Academy of Sciences, Washington, DC.

NRC (2007a) *Nutrient Requirements of Horses*, 6th edn. National Academy of Sciences, Washington, DC.

NRC (2007b) *Nutrient Requirements of Small Ruminants*. National Academy of Sciences, Washington, DC.

NRC (2012) *Nutrient Requirements of Swine*, 11th edn. National Academy of Sciences, Washington, DC.

Oetzel, G.R. (2011) Feed supplements: anionic salts. In: *'Encyclopaedia of Dairy Sciences,* 2nd edn. Academic Press, New York, pp. 356–362.

Oikawa, S. and Kato, N. (2002) Decreases in serum apolipoprotein B-100 and A-1 concentrations in cows with milk fever and downer cows. *Canadian Journal of Veterinary Research* 66, 31–34.

Olukosi, O.A. and Fru-Nji, F. (2014) The interplay of dietary nutrient level and varying calcium to phosphorus ratios on efficacy of a bacterial phytase: 2. Ileal and total tract nutrient utilization. *Poultry Science* 93, 3044–3052.

Olukosi, O.A., Cowieson, A.J. and Adeola, O. (2007) Age-related influence of a cocktail of xylanase, amylase and protease or phytase individually or in combination in broilers. *Poultry Science* 86, 77–86.

Pagan, J.D. (2001) Mineral digestibility in horses. In: Pagan, J.D. and Geor, R.J. (ed.) *Advances in Equine Nutrition II*, pp. 13–27. Nottingham University Press, Nottingham, UK.

Pagan, J.D., Petroski-Rose, L., Mann, A. and Hauss, A. (2020) Omeprazole reduces calcium digestibility in Thoroughbred horses. *Journal of Equine Veterinary Science* 86, 102851, ISSN 0737-0806.

Paiva, D.M., Walk, C.L. and McElroy, A.P. (2013) Influence of dietary calcium level, calcium source, and phytase on bird performance and mineral digestibility during a natural necrotic enteritis episode. *Poultry Science* 92, 3125–3133.

Payne, J.M. and Manston, R. (1967) The safety of massive doses of vitamin D_3 in the prevention of milk fever. *Veterinary Record* 81, 214–216.

Pérez, A.V., Picotto, G., Carpentieri, A.R., Rivoira, M.A., Peralta López, M.E. and Tolosa de Talamoni, N.G. (2008) Minireview on regulation of intestinal calcium absorption: emphasis on molecular mechanisms of transcellular pathway. *Digestion* 77, 22–34.

Perhson, B., Svensson, C., Gruvaeus, I. and Virkki, M. (1999) The influence of acidic diets on the acid–base balance of dry cows and the effect of fertilisation on the mineral content of grass. *Journal of Dairy Science* 82, 1310–1316.

Phillippo, M., Reid, G.W. and Nevison, I.M. (1994) Parturient hypocalcaemia in dairy cows: effects of dietary acidity on plasma minerals and calciotropic hormones. *Research in Veterinary Science* 56, 303–309.

Pryce, J.E., Veerkamp, R.F., Thompson, R., Hill, W.G. and Simm, G. (1997) Genetic aspects of common health disorders and measures of fertility in Holstein Friesian herds. *Animal Science* 65, 353–360.

Qian, H., Kornegay, E.T. and Denbow, D.M. (1997) Utilisation of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol and the calcium:total phosphorus ratio in broiler diets. *Poultry Science* 76, 37–46.

Rahman, M.Z., Ali, M.Y., Huque, K.S. and Talukder, M.A.I. (2014) Effect of di-calcium phosphate on calcium balance and body condition score of dairy cows fed Napier grass. *Bangladesh Journal of Animal Science* 43, 197–201.

Rajala-Schulz, P.J., Grohn, Y.T. and McCulloch, C.E. (1999) Effects of milk fever, ketosis and lameness on milk yield in dairy cows. *Journal of Dairy Science* 82, 288–294.

Ramberg, C.F., Johnson, E.K., Fargo, R.D. and Kronfeld, D.S. (1984) Calcium homeostasis in dairy cows with special reference to parturient hypocalcaemia. *American Journal of Physiology* 246, 698–704.

Ray, K. (2015) Calcium-sensing receptor: trafficking, endocytosis, recycling, and importance of interacting proteins. *Progress in Molecular and Biological Translational Sci*ence 13, 127–150.

Regmi, N., Nelson, J.P., Steibel, K.E., Anderson, D.M. and Karcher, D.M. (2016) Comparisons of bone properties and keel deformities between strains and housing systems in end-of-lay hens. *Poultry Science* 95, 2225–2234.

Reinhardt, T.A., Lippolis, J.D., McCluskey, B.J., Goff, J.P. and Horst, R.L. (2011) Prevalence of subclinical hypocalcemia in dairy herds. *Veterinary Journal* 188, 122–124.

Roche, J.R. and Berry, D.P. (2006) Periparturient climatic, animal, and management factors influencing incidence of milk fever in grazing systems. *Journal of Dairy Science* 89, 2775–2783.

Roche, J.R., Dalley, D., Moate, P., Grainger, C., Rath, M. and O'Mara, F. (2003a) Dietary cation–anion difference and the health and production of pasture-fed dairy cows. 1. Dairy cows in early lactation. *Journal of Dairy Science* 86, 970–978.

Roche, J.R., Dalley, D., Moate, P., Grainger, C., Rath, M. and O'Mara, F. (2003b) Dietary cation–anion difference and the health and production of pasture-fed dairy cows. 2. Nonlactating periparturient cows. *Journal of Dairy Science* 86, 979–987.

Rodney, R.M., Martinez, N., Block, E., Hernandez, L.L., Celi, P., Nelson, C.D., Santos J.E.P. and Lean, I.J. (2018) Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Vitamin D, mineral, and bone metabolism. *Journal of Dairy Science* 101, 1–25.

Rodney, R.M., Celi, P., McGrath, J.J., Golder H.M., Anderson, S.T., McNeill, D.M., Fraser, D.R. and Lean, I.J. (2019) Metabolic and production responses to calcidiol treatment in mid-lactation dairy cows. *Animal Production Science* 59, 449–460.

Rodrigues, R., Cooke, R.F., Ferreira, H.A.O., Florido, R.R., Camargo, V., de Godoy, H.O., Bruni, G.A. and Vasconcelos, J.L.M. (2020) Impacts of subclinical hypocalcemia on physiological, metabolic, and productive responses of Holstein × Gir dairy cows, *Translational Animal Science* 4, 1060–1069.

Roland, D.A. (1986) Calcium and phosphorus requirements of commercial Leghorns. *World Poultry Science Journal* 42, 154–165.

Roque, A.P., Dias, R.S., Vitti, D.M.S.S., Bueno, I.C.S., Cunha, E.A., Santos, L.E. and Bueno, M.S. (2007) True digestibility of calcium from sources used in lamb finishing diets. *Small Ruminant Research* 71, 243–249.

Rousseau, X., Valable, A.S., Létourneau-Montminy, M.P., Meme, N., Godet, E., Magnin, L., Nys, Y., Duclos, M. and Narcy, A. (2016) Adaptive response of broilers to dietary phosphorus and calcium restrictions. *Poultry Science* 95, 2849–2860.

Saborio-Montero, A., Vargas-Leitón, B., Romero-Zúñiga, J.J. and Sánchez, J.M. (2017) Risk factors associated with milk fever occurrence in grazing dairy cattle. *Journal of Dairy Science* 100, 9715–9722.

Sandilands, V., Moinard, C. and Sparks, N.H.C. (2009) Providing laying hens with perches: fulfilling behavioural needs but causing injury? *British Poultry Science* 50, 395–406.

Santos, J.E.P., Lean, I.J., Golder, H. and Block, E. (2019) Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. *Journal of Dairy Science* 102, 2134–2154.

Sath, K., Pauly, T. and Holtenius, K. (2013) Mineral status in cattle fed rice straw and para grass combined with different levels of protein derived from cassava foliage. *Asian-Australasian Journal of Animal Science* 26, 59–64.

Sato, R., Onda, K., Kato, H., Ochiai, H., Kawai, K., Iriki, I., Kaneko, K., Yamazaki, Y. and Wada, Y. (2013) An evaluation of the effect of age and the peri-parturient period on bone metabolism in dairy cows as measured by serum bone-specific alkaline phosphatase activity and urinary deoxypyridinoline concentration. *Veterinary Journal* 197, 1358–1362,

Sauer, W.C., Cervantes, M., He, J.M.M. and Schulze, H. (2003) Effect of phytase supplementation to barley-canola meal and barley-soybean meal diets on phosphorus and calcium balance in growing pigs. *Interciencia* 28(8).

Schlegel, P. and Gutzwiller, A. (2020) Dietary calcium to digestible phosphorus ratio for optimal growth performance and bone mineralization in growing and finishing pigs. *Animals* 10, 178.

Scott, T.A., Kampen, R. and Silversides, F.G. (2000) The effect of phosphorus, phytase enzyme and calcium on the performance of layers fed wheat-based diets. *Canadian Journal of Animal Science* 80, 183–190.

Seifi, H.A. and Kia, S. (2017) Hypocalcemia in Dairy Cows: pathophysiology, consequences and monitoring. *Iranian Journal of Veterinary Science and Technology* 9, 2–15.

Selle, P.H., Cowieson, A.J. and Ravindran, V. (2009) Consequences of calcium interactions with phytate and phytase for poultry and pigs, *Livestock Science* 124, 126.

She, Y., Li, D. and Stein. H.H. (2016) Effects of microbial phytase on the apparent and standardized total tract digestibility of calcium in milk co-products fed to growing pigs. *Journal of Animal Science* 94, 436–437.

Simons, P.C.M. (1986) Major minerals in the nutrition of poultry. In: Fisher, C. and Boorman, K.N. (eds) *Nutrient Requirements of Poultry and Nutritional Research*. Butterworths, London, pp. 141–145.

Smith, B.S.W. and Wright, H. (1981) Seasonal variation in serum 25-hydroxyvitamin D concentration in sheep. *Veterinary Record* 109, 139–141.

Soares, J.H. (1995) Calcium bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 1195–1198.

Spence, J.A., Sykes, A.R., Atkinson, P.J. and Aitchison, G.U. (1985) Skeletal and blood biochemical characteristics of sheep during growth and breeding: a comparison of flocks with and without broken mouth. *Journal of Comparative Pathology* 95, 505–522.

Stein, H.H., Adeola, O., Cromwell, G.L., Kim, S.W., Mahan, D.C. and Miller, P. S. (2011) Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. *Journal of Animal Science* 89, 2139–2147.

Stein, H.H., Merriman L.A. and González-Vega, J.C. (2016) Chapter 13: Establishing a digestible calcium requirement for pigs. In: Walk, C.L., Kuhn, I., Stein, H.H., Kidd, M. and Rodehutscord, M. (eds) *Phytate Destruction – Consequences for Precision Animal Nutrition*. Wageningen Academic Publishers, pp. 207–216.

Stevenson, M.A., Williamson, N.B. and Russell, D.J. (2003) Nutrient balance in the diet of spring-calving, pasture-fed dairy cows. *New Zealand Veterinary Journal* 51, 81–88.

Sulabo, R.C. and Stein, H.H. (2013) Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs, *Journal of Animal Science* 91,1285–1294.

Suttle, N.F. and Wadsworth, I.R. (1991) Physiological responses to vaccination in sheep. *Proceedings of the Sheep Veterinary Society* 15, 113–116.

Sykes, A.R. (1987) Endoparasites and herbivore nutrition. In: Hacker, J.B. and Ternouth, J.H. (eds) *The Nutrition of Herbivores*. Academic Press, Canberra, pp. 211–232.

Sykes, A.R. and Field, A.C. (1972) Effects of dietary deficiencies of energy, protein and calcium on the pregnant ewe. I. Body composition and mineral content of the ewes. *Journal of Agricultural Science, Cambridge* 78, 109–117.

Sykes, A.R. and Geenty, K.C. (1986) Calcium and phosphorus balances of lactating ewes at pasture. *Journal of Agricultural Science, Cambridge* 106, 369–375.

Tamin, N.M., Angel, R. and Christman, M. (2004) Influence of dietary calcium and phytase on phytate phosphorus utilisation in broiler chickens. *Poultry Science* 83, 1358–1367.

Taylor, M.S., Knowlton, K.F., McGilliard, M.L., Swecker, W.S., Ferguson, J.D., Wu, Z. and Hanigan, M.D. (2009) Dietary calcium has little effect on mineral balance and bone mineral metabolism through twenty weeks of lactation in Holstein cows. *Journal of Dairy Science* 92, 223–237.

Thilsing-Hansen, T., Jorgensen, R.J. and Ostergaard, S. (2002) Milk fever control principles: a review. *Acta Veterinaria Scandinavica* 43, 1–19.

Thompson, J.R., Gelman, A. and Weddell, J.R. (1988) Mineral retention and body composition of grazing lambs. *Animal Production* 46, 53–62.

Traylor, S.L., Cromwell, G.L., Lindemann, M.D. and Knabe, D.A. (2001) Bioavailability of phosphorus in meat and bone meal for swine. *Journal of Animal Science* 79, 2634–2642.

Tsiamadis, V., Banos, G., Panousis, N., Kritsepi-Konstantinou, M., Arsenos, G. and Valergakis, G.E. (2016) Genetic parameters of subclinical macromineral disorders and major clinical diseases in postparturient Holstein cows. *Journal of Dairy Science* 99, 8901–8914.

Umana Sedó, U., Rosa, S., Mattioli, D., Luzbel de la Sota, G. and Giuliodori, R. (2018) Associations of subclinical hypocalcemia with fertility in a herd of grazing dairy cows. *Journal of Dairy Science* 101, 10469–10477.

Underwood, E.J. and Suttle, F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Valable, A.S., Narcy, A., Duclos, M.J., Pomar, C., Page, G., Nasir, Z., Magnin, M. and Létourneau-Montminy, M.P. (2018) Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. *Animal* 12, 1555–1563.

Van Saun, R.J. (2004) Vitamin D-responsive rickets in neonatal lambs. *Canadian Veterinary Journal* 45, 841–844.

Venjakob, P.L., Pieper, L., Heuwieser, S. and Borchardt,W. (2018) Association of postpartum hypocalcemia with early-lactation milk yield, reproductive performance, and culling in dairy cows. *Journal of Dairy Science*, 101, 9396–9405.

Venjakob, P.L., Staufenbiel, R., Heuwieser, S. and Borchardt, W. (2019) Serum calcium dynamics within the first 3 days in milk and the associated risk of acute puerperal metritis. *Journal of Dairy Science* 102, 11428–11438.

Vieira-Neto, A., Lima, I.R.P., Lopes Jr, F., Lopera, C., Zimpel, R. *et al.* (2017) Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. *Journal of Dairy Science* 100, 1–19.

Wallace, I.J., Demes, B., Mongle, C., Pearson, O.M., Polk, J.D. *et al.* (2014) Exercise-induced bone formation is poorly linked to local strain magnitude in the sheep tibia. *PLOS ONE* 9, e99108.

Weich, W., Block, E. and Litherland, N.B. (2013) Extended negative dietary cation-anion difference feeding does not negatively affect postpartum performance of multiparous dairy cows. *Journal of Dairy Science* 96, 5780–5792.

Whitehead, C.C. (1995) Nutrition and skeletal disorders in broilers and layers. *Poultry International* 34, 40–48.

Whitehead, C.C. (2004) Overview of bone biology in the egg-laying hen. *Poultry Science* 83, 193–199.

Wilkens, M.R., Mrochen, N., Breves, G. and Schröder, B. (2011) Gastrointestinal calcium absorption in sheep is mostly insensitive to an alimentary induced challenge of calcium homeostasis. *Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology* 158, 199–207.

Wilkens, M.R., Richter, J., Fraser, D.R., Liesegang, A., Breves, G. and Schroder, B. (2012) In contrast to sheep, goats adapt to dietary restriction by increasing intestinal absorption of calcium. *Comparative Biochemistry and Physiology.* Part A, 163, 395–406.

Wilkens, M.R., Liesegang, A., Richter, J., Fraser, D.R., Breves, G. and Schroder, B. (2014) Differences in peripartal plasma parameters related to calcium homeostasis of dairy sheep and goats in comparison with cows. *Journal of Dairy Research* 81, 325–332.

Wilkens, M., Praechter, C., Breves, G. and Schröder, B. (2016) Stimulating effects of a diet negative in dietary cation–anion difference on calcium absorption from the rumen in sheep. *Journal of Animal Physiology and Animal Nutrition* 100, 156–166.

Wilkens, M.R., Firmenich, C.S., Schnepel, N. and Muscher-Banse, A.S. (2019) A reduced protein diet modulates enzymes of vitamin D and cholesterol metabolism in young ruminants*, Journal of Steroid Biochemistry and Molecular Biology* 186, 196–220.

Wilson, J.H. (1991) Bone strength of caged layers as affected by dietary Ca and phosphorus concentrations, reconditioning and ash content. *British Poultry Science* 32, 501–508.

WPSA (2013) Working Group No 2: Nutrition of the European Federation of Branches of World Poultry Science Association. Determination of phosphorus availability in poultry. *World's Poultry Science Journal* 69, 687–698.

Wuthier, R.E. (1993) Involvement of cellular metabolism of calcium and phosphate in calcification of avian growth plate. *Journal of Nutrition* 123, 301–309.

Wynn, S., Teramura, M., Sato, T. and Hanada, M. (2015) Changes of serum calcium concentration, frequency of ruminal contraction and feed intake soon after parturition of dairy cows fed difructose anhydride III. *Asian-Australasian Journal of Animal Sciences* 28, 58–68.

Yamagishi, N., Ogawa, K. and Naito, Y. (1999) Pathological changes in the myocardium of hypocalcaemic parturient cows. *Veterinary Record* 144, 67–72.

Yan, F., Angel, R., Ashwell, C., Mitchell, A. and Christman, M. (2005) Evaluation of the broiler's ability to adapt to an early moderate deficiency of phosphorus and calcium. *Poultry Science* 84, 1232–1341.

Yuangklang, C., Wensing, T., Van den Broek, L., Jittakhot, S. and Beynen, A.C. (2004) Fat digestion in veal calves fed milk replacers low or high in calcium and containing either casein or soy protein isolate. *Journal of Dairy Science* 87, 1051–1056.

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle, T.E., Silva, B.C. et al. (2017a) Estimating mineral requirements of Nellore beef bulls fed with or without inorganic mineral supplementation and the influence on mineral balance. *Journal of Animal Science* 95, 1696–1706.

Zanetti, D., Valadares Filho, S.C., Prados, L.F., Detmann, E., Pacheco, M.V.C., Godoi, L.A., Rennó, L.N. and Engle, T.E. (2017b) Impacts of reduction of phosphorus in finishing diets for Holstein×Zebu steers. *Livestock Science* 198, 45–51.

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle T.E., Pacheco, M.V.C., Pereira, J.M.V., Detmann, E., Paulino, M.F. and Valadares Filho, S.C. (2019) Influence of a mineral supplement containing calcium, phosphorus and micronutrients on intake, digestibility, performance and mineral status of young Nellore bulls in a feedlot. *Animal Production Science* 60, 277–287.

Zhang, B., Guo, H., Yang, W., Li, M., Zou, Y., Loor, J.J., Xia, C. and Xu, C. (2019) Effects of ORAI calcium release-activated calcium modulator 1 (ORAI1) on neutrophil activity in dairy cows with subclinical hypocalcemia. *Journal of Animal Science* 97, 3326–3336.

Zhang, F., Ragland, D. and Adeola O. (2016) Comparison of apparent ileal and total tract digestibility of calcium in calcium sources for pigs. *Canadian Journal of Animal Science* 96, 563–569.

5 Magnesium

Introduction

By 1930, lack of dietary Mg was known to cause hyperirritability and convulsions in laboratory rats. Coincidentally, similar abnormalities in grazing cows were being associated with subnormal serum Mg and termed 'grass tetany' (Sjollema and Seekles, 1930). Studies of magnesium metabolism in ruminants were keenly pursued, sustained by the fact that intensive use of inorganic fertilizers on grassland seemed to increase incidence of a disorder that remains problematic for cattle grazing lush green forage (Martens, 2016). By contrast, there was little research on pigs and poultry because common feedstuffs contained sufficient Mg to meet requirements and natural deficiencies were unheard of. The discovery that Mg supplementation prior to slaughter can improve meat quality in stressed pigs led to an upsurge of research activity, which may be reignited by responses reported recently in the farrowing sow. However, there has been relatively little research on magnesium in either human or animal nutrition and it has been described as 'the forgotten electrolyte' (Ahmed and Mohammed, 2019). The focus of the chapter remains on magnesium nutrition of ruminants.

Functions

The skeleton contains 60–70% of Mg in the body but Mg is second only to K in abundance in soft tissues, which contain 0.1–0.2g Mg kg^{-1} FW. Unlike K, Mg is largely (80%) protein bound and found predominantly in microsomes, in which it catalyses activity of many enzymes by facilitating union of substrate and enzyme, first binding to one or the other (Ebel and Günther, 1980). Thus, Mg is required for: oxidative phosphorylation leading to ATP formation, fuelling Na^+/K^+ pumps; pyruvate oxidation and conversion of α-oxoglutarate to succinyl coenzyme A; hexokinases and deoxyribonucleases; β oxidation of fatty acids and transketolase reactions of the pentose monophosphate shunt (Shils, 1997). Topoisomerases, helicases and cyclases are all Mg-dependent (Ahmed and Mohammed, 2019). Magnesium also performs non-enzymic functions: folding ribonucleotide chains by binding to phosphate groups (Ahmed and Mohammed, 2019); exchanging with Ca^{2+} to facilitate muscle contraction and recovery (Ebel and Günther, 1980; Shils, 1997); binding to phospholipids in cell membranes, contributing to their integrity. For example, lack of Mg reduces the fluidity of red cell membranes (Tongyai *et al*., 1989). Rumen microorganisms require Mg to catalyse many of the above functions and severe deprivation rapidly impairs cellulolytic activity in the rumen. Magnesium occurs in relatively low but life-sustaining concentrations in extracellular fluids, including cerebrospinal fluid (CSF). Lack of Mg affects the transmission of nerve impulses by decreasing the threshold for axon stimulation and increasing nerve conduction velocity. In hypomagnesemic individuals, entry of Ca into presynaptic nerve terminals, influencing the release of neurotransmitters at the neuromuscular junction, is restricted, causing hyper-responsive neuromuscular activity and affecting autonomic control in the heart (Ahmed and Mohammed, 2019).

Consequences of Magnesium Insufficiency

Tetany in cattle and sheep

In the UK, the disorder is most common in recently calved beef cows >5 years old. Initial signs are those of nervous apprehension with head held high, ears pricked and flicked, eyes staring and a readiness to kick out. Animals stagger when walking and there is muscular twitching, especially of face and ears. Within hours or days, extreme excitement and violent convulsions may develop, culminating in the animal lying on its side and 'pedalling' its forelegs. The presence of a scuffed arc of pasture by the feet of a casualty is an important diagnostic feature.

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Response to treatment with Mg is rapid and confirms diagnosis but mortality among untreated clinical cases can be as high as 30% (Donovan *et al*., 2004). Pre-convulsive clinical signs in sheep are less clearly defined than in cattle and can be confounded with those of hypocalcaemia and pregnancy toxaemia. Vulnerability in ewes also increases with age. Affected ewes breathe rapidly and facial muscles tremble: some cannot move, while others move awkwardly, eventually collapsing and showing repeated tetanic spasms, with legs rigidly extended.

Grass tetany in horses

Clinical signs of hypomagnesaemia in horses are similar to those in cattle and ewes (Stewart, 2011) but rarely reported, even on pastures that cause disorder in ruminants. Signs were induced experimentally in 2 out of 11 foals after 90 days on a diet containing only 7–8 mg Mg kg^{-1}DM and serum Mg decreased from 19 ± 1 to 7 ± 4 mg l^{-1}. Elastic fibres of the aorta and pulmonary artery were severely calcified in all foals.

Subclinical disorders in ruminants

Lack of dietary Mg may cause loss of production and signs of 'irritability' in herds (Whittaker and Kelly, 1982) and flocks (Dove and McMullen, 2009) without overt clinical disease. Moderate hypomagnesaemia was present in affected herds in Northern Ireland and estimated to cost the small dairy industry £4 million p.a. (McCoy *et al*., 1993). Supplements of 10 g Mg day^{-1} produced small improvements in milk-fat yield in New Zealand, particularly in early lactation in underfed, grazing herds (Young *et al*., 1981): digestibility of low-quality forage may have been improved (Wilson, 1980). Comparable subclinical disorders have been reported in sheep in South Australia, while grazing cereal and rapeseed crops in a vegetative state, when pasture is scarce. Growth rates of twin lambs were improved by supplementing asymptomatic ewes with Ca, Mg and Na (McGrath *et al*., 2011): the latter elements were thought to have elicited responses and high cereal K to have induced a largely asymptomatic dysfunction (Dove and McMullen, 2009; Dove *et al*., 2016). Supplements of MgO can raise milk-fat yields in dairy cows on grain-based diets (Xin *et al*., 1989). Other minerals, with (e.g. $NaHCO_3$) or without (e.g. NaCl) buffering capacity, can be equally effective but 'low milk fat' syndrome' is not caused by lack of Mg.

Occurrence of Hypomagnesaemia and Tetany

National estimates of incidences of hypomagnesaemic tetany in dairy herds are much lower than the 1–4% reported in the previous century.[i] Incidence from 1989 through 2005 in Norwegian dairy cattle was 0.19% (Osteras *et al*., 2007) and only 0.04% in UK Holsteins over a slightly earlier period (Kadarmideen *et al*., 2000). Mortality rate amongst clinical cases could be high (25% in beef cows, 55% in dairy cows) (Harris *et al*., 1983) and is unlikely to have changed. Incidence of clinical and subclinical disorder varied between breeds (Greene *et al*., 1985), seasons (McCoy *et al*., 1993, 1996) and years (Whittaker and Kelly, 1982) (Fig. 5.1). Incidence of hypomagnesaemia was far higher in beef than in dairy herds in Northern Ireland (McCoy *et al*., 1993) and in non-lactating than lactating cows in the UK (Whittaker and Kelly, 1982), probably because beef cows and dry cows receive less concentrates and Mg supplements. In New Zealand, where dairy cows are pastured throughout the year, incidence was highest in late winter and early spring: almost half the herds, in some areas, were hypomagnesaemic (Young *et al*., 1981). Outwintered beef cattle, often given diets low in Mg and containing a large proportion of straw, can develop hypomagnesaemic tetany (Menzies *et al*., 1994) (Fig. 5.1b). The disease can occur when early growths of cereals and dual-purpose rape are grazed (e.g. 'wheat' staggers; Littledike and Goff, 1987) and occasionally affects stall-fed animals. In ewes, the disorder is more likely to develop after lambing, ewes rearing twins being particularly vulnerable (Sansom *et al*., 1982): 20–40 incidents were reported annually in the UK. In southern NSW, a producer questionnaire found fewer deaths attributable to metabolic diseases (0.6% versus 3.6%) amongst lambing ewes grazing dual-purpose wheat with than without access to supplementary Mg and Na: feeding grain prior to lambing was also a risk factor (McGrath *et al*., 2013). However, specific responses to Mg have yet to be reported in sheep grazing cereals.

Role of potassium fertilizers

Historically, incidence of grass tetany was associated with heavy use of fertilizers. In an early Dutch

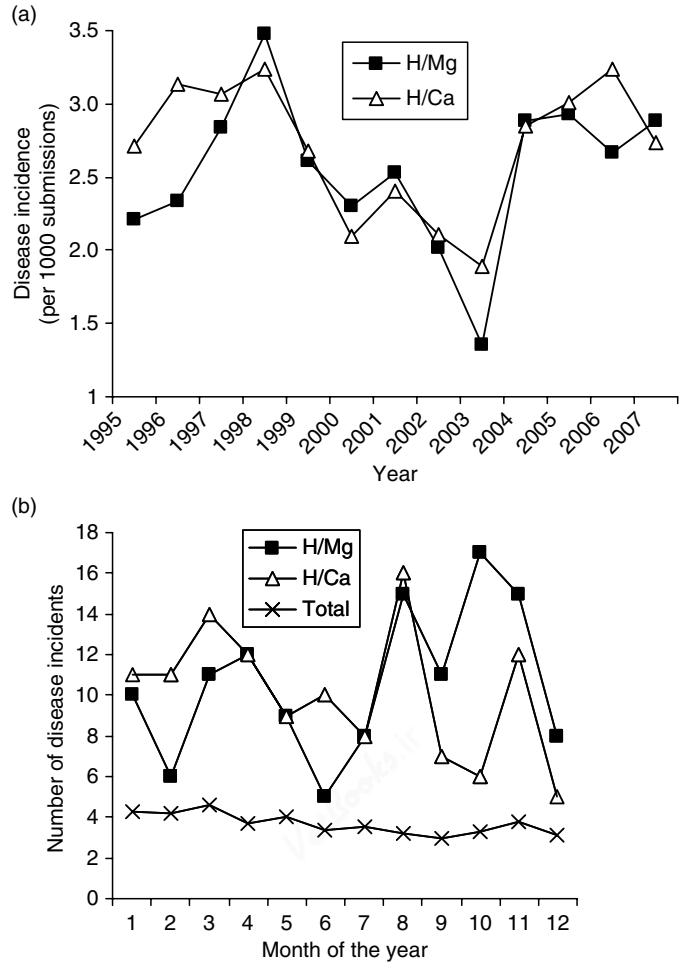

Fig. 5.1. Annual relative (a) and actual monthly incidence (b) of 'hypomagnesaemia' (H/Mg) and 'hypocalcaemia' (H/Ca) in UK cattle: in (a), results are expressed relative to total submissions for all diseases to minimize confounded effects of disease outbreaks (e.g. foot-and-mouth disease in 1998) and structural changes in national disease surveillance; in (b) total monthly submissions (x) in 2007 are given in thousands.

study, applying 50 kg K ha^{-1} to pastures increased incidence of tetany from 0.5% to 4.3–6.5% ('t Hart and Kemp, 1956). Such treatments can decrease herbage Mg, especially if legumes are suppressed, but lower Mg status more by lowering A_{Mg} (p. 112). High dietary K and N were also implicated in 'wheat staggers' (Mayland *et al.*, 1976). The two plant nutrients were usually applied together but K is probably the more influential. There is no evidence that N-rich diets *per se* are a risk factor, whereas hypomagnesaemia and tetany were induced by simply increasing dietary K in sheep (Suttle and Field, 1969) and cattle (Bohman *et al.*, 1969). Applying 150 or 225kg K ha^{-1} as KCl decreased urinary Mg in cows (Roche *et al.*, 2002). Furthermore, K lowers the responsiveness of bone to PTH (p.) and may reduce the supply of Mg from resorbing bone.

Transition to pasture

Other dietary anomalies coincide with high dietary K in spring grass and have been investigated as risk factors but their roles remain ill-defined (Gabel and Martens, 1986) and effects often transient (Martens and Schweigel, 2000).[ii] Rumen composition gradually

returns towards normal (Johnson *et al*., 1988; Johnson and Aubrey Jones, 1989) as both the rumen microflora and host adjust and there is usually gradual recovery in plasma Mg in the weeks following turnout. Other confounded effects include:

- reductions in sodium intake, particularly when cereals or rape are grazed (Masters *et al*., 2019);
- increases in milk yield and demand for Mg, due to higher digestible organic matter intake;
- infection by newly hatched nematode larvae that invade the gut of immunosuppressed dams that have recently given birth; and
- increased net requirements associated with increased muscular activity at turnout.

Dietary change at parturition

The housed dairy cow experiences dietary change at calving and transition diets (i.e. covering the pre- to post-calving period) with a low DCAD, fed for the primary purpose of preventing milk fever, raised plasma Mg in early lactation (Roche *et al*., 2005). However, the effect is again confounded with that of K, the major contributor of cations.

- Inclusion of KCl in an acidogenic diet will reduce the otherwise abrupt change in K intakes at turnout.

Conditions that increase risk of milk fever in herds at pasture (Roche and Berry, 2006) also increase risk of grass tetany and incidences of the two disorders may be related (Table 5.1; Fig. 5.1a).

Adverse weather

A sudden rise in grass temperature in spring is a risk factor because pasture K can rise rapidly as soil temperature rises (Dijkshoorn and 't Hart, 1957; cited by Kemp *et al*., 1961) and allows rapid grass growth. Frequency distributions of pasture K:Mg may be better indicators of risk than mean herbage K when change is rapid (Dove *et al*., 2016) (p. 116). Outbreaks can be precipitated in outwintered beef cattle when adverse weather disrupts grazing and feeding (Menzies *et al*., 1994; see Fig. 5.1b). In Australia, outbreaks in breeding ewes were associated with periods of rapid winter growth of pastures (Blumer *et al*., 1939). In the UK, extreme spring weather conditions – either mild and wet or cold, with snowfall – have caused outbreaks of hypomagnesaemic tetany.

- Global warming may increase incidence of hypomagnesaemic tetany by increasing the frequency of extreme weather events and widen the interval between seasonal peaks in spring and autumn.

Transportation

Hypomagnesaemic tetany can occur when cattle and sheep are subjected to intense excitement, as in transit or sale-yard conditions, giving rise to the term 'transit tetany'. Lack of food and the effect of stressors on the distribution of Mg between extracellular and intracellular pools may compound the problem (Martens and Schweigel, 2000). Concurrent hypocalcaemic and hypomagnaesemic tetany was reported in two Thoroughbred broodmares that had been transported for breeding. The mares were nursing foals that were 4 and 7 weeks old. Serum Ca was 40 and 54 mg l^{-1}, serum Mg 10 and 19 mg l^{-1} and both responded to i.v. calcium borogluconate and $MgCl_2$ (Stewart, 2011). Magnesium supplementation of sheep for ten days prior to bouts of exercise and for four days before transportation for slaughter reduced the depletion of muscle glycogen and increased the subsequent recovery of glycogen (Gardner *et al*., 2001). Brief magnesium supplementation might reduce the stress of transportation but requirements may be increased by intense muscular activity, triggering tetany (Masters, 2018). Stricter regulation of animal transport and lairage on welfare grounds may have reduced incidence of transport tetany in sheep and cattle.

Table 5.1. Similarity between causative or associated factors in hypomagnesaemic tetany (HMT) and hypocalcaemia (HC) in cows and ewes suggests a common aetiology, with poor mobilization of calcium *and* magnesium from the skeleton playing a unifying role. Proximity to parturition promotes HC in cows but not in twin-bearing ewes, whose demand for both minerals is already high; preventive measures for one disease should work for both.

	Plasma Ca	Plasma Mg	Dietary K	Temperature	Parturition	Age
HMT	↓	↓	↑	↓	↓	↑
HC	↓	↓	↑	↓	↑	↑

Physiochemistry of Magnesium Insufficiency

Biochemical changes depend on the stage of development at which dietary Mg fails to meet requirement and the margin between supply and demand. Changes in the young growing animal appear typical of a Type 1 mineral in showing tolerance of deprivation but responses in the adult animal, faced with an acute dietary deficit, are those of a Type 2 mineral (Fig. 3.2), with rapid onset of neurological dysfunction. Differences are attributable to the contrasting lability of Mg in the skeletons of young and mature animals until lactation begins.

Chronic depletion

Bone provides about 60% of total body Mg, held in a 1:50 ratio with Ca in the hydration shell of hydroxyapatite crystals. In Mg-deprived calves, Ca:Mg ratios in bone increase to 150:1 probably due to decreased deposition of Mg in new bone rather than depletion of stored Mg. Rates of accretion of Mg in growing kids vary widely, due partly to duration of milk feeding and possibly dietary K:Mg ratio (Fig. 1.4).

Acute depletion

Bone Mg was normal in cases of grass tetany (Rook and Storry, 1962) and modelling studies suggested that mobilization of Mg from the adult bovine skeleton is too slow to delay the hypomagnesaemic response to acute and severe reductions in Mg supply (Robson *et al*., 2004). However, further studies showed that hypomagnesaemia was less severe in the model cow than in reality (Bell *et al*., 2006): the difference may have been attributable to mobilization of Mg from the skeleton. Bone Mg can be mobilized by experimental procedures such as haemodialysis (Gardner, 1973) and intravenous chelation (Robson *et al*., 2004): inhibition of bone resorption lowers plasma Mg (Matsui *et al*., 1994). The oldest cows in a suckler herd had the lowest plasma Mg values (Fig. 5.2): slower bone turnover and/or cumulative depletion in older animals might have been responsible. Pronounced seasonal cycling of bone Mg was reported in grazing cows in association with lactation (Fig. 5.3), while plasma Mg remained normal. Such changes may be a secondary consequence of obligatory bone resorption (p. 58). Of soft tissues examined, only kidney Mg was reduced in sheep experimentally deprived of Mg (McCoy *et al*., 2001a).

Deficiency

Plasma normally contains about 1 mmol (23mg) Mg l^{-1}, partitioned between two forms, one tightly bound to protein (32%), the other free and ionized (68%). Plasma Mg reflects equilibria attained between extracellular, intracellular and skeletal pools of magnesium and is not hormonally regulated (Martens and Schweigel, 2000). In the young, lack of dietary Mg causes a gradual decline in plasma Mg but factors other than dietary Mg affect plasma Mg in the adult animal.[iii] Adult sheep and cattle, whether non-lactating or lactating (Suttle and Field, 1969; McCoy *et al*., 2001a, b), show rapid decreases in plasma Mg that reflect the degree of dietary deprivation (Fig. 5.4). Urinary Mg

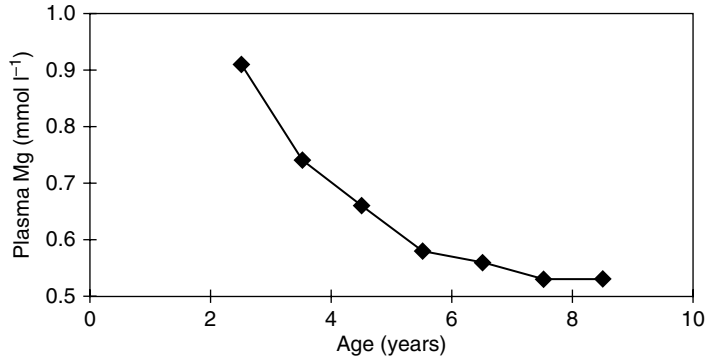

Fig. 5.2. Plasma magnesium concentrations decreased markedly with age in a beef suckler herd but cumulative nutritional deficits may have contributed to the apparent 'age' effect. (From Suttle *et al*., 1980)

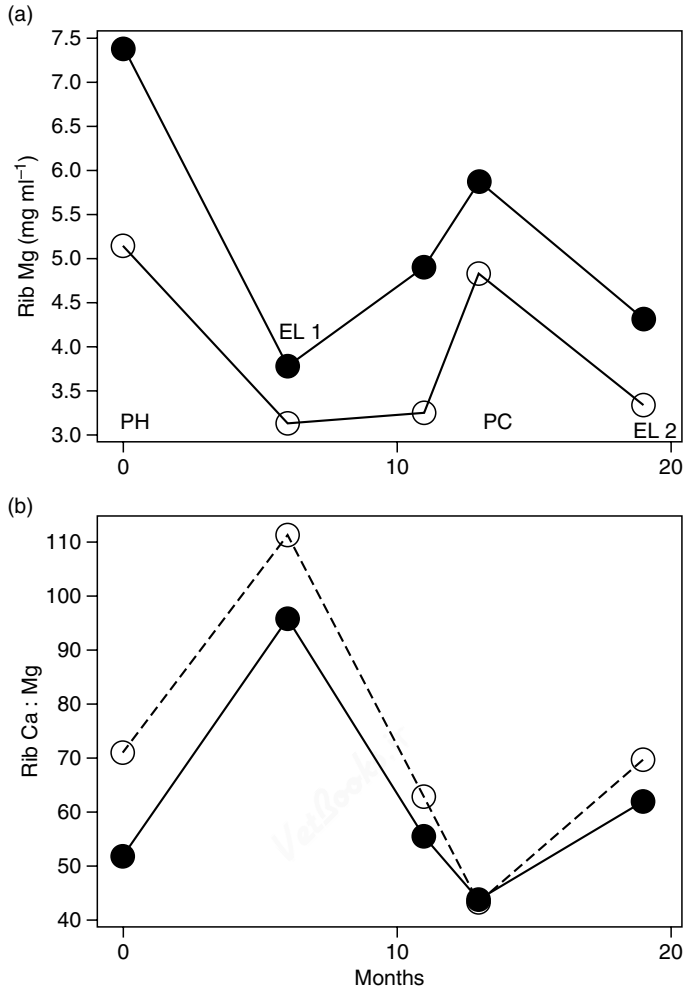

Fig. 5.3. Effects of lactation and phosphorus (P) status on (a) rib Mg and (b) rib Ca:Mg ratio in beef cows, either receiving (●) or not receiving (○) supplementary P while grazing P-deficient pasture. (Data from Engels, 1981; PH or PC, pregnant heifer or cow; EL, early lactation)

losses fall close to zero and losses via secretions into the gut (FEL_{Mg}) follow the decline in plasma Mg (Bell *et al*., 2006). Cerebrospinal fluid (CSF) Mg also declines but at a much slower rate (Pauli and Allsop, 1974), suggesting an active transport mechanism at the blood–brain barrier. There are probably gradual changes in other body fluids, such as vitreous humour in sheep (McCoy *et al*., 2001a) and cattle (McCoy *et al*., 2001b) but not in milk (Rook and Storry, 1962; McCoy *et al*., 2001c). In the early stages of deprivation, hypomagnesaemia is not accompanied by hypocalcaemia in ewes, whether dry (Suttle and Field, 1969) or lactating (McCoy *et al*., 2001a).

Dysfunction

Mild behavioural changes in severely hypomagnesaemic individuals indicate that neuromuscular activity increases when CSF Mg falls below 0.6 mmol l^{-1} but transition from deficiency to disorder is often so rapid in the adult that a period of dysfunction is by-passed. Severe hypomagnesaemia can delay rumen contractions in response to feeding and impair motility of the lower intestine (Bueno *et al*., 1980): this may explain the loss of appetite seen in some ewes (Suttle and Field, 1969) and cows (van de Braak *et al*., 1987; McCoy *et al*., 2001c), deprived of Mg, and exacerbates

hypomagnesaemia (Herd, 1966). Adult sheep, given a grossly deficient diet, showed an immediate, marked loss of appetite due to rumen dysfunction but inappetance was delayed and less pronounced in young lambs (Chicco *et al*., 1973). In parallel studies with non-lactating and lactating ewes deprived of magnesium, individuals that subsequently developed tetany showed not only a more rapid fall in plasma Mg than those that did not succumb (McCoy *et al*., 2001a) but also faster development of hypocalcaemia, 48 h before the onset of disorder. Severe hypomagnesaemia and any associated loss of appetite may inhibit the customary PTH response to mild hypocalcaemia (Littledike and Goff, 1987). In experimentally depleted cattle, haematocrit, white cell and platelet counts increased between 11 and 26 days but red-cell fragility was not compromised (McCoy *et al*., 2002). Decreases in plasma triglycerides and increases in tissue iron (in bovine heart and ovine liver) may have been partly attributable to inappetence. Abnormalilities such as peripheral vasodilatation, calcification of the kidneys, seen in severely deprived young laboratory animals (Shils, 1997), were not seen in cows (McCoy *et al*., 2002) but were probably pre-empted by acute disorder. Severe Mg deprivation in piglets induced convulsions and cardiomyopathy: the possibility that a supplement of only 52 mg Mn kg^{-1} DM exacerbated Mg deprivation is intriguing but remote. Marginal dietary Mg reduced activity of cardiac sarcoplasmic reticulum calcium-ATPase independently of Mn in rats (Miller *et al*., 2006).

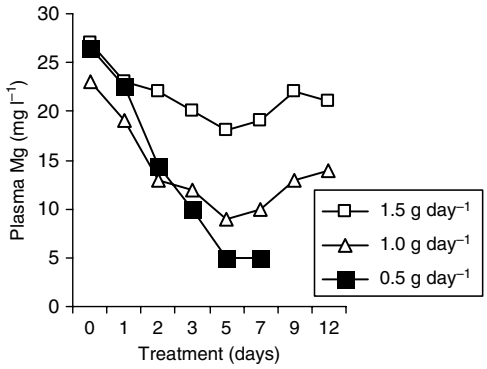

Fig. 5.4. Effect of magnesium intake on plasma Mg in dry ewes given a semi-purified diet, providing 47 g K day^{-1} (Suttle and Field, 1967): recovery of plasma Mg after day 5 may indicate mobilization of skeletal Mg reserves.

Diagnosis of Hypomagnesaemia and Tetany

Diagnoses of hypomagnesaemic tetany are made instantaneously on the basis of presenting signs, confirmed initially by response to magnesium treatment and later by low plasma Mg in samples taken prior to treatment. Although tetany can occur when serum Mg falls below 0.5mmol l^{-1}, individual ewes and cows can survive with values <0.2mmol l^{-1} (McCoy *et al*., 2001a, b). Subclinical disease in cattle has been associated with mean serum Mg <0.6 mmol l^{-1} and values of 0.6–0.8mmol l^{-1} are marginal (Sutherland *et al*., 1986; McCoy *et al*., 1996); marginal ranges are slightly lower in sheep (Table 5.2). Diagnostic thresholds of 0.5 and 0.6 mmol Mg l^{-1} in CSF were indicated for cattle (McCoy *et al*., 2001b) and sheep (McCoy *et al*., 2001a), respectively, although correlations with plasma Mg were relatively weak. In the dead animal, vitreous humour Mg correlated well with plasma Mg in sheep and cattle; values of <0.6 and <0.5mmol Mg l^{-1}, respectively, in fresh[iv] samples were indicative of hypomagnesaemic tetany. However, tetanic and non-tetanic cows were most clearly differentiated by lower aqueous humour Ca (mean 0.89 versus 1.33 mmol Ca l^{-1}) (McCoy *et al*., 2001c): a distinction was less evident in ewes (McCoy *et al*., 2001a). Ca:Mg ratios of >50:1 in rib bone indicate a probability of hypomagnesaemic tetany in sucking or milk-fed calves and lambs, but are unreliable in the lactating animal because values fluctuate in synchrony with other minerals, including P (Fig. 5.3).

Treatment

Subcutaneous injection of a single dose of 200–300ml 20% $MgSO_4$ and i.v. injection of a similar dose of magnesium lactate both restore serum Mg of affected cows to near normal within about ten minutes and usually alleviate signs of tetany. Artificially depleted calves responded rapidly to rectal perfusion of 51mg Mg kg^{-1}LW as $MgCl_2$ (Bacon *et al*., 1990). Serum Mg may fall again unless the diet is immediately changed to hay and magnesium-rich concentrates, or the animal is given oral Mg. Affected ewes respond quickly to i.v. Mg, invariably given with Ca, usually as 50 ml calcium borogluconate (CaBG) solution containing 25 g

magnesium hypophosphite (Mg $(H_2PO_2)_2$. Calcium should also be given to the recovering cow using a dose of 500ml to correct any hypocalcaemia.

Magnesium in Feeds

Milk and milk replacers

Main milks are relatively low in Mg (0.1–0.2g l^{-1}) but colostrums are three times richer (Table 2.4). Milk Mg cannot be raised by dietary Mg supplements but is absorbed very efficiently from milk by young lambs and calves, due partly to extensive absorption from the large intestine (Dillon and Scott, 1979). However, A_{Mg} coefficients fell in calves from 0.87 at 2–3 weeks to 0.32 at 7–8 weeks of age (ARC, 1980). Prolonged rearing of calves (Blaxter *et al*., 1954) and adult ruminants (Baker *et al*., 1979) on simulated milk diets induced hypomagnesaemic tetany. Milk and milk products are unsuitable for prolonged use as predominant sources of dietary Mg.

Forages

Forage Mg is affected by genotype and seasonal and climatic conditions but soil origin is of little influence (Jumba *et al*., 1995). Grasses in temperate and tropical pastures contained on average 1.8 and 3.6g Mg kg^1 DM, respectively, and legumes 2.6 and 2.8g Mg kg^1DM, respectively (results for 930 forage samples; Minson, 1990). In temperate grasses, 65% of samples contained <2g Mg kg^1DM. Ajax and S24 perennial ryegrass contained 30% less Mg than Augusta (hybrid ryegrass), Melle (perennial ryegrass) and average UK pastures (Underwood and Suttle, 1999; Table 5.3). Seasonal variations within species are relatively small (Minson, 1990) but larger in mixed swards than monocultures and there are important, concurrent changes in herbage K (Fig. 5.5). Higher pasture Mg in New Zealand pastures (Fig. 5.5) than in UK pastures (Table 5.3) probably reflect higher legume contributions (about 20%) in New Zealand. Legume

Table 5.2. Marginal bands for assessing the magnesium status of ruminant livestock.

	Serum[a] (mmol l^{-1})[b]	CSF[c] (mmol l^{-1})[b]	Vitreous humour (fresh)[c] (mmol l^{-1})[b]	Urine[a] (mg creatinine^{-1}, molar ratio)	Rib (mg g^{-1} ash)[b]
Cattle	0.5–0.75	0.4–0.6	0.5–0.75	0.4–0.8	<12 (6–12 months) <7 (>36 months)
Sheep	0.6–0.75	0.6–0.8	0.4–0.8	0.4–0.8	–

CSF, cerebrospinal fluid.
[a]When mean values for sampled populations fall below these ranges, the flock or herd will probably benefit from supplementation. Values within bands indicate a possibility of current or future responses in some individuals that increases with proximity to the lower limit.
[b]Multiply values by 24.3 to convert units from mmol to mg and divide rib data by 24.3 to convert mg to mmol.
[c]When the mean *post-mortem* value is below the ranges given, hypomagnesaemic tetany is a probable cause of death.

Table 5.3. Mean magnesium (± standard deviation) concentrations (mg kg^1DM) in livestock feeds commonly used in the UK. (From MAFF, 1990)

Roughages	Mg	Concentrates	Mg	By-products	Mg
Barley straw	0.7 (0.31)	Barley	1.2 (0.20)	Wheat bran	6.2 (2.70)
Oat straw	0.9 (0.31)	Cassava	1.1 (0.57)	Brewers' grains	1.7 (0.36)
Grass	1.6 (0.56)	Maize	1.3 (0.13)	Distillers' grains[a]	3.3 (0.34)
Kale	1.6 (0.18)	Oats, winter	1.0 (0.06)	Citrus pulp	1.7 (0.50)
White clover	2.2 (0.50)	Wheat	1.1 (0.13)	Sugarbeet pulp	1.1 (0.19)
Grass silage	1.7 (0.54)	Cottonseed meal	5.8 (0.43)	Maize gluten	4.1 (0.70)
Grass hay	1.4 (0.52)	Fish meal, white	2.3 (0.31)	Maize-germ meal	2.1 (0.69)
Clover silage	2.3 (0.75)	Groundnut meal	3.5 (0.21)	Palm-kernel meal	3.0 (0.41)
Lucerne hay	1.7 (0.27)	Linseed meal	5.4 (0.09)		
Lucerne silage	1.8 (0.23)	Rapeseed meal	4.4 (0.53)		
Maize silage	2.2 (0.69)	Soybean meal	3.0 (0.23)		
Fodder beet	1.6 (0.30)	Sunflower meal	5.8 (0.49)		
Swedes	1.1 (0.07)				

[a]Barley-based.

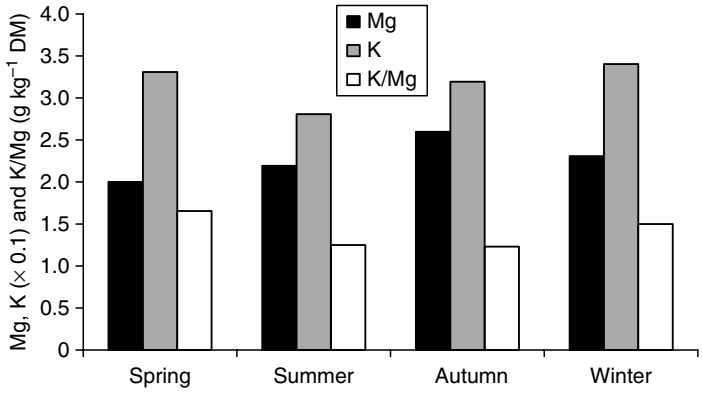

Fig. 5.5. Effect of season on mean magnesium and potassium concentrations in mixed (*Lolium perenne* and *Trifolium repens*) pastures on seven farms in Manawatu, New Zealand, with fertilizer applied in autumn. (From Stevenson *et al.*, 2003)

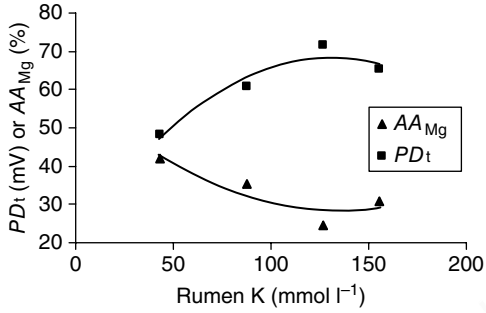

Fig. 5.6. Adding $KHCO_3$ to a concentrate diet for sheep increased potential difference across the ovine rumen mucosa (*PDt*) while decreasing apparent absorption of magnesium (AA_{Mg}): both relationships were curvilinear. (Data from Jittakhot *et al.*, 2004b)

(alfalfa) hay and silage (clover) Mg was higher than grass hay and silage Mg in UK. Fresh grass generally contains less Mg than conserved grass (Table 5.3). Other roughages, such as straws, were low in Mg, while root and leafy brassica crops were similar to grasses (Table 5.3). Cereal crops, sometimes grazed in the vegetative state for short periods, are often low in Mg but high in K (Dove *et al.*, 2016; Masters *et al.*, 2019).

Magnesium in forage supplements

Energy and protein supplements vary widely in magnesium concentration (Table 5.3), with, cereal grains and root crops low (1.1–1.6), oilseed meals high (3.0–5.8) and fish meals intermediate (1.7–2.5g Mg kg¹DM) (MAFF, 1990). Newer data from the USA (Kerr *et al.*, 2008), showed broad agreement for six staple feeds but large differences between maize by-products, corn gluten feed and meal containing 5.5 ± 0.29 and 0.73 ± 0.09g (s.d.) Mg kg^{-1}DM, respectively. Animal by-products vary with bone content, values increasing from 0.4g Mg kg¹DM in pure meat meal to 2g Mg kg¹DM in the average meat-and-bone meal but are no longer permitted for use as protein supplements in Europe.

Magnesium Value of Feedstuffs for Ruminants

Apparent availability of magnesium ($AA_{Mg,}$ p.22) in forages, fresh or conserved, can vary >tenfold and determines risk of disorder, requirement and the success of preventive measures (ARC, 1980). Coping with such wide variation requires an understanding of Mg metabolism, particularly the processes involved in absorption.

Absorption of magnesium from the rumen

The rumen is the major site of absorption in sheep (Tomas and Potter, 1976b; Field and Munro, 1977) and cattle (Greene *et al.*, 1983b; Khorasani *et al.*, 1997); here, efficiencies of overall absorption (A_{Mg}) are largely determined. Absorption begins at the apical membrane of the mucosa, where Mg uptake is driven by negative potential

differences (*PDa*). *A* secondary, active but saturable process, located in the basolateral membrane, controls efflux to the bloodstream (Dua and Care, 1995). Magnesium uptake from the rumen is affected by:

- *PD* across the whole mucosa (*PDt*);
- soluble Mg concentrations, which are influenced by rumen pH; and
- antagonism from K which depolarizes the apical membrane.[v]

Potassium inhibits absorption from the rumen

As rumen K rises, PD_t increases (Care *et al*., 1984; Martens and Schweigel, 2000) but the relationship is curvilinear and causes a curvilinear decrease in A_{Mg} from the rumen (Fig. 5.6). With rumen Mg >3–4 mmol Mg l^{-1} in sheep, Mg is absorbed passively without affecting *PDt* (Jittakhot *et al*., 2004b; Martens and Schweigel, 2000). High dietary K may also reduce A_{Mg} by increasing rate of outflow of digesta from the rumen (Dalley *et al*., 1997a; Ram *et al*., 1998) but does not completely explain the low A_{Mg} from all K-rich forages (Schonewille *et al*., 2002). Succulent forages induce post-prandial salivary flow, which may change the rumen K profile. Continuously infusing large volumes of water (10 l d^{-1}) into the rumen of sheep, on K-rich, pelleted dried grass diet, increased AA_{Mg} from 0.02 to 0.35 (Suttle and Field, 1966). Rumen K may have been reduced by a combination of simple dilution, solvent drag and decreased salivary flow, normally copious on such a diet.

- High water intakes from lush forages may counteract antagonism from K.

Dietary K supplements decrease $AA_{Mg,}$ unless Mg intakes are very high (Martens, 2016) but more Mg is needed to saturate absorption from the rumen in cattle than in sheep (12.5 versus 4 mmol Mg l^{-1}) (Martens, 1983).

Effects of other cations and anions on absorption from the rumen

Lack of dietary Na increases salivary and rumen K by inducing secretion of aldosterone (p. 188) and may thus lower A_{Mg} from the rumen (Martens *et al*., 1987). Anion supplements increased AA_{Mg} in cows (Roche *et al*., 2003) and the anion that accompanies K in simulating high forage K may influence the effect on A_{Mg}. Adding K as acetate rather than chloride salt decreased AA_{Mg} in sheep (Suttle and Field, 1967). *In vitro*, sulfate inhibited Mg uptake across rumen epithelium more than chloride, due possibly to a rise in lumenal pH (Martens and Blume, 1986). Supplements of $KHCO_3$ raised rumen pH by 15% (Wylie *et al*., 1985; Ram *et al*., 1998) and inhibited AA_{Mg} more than K given as KCl (Schonewille *et al*., 1997a) but to the same extent as K in dried grass (Schonewille *et al*., 1999a) or given as citrate (Schonewille *et al*., 1999b).

- Studies using KCl may have underestimated effects of herbage K on AA_{Mg}.

Absorption of magnesium beyond the rumen

Small but variable amounts of Mg are absorbed from the hind gut of sheep (Tomas and Potter, 1976a; Dalley *et al*., 1997a), depending on pH of caecal digesta (Dalley *et al*., 1997b) and dietary K:Mg ratios.[vi] However, hind gut absorption is not directly affected by K in sheep (Wylie *et al*., 1985) or cattle (Greene *et al*., 1983b). Post-ruminal A_{Mg} was higher from forages fed once daily rather than continuously (Grace and MacRae, 1972), possibly reflecting faster digesta flow from the rumen, which lowered A_{Mg}. There are net *losses* of Mg from the hind gut in cattle, where soluble Mg concentrations are relatively low (Bell *et al*., 2006). Low AA_{Mg} in dried grass indicates limited scope for compensatory hind gut absorption in small and large ruminants. However, models of Mg metabolism that allowed for hind gut absorption improved the prediction of experiment outcomes in sheep (Robson *et al*., 1997) and cattle (Robson *et al*., 2004). Absorption can occur from the bovine hind gut because rectal perfusion of Mg prevented hypomagnesaemia in cattle (Bacon *et al*., 1990).

Measuring absorbability

Provided that appropriate protocols are used, AA_{Mg} can be an attribute of feed rather than animal (p. 26) and is conveniently expressed as a fraction of intake (coefficient). Although rarely measured directly, true absorption (A_{Mg}) has been estimated indirectly from balance data by correcting AA_{Mg} for a constant endogenous loss (ARC,

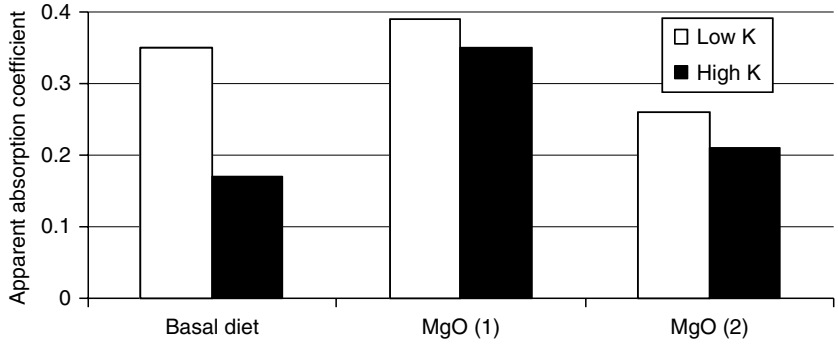

Fig. 5.7. Contrasting effects of high dietary potassium on apparent absorption of magnesium (AA_{Mg}) inherent in the diet and added as magnesium oxide to provide approximately 2.6 (MgO(1)) or 3.4g d^{-1}(MgO(2)): AA_{MgO} coefficients were calculated as increments in faecal Mg in supplemented sheep over those on the basal diet. (Data from Ram *et al*., 1988)

1980; Shonewillie *et al*., 2008; Martens and Stumpff, 2019). Validity of that assumption was questioned following a study in which AA_{Mg} varied with supplementary Mg intake but intakes were taken to levels never found in natural feeds (Fig. 5.7). The linear relationship between urine Mg and Mg intake above requirement has been confirmed in sheep (Martens and Schweigel, 2000)[vii] and the slope can indicate A_{Mg} in mature ruminants, provided that FEL_{Mg} has remained constant and there was no change in body Mg (i.e magnesium balance is zero (Fig. 5.8).

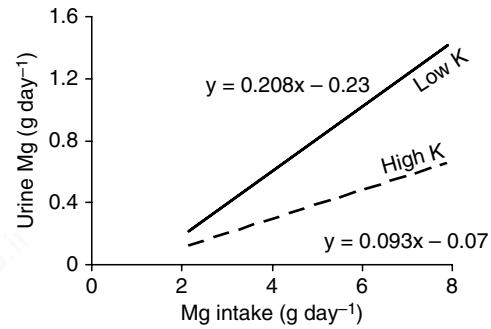

Fig. 5.8. Linear relationships between intake and urinary excretion of magnesium in identical twin dairy heifers, indicated that efficiency of magnesium absorption (A_{Mg}) was reduced from 0.21 to 0.09 by increasing dietary potassium from 20 (solid line) to 40 (broken line) g K kg^{-1}DM). (Data from Field and Suttle, 1979)

Individuality of absorptive efficiency

Wide, repeatable individual variation in AA_{Mg} has often been reported and must be minimized by appropriate experimental designs (e.g. Latin square) (Schonewille *et al*., 1999a, b) to increase the likelihood of obtaining significant treatment effects. Early studies in the Netherlands used groups of only two cows, some of which were used repeatedly (see Schonewille *et al*., 2008) and may have yielded estimates of 'magnesium value' that were unrepresentative of larger populations. The three pairs of twin heifers featured in Fig. 5.8 had significantly different AA_{Mg} (ranging from 0.07 to 0.23; Field and Suttle, 1979). Rates of Mg transport across the isolated rumen epithelium differ between breeds (Martens and Schweigel, 2000) but individuality in AA_{Mg} was also seen in sheep fed by abomasal infusion (Baker *et al*., 1979).

Availability of magnesium in forages

Relationships between AA_{Mg} and dietary K in pooled data from the literature are summarized in Fig. 5.9 (Adediji and Suttle, 1999). As K increased, AA_{Mg} decreased in cattle and sheep, whether forage or mixed diets were fed, but slopes and intercepts differed. In forages, increases of 10g K^{-1}DM were associated with decreases in AA_{Mg} of –0.112 and –0.016 in sheep and cattle, respectively, but the intercept was far higher in sheep (0.67 versus 0.21).

- Higher sensitivity to K in sheep may reflect the higher ratio of mucosal surface area to volume in a much smaller rumen and lower rumenal Mg needed to saturate mucosal transport.
- The lower intercept in cattle may also reflect lack of compensatory A_{Mg} from the caecum (p. 109).

A subsequent meta-analysis of cattle data (Schonewille *et al*., 2008)[viii] yielded the following equation:

$$A_{Mg}(g\,d^{-1}) = 3.6(0.67) + 0.2(0.01)Mg_i - 0.08K$$

where Mg_i = Mg intake ($g\,d^{-1}$) and K = %K in DM.

The weighting given for dietary K was closer to the estimate for cattle given grass or roughage (Fig. 5.9c) than concentrates (Fig. 5.9d). In sheep given K-rich, dried grasses, AA_{Mg} is as low as in cattle (0.02–0.12 AA_{Mg} (Suttle and Field, 1966; Adams *et al*., 1996; Schonewille *et al*., 2002).

Availability of magnesium in mixed, dry diets

With mixed, dry (R/C) rations, relationships between AA_{Mg} and dietary K in sheep and cattle were much closer than with forage diets (compare intercepts and slopes in Fig. 5.9b and 5.9c). The relationship for cattle was confirmed by subsequent meta-analysis of data for cattle fed R/C diets at a single research centre (Weiss, 2004), in which

$$AA(g/d) = 4.5(4.0) + 0.24(0.07)Mg_i - 4.4(2.2)K$$

The coefficient for K is closer to that of Fig. 5.6d than 5.6c. The contrast between the Schoneville and Weiss equations resembles that between Figs 5.6b and 5.6d. Higher absorbability of Mg from R/C diets may simply give scope for antagonism from K to be expressed. When dietary K was reduced by replacing dried grass with up to 60% concentrates, the antagonistic effect of K on AA_{Mg} was greatly increased (Schonewille *et al*., 2002). In goats, reducing dietary K from 34 to 7.8g kg^{-1}DM, by substituting starch-rich concentrate for roughage, increased AA_{Mg} from 0.22 to 0.30 (Schonewille *et al*., 1997b), a response closer to that for sheep than for cattle on mixed diets (Fig. 5.9b versus 5.9d). Variability of relationships for mixed, dry (Fig. 5.9b and d) may be partly due to differences in concentrate inclusion rates.

Availability of magnesium from diets containing silages

With cows given 50:50 mixtures of whole-crop cereal and alfalfa silages with concentrates, relatively low in K (<16g kg^{-1}DM), AA_{Mg} was 0.17–0.24 (Khorasani *et al*., 1997), similar to values on mixed dry diets (Fig. 5.9d). Higher AA_{Mg} of 0.56 and 0.42 were recorded for diets based on sugarcane bagasse and corn silage, containing 5.5 and 10.4g K kg^{-1}DM, respectively (Zanetti *et al*., 2017). When Mg and K supplements were sprinkled on grass silage and offered to cows separately from but simultaneously with concentrate, no K × Mg antagonism was found (Holtenius *et al*., 2008). Cows may have filled their rumens with concentrate before consuming the supplements, briefly creating higher dietary Mg and K concentrations than intended. In sheep, the K × Mg antagonism was attenuated with high dietary Mg (Fig. 5.7). The manner in which feeds are presented to ruminants may influence AA_{Mg} and

- Mg supplements may be more effective when parlour-fed than in either a K-rich, TMR or presented at pasture in free-access minerals.

Other factors that have influenced AA_{Mg} include ionophore supplements and dietary Ca and P but effects were either small or inconsistent.[ix]

Magnesium Value of Feeds for Non-ruminants

Monogastric species absorb magnesium mostly from the small and large intestine (Güenter and Sell, 1973; Partridge, 1978). Knowledge of A_{Mg} is meagre for pigs and poultry but values for older animals are much higher than those for ruminants. A combination of comparative balance and isotope dilution techniques estimated A_{Mg} in chickens to be 0.55–0.58 from barley, maize and wheat, 0.83 from oats, 0.60 from soybean meal, 0.62 from dried skimmed milk and 0.43 from rice (Güenter and Sell, 1974). In newly weaned pigs, AA_{Mg} was 0.29 and reduced to 0.19 ± 0.02 by adding 5 g phytate kg^{-1}DM to a semi-purified diet: 20 g induced negative Mg balance and must have increased FEL_{Mg} (Woyengo *et al*., 2009).

- Pigs may absorb feed Mg less efficiently than poultry because they degrade phytate less efficiently and are more prone to Mg × phytate antagonism.

Phytate supplements decreased soluble Mg in jejunal digesta in pigs, unless phytase supplements were also added (Woyengo *et al*., 2010). The AA_{Mg} for an unsupplemented corn/SBM blend was only

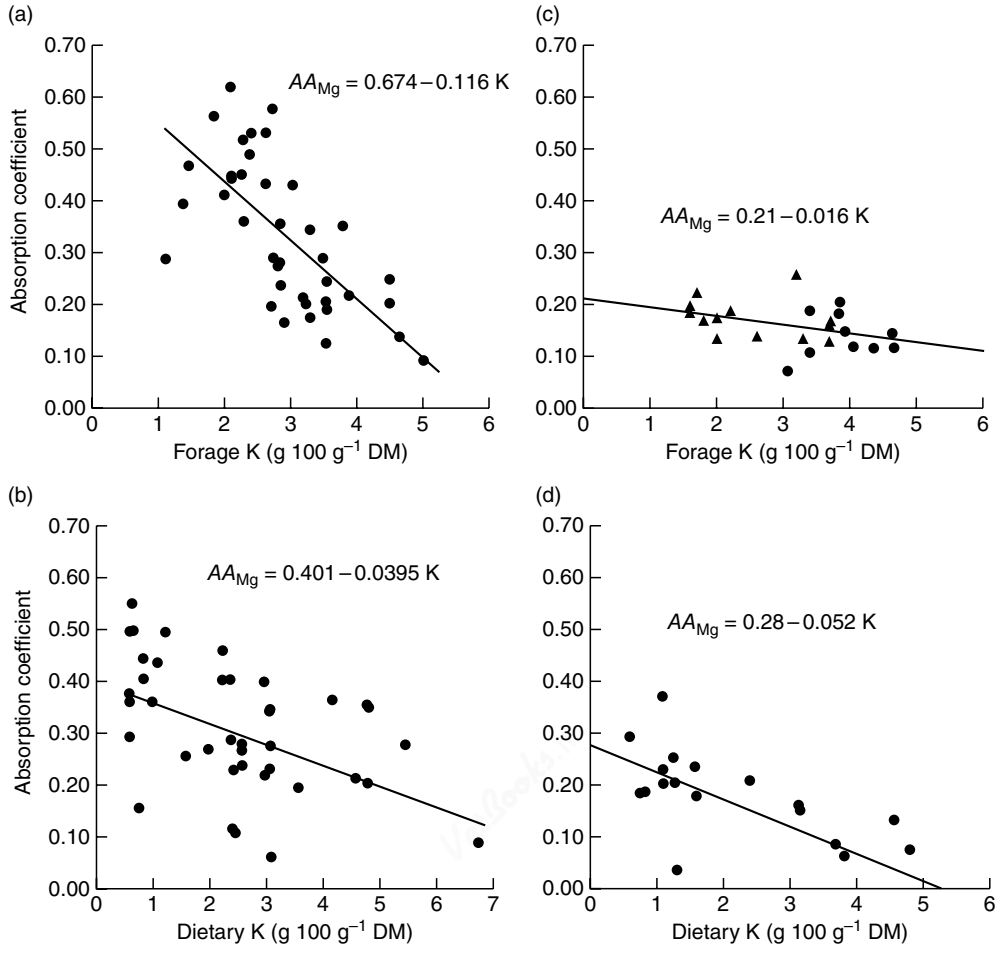

Fig. 5.9. Published apparent magnesium absorption coefficients are inversely related to dietary potassium concentration but the interaction differs between sheep and cattle on different types of diet (from Adediji and Suttle, 1999). (a) Sheep given fresh grasses; (b) sheep given roughage plus concentrates; (c) cattle given hays or grasses; (d) cattle given hay plus concentrates.

0.24 (Merriman *et al*., 2016) and agreed with two earlier figures for pigs given European diets. Fat supplements did not decrease AA_{Mg} and oil supplements increased AA_{Mg}. Guinea pigs absorbed Mg from grass and alfalfa hays more than twice as efficiently as lambs given the same hays (Reid *et al*., 1978). The horse, a hind gut fermenter, absorbs Mg well from grass. Mean (standard error) AA_{Mg} values for cut grass given to Thoroughbred yearlings and lactating mares were 0.50 (0.035) and 0.63 (0.04), respectively, three to four times higher than in cattle, but reduced to 0.38 (0.04) by large $CaCO_3$ supplements (Grace *et al*., 2003).

Magnesium Requirements of Ruminants

Net requirements for maintenance

Although faeces do not provide the major route of excretion, ARC (1980) estimated maintenance requirement (M, $^{o}FEL_{Mg}$) (p. 6) to be 3mg Mg kg^{-1}LW, a far higher fraction of total body content than for either $^{o}FEL_{Ca}$ or $^{o}FEL_{P}$. Salivary secretion was 0.85 and 0.30mg Mg kg^{-1}LW respectively, in sheep given fresh ryegrass and lucerne hay (Grace *et al*., 1985) may have been prematurely dismissed as a source of variation in FEL_{Mg}. Reanalysis of published data revealed a linear relationship between FEL_{Mg} and plasma Mg,

with values declining to approximately 1mg kg^{-1}LW in severe hypomagnesaemia (Bell *et al.*, 2006). Allowing FEL_{Mg} to decrease improved predicted outcomes of experimental depletion in cattle (Bell *et al.*, 2006). In livestock consuming forages with high K:Mg ratios, *M* may be reduced. The 'x' intercepts of relationships in Fig. 5.8, indicating Mg intake to achieve zero balance, was 0.78 and 1.11 mg Mg d^{-1}, respectively, with and without addition of K to pelleted, complete diets. Potassium may decrease salivary Mg secretion and/or reabsorption. In correcting AA_{Mg} data for purposes of meta-analysis (Schonwillie *et al.*, 2008) and revaluating requirements (Martens and Stumpff, 2019), FEL_{Mg} was assumed to be 4 mg kg^{-1}LW: corrected A_{Mg} was often twofold higher than AA_{Mg} but arguably irrelevant (p. 11). Using debatable comparative slaughter methods (p. 6), *M* (mg Mg kg^{-1} LW) was estimated to be 8.2 in cattle (Zanetti *et al.*, 2017), 0.2 in lambs (Costa e Siva *et al.*, 2015) and as high as 1.6 in goat kids (Texeira *et al.*, 2013): errors in such estimates were large (e.g. 1.31 ± 0.51; Fernandes *et al.*, 2012). Genetic variation in M_{Mg} was often invoked without evidence. Urinary Mg losses are negligible at marginal Mg intakes, irrespective of K intake (Fig. 5.8).

Net requirements for growth and milk production

Net requirements for growth can no longer be equated to carcase mineral concentration (p. 9). Housed Nellore bulls, given various diets to maintain or gain liveweight (1.1 kg d^{-1}), were predicted to require 0.9–1g Mg kg^{-1}LWG (Zanetti *et al.*, 2017) by unvalidated comparative slaughter methods (p. 9): this was twice the estimate of ARC (1980) and probably attributable to provision of excess Mg, since 2.8–5.8g Mg was excreted in urine each day. By contrast, similar methods estimated Mg accretion in confined cattle to be only 0.22–0.29 g Mg kg^{-1}EBWG, decreasing as animals grew (Chizzoti *et al.*, 2009): accretion may have been reduced by confinement (p. 74). Accretion of Mg also varied in early-weaned goat kids (Fig. 1.4) and lambs (from 0.26 in Ji *et al.*, 2015 to 0.47g Mg kg^{-1}EBWG in Texeira *et al.*, 2013). Milk Mg is low (4mmol l^{-1}), compared to Ca, but maintained during depletion and represents a continuous drain on maternal reserves. Furthermore, colostrum contains two to three times more Mg than main milk and adds to maternal demand around calving (Goff and Horst, 1997). New Zealand studies suggest that milk Mg has declined over the years (Theilin *et al.*, 2001), probably reflecting widespread crossing of Holstein and Jersey breeds, long known to differ in milk Mg.

Gross requirements

Gross requirements vary widely with species, age, diet type and interactions between those factors, due largely to variation in AA_{Mg}. ARC (1980) set a precedent by recommending allowances that exceeded estimates of minimum requirement to protect the most vulnerable individuals in a herd or flock. Refining that strategy, AA_{Mg} values derived from Fig. 5.9 are again used to generate gross needs for two levels of K and two types of diet (forage and roughage/concentrate mixture (1:1) for sheep and cattle) (Tables 5.4 and 5.5, respectively). Alternative factorial estimates of requirements, based on dubious estimates of net requirements for *M* and *P* (p. 10), described above, are disregarded.
Note that:

- cattle have higher requirements than sheep;
- cows need more Mg at pasture (e.g. in New Zealand) than housed (e.g. in USA);
- average pasture Mg in UK (Table 5.3) and New Zealand (Fig. 5.5) is close to need;
- lactation increases Mg requirement;
- sheep given mixed rations as opposed to forage need 50% less Mg;
- required dietary Mg concentration does not increase with level of performance.

Support for the cattle requirements was given by induction of hypomagnesaemia with mixed diets containing 1.7g Mg and 40g K kg^{-1}DM (van de Braak *et al.*, 1987) and the recommendation that an additional 18g Mg day^{-1} should be allowed for every increase of 10g K kg^{-1}DM in mixed diets for USA herds (Weiss, 2004).

- Suggestions that lesser allowances for high K should be made for cows on a 'European diet' (Martens and Stumpff, 2019) ignore real differences in dairy cow management across Europe.
- Requirements of goats may be closer to those of sheep than cattle.

Prevention of Disorders

Magnesium fertilizers

Prevention is far better than cure for an acute disorder that requires immediate, skilful treatment. Foliar

Table 5.4. Factorially derived estimates of magnesium requirements for grazing sheep.[a]

			Dietary requirement (g kg^{-1}DM)		
	Liveweight (kg)	Growth/production (kg day^{-1})	High K[c]	Low K[c]	DMI[b] (kg day^{-1})
Growth	20	0.1	1.0	0.50	0.50
		0.2	0.9	0.45	0.76
	30	0.1	1.0	0.50	0.67
		0.2	0.9	0.45	1.00
		0.3	0.8	0.40	UA
	40	0.1	1.0	0.50	0.83
		0.2	0.9	0.45	1.23
		0.3	0.7	0.35	1.80
		Fetuses (–12 weeks to term)			
Pregnancy	40	1	0.9	0.45	0.64–0.96
		2	0.9	0.45	0.74–1.25
	75	1	1.1	0.55	1.03–1.51
		2	0.9	0.45	1.17–1.93
		Milk (kg day^{-1})			
Lactation	40	1	1.2	0.60	1.18
		2	1.2	0.60	1.90
	75	1	1.4	0.70	1.48
		2	1.3	0.65	2.18
		3	1.3	0.65	2.90

UA, unattainable performance on such diets.
[a]For housed sheep on mixed rations, requirements fall between those shown for high- and low-K diets.
[b]Dry matter intakes for diets of intermediate nutritive value, q = 0.6 (see ARC, 1980, which also gives the net requirements for maintenance (3 mg kg^{-1} LW), growth (450 mg kg^{-1} LWG) and milk production (170 mg kg^{-1})).
[c]Absorption coefficients were 0.20 for sheep grazing pasture high in K (40 g kg^{-1} DM) and 0.40 for pastures low in K (20 g kg^{-1} DM).

Table 5.5. Factorially derived estimates of magnesium requirements of grazing cattle.

			Dietary requirement(g kg^{-1}DM)		
	Liveweight (kg)	Growth/production (kg day^{-1})	High K[b]	Low K[b]	DMI[a](kg day^{-1})
Growth	100	0.5	1.5	1.1	2.1
		1.0	1.4	1.1	3.2
	200	0.5	1.6	1.2	3.3
		1.0	1.4	1.1	4.7
	400	0.5	1.7	1.3	5.2
		1.0	1.4	1.1	7.3
		Fetuses (weeks to term)			
Pregnancy	600	12	2.1	1.6	5.8
		4	1.8	1.4	7.3
		Milk (kg day^{-1})			
Lactation	600	10	2.4	1.8	9.4
		20	2.1	1.7	14.0
		30	2.0	1.6	18.8
		40	23	1.8	21.0

[a]Assuming a diet of moderate digestibility, with a 'q' value of 0.6 (see ARC, 1980, which also gives the net requirements for maintenance (3mg kg^{-1} LW), growth (450mg kg^{-1} LWG) and milk production (125mg kg^{-1})).
[b]Based on apparent absorption coefficients of 0.14 and 0.18 for grass high (40g kg^{-1} DM) and low (20 g kg^{-1}DM) in K. Dutch authorities make a similar distinction between grass products and corn silage (Schonewille *et al*., 2008).

dusting and spraying uses pasture as a short-term carrier for the supplement. Dusting pastures with fine calcined magnesite (cMgO), either before or during tetany-prone periods, increased pasture Mg but effects were short-lived, regardless of the amount applied (Fig. 5.10) (Kemp, 1971). Dustings with at least 17 kg ha^{-1}, repeated after heavy rainfall and at ten-day intervals, were recommended (Rogers, 1979). Adhesion to the leaf can be improved by applying cMgO in a slurry with bentonite. Pasture dusting is more reliable when integrated with a 'strip-grazing' system and remains a popular preventive method in New Zealand. Magnesium fertilizers remained popular on large dairy farms in Northern Ireland (McCoy *et al*., 1996). Dolomitic limestone has been used but much higher rates of application are needed.[x] Spray application of $Mg(OH)_2$ in fine suspension briefly increased herbage and serum Mg at low application rates (5 kg Mg ha^{-1}) (Parkins and Fishwick, 1998). Chelated magnesium is commercially available for spray application but claimed advantages in terms of increased herbage Mg have yet to receive peer-reviewed support.

Dietary magnesium supplements

Supplementation with Mg-rich concentrates was preferred by many small dairy farmers (McCoy *et al*., 1996) but a recent survey found most UK farmers reliant on free-access mineral mixes containing Mg for sheep and cattle (Kumssa *et al*., 2019). Historic prophylactic doses of cMgO, providing 25–30g Mg day^{-1} for adult dairy cattle, 7–15 g day^{-1} for calves and 7 g day^{-1} for lactating ewes, were over-generous, being sufficient to raise urine pH (Xin *et al*., 1989) and precipitate milk fever. Smaller doses, providing 12 g Mg day^{-1} for cows, became routinely used in New Zealand in the 1980s and may have reduced incidence of milk fever (Roche and Berry, 2006) but chloride or sulfate salts are now preferred. Commercial sources of cMgO vary widely in nutritive value, due to differences in origin, particle size and processing (e.g. calcination temperature) (Adams *et al*., 1996; Xin *et al*., 1989). Coarse particles (>500 μm) and low calcination temperatures (<800°C) are associated with low AA_{Mg}. Magnesium compounds can also be either included in drenches, salt- and molasses-based free-choice licks and blocks or sprinkled on concentrates, chopped roots, hays and silages, to varying effect. Provision of Mg-containing blocks was common amongst beef suckler herds in Northern Ireland but did not reduce hypomagnesaemia (McCoy *et al*., 1996). Soluble magnesium salts can be effective when added to drinking water (see Rogers and Poole, 1976), but water intake varies widely between individuals and with weather and pasture conditions (p. 22). Magnesium salts are metered into the water supply during bail milking in New Zealand (Dosatron, Florida, USA) during the tetany season. Uniform individual treatment can be ensured without additional labour costs by adding 10–20 g Mg as chloride or sulfate to bloat control drenches, given daily from the milking platform. Magnesium alloy bullets that lodged in the rumen did not prevent hypomagnesaemia in dairy cows but may be more effective in beef cattle, which require less Mg (Stuedemann *et al*., 1984).

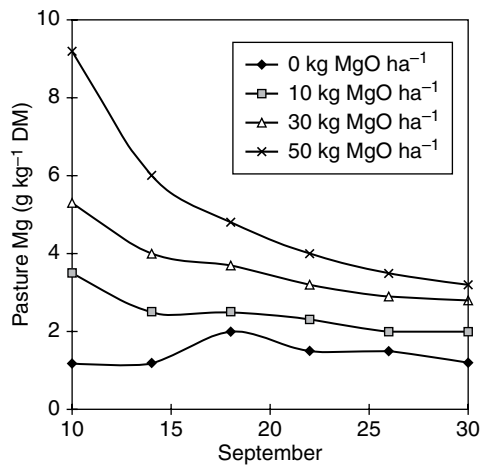

Fig. 5.10. Control of hypomagnesaemic tetany by dusting pastures with magnesite (MgO) is short-lived because of waning surface adherence (Kemp, 1971). Repeated applications are necessary and a single treatment with a suspension of magnesium hydroxide may be more effective.

Merits of different magnesium sources

Comparisons with $MgSO_4$ in sheep accorded average *relative* availabilities of 0.75 (n = 18) and 0.60 (n = 3) to MgO and $Mg(OH)_2$, respectively (Henry and Benz, 1995), but the hydroxide can be superior to MgO (Hurley *et al*., 1990). Hind gut absorption allowed a source of low solubility in the rumen (magnesium-mica) to equal rumen-soluble $Mg(OH)_2$ in terms of total tract A_{Mg} (Hurley *et al*.,

1990), probably by by-passing antagonism from K in the rumen. Rankings in individual studies may depend on rumen solubility of a particular source. Values for A_{Mg} in supplements are influenced by attributes of the diet being supplemented. For example, A_{Mg} for $MgCl_2$ was four times higher when infused into the rumen of sheep given a pelleted semi-purified diet rather than chopped hay (0.55 versus 0.13) (Field and Munro, 1977). More available Mg was provided by cMgO added to maize-based rather than dried-grass pellets for sheep (Adams *et al*., 1996). The AA_{Mg} from a supplement such as cMgO can exceed that of a basal diet, although the advantage can be masked at high Mg intakes in sheep (Fig. 5.7). Availability of cMgO to cattle can be halved by raising dietary K (Jittakhot *et al*., 2004a). Magnesium phosphates can also provide absorbable magnesium (Underwood and Suttle, 1999).

Predictors

Incidence of disorder can be reduced by pre-empting the risk factors listed earlier (p. 101 *et seq*.) Emphasis was initially placed on chemical composition of pasture; K:Ca+Mg ratios >2.2 indicated heightened risk of tetany in the Netherlands (incidence 6.7%; Kemp and 't Hart, 1957) and the relationship was confirmed in many countries. However, relationships with disease incidence are likely to be linear rather than threshold in nature. A relationship between plasma Mg, pasture K and crude protein was also devised in the Netherlands but never widely adopted. Emphasis shifted to indices of magnesium status in the animal, including the curvilinear relationship between plasma and urinary Mg (Fig. 5.11). Individual urinary Mg: creatinine ratios (molar) of <0.4 were suggested to be 'critical' (van de Braak *et al*., 1987) and mean values <1.0 to indicate possible benefit from supplementary Mg (Sutherland *et al*., 1986). The marginal bands given in Table 5.2 are based on the above findings. A modelling approach to prediction of hypomagnesaemia was investigated in New Zealand using, among other things, easily recorded variables such as an individual cow's milk Mg output (Bell *et al*., 2006), but has yet to be tested. Cereals and canola grazed in the vegetative state frequently have K/Ca + Mg ratios >2.2. Extreme K/Na+Mg values would allow for a protective effect of sodium (Fig. 5.12). Prevalence of abnormal ratios was higher on triticale and wheat than on barley and oats but no clinical signs of tetany were seen. Production responses were obtained to combined supplements of Mg, Na and Ca but could not be attributed specifically to magnesium and may be linked to high pasture DCAD (Dove *et al*., 2016)

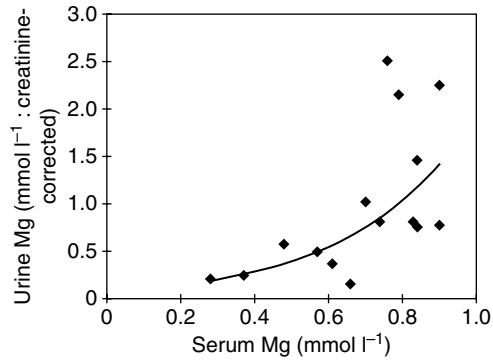

Fig. 5.11. The relationship between urine and plasma magnesium is curvilinear because urine Mg reflects the dietary supply in excess of need, while plasma Mg reflects the degree to which need is not met. (Data from Sutherland *et al*., 1986)

Restricting use of potassium fertilizers

Conservative use of fertilizer K, including K-rich dairy slurry (Soder and Stout, 2003), will weaken the K × Mg antagonism but scope is limited by the need to maximize pasture yield and the need to dispose of slurry. Slurries should be applied to fields intended for conservation but where this is impracticable, recently treated pastures should not be grazed at times of high risk. Use of inorganic fertilizer K can be similarly restricted, and K-rich feeds such as molasses and molassed beet pulp should not be used at vulnerable times. The requirements presented in Tables 5.4 and 5.5 suggest that the K:Mg ratio in herbage should be <16 for cattle and <30 for sheep. With fertilizer applied in autumn, mean K:Mg ratio in Manawatu (New Zealand) pasture stayed just above the cattle threshold (Fig. 5.5).

Managing turnout

By gradually extending the daily grazing period in spring, risks of inducing hypomagnesaemia and tetany decrease and supplementary feed costs can be reduced. Similarly, sheep can be gradually introduced to vegetative cereal grazing and both Mg and Na intakes maintained by providing mineral

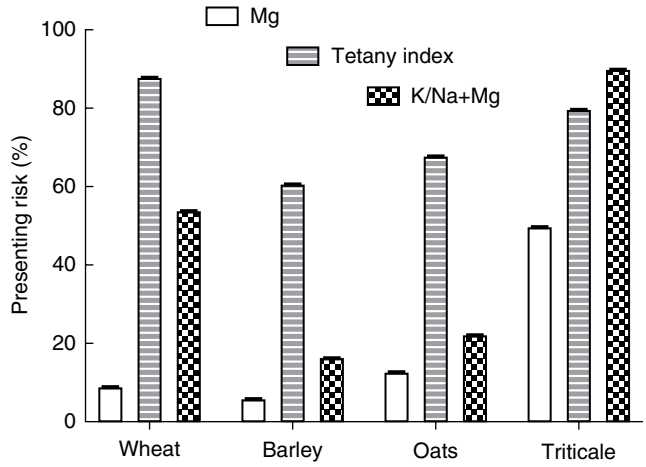

Fig. 5.12. Proportion of four vegetative cereal samples collected in New South Wales that contained <1g Mg kg^{-1}DM had a K/(Ca + Mg) ratio >2.2 and K/(Na + Mg) ratio >5.0). (Data from Dove *et al*., 2016)

supplements (Dove and McMullen, 2009; Dove *et al*., 2016). If weather forecasts indicate an imminent, abrupt change in temperature, wind chill or heavy rainfall, turnout should be delayed and livestock already outdoors should be sheltered, if possible. Carbohydrate supplements lower rumen pH and improve A_{Mg} (Schonewille *et al*., 1997b) and can be provided in liquid mixtures of molasses and Mg salts. Providing grain as a preventive measure for milk fever (p. 64) should also improve magnesium status. A short period of grain feeding at times of risk in an otherwise pasture-based system may save lives and inconvenient treatments.

Genetic selection

Studies with identical twin cows showed that variation in A_{Mg} was partly heritable (Field and Suttle, 1979). Repeatable differences in milk Mg have been reported between individual cows and these may be partly heritable and affect individual susceptibility to grass tetany (Theilin *et al*., 2001). In a comparison of beef breeds on a common diet, Aberdeen Angus had relatively low serum Mg (Littledike *et al*., 1995). Marked breed differences in Mg metabolism were linked to differences in susceptibility to grass tetany in cattle (Greene *et al*., 1986) but representation was small (five per breed or cross) and rankings for crosses were not always intermediate between those of parent breeds. Effects of sire on urinary Mg excretion in lambs confirmed genetic control in sheep (Field *et al*., 1986). Selecting for high magnesium status should reduce susceptibility to hypomagnesaemia and tetany.

- A selection strategy targets the vulnerable minority of any population, whereas supplementation strategies treat the whole herd or flock to ensure survival of that minority.
- Ratios of Mg:creatinine in urine of bulls held at artificial insemination centre on a standard ration and might allow selection for resistance to hypomagnesaemia and tetany.

Progress can also be made by selecting for plant cultivars rich in Mg (Moseley and Baker, 1991). The quest for low K and low DCAD genotypes that decrease risk of milk fever (p. 64) should yield cultivars that reduce risk of hypomagnesaemia and tetany.

Magnesium Requirements for Non-ruminants

Horses

A 200-kg horse gaining 1 kg day^{-1} has been estimated by factorial modelling to require only 0.7g Mg kg^{-1}DM in its pasture (Grace *et al*., 2002b), while lactating mares require 1.7 g Mg kg^{-1}DM (Grace *et al*., 2002a). The models used an FEL_{Mg} of 6 mg kg^{-1} LW – at least twice that of the ruminant – and an A_{Mg} of 0.4, *less* than the AA_{Mg} observed in the group's balance studies (0.5–0.6), and are likely to have overestimated needs that are closer to those of

pigs and poultry. Natural diets should invariably meet the low needs of the weanling or mature equine. If there is a problem it might lie with the suckling foal, as mare's milk is relatively low in Mg (2 mmol l^{-1}) (Table 2.4).

Pigs

The minimum requirement of baby pigs receiving a purified diet was 325mg Mg kg^{-1}DM (Miller *et al.*, 1965) but weaner pigs needed 400–500mg Mg kg^{-1}DM (Mayo *et al.*, 1959). Factorially derived requirements were of the same order (ARC, 1981), despite using model components now known to be inaccurate. Net growth requirement is much lower than ARC (1981) assumed, does not decline with age and may be influenced by diet, as in goats (Fig. 1.4). Magnesium accretion rate has varied between studies (251, Mahan and Shields, 1998; 174, Mahan *et al.*, 2009; and 280 mg kg^{-1} EBW, Ruiz-Ascacibar *et al.*, 2019): diets in the last two studies contained 0.6g and 1.0-1.4g Mg kg^{-1}DM, respectively, indicating that accretion may vary with dietary Mg, as in goat kids (Fig. 1.4). Magnesium concentration at birth was 171 mg kg^{-1}EBW and subsequent accretion was unaffected by dietary protein concentration (Ruiz-Ascacibar *et al.*, 2019).

- The best estimate of minimum net need for growth is 174 mg kg^{-1}LWG.

Magnesium availability from feeds may be only one third that assumed by ARC (1981), whose chosen A_{Mg} coefficient (0.7) was probably for inorganic Mg supplements. By luck rather than judgement, ARC (1981) got close to a realistic requirement for growth. Gross magnesium requirements for growth should be met by any commercial ration. Supplementation with mica improved feed conversion efficiency during the early finishing phase in one study (Apple *et al.*, 2005) but performance was rarely improved in others. However,

- farrowing sows may require magnesium supplements of diets lacking phytase.

Some 50% of Mg in a newborn litter accumulates during the last two weeks of gestation (Mahan *et al.*, 2009), when a litter of ten can accumulate 4.9 g Mg d^{-1}. The sow may not eat for several hours around parturition but inactivity may increase Mg resorption from her own skeleton (p. 76). Continuous supplementation of a corn-SBM diet containing 2100mg Mg kg^{-1}DM with a further 150-450 mg Mg as $MgSO_4$ from mating to weaning reduced weaning-to-oestrus interval in gilts and sows (Zang *et al.*, 2014). Furthermore, number of piglets born and weaned by sows was increased. Serum prolactin, Mg and colostrum Mg were increased in sows and plasma growth hormone increased in piglets: the increase in serum Mg was maximal with intermediate supplementation, which raised values from 17.6 to 19.9 mg l^{-1}. It has been hypothesized that providing extra Mg at parturition minimizes risk of piglet hypoxia (Plush *et al.*, 2018). Supplementing a conventional sow diet with 0.7% $MgSO_4$ for five days before farrowing did not affect piglet survival but increased plasma glucose and weight gain in the first 24 hours, suggesting higher colostrum intake. Colostrum contains no more magnesium (or calcium) than main milk (Hu *et al.*, 2019) and net requirement for lactation is 118 mg Mg l^{-1} (from Plush *et al.*, 2018), rising from about 0.6 to 1.2g d^{-1} in the first ten days. Lactation needs appeared to be met by 325 but not 130mg Mg kg^{-1}DM in the diet (Harmon *et al.*, 1976): an intake of 5kg DM would have provided 1.6 and 0.65g Mg, respectively, which was only partially absorbed.

Pork quality

Magnesium supplementation prior to slaughter can moderate some biochemical changes associated with transport stress (Apple *et al.*, 2005), as in sheep (p. 103). Magnesium also reduces *post mortem* (PM) changes associated with muscle glycolysis: these cause pork to become pale, soft and exudative ('PSE pork') (D'Souza *et al.*, 1999: Chen *et al.*, 2013). Incidence of PSE pork is a major concern amongst meat retailers. Numerous comparisons have been made of the effects of organic and inorganic magnesium sources, measuring multiple biochemical indices, tentatively related to pork quality (e.g. Apple *et al.*, 2005). Responses to Mg have varied widely because many factors can increase incidence of PSE, such as genotype, length of transportation, goading and lack of lairage. Short-term supplementation prior to slaughter reduced PM muscle glycolysis, 1.6g Mg for one day being as effective as 3.2g for five days, regardless of source (sulfate, propionate or proteinate) (Hamilton *et al.*, 2003). In pigs of the halothane-sensitive genotype, 15g Mg kg^{-1}DM, given for five days as acetate or sulfate reduced serum lactate, serum creatine kinase activity and slowed the rise in muscle lactate, particularly in the first two days of display (Chen *et al.*, 2013). Magnesium can be

conveniently provided via the drinking water (Frederick *et al*., 2004). When transport and slaughterhouse stresses are minimized, benefits from Mg supplementation become less likely. Comparable protection may also be afforded by allowing sheep and cattle access to magnesium-supplemented drinking water for 24 hours prior to transportation.

Poultry requirements

The magnesium nutrition of poultry has been reviewed since the last edition (Shastak and Rodehutsecord, 2015) but there had been few new studies. Requirement appeared to increase with growth rate in an early study: broiler chicks had needed 0.5g Mg kg^{-1}DM, whereas slower-growing White Leghorn chicks needed 60% less Mg (McGillivray and Smidt, 1975). Adding Mg to high-energy diets increased weight gain to 3 weeks of age but not to market weight, (McGillivray and Smidt, 1977). Dietary Ca and P may interact with Mg (Shashtak and Rodehutsecord, 2015) but had small effects on chick requirements (Nugara and Edwards, 1963; Wu and Britton, 1974). Requirements of laying hens (g Mg kg^{-1}DM) depended on criterion of adequacy and type of diet: 0.16 to maintain normal serum Mg, 0.26 to support maximal egg production and 0.36 to obtain a normal egg Mg, weight and hatchability (Hajj and Sell, 1969: Sell, 1979), a finding supported in hens on a practical ration (Bastien *et al*., 1978). The requirement for maximum egg weight was ≤ 0.3 g Mg kg^{-1}DM in a recent study and not increased by heat stress (Sedgh-Gooya and Torki, 2018). Inorganic sources, oxide, carbonate and sulfate, all provided highly available Mg for broiler chicks (McGillivray and Smidt, 1975). Responses in liver Mg and catalase gene expression to increases in dietary Mg, 3- to 10-fold above requirement, as Mg aspartate or MgO, indicated greater retention for the organic source (Liu *et al*., 2007) but may lack commercial significance. The A_{Mg} value for reagent grade $MgSO_4$ was 0.57 in a semi-purified diet for chickens (Güenter and Sell, 1974). Corn/soybean blends can contain as little as 26mg Mg kg^{-1}DM and need supplementation but the recommendations of NRC (1994) for a uniformly high 0.5g Mg kg^{-1}DM for broilers, turkey poults and laying hens (with food intake 100g day^{-1}) contains a generous safety margin, particularly if Ca and P are fed to minimum requirements. Heat stress may increase the requirement for Mg in poultry and be better met by Mg proteinate than MgO supplements (Sahin *et al*., 2005)

Magnesium Toxicity

Lack of homeostatic mechanisms for control of metabolism for a given mineral is usually associated with tolerance of extreme intakes but this is barely true of magnesium. The NRC (2005) gave tolerable limits for pigs, poultry, ruminants and horses at 2.4, 5.0–7.5, 6 and 8g Mg kg^{-1}DM, respectively. In laying hens, 8–12g Mg kg^{-1}DM has reduced egg production and eggshell strength (Hess and Britton, 1997) and chicks develop diarrhoea with >30g Mg (as MgO) kg^{-1}DM (Lee and Britton, 1987). Tolerance is of most practical importance in forage-fed ruminants, often given large magnesium supplements to prevent disorder. In sheep and steers given roughage/concentrate mixtures, 14g Mg kg^{-1}DM was mildly toxic (Chester-Jones *et al*., 1989, 1990). The first abnormalities were loosening of the surface (stratum corneum) of rumen papillae. Higher levels (25 and 47g Mg kg^{-1}DM) caused severe diarrhoea, drowsiness and severe degeneration of the stratified squamous rumen epithelium in steers. Serum Mg was linearly related to magnesium intakes and rose to >3 mmol l^{-1} after 71 days supplementation in steers (Chester-Jones *et al*., 1990). At such levels there is likely to be loss of muscle tone, deep tendon reflexes and muscle paralysis (Ebel and Günther, 1980). Increases in soft tissue Mg were, however, small and mostly non-significant. Early loss of appetite occurred with >13g Mg kg^{-1}DM (Gentry *et al*., 1978) but this may have been attributable to unpalatability of the source, MgO. Providing grazing animals with free access to magnesium-rich mineral mixtures containing 130g Mg kg^{-1}DM exacerbated diarrhoea in lambs (Suttle and Brebner, 1995).

- Concurrent, high intakes of K may increase laxative effects of Mg-rich, free-access minerals in ruminants by increasing digesta Mg in the large intestine.
- Individual intakes of Mg-rich mineral may be high enough to disturb gut function.

Excessive magnesium intakes increased risk of urolithiasis in goats (James and Chandron, 1975), sheep (Chester-Jones *et al*., 1989) and calves (Chester-Jones *et al*., 1990), particularly if phosphorus intakes are also high and raise urinary P. Addition of magnesium-mica to feeds as a pelleting agent may increase risk of urolithiasis. Addition of 0.2–0.6g $MgSO_4$ kg^{-1}DM to the sows diet relieved constipation but linearly decreased piglet survival (Hou *et al*., 2014).

Notes

[i] On average, hypomagnesaemic tetany affected 1–4% of dairy cows in New Zealand, USA and Europe (Cairney, 1964; Grunes *et al*., 1970; Whittaker and Kelly, 1982) and incidence could be as high as 20–30% in individual herds.

[ii] Transition from winter housing to spring pastures is accompanied by manifold changes in dietary composition, including marked increases in crude protein and fatty acid concentrations, which may inhibit spring pasture inhibit A_{Mg}. Transfer of ruminants from hay plus concentrates to grass or grass products raised rumen NH_3 as well as pH (Johnson *et al*., 1988; Johnson and Aubrey Jones, 1989) and infusion of NH_4Cl into the rumen lowered A_{Mg} without affecting *PDt* (Care *et al*., 1984). Supplementing diets with fat lowered AA_{Mg} and was thought to have formed insoluble magnesium soaps in the rumen, which were excreted in faeces (Kemp *et al*., 1966; Rahnema *et al*., 1994). However, calcium rather than magnesium soaps form at high fat intakes (Ferlay and Doreau, 1995) and degradation of soaps in the small intestine would release some bound Mg.

[iii] Fasting can cause a rapid fall in serum Mg while cessation of milking causes abrupt increases (Littledike and Goff, 1987). Plasma Mg is briefly increased, by about 25% after parturition (e.g. Goff and Horst, 1997), probably due to mobilization of Mg from resorbing bone, but the ionized proportion remains unchanged (Riond *et al*., 1995). Plasma Mg may be influenced indirectly by calcium-regulating hormones (calcitonin and PTH), by aldosterone (p. 188) (Littledike and Goff, 1987; Charlton and Armstrong, 1989), thyroxine and insulin (Ebel and Günther, 1980).

[iv] Values for vitreous humour Mg increase within 24 h of death but addition of a small volume (3% v/v) of 4% formaldehyde reduced anomalies for 72 h (McCoy *et al*., 2001b): anomalies can be caused by bacterial growth during sample storage.

[v] At high luminal magnesium concentrations an active process mediated by an Mg+/H+ exchanger, becomes the dominant process (Martens, 2016).

[vi] Contributions to total A_{Mg} are small when the AA_{Mg} coefficient is high (Field and Munro, 1977) but when high dietary K decreased absorption from the rumen, A_{Mg} from the hind gut increased on Mg-rich diets (2.5 or 3.4g kg^{-1}DM) (Dalley *et al*., 1997a): K supplementation had no such effect in cattle on diets with (1 or 2g Mg kg^{-1}DM) (Greene *et al*., 1983a).

[vii] Magnesium is less readily filtered at the glomerulus than most macro-minerals but sufficient escapes tubular reabsorption – once the renal threshold of 0.92 mmol l^{-1} is exceeded – to allow urine to become the major route of excretion (Ebel and Günther, 1980).

[viii] additional data came from experiments using dried grass diets (Schonewille *et al*., 1997a; Schonewille *et al*., 1999a, b; Jittakhot *et al*., 2004a), with and without concentrates: values for A_{Mg} were derived from AA_{Mg} by correcting for a fixed endogenous loss of 4mg kg^{-1}LW d^{-1}.

[ix] High dietary Ca and P may suppress A_{Mg} (Littledike and Goff, 1987). A supplement of 150g $CaCO_3$ d^{-1} for 4 days post-calving lowered serum Mg in cows (Roche *et al*., 2002) but no evidence for a Ca × Mg antagonism was found in other cattle studies (Weiss, 2004). In sheep, effects of adding Ca and P on AA_{Mg} were small (Chicco *et al*., 1973) but addition of DCP raised urine Mg on a diet based on sugarcane and soybean hulls (Zanetti *et al*., 2017). Addition of an ionophore, monensin, to concentrate-based rations for sheep and cattle usually increased A_{Mg}, chiefly from the rumen, by about 10% (Kirk *et al*., 1994).

[x] When calcined magnesite (cMgO), kieserite ($MgSO_4.H_2O$) and dolomitic limestone ($CaCO_3.MgCO_3$) were compared in early studies, the latter was least effective. The former sources increased pasture Mg from 2 to about 3g kg^{-1}DM and recommended application rates were much lower than for dolomitic limestone (1125 and 5600kg ha^{-1}, respectively) (Underwood and Suttle, 1999).

References

Adams, C.L., Hemingway, R.G. and Ritchie, N.S. (1996) Influence of manufacturing conditions on the bioavailability of magnesium in calcined magnesites measured *in vivo* and *in vitro. Journal of Agricultural Science, Cambridge* 127, 377–386.

Adediji, O. and Suttle, N.F. (1999) Influence of diet type, K and animal species on the absorption of magnesium by ruminants. *Proceedings of the Nutrition Society* 58, 31A.

Ahmed, F. and Mohammed, A. (2019) Magnesium: the forgotten electrolyte: a review on hypomagnesemia. *Medical Science* 7, 56.

Apple, J.K., Kegley, E.B., Maxwell, C.C., Rakes, L.K., Galloway, D. and Wistuba, T.J. (2005) Effects of magnesium and short-duration transportation on stress response, post-mortem muscle metabolism and meat quality in finishing swine. *Journal of Animal Science* 83, 1633–1645.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

ARC (1981) *The Nutrient Requirements of Pigs*. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Bacon, J.A., Bell, M.C., Miller, J.K., Ramsey, N. and Mueller, F.J. (1990) Effect of magnesium administration route on plasma minerals in Holstein calves receiving either adequate or insufficient magnesium in their diets. *Journal of Dairy Science* 73, 470–473.

Baker, R.M., Boston, R.C., Boyes, T.E. and Leever, D.D. (1979) Variations in the response of sheep to

experimental magnesium deficiency. *Research in Veterinary Science* 26, 129–133.

Bastien, R.W., Bradley, J.W., Pennington, B.L. and Ferguson, T.M. (1978) Bone strength and egg characteristics as affected by dietary minerals. *Poultry Science* 57, 1117.

Bell, S.T., McKinnon, A.E. and Sykes, A.R. (2006) Estimating the risk of hypomagnesaemic tetany in dairy herds. In: Kebreab, E., Dijkstra, J., Bannink, A., Gerrits, W.J.J. and France, J. (eds) *Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches.* CAB International, Wallingford, UK, pp. 211–228.

Blaxter, K.L., Rook, J.A.F. and MacDonald, A.M. (1954) Experimental magnesium deficiency in calves. 1. Clinical and pathological observations. *Journal of Comparative Pathology* 64, 157–175.

Blumer, C.C., Madden, F.J. and Walker, D.J. (1939) Hypocalcaemia, grass tetany or grass staggers in sheep. *Australian Veterinary Journal* 15, 2–27.

Bohman, V.R., Lesperance, A.L., Harding, G.D. and Grunes, D.L. (1969) Induction of experimental tetany in cattle. *Journal of Animal Science* 29, 99–102.

Bueno, L., Fioramonti, J., Geux, E. and Raissiguer, Y. (1980) Gastrointestinal hypomotility in magnesium deficient sheep. *Canadian Journal of Animal Science* 60, 293–301.

Cairney, I.M. (1964) Grass staggers in beef cattle: results of survey of disease in Hawke's Bay. *New Zealand Journal of Agriculture* 109, 45–49.

Care, A.D., Brown, R.C., Farrar, A.R. and Pickard, D.W. (1984) Magnesium absorption from the digestive tract of sheep. *Quarterly Journal of Experimental Physiology* 69, 577–587.

Charlton, J.A. and Armstrong, D.G. (1989) The effect of an intravenous infusion of aldosterone upon magnesium metabolism in the sheep. *Quarterly Journal of Experimental Physiology* 74, 329–337.

Chen, J., Liu, X. and Bian, L. (2013) Effects of short-term feeding magnesium before slaughter on blood metabolites and postmortem muscle traits of halothane-carrier pigs. *Asian-Australasian Journal of Animal Science* 26, 879–885.

Chester-Jones, H., Fontenot, J.P., Veit, H.P. and Webb, K.E. (1989) Physiological effects of feeding high levels of magnesium to sheep. *Journal of Animal Science* 67, 1070–1081.

Chester-Jones, H., Fontenot, J.P., Veit, H.P. and Webb, K.E. (1990) Physiological effects of feeding high levels of magnesium to steers. *Journal of Animal Science* 68, 4400–4413.

Chicco, C.F., Ammerman, C.B., Feaster, J.P. and Dunavant, B.G. (1973) Nutritional interrelationships of dietary calcium, phosphorus and magnesium in sheep. *Journal of Animal Science* 36, 986–993.

Chizzotti, M.L., Valadares Filho, S.-C., Tedeschi, L.O., Paulino, P.V.R., Paulino, M.F. *et al*. (2009) Net requirements of calcium, magnesium, sodium, phosphorus, and potassium for growth of Nellore×Red Angus bulls, steers, and heifers. *Livestock Science* 124, 242–247.

Costa e Siva, L.F., Valdares Filho, S.C., Rotta, P.F., Marcandes, M.I., Zanetti, D. *et al*. (2015) Mineral requirements for beef cattle. Section 9 in *Nutrient Requirements of Zebu and Crossbred Cattle,* BR-Corte, Viscondo do Rio Branco, Brazil, pp. 213–249.

Dalley, D.E., Isherwood, P., Sykes, A.R. and Robson, A.B. (1997a) Effect of intraruminal infusion of potassium on the site of magnesium absorption within the digestive tract of sheep. *Journal of Agricultural Science, Cambridge* 129, 99–106.

Dalley, D.E., Isherwood, P., Sykes, A.R. and Robson, A.B. (1997b) Effect of *in vitro* manipulation of pH on magnesium solubility in ruminal and caecal digesta in sheep. *Journal of Agricultural Science, Cambridge* 129, 107–112.

Dillon, J. and Scott, D. (1979) Digesta flow and mineral absorption in lambs before and after weaning. *Journal of Agricultural Science, Cambridge* 92, 289–297.

Donovan, G.A., Steenholdt, C., McGehee, K. and Lunduist, R. (2004) Hypomagnesaemia among cows in a confinement-housed herd. *Journal of the American Veterinary Medicine Association* 204, 96–99.

Dove, H., Masters, D.G. and Thompson, A.N. (2016) New perspectives on the mineral nutrition of livestock grazing cereal and canola crops. *Animal Production Science* 56.

Dove, H. and McMullen, K.G. (2009) Diet selection, herbage intake and liveweight gain in young sheep grazing dual-purpose wheats and sheep responses to mineral supplement. *Animal Production Science* 49, 749–775.

D'Souza, D.N., Warner, R.D., Dunshea, F.R. and Leury, B.J. (1999) Comparison of different dietary magnesium supplements on pork quality. *Meat Science* 51, 221–225.

Dua, K. and Care, A.D. (1995) Impaired absorption of magnesium in the aetiology of grass tetany. *British Veterinary Journal* 151, 413–426.

Ebel, H. and Günther, T. (1980) Magnesium metabolism: a review. *Journal of Clinical Chemistry and Clinical Biochemistry* 18, 257–270.

Engels, E.A.N. (1981) Mineral status and profiles (blood, bone and milk) of the grazing ruminant with special reference to calcium, phosphorus and magnesium. *South African Journal of Animal Science* 11, 171–182.

Ferlay, A. and Doreau, M. (1995) Influence of method of administration of rapeseed oil in dairy cows. 2. Status of divalent cations. *Journal of Dairy Science* 78, 2239–2246.

Fernandes, M.H.M.R., Resende, K.T., Tedeschi, L.O., Teixeira, I.A.M.A. and Fernandes, J.S. (2012) Macromineral requirements for the maintenance and growth of Boer crossbred kids. *Journal of Animal Science* 90, 4458–4466.

Field, A.C. and Munro, C.S.M. (1977) The effect of site and quantity on the extent of absorption of Mg infused into the gastro-intestinal tract of sheep. *Journal of Agricultural Science, Cambridge* 89, 365–371.

Field, A.C. and Suttle, N.F. (1979) Effect of high and low potassium intakes on the mineral metabolism of monozygotic twin cows. *Journal of Comparative Pathology* 89, 431–439.

Field, A.C., Woolliams, J.A. and Woolliams, C. (1986) The effect of breed of sire on the urinary excretion of phosphorus and magnesium in lambs. *Animal Production* 42, 349–354.

Frederick, B.R., van Heugten, E. and See, M.T. (2004) Timing of magnesium supplementation administered through drinking water to improve fresh and stored pork quality. *Journal of Animal Science* 82, 1454–1460.

Gabel, G. and Martens, H. (1986) The effect of ammonia on the magnesium metabolism of sheep. *Journal of Animal Physiology and Animal Nutrition* 55, 268–277.

Gardner, G.E., Jacob, R.H. and Pethick, D.W. (2001) The effect of magnesium oxide supplementation on muscle glycogen metabolism before and after exercise and at slaughter in sheep. *Australian Journal of Agricultural Research* 52, 723–729.

Gardner, J.A.A. (1973) Control of serum magnesium levels in sheep. *Research in Veterinary Science* 15, 149–157.

Gentry, R.P., Miller, W.J., Pugh, D.G., Neathery, M.W. and Bynum, J.B. (1978) Effects of feeding high magnesium to young dairy calves. *Journal of Dairy Science* 61, 1750–1754.

Goff, J.P. and Horst, R.L. (1997) Physiological changes at parturition and their relationship to metabolic disorders. *Journal of Dairy Science* 80, 1260–1268.

Grace, N.D. and MacRae, J.C. (1972) Influence of feeding regimen and protein supplementation on the sites of net absorption of magnesium in sheep. *British Journal of Nutrition* 27, 51–55.

Grace, N.D., Carr, D.H. and Reid, C.S.W. (1985) Secretion of sodium, potassium, phosphorus, calcium and magnesium via the parotid and mandibular saliva in sheep offered chaffed lucerne hay or fresh 'Grasslands Ruanui' ryegrass. *New Zealand Journal of Agricultural Research* 28, 449–455.

Grace, N.D., Shaw, H.L., Firth, E.C. and Gee, E.K. (2002a) Determination of digestible energy intake, and apparent absorption of macroelements of grazing, lactating Thoroughbred mares. *New Zealand Veterinary Journal* 50, 182–185.

Grace, N.D., Shaw, H.L., Firth, E.C. and Gee, E.K. (2002b) Determination of digestible energy intake, dry matter digestibility and mineral status of grazing, New Zealand Thoroughbred yearlings. *New Zealand Veterinary Journal* 50, 63–69.

Grace, N.D., Rogers, C.W., Firth, E.C., Faram, T.L. and Shaw, H.L. (2003) Digestible energy intake, dry matter digestibility and effect of calcium intake on bone parameters of Thoroughbred weanlings in New Zealand. *New Zealand Veterinary Journal* 51, 165–173.

Greene, L.W., Fontenot, J.P. and Webb, K.E. Jr (1983a) Site of magnesium and other macro-mineral absorption in steers fed high levels of potassium. *Journal of Animal Science* 57, 503–513.

Greene, L.W., Webb, K.E. and Fontenot, J.P. (1983b) Effect of potassium level on site of absorption of magnesium and other macroelements in sheep. *Journal of Animal Science* 56, 1214–1221.

Greene, L.W., Baker, J.F., Byers, F.M. and Schelling, G.T. (1985) Incidence of grass tetany in a cow herd of a five-breed diallel during four consecutive years. *Journal of Animal Science* 61 (Suppl. 1), 60.

Greene, L.W., Solis, J.C., Byers, F.M. and Schelling, G.T. (1986) Apparent and true digestibility of magnesium in mature cows of five breeds and their crosses. *Journal of Animal Science* 63, 189–196.

Grunes, D.L., Stout, P.R. and Brownell, J.R. (1970) Grass tetany of ruminants. *Advances in Agronomy* 22, 331.

Güenter, W. and Sell. J.L. (1973) Magnesium absorption and secretion along the gastrointestinal tract of the chicken. *Journal of Nutrition* 103, 875–881.

Güenter, W. and Sell, J.L. (1974) A method for determining 'true' availability of magnesium from foodstuffs using chickens. *Journal of Nutrition* 104, 1446–1457.

Hajj, R.N. and Sell, J.L. (1969) Magnesium requirement of the laying hen for reproduction. *Journal of Nutrition* 97, 441–448.

Hamilton, D.N., Elis, M., McKeith, F.K. and Eggert, J.M. (2003) Effect of level, source and time of feeding prior to slaughter of supplementary dietary magnesium on pork quality. *Meat Science* 65, 853–857.

Harmon, B.G., Liu, C.T., Jensen, A.H. and Baker, D.H. (1976) Dietary magnesium levels for sows during gestation and lactation. *Journal of Animal Science* 42, 860–865.

Harris, D.J., Lambell, R.G. and Oliver, C.J. (1983) Factors predisposing beef and dairy cows to grass tetany. *Australian Veterinary Journal* 60, 230–234.

Henry, P.R. and Benz, S.A. (1995) Magnesium bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 239–256.

Herd, R.P. (1966) Fasting in relation to hypocalcaemia and hypomagnesaemia in lactating cows and ewes. *Australian Veterinary Journal* 42, 269–272.

Hess, J.B. and Britton, W.M. (1997) Effect of dietary magnesium excess in white Leghorn hens. *Poultry Science* 76, 703–710.

Holtenius, K., Kronqvist, C., Briland, E. and Spörndly, R. (2008) Magnesium absorption by lactating dairy cows on a grass silage-based diet supplied with different potassium and magnesium levels. *Journal of Dairy Science* 91, 743–748.

Hou, W.X., Cheng, S.Y., Liu, S.T., Shi, B.M. and Shan, A.S. (2014) Dietary supplementation of magnesium

sulfate during late gestation and lactation affects the milk composition and immunoglobulin levels in sows. *Asian-Australasian Journal of Animal Sciences* 27, 1469–1477.

Hu, P., Yang, H., Lv, B., Zhao, D., Wang, J. and Zhu, W. (2019) Dynamic changes of fatty acids and minerals in sow milk during lactation. *Journal of Animal Physiology and Animal Nutrition* 103, 603–611.

Hurley, L.A., Greene, L.W., Byers, F.M. and Carstens, G.E. (1990) Site and extent of apparent magnesium absorption by lambs fed different sources of magnesium. *Journal of Animal Science* 68, 2181–2187.

James, C.S. and Chandran, K. (1975) Enquiry into the role of minerals in experimental urolithiasis in goats. *Indian Veterinary Journal* 52, 251–258.

Ji, S., Xu, G., Diao, Q., Deng, K., Jiang, C., Tu, Y. and Zhang, N. (2015) Macromineral requirements of Dorper×thin-tailed Han crossbred female lambs. *Journal of Intergrative Agriculture* 14, 1617–1626.

Jittakhot, S., Schonewille, J., Wouterse, H.S., Yuangklang, C. and Beynen, A.C. (2004a) Apparent magnesium absorption in dry cows fed at 3 levels of potassium and two levels of magnesium intake. *Journal of Dairy Science* 87, 379–385.

Jittakhot, S., Schonewille, J., Wouterse, H.S., Yuangklang, C. and Beynen, A.C. (2004b) The relationships between potassium intakes, transmural potential difference of the rumen epithelium and magnesium absorption in wethers. *British Journal of Nutrition* 91, 183–189.

Johnson, C.L. and Aubrey Jones, D.A. (1989) Effect of change of diet on the mineral concentration of rumen fluid, on magnesium metabolism and on water balance in sheep. *British Journal of Nutrition* 61, 583–594.

Johnson, C.L., Helliwells, S.H. and Aubrey Jones, D.A. (1988) Magnesium metabolism in the rumens of lactating dairy cows fed on spring grass. *Quarterly Journal of Experimental Physiology* 73, 23–31.

Jumba, I.O., Suttle, N.F., Hunter, E.A. and Wandiga, S.O. (1995) Effects of soil origin and composition and herbage species on the mineral composition of forages in the Mount Elgan region of Kenya. 1. Calcium, phosphorus, magnesium and sulfur. *Tropical Grasslands* 29, 40–46.

Kadarmideen, H.N., Thompson, R. and Simm, G. (2000) Linear and threshold model genetic parameters for disease, fertility and milk production in dairy cattle. *Journal of Animal. Science* 71, 411–419.

Kemp, A. (1971) The effects of K and N dressings on the mineral supply of grazing animals. In: *Proceedings of the 1st Colloquium of the Potassium Institute*. IBS, Wageningen, The Netherlands, pp. 1–14.

Kemp, A. and 't Hart, M.L. (1957) Grass tetany in grazing milking cows. *Netherlands Journal of Agricultural Science* 5, 4–17.

Kemp, A., Deijs, W.B., Hemkes, O.J. and Van Es, A.J.H. (1961) Hypomagnesaemia in milking cows: intake and utilization of magnesium from herbage by lactating cows. *Netherlands Journal of Agricultural Science* 9, 134–149.

Kemp, A., Deijs, W.B. and Kluvers, E. (1966) Influence of higher fatty acids on availability of magnesium in milking cows. *Netherlands Journal of Agricultural Science* 14, 290–295.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S.L., Bearson, B.L, Shurson, G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Khorasani, G.R., Janzen, R.A., McGill, W.B. and Kenelly, J.J. (1997) Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage or alfalfa silage. *Journal of Animal Science* 75, 239–248.

Kirk, D.J., Fontenot, J.P. and Rahnema, S. (1994) Effects of lasalocid and monensin on digestive tract flow and partial absorption on minerals in sheep. *Journal of Animal Science* 72, 1029–1103.

Kumssa, D.B., Penrose, B., Bone, P.A., Lovatt, J.A., Broadley, M.R., Kendall, N.R. and Ander, E.L. (2019) A reconnaissance survey of farmers' awareness of hypomagnesaemic tetany in UK cattle and sheep farms. *PLOS ONE* 14, e0223868. https://doi.org/10.1371/journal.pone.0223868

Lee, S.R. and Britton, W.M. (1987) Magnesium-induced catharsis in chicks. *Journal of Nutrition* 117, 1907–1912.

Littledike, E.T. and Goff, J.P. (1987) Interactions of calcium, phosphorus and magnesium and vitamin D that influence their status in domestic meat animals. *Journal of Animal Science* 65, 1727–1743.

Littledike, E.T., Wittam, J.E. and Jenkins, T.G. (1995) Effect of breed, intake and carcass composition on the status of several macro and trace minerals of adult beef cattle. *Journal of Animal Science* 73, 2113–2119.

Liu, Y., Guo, Y., Wang, Z. and Nie, W. (2007) Effects of source and level of magnesium on catalase activity and its gene expression in livers of broiler chickens. *Archives of Animal Nutrition* 61, 292–300.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.). Rowett Research Services, Aberdeen, UK.

Mahan, D.C. and Shields, R.G., Jr (1998) Macro- and micromineral composition of pigs from birth to 145 kilograms of body weight. *Journal of Animal Science* 76, 506–512.

Mahan, D.C., Watts, M.R. and St-Pierre, N. (2009) Macro- and micromineral composition of fetal pigs and their accretion rates during fetal development. *Journal of Animal Science* 87, 2823–2832.

Martens, H. (1983) Saturation kinetics of magnesium efflux across the rumen wall in heifers. *British Journal of Nutrition* 49, 153–158.

Martens, H. (2016) Diseases of dairy animals: non-infectious diseases: grass tetany. In: *Reference Module in Food Science,* Elsevier, pp. 1–7.

Martens, H. and Blume, I. (1986) Effect of intraruminal sodium and potassium concentrations and of the transmural potential difference on magnesium absorption from the temporarily isolated rumen of sheep. *Quarterly Journal of Experimental Physiology* 71, 409–415.

Martens, H. and Schweigel, M. (2000) Pathophysiology of grass tetany and other hypomagnesemias: implications for clinical management. *Veterinary Clinics of North America: Food Animal Practice* 16, 339–368.

Martens, H. and Stumpff, F. (2019) Assessment of magnesium intake according to requirement in dairy cows. *Animal Physiology and Animal Nutrition* 103, 1023–1029.

Martens, H., Kubel, O.W., Gabel, G. and Honig, H. (1987) Effects of low sodium intake on magnesium metabolism of sheep. *Journal of Agricultural Science, Cambridge* 108, 237–243.

Masters, D.G. (2018) Practical implications of mineral and vitamin imbalance in grazing sheep. *Animal Production Science* 58, 1418–1450.

Masters, D.G., Norman, H.C. and Thomas, D.T. (2019) Minerals in pastures: are we meeting the needs of livestock? *Crop and Pasture Science.* https://doi.org/10.1071/CP18546

Matsui, T., Yans, H., Kawabata, T. and Harumoto, T. (1994) The effect of suppressing bone resorption on magnesium metabolism in sheep (*Ovis aries*). *Comparative Biochemistry and Physiology* 107A, 233–236.

Mayland, H.F., Grunes, D.L. and Lazar, V.A. (1976) Grass tetany hazard of cereal forages based upon chemical composition. *Agronomy Journal* 68, 665–667.

Mayo, R.H., Plumlee, M.P. and Beeson, W.M. (1959) Magnesium requirement of the pig. *Journal of Animal Science* 18, 264–274.

McCoy, M.A., Goodall, E.A. and Kennedy, D.G. (1993) Incidence of hypomagnesaemia in dairy and suckler cows in Northern Ireland. *Veterinary Record* 132, 537.

McCoy, M.A., Goodall, E.A. and Kennedy, D.G. (1996) Incidence of bovine hypomagnesaemia in Northern Ireland and methods of supplementation. *Veterinary Record* 138, 41–43.

McCoy, M.A., Bingham, V., Hudson, J., Cantley, L., Hutchinson, T. *et al*. (2001a) Postmortem biochemical markers of experimentally induced hypomagnesaemic tetany in sheep. *Veterinary Record* 148, 233–237.

McCoy, M.A., Hudson, J., Hutchinson, T., Davison, G., Fitzpatrick, D.A. and Kennedy, D.G. (2001b) Postsampling stability of eye fluid magnesium concentrations in cattle. *Veterinary Record* 148, 312–313.

McCoy, M.A., Hutchinson, T., Davison, G., Fitzpatrick, D.A., Rice, D.A. and Kennedy, D.G. (2001c) Postmortem biochemical markers of experimentally induced hypomagnesaemic tetany in cattle. *Veterinary Record* 148, 268–273.

McCoy, M.A., Young, P.B., Edgar, E.M., McCarville, G., Fitzpatrick, D.A. and Kennedy, D.G. (2002) Biochemical changes induced by hypomagnesaemia in lactating cows and ewes. *Veterinary Record* 150, 176–181.

McGillivray, J.J. and Smidt, M.J. (1975) Biological evaluation of magnesium sources. *Poultry Science* 54, 1792–1793.

McGillivray, J.J. and Smidt, M.J. (1977) Energy level, potassium, magnesium and sulfate interaction in broiler diets. *Poultry Science* 56, 1736–1737.

McGrath, S., Bhanugopan, M., Virgona, J., Dove, H. and Friend, M. (2011) Mineral supplementation of lambing merino ewes grazing dual-purpose wheat under Australian conditions increases twin-lamb growth rate. *Advances in Animal Biosciences* 2, 247.

McGrath, S.R., Lievaart, J.J., Virgona, A.J., Bhanugopan, M.S. and Friend, M.A. (2013) Factors involved in high ewe losses in winter lambing flocks grazing dual-purpose wheat in southern New South Wales: a producer survey. *Animal Production Science* 53.

Menzies, F.D., Bryson, D.G. and McCallion, T. (1994) Bovine mortality survey. *Department of Agriculture for Northern Ireland Publication*.

Merriman, L.A., Walk, C.L., Parsons, C.M. and Stein H.H. (2016) Effects of tallow, choice white grease, palm oil, corn oil, or soybean oil on apparent total tract digestibility of minerals in diets fed to growing pigs. *Journal of Animal Science* 94, 4231–4238.

Miller, E.R., Ullrey, D.E., Zutaut, C.L., Baltzer, B.V., Schmidt, D.A., Hoefer, J.A. and Luecke, R.W. (1965) Magnesium requirement of the baby pig. *Journal of Nutrition* 85, 13–20.

Miller, K.B., Caton, J.S. and Finley, J.W. (2006) Manganese depresses rat heart muscle respiration. *BioFactors* 28, 33–46.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Moseley, G. and Baker, D.H. (1991) The efficacy of a high magnesium grass cultivar in controlling hypomagnesaemia in grazing animals. *Grass and Forage Science* 46, 375–380.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2005) *Mineral Tolerances of Animals,* 2nd edn. National Academy of Sciences, Washington, DC.

Nugara, D. and Edwards, H.M. Jr (1963) Influence of dietary Ca and P levels on the Mg requirements of the chick. *Journal of Nutrition* 80, 181–184.

Osteras, O., Solbu, H., Refsdal, A.O., Roalkvam, T., Filseth, O. and Minsaas, A. (2007) Results and evaluation of thirty years of health recordings in the Norwegian dairy cattle population. *Journal of Dairy Science* 90, 4483–4497.

Parkins, J.J. and Fishwick, G. (1998) Magnesium hydroxide as a novel herbage spray supplement for lactating cows and ewes. *Animal Science* 66, 93 (abstract).

Partridge, I.G. (1978) Studies on digestion and absorption in the intestines of growing pigs. 3. Net movements of mineral nutrients in the digestive tract. *British Journal of Nutrition* 39, 527–537.

Pauli, J.V. and Allsop, T.P. (1974) Plasma and cerebrospinal fluid magnesium, calcium and potassium in dairy cows with hypomagnesaemic tetany. *New Zealand Veterinary Journal* 22, 227–231.

Plush, K., Weaver, A., Staveley, L. and van Wettere, W. (2018) Maternal magnesium sulfate supplementation in a pre-farrow diet improves factors important for piglet viability. *Animals (Basel)* 8, 185. DOI:10.3390/ani8100185.

Rahnema, S., Wu, Z., Ohajuruka, O.A., Weiss, W.P. and Palmquist, D.L. (1994) Site of mineral absorption in lactating cows fed high-fat diets. *Journal of Animal Science* 72, 229–235.

Ram, L., Schonewille, J. Th, Martens, H., Van't Klooster, A. Th, Wouterse, H. and Beynen, A.C. (1998) Magnesium absorption by wethers fed potassium bicarbonate in combination with different magnesium concentrations *Journal of Dairy Science* 81, 2485–2492.

Reid, R.L., Jung, G.A., Roemig, I.J. and Kocher, R.E. (1978) Mineral utilisation in lambs and guinea pigs fed Mg-fertilized grass and legume hays. *Agronomy Journal* 70, 9–14.

Riond, J.-L., Kocabagli, N., Spichiger, V.E. and Wanner, M. (1995) The concentration of ionized magnesium in serum during the peri-parturient period of non-paretic cows. *Veterinary Research Communications* 19, 195–203.

Robson, M.R., Field, A.C. and Sykes, A.R. (1997) A model for magnesium metabolism in sheep. *British Journal of Nutrition* 78, 975–992.

Robson, M.R., Sykes, A.R., McKinnon, A.E. and Bell, S.T. (2004) A model for magnesium metabolism in young sheep. *British Journal of Nutrition* 91, 73–79.

Roche, J.R. and Berry, D.P. (2006) Periparturient climatic, animal, and management factors influencing incidence of milk fever in grazing systems. *Journal of Dairy Science* 89, 2775–2783.

Roche, J.R., Morton, J. and Kolver, E.S. (2002) Sulfur and chlorine play a non-acid base role in periparturient calcium homeostasis. *Journal of Dairy Science* 85, 3444–3453.

Roche, J.R., Dalley, D., Moate, P., Grainger, C., Rath, M. and O'Mara, F.O. (2003) Dietary cation–anion difference and the health and production of pasture-fed dairy cows. 2. Non-lactating peri-parturient cows. *Journal of Dairy Science* 86, 979–987.

Roche, J.R., Petch, S. and Kay, J.K. (2005) Manipulating the dietary cation–anion difference via drenching to early-lactation dairy cows grazing pasture. *Journal of Dairy Science* 88, 264–276.

Rogers, P.A.M. (1979) Hypomagnesaemia and its clinical syndromes in cattle: a review. *Irish Veterinary Journal* 33, 115–124.

Rogers, P.A.M. and Poole, D.B.R. (1976) Control of hypomagnesaemia in cows: a comparison of magnesium acetate in the water supply with magnesium oxide in the feed. *Irish Veterinary Journal* 30, 129–136.

Rook, J.A.F. and Storry, J.E. (1962) Magnesium in the nutrition of ruminants. *Nutrition Abstracts and Reviews* 32, 1055–1076.

Ruiz-Ascacibar, I., Stoll, P., Bee, G. and Schlegel, P. (2019) Dynamics of the mineral composition and deposition rates in the empty body of entire males, castrates and female pigs. *Animal* 13, 50-58.

Sahin, N., Onderci, M., Sahin, K., Cikim, G. and Kucuk, O. (2005) Magnesium proteinate is more protective than magnesium oxide in heat-stressed quail. *Journal of Nutrition* 135, 1732–1737.

Sansom, B.F., Bunch, K.G. and Dew, S.M. (1982) Changes in plasma calcium, magnesium, phosphorus and hydroxyproline concentrations in ewes from twelve weeks before until three weeks after lambing. *British Veterinary Journal* 138, 393–401.

Schonewille, J., Ram, L., Van Klooster, A., Wouterse, H. and Beynen, A.C. (1997a) Intrinsic potassium in grass silage and magnesium absorption in dry cows. *Livestock Production Science* 48, 99–110.

Schonewille, J., Ram, L., Van Klooster, A., Wouterse, H. and Beynen, A.C. (1997b) Native corn starch versus either cellulose or glucose in the diet and the effects on apparent magnesium absorption in goats. *Journal of Dairy Science* 80, 1738–1743.

Schonewille, J., Beynen, A.C., Van Klooster, A. and Wouterse, H. (1999a) Dietary potassium bicarbonate and potassium citrate have a greater inhibitory effect than does potassium chloride on magnesium absorption in wethers. *Journal of Nutrition* 129, 2043–2047.

Schonewille, J., t'Klooster, A.T., Wouterse, H. and Beynon, A.C. (1999b) Effects of intrinsic potassium in artificially dried grass and supplemental potassium bicarbonate on apparent magnesium absorption in dry cows. *Journal of Dairy Science* 82, 1824–1830.

Schonewille, J., Wouterse, H. and Beynen, A.C. (2002) The effect of iso-energetic replacement of artificially dried grass by pelleted concentrate on apparent magnesium absorption in dry cows. *Livestock Production Science* 76, 59–69.

Schonewille, J., Everts, H., Jittakhot, S. and Beynen, A.C. (2008) Quantitative prediction of magnesium absorption in dairy cows. *Journal of Dairy Science* 91, 271–278.

Sedgh-Gooya, S. and Torki, M. (2018) Influence of dietary supplemental chromium and magnesium on performance and metabolic parameters of laying hens subjected to heat stress. *Journal of Applied Animal Research* 46, 1469–1477.

Sell, J.L. (1979) Magnesium nutrition of poultry and swine. In: *Proceedings of the Second Annual International Minerals Conference, St Petersburg Beach, Florida, Illinois*. International Minerals and Chemical Corporation.

Shastak, Y., and Rodehutscord, M. (2015) A review of the role of magnesium in poultry nutrition. *World's Poultry Science Journal* 71, 125–138.

Shils, M.E. (1997) Magnesium. In: O'Dell, B.L. and Sunde, R.A. (eds) *Handbook of Nutritionally Essential Mineral Elements*. Marcel Dekker, New York, pp. 117–152.

Sjollema, B. and Seekles, L. (1930) Uber Storungen des mineralen Regulationsmechanismus bei Krankheiten des Rindes. (Ein Beitrag zur Tetaniefrage.) *Biochemische Zeitschrift* 229, 338–380.

Soder, K.J. and Stout, W.L. (2003) The effect of soil type and fertilisation level on mineral concentration in pasture: potential relationships to ruminant performance and health. *Journal of Animal Science* 81, 1603–1610.

Stevenson, M.A., Williamson, N.B. and Russell, D.J. (2003) Nutrient balance in the diet of spring-calving, pasture-fed dairy cows. *New Zealand Veterinary Journal* 51, 8188.

Stewart, A.J. (2011) Magnesium disorders in horses Veterinary Clinics of North America. *Equine Practice* 27, 149–163.

Stuedemann, J.A., Wilkinson, S.R. and Lowrey, R.S. (1984) Efficacy of a large magnesium alloy rumen bolus in the prevention of hypomagnesaemic tetany in cows. *American Journal of Veterinary Research* 45, 698–702.

Sutherland, R.J., Bell, K.C., McSporran, K.D. and Carthew, G.W. (1986) A comparative study of diagnostic tests for the assessment of herd magnesium status in cattle. *New Zealand Veterinary Journal* 34, 133–135.

Suttle, N.F. and Brebner, J. (1995) A putative role for larval nematode infection in diarrhoeas which did not respond to anthelmintic drenches. *Veterinary Record* 137, 311–316.

Suttle. N.F. and Field, A.C. (1966) Studies on magnesium in ruminant nutrition. 6. Effect of intake of water on the metabolism of magnesium, calcium, sodium, potassium and phosphorus in sheep. *British Journal of Nutrition* 20, 609–619.

Suttle. N.F. and Field, A.C. (1967) Studies on magnesium in ruminant nutrition. 8. Effect of increased intakes of potassium and water on the metabolism of magnesium, phosphorus, sodium, potassium and calcium in sheep. *British Journal of Nutrition* 21, 819–831.

Suttle. N.F. and Field, A.C. (1969) Studies on magnesium in ruminant nutrition. 9. Effect of potassium and magnesium intakes on development of hypomagnesaemia in sheep. *British Journal of Nutrition* 23, 81–90.

Suttle, N.F., Field, A.C., Nicolson, T.B., Mathieson, A.O., Prescott, J.H.D., Scott, N. and Johnson, W.S. (1980) Some problems in assessing the physiological and economic significance of hypocupraemia in beef suckler herds. *Veterinary Record* 106, 302–304.

Texeira, I.A.M.A., Resende, K.T., Silva, A.M.A., Sobrinho, A.G.S., Harter, C.J. and Sader, A.P.O. (2013) Mineral requirements of wool and hair lambs. *Revista Brasiliera de Zootechnica* 42, 347–53.

't Hart, M.L. and Kemp, A. (1956) De invloed van de weersomstandigheden op het optreden van kopziekte bij rundvee. *Tijdschrift voor Diergeneeskunde* 81, 84–95.

Theilin, M., Sedcole, J.R. and Sykes, A.R. (2001) Changes in plasma and milk magnesium concentrations in pasture-fed dairy cows in early lactation. *Proceedings of the New Zealand Society of Animal Production* 61, 152–155.

Tomas, F.M. and Potter, B.J. (1976a) Interaction between site of magnesium absorption in the digestive tract of the sheep. *Australian Journal of Agricultural Research* 27, 437–446.

Tomas, F.M. and Potter, B.J. (1976b) The site of magnesium absorption from the ruminant stomach. *British Journal of Nutrition* 36, 37–45.

Tongyai, S., Raysigguer, Y., Molta, C., Gueux, E., Maurois, P. and Heaton, F.W. (1989) Mechanism of increased erythrocyte membrane fluidity during magnesium deficiency in rats. *American Journal of Physiology* 257, 270–276.

Underwood, E.J. and Suttle, F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

van de Braak, A.E., van't Klooster, A.T. and Malestein, A. (1987) Influence of a deficient supply of magnesium during the dry period on the rate of calcium mobilisation by dairy cows at parturition. *Research in Veterinary Science* 42, 101–108.

Weiss, W.P. (2004) Macromineral digestion by lactating dry cows: factors affecting digestibility of magnesium. *Journal of Dairy Science*, 87, 2167–2171.

Whittaker, D.A. and Kelly, J.M. (1982) Incidence of clinical and subclinical hypomagnesaemia in dairy cows in England and Wales. *Veterinary Record* 110, 450–451.

Wilson, G.F. (1980) Effects of magnesium supplements on the digestion of forages and milk production of cows with hypomagnesaemia. *Animal Production* 31, 153–157.

Wu, C.L. and Britton, W.M. (1974) The influence of phosphorus on magnesium metabolism in the chick. *Poultry Science* 53, 1645 (abstract).

Wylie, M.J., Fontenot, J.P. and Greene, L.W. (1985) Absorption of magnesium and other macrominerals in sheep infused with potassium in different parts of the digestive tract. *Journal of Animal Science* 61, 1219–1229.

Woyengo, T., Cowieson, A., Adeola, O. and Nyachoti, C. (2009) Ileal digestibility and endogenous flow of minerals and amino acids: responses to dietary phytic acid in piglets. *British Journal of Nutrition* 102, 428–433.

Woyengo, A., Adeola, O., Udenigwe, C.M. and Nyachoti, C.C. (2010) Gastro-intestinal digesta pH, pepsin activity and soluble mineral concentration responses to supplemental phytic acid and phytase in piglets. *Livestock Science* 134, 91–93.

Xin, Z., Tucker, W.B. and Hemken, R.W. (1989) Effect of reactivity rate and particle size of magnesium oxide on magnesium availability, acid–base balance, mineral metabolism and milking performance of dairy cows. *Journal of Dairy Science* 72, 462–470.

Young, P.W., Rys, G. and O'Connor, M.B. (1981) Hypomagnesaemia and dairy production. *Proceedings of the New Zealand Society of Animal Production* 41, 61–67.

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle, T.E., Silva, B.C. *et al*. (2017) Estimating mineral requirements of Nellore beef bulls fed with or without inorganic mineral supplementation and the influence on mineral balance. *Journal of Animal Science* 95, 1696–1706.

Zang, J., Chen, J., Tian, J., Wang, A., Liu, H. et al. (2014) Effects of magnesium on the performance of sows and their piglets. *Journal of Animal Science and Biotechnology* 5(1), 39.

6 Phosphorus

An early indication that ruminants needed phosphorus (P) in their diet to remain healthy came from pioneering work on two debilitating diseases of cattle and sheep ('styfziekte' and 'lamziekte'), occurring on veldt pastures in South Africa's Northern Cape. Both diseases were characterized by high mortality, poor growth, infertility, fragile bones and periodic osteophagia (Theiler, 1912) and prevented by feeding P-containing supplements (Theiler and Green, 1932; DuToit *et al.*, 1940). Prolonged P-deprivation in experiments demineralized bones (Stewart, 1934–35). Pastoral areas lacking in P were subsequently identified on many continents, including Australia and North America and requirements for P were estimated for lambs (Beeson *et al.*, 1944). However, the veldt supplements had provided protein as well as P and the specific role of P was exaggerated. Fifty years later, optimal P supplementation for cattle was still under investigation on veldt pastures (De Waal *et al.*, 1996; de Brouwer *et al.*, 2000) and elsewhere (Karn, 2001). Provision of supplementary cereals, outdoors or indoors, alleviates all risk of inadequacy because rumen microbes secrete phytases that release P from phytate, abundant in all grains. The discovery that adding exogenous phytase to chick diets could liberate P (Nelson *et al.*, 1971) initiated intense research activity, which continues unabated. The wise use of phytase supplements promises an era of non-ruminant nutrition no longer constrained by phytate (Cowieson *et al.*, 2016). Indeed, the outstanding challenge is the management of P excess, not deficit. Profligate use of inorganic P (P_i) supplements in feeds and fertilizers enriched soils and watercourses with P in intensively farmed areas, even leading to mandatory restrictions on farm 'inputs' of P (Jongbloed and Henkens, 1996). Animal requirements for P and the capacity of feeds to meet those needs must be defined more precisely to minimize pollution. However, describing needs and feeds for pigs and poultry in terms of standardized P digestibility (NRC, 2012; WPSA, 2013) is unlikely to help, for reasons explained later.

Functions of Phosphorus

Phosphorus is the second most abundant mineral in the animal body and found predominantly (80%) in bones and teeth. Phosphorus deprivation causes changes in bone composition that are similar to those described for calcium deprivation (see p. 133) but structural differences arise because phosphate (PO_4^{2-}) is required to form as well as mineralize organic bone matrix. In the young, PO_4^{2-} is essential for apoptosis of chondrocytes and closure of the growth plate (Goretti *et al.*, 2017). The remaining P (20%) is widely distributed in fluids and soft tissues, performing a range of essential, life-sustaining functions. Phosphorus is a component of deoxy- and ribonucleic acids, which are essential for cell growth and differentiation. As phospholipid, phosphorus contributes to cell membrane fluidity and integrity and the myelination of nerves; PO_4^{2-} helps to maintain osmotic and acid–base balance. Exchanges of energy-rich PO_4 bonds between adenosine mono- di- and triphosphates (AMP, ADP and ATP), allow phosphorus to drive a host of metabolic functions, including cell-signalling glucogenesis, gluconeogenesis, fatty acid transport, amino acid synthesis, muscle growth and mineral metabolism via sodium/potassium ion pumps (Goretti *et al.*, 2017). Phosphorus regulates activity of fibroblast growth factor (FGF23) (Wang *et al.*, 2018). Rumen and caeco-colonic microflora require phosphorus for many of the above functions and microbial protein synthesis may be impaired on low-P diets (Petri *et al.*, 1989; Ternouth and Sevilla, 1990b). Glycolytic metabolism is abnormal in erythrocytes from P-deficient cattle (Wang *et al.*, 1985).

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In a fascinating twist, phytate derivatives became regarded as important constituents of basolateral membranes, where they 'anchored' alkaline phosphatases (Vucenik *et al.*, 2004), rather than anti-nutrients. Phytate is synthesized in tissues, where receptors for inositol triphosphate in the sarcoplasmic reticulum provide Ca^{2+} channels: ion affinities for Fe3+ provide antioxidant capacity and stimuli for cell replication and death, gene regulation, RNA export, DNA repair and cell signalling (Bohn *et al.*, 2008; Narayanan *et al.*, 2012).

Signs that Diets Lack Phosphorus

Poor appetite

Appetite is lost in both growing and mature animals of all species and illustrated by early data for yearling cattle in Table 6.1 (Little, 1968) and young pigs (Table 6.2). Anorexia usually takes weeks or months to develop in lambs (Wan Zahari *et al.*, 1990), beef cattle (Call *et al.*, 1978, 1986; Gartner *et al.*, 1982) and dairy cows (Call *et al.*, 1986). Rumen-based as well as systemic mechanisms (Ternouth and Sevilla, 1990a, b) may become involved because rumen P can fall below 3 mmol l^{-1}, the minimum required by rumen microbes (Wan Zahari *et al.*, 1990). Earlier reductions in appetite occurred in ruminants given semi-purified diets, very low in P (Fig. 4.8; Miller *et al.*, 1987; Ternouth and Sevilla, 1990a), which stimulate little or no rumination, thus depriving rumen microbes of salivary as well as dietary P. Reduced egg production in P-deprived hens was attributed to reduced food intake (Bar and Hurwitz, 1984). However, growth in newly weaned pigs deprived of P can be retarded before appetite is impaired (Reinhart and Mahan, 1986).

- Mechanisms by which appetite is reduced have yet to be elucidated but may involve regulation by insulin (Yan *et al.*, 2011).

Pica

Loss of appetite is often accompanied by a craving for and consumption of abnormal materials such as soil, wood, flesh and bones. The depraved appetite (pica) may be general (allotriophagia) or specific (osteophagia, a craving for bones; sarcophagia, a craving for flesh). Pica is not a specific sign and also seen in animals suffering from lack of Na, K, energy and protein (Underwood *et al.*, 1940). However, bone-chewing was induced in cows by feeding a low-P diet and simultaneously exteriorizing the parotid salivary duct to prevent recycling of P. Osteophaghia stopped within an hour of intravenously infusing Na_2PO_4 (Underwood and Suttle, 1999). Pica had disastrous consequences in areas such as South Africa, where carcases became infected with *Clostridium botulinum* and toxin formation during putrefaction caused botulism (toxic paralysis) and death. The need to prevent botulism in grazing livestock revealed the underlying need for P (Theiler, 1912). Losses of sheep and cattle from botulism, once widespread (Underwood *et al.*, 1939) are now controllable by vaccination.

Abnormalities of bones and teeth

Skeletal abnormalities are almost identical to those described for Ca deprivation (p. 66 *et seq.*) because bone mineral cannot be produced if either mineral is lacking. This was illustrated by parallel studies of phosphorus and calcium requirements in baby pigs (compare Table 6.2 with Table 4.2) (Miller *et al.*, 1964), in which rickets was induced, whichever element was lacking.

Table 6.1. Effect of phosphorus supplementation on growth and feed consumption of yearling cattle given Townsville lucerne (*Stylosanthes humilis*) containing 10% crude protein and 0.07% P for 8 weeks (mean values ± standard error). (From Little, 1968)

Rate of supplementation with P	P intake (g day^{-1})	Weight gain (kg $week^{-1}$)	DMI (kg day^{-1})	Blood P (mg l^{-1})[a]
Nil	3.3 ± 0.1	0.9 ± 0.2	5.2 ± 0.1	33 ± 1.0
Low	7.4 ± 0.1	1.7 ± 0.2	5.8 ± 0.4	60 ± 1.0
Medium	11.4 ± 0.1	1.7 ± 0.2	6.0 ± 0.2	63 ± 3.0
High	15.5 ± 0.3	2.7 ± 0.7	6.0 ± 0.5	67 ± 0.4

[a]Divide by 3 l to give mmol l^{-1}.

Table 6.2. Effects of dietary phosphorus (P) concentration on selected performance, blood and skeletal and indices of P status in baby pigs given a synthetic milk diet from 0–6 weeks of age (Trial 1; Miller *et al.*, 1964).

		Dietary P (g kg^{-1} DM)		
Index of P status	Criterion	2	4	6
Performance	Liveweight gain (g d^{-1})	130	290	290
	Dry matter intake (g)	240	380	370
	Feed/gain (g g^{-1})	1.85	1.31	1.28
Blood serum	P (mmol l^{-1})	1.03	2.71	3.03
	Ca (mmol l^{-1})	3.20	3.05	2.58
	Alkaline phosphatase (U l^{-1})	20.5	8.3	4.5
Bone serum	Humerus ash (%)	33.4	44.1	47.5
	Femur (specific gravity)	1.11	1.15	1.17
	Eighth rib weight (g)	3.30	4.49	4.67
	Femur breaking load (kg)	24	61	81

In broiler chicks, 'phosphorus rickets' can be distinguished from 'calcium rickets' by an elongated primary spongiosa beneath growth plate, which is characteristically enlarged in depth and width (Edwards and Veltman, 1983). Hens given insufficient P can become lethargic, unwilling to stand and eventually die, events occurring five weeks earlier in older hens (70–76 versus 20–70 weeks) (Boling *et al.*, 2000a). The condition fits the description of 'cage layer fatigue', but lack of P is probably not the only cause. Rickets occurred in newborn lambs, offspring of outwintered ewes that had to rely heavily on grazed fodder beet, containing only 1.5g P kg^{-1}DM, during gestation (Dittmer *et al.*, 2017). Replacement ewe lambs, similarly reared and fed, had unerupted molar teeth lacking enamel and with irregular dentine.

Infertility in ruminants

Poor lamb and calf crops were recurring features of early studies on the severely P- and protein-deficient veldt pastures (p.128). Subnormal fertility, depressed or irregular oestrus and delayed conception, responsive to P alone, were subsequently reported in cattle but sheep were not affected (Read *et al.*, 1986a, b). However, infertility is a late sign of deprivation, not developing until the seventh year of long-term studies, commencing with heifers given a diet with 1.4g P kg^{-1}DM; by that time other symptoms of deprivation had developed (Call *et al.*, 1978, 1986, 1987). Improved reproduction was rarely recorded in Australia and probably a secondary effect of poor condition at conception (Winks, 1990). Delayed conception was observed in dairy cows, mated during full lactation with marginal hypophosphataemia (Snook, 1958) and fertility was similarly improved by adding superphosphate to the drinking water (Scharp, 1979). First-service pregnancy rate increased from 36.5% to 63.2%, mean calving to conception interval decreased from 109 to 85 days and numbers culled annually for infertility decreased from 15 to 5. However, infertility has many causes and P insufficiency is rarely one of them, except in the minds of those with supplements to sell.

Occurrence of Insufficiencies

In ruminants

The difficulty of reducing productivity in experiments by feeding diets low in P suggested that natural occurrences will be rare. Severe deprivation during late pregnancy and early lactation lowered milk yield in beef cows (Fishwick *et al.*, 1977). Second-parity dairy cows, given diets low in P (2.4g kg^{-1}DM) but high in Ca (7.2g kg^{-1}DM) from the seventh month of gestation, showed loss of milk yield (32–35%) between 18 and 34 weeks but proportionate reductions in food intake (Call *et al.*, 1987). A similar low P supply throughout 1.3 lactations reduced milk yield, body weight and appetite during the second lactation (Valk and Sebek, 1999). In both dairy cow studies, wheat straw was fed during intervening dry periods to exacerbate P deprivation.[i] Had those diets provided sufficient Ca to replenish skeletal reserves, milk yield may have been sustainable in second lactations. Transfer of goats to a low-P diet after 6 weeks' lactation reduced milk yield (Muschen *et al.*, 1988) but such reductions are

unlikely to occur on farms. Reductions in food conversion efficiency (FCE) were rare (Ternouth, 1990) but occurred when calves in experiments were continuously offered restricted amounts of food, differing in P content but totally consumed (Challa and Braithwaite, 1988). Reductions in FCE have been reported in P-deprived steers (Long *et al.*, 1957), lactating beef cows (Fishwick *et al.*, 1977) and goats (Muschen *et al.*, 1988), they were in all cases appetite was reduced. Improvements in milk yield or weaning weight were rare consequences of P deprivation in grazing dairy and beef herds in Australia (Winks, 1990) and North America (Karn, 1997, 2001); they were most likely to occur during a first lactation, when animals are still growing. After successive lactations on P-deficient veldt pastures, in South Africa, improvements of up to 27.5% in weaning weight in beef herds given P supplements (Read *et al.*, 1986a, b) probably reflected improved milk yields. Most of the native Campos grasslands of Uruguay contain <2.0g P kg^{-1}DM but are also low in N (<17g) (Cardozo *et al.*, 2017). Post-weaning weight gain increased with applications of fertilizer P in conjunction with over-sowing of legumes (del Pino *et al.*, 2016).

In pigs and poultry

Phosphorus insufficiency would be endemic if P_i was not routinely added to commercial rations to compensate for poor availability of dietary P. Excess dietary Ca exacerbates P insufficiency by lowering the availability of phytate P (*Py*P) (Lei *et al.*, 1994; Eekhout *et al.*, 1995; Liu *et al.*, 1998) and is widely overfed. The dominant effect on performance is that of high dietary Ca, not high Ca:P ratio (Schlegel and Guttzwiller, 2020). Bone deformities (rickets) and fragilities are rarely seen and may be pre-empted by reductions in food intake and FCE, which slow growth (Table 6.2) (Koch and Mahan, 1985; Reinhart and Mahan, 1986). Vulnerability of poultry is also increased by feeding dietary Ca in excess of levels already higher than those of pig rations (e.g. Qian *et al.*, 1997; Driver *et al.*, 2005). Mortality was high in broiler chicks given corn/mixed-vegetable protein diet containing 4.5g P kg^{-1}DM without added Pi (Simons *et al.*, 1990). The drive to lower imports of P_i on farms by lowering dietary P will increase risk of insufficiency. A study of lame, 35-day-old broiler chicks from Holland revealed a 44% incidence of hypophosphataemic rickets, which can increase susceptibility to tibial dyschrondroplasia (p. 70) and bacterial chondronecrosis (Thorp and Waddington, 1997). Since around a third of broilers can be lame (p. 57), incidence of P insufficiency could have been as high as 15%. In hens, P deprivation reduces egg yield, hatchability and shell thickness but lack of Ca is far more likely to limit performance in these respects. Egg production and hatchability of fertile eggs decreased rapidly in caged hens on diets containing 3.4–5.4 g P of plant origin but adding 0.9g P_i kg^{-1}DM improved performance (Waldroup *et al.*, 1967), as in a later study (Boling *et al.*, 2000a). Egg weight and body weight were improved by adding phytase to a diet rich in *Py*P (Barkley *et al.*, 2004). Offering limestone separately from feed to increase *ad libitum* food intake (Barkley *et al.*, 2004) may improve P availability and lower risk of insufficiency.

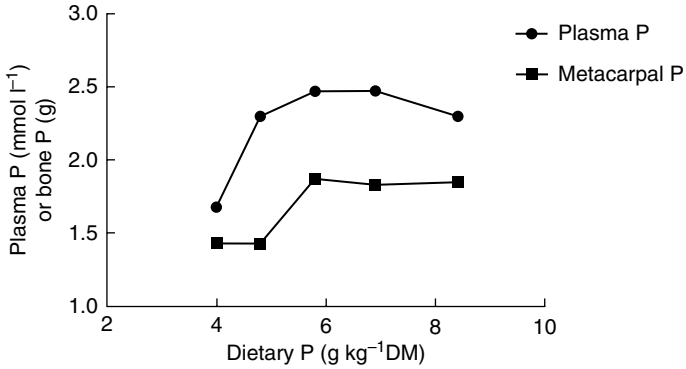

Fig. 6.1. Adding inorganic phosphorus to a cereal diet, rich in phytate P, increased phosphorus concentrations in plasma and bone in pigs. (Data from Ekpe *et al.*, 2002)

Physiochemical Changes on Diets Lacking Phosphorus

In non-ruminants, failure of the diet to meet P requirements causes a sequence of biochemical, physiological and morphological changes that bear the characteristics of a Type 1 mineral in growing animals and a Type 2 mineral after growth has ceased (p. 40): in ruminants, that distinction is blurred.

Depletion

Bone mineralization can increase substantially even while growth is being retarded in P-depleted weanling pigs (Reinhart and Mahan, 1986), whereas the large pool of P in the skeleton can probably be denuded in the adult before biochemical changes occur elsewhere. In broiler chicks, tibia ash increased by 25% between the 16th and 32nd days of P deprivation (Driver *et al.*, 2005): corresponding changes in turkey poults were illustrated in Fig. 1.6. In all species, P is absorbed principally as PO_4^{2-} from the proximal small intestine but weakly regulated. Specific Na^+-dependent PO_4^{2-} transporters have been identified in the jejunum of broilers (Huber *et al.*, 2006) and pigs (Pokharel *et al.*, 2017) but were only upregulated by P-deprivation in pigs and not until other abnormalities were evident.[ii] Upregulation of Na^+-dependent transporters in the renal tubule allows P, secreted by the kidney, to be conserved at low P intakes in broiler chicks (Huber *et al.*, 2006) and pigs (Pokharel *et al.*, 2017). In ruminants, young and old, the rumen provides a pool of P which can be depleted and delay systemic biochemical anomalies but the microbial population becomes depleted of P. Lambs grew normally for three weeks on a diet low in P (see Fig. 4.8) before plasma P_i began to decrease (Fig. 6.2).

Deficiency

The point of transition from depletion to deficiency is ill-defined, particularly in ruminants. Once plasma P_i begins to fall, salivary P secretion falls and rumen P falls further in young (Challa *et al.*, 1989) and mature ruminants (Wright *et al.*, 1984; Valk *et al.*, 2002). Bone P decreases in the young of all species (e.g. pigs, Table 6.2 and Fig. 6.1; lambs, Table 4.1), lost from hydroxyapatite during bone remodelling: there are obligatory, parallel decreases in bone Ca (e.g in broiler chicks; Fig. 6.3). In weanling lambs deprived of P, skeletal size increased but ash and organic matter content of bones decreased (Table 4.1; Field *et al.*, 1975). Interactions between dietary Ca and P can affect the course of events. In young lambs, hypophosphataemia was delayed from 5 to 10 weeks when dietary Ca was also low (Fig. 6.2), partly due to lower P retention in the skeleton (Braithwaite, 1984; Field *et al.*, 1985; Rajaratne *et al.*, 1994). Rumen P_i may be reduced on diets high in Ca through precipitation of insoluble calcium phosphates (Wan Zahari *et al.*, 1990). Milk P does not decrease in lactating goats and cows deprived of P (Muschen *et al.*, 1988; Valk *et al.*, 2002).

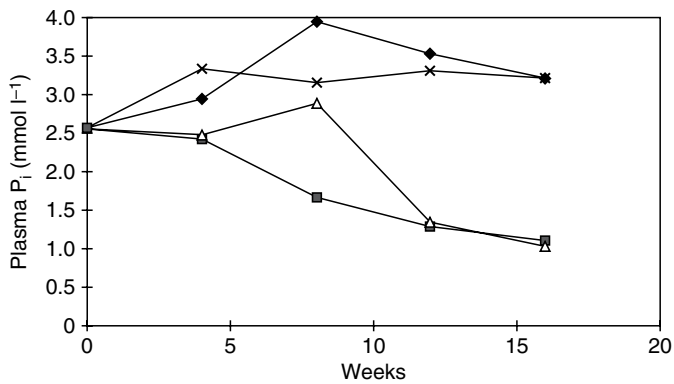

Fig. 6.2. Effects of diets low in phosphorus and/or calcium on plasma inorganic P (P_i) in lambs. Note that feeding a diet low in both minerals (△) delays the fall in Pi compared to a diet low only in phosphorus (■); feeding a diet low in calcium but adequate in phosphorus (◆) raises Pi when compared with a diet adequate in both minerals (×). (Field *et al.*, 1975)

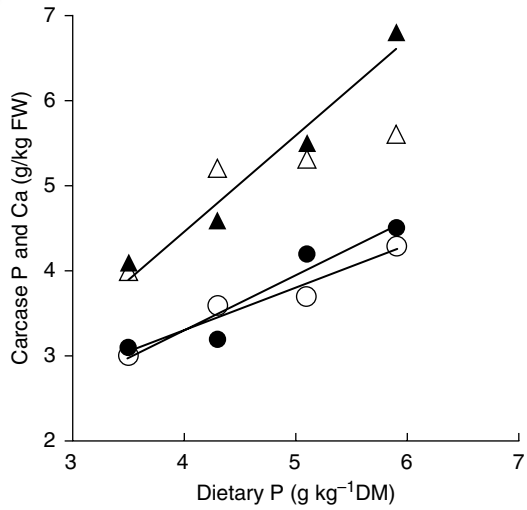

Fig. 6.3. Increases in dietary phosphorus in broiler chicks raised carcase concentrations of both phosphorus (circles) and calcium (triangles), irrespective of age (15- (open circle) and 38-day-old (solid circle). (From Shastak *et al*., 2012)

Dysfunction

Growth becomes limited by a reduction in appetite and FCE: together they mark onset of dysfunction (see Fig. 4.8). In the absence of regulators of P metabolism, appetite regulation involving plasma insulin (Lin *et al*., 2018) may represent a first line of defence against insufficiency, reducing demand for P by restricting growth. Dysfunctional bone development is shown by histological changes in the epiphyseal growth plate of lambs, particularly in ribs (Field *et al*., 1975), similar to those found in non-ruminants (p. 130). Low specific gravity, mineral:volume and ash:volume ratios in entire bones indicate poor mineralization (e.g. lambs, Field *et al*., 1975; cattle, Williams *et al*., 1991a). Severe, abrupt P deprivation can decrease performance without significant reductions in bone quality (Wan Zahari *et al*., 1990; Coates and Ternouth, 1992) because appetite rather than bone strength is the limiting factor.

Disorder

There are limits to the tolerance of demineralization, particularly in species with high relative growth rates, such as the pig. Eventually, breaking and shear strength of bone are reduced in all species, regardless of age (e.g. growing pigs: Combs *et al*., 1991) and bones may fracture. Increases in the width and depth of epiphyseal growth plates in growing animals create weakened zones at the ends of long bones. Pressures then cause distortions, deformities, such as bowing, and lameness. Swollen chostrochondral junctions in ribs created a 'beaded' appearance.

Differential diagnosis

Although plasma and serum P_i are used in 'metabolic profiles' to indicate P status, values are affected by factors other than dietary P and therefore of limited diagnostic use. Weak regulation of P metabolism means that plasma P_i reflects the hourly equilibrium between inflow from diet, saliva and resorbing bone and outflow to mineralize bone growth, eggshell and produce milk. Serum P_i rises after feeding and is lowered by handling stress and parturition. For ruminants, normal ranges for plasma P_i change with stage of development. The suckling lamb has a higher serum P_i than its dam until it is weaned and should be accorded a higher normal range (>1.7 mmol l^{-1}) (Sharifi *et al*., 2005), as should the milk-fed calf. With little 'drain' of P into saliva, a higher plasma P_i is sustainable from a given P intake, prior to weaning. Increased outflow in milk may lower the normal range during early lactation. Marginal bands for serum P_i are suggested in Table 6.3 and carry most diagnostic weight in young, rapidly growing stock and high-yielding, lactating animals: 1.2–1.5 mmol l^{-1} is an appropriate marginal band (p. 41) for weanling ruminants and milking cows (Valk *et al*., 2002).

- Neither plasma nor serum P_i should be interpreted using a single threshold value.

Numerous biochemical markers of bone resorption or growth, in serum and urine, rise or fall due to lack of dietary P but none are specific indicators of lack of dietary P and some do not change in certain species.[iii] Demineralization of bone around parturition (p. 58) (e.g. Wu *et al*., 2001) may be obligatory and masquerade as P deficiency in most species, including the sow (Lee *et al*., 2019). In cows, rib biopsy P changed markedly in early lactation (Fig. 6.4) without phosphorus deprivation.[iv] Indices of bone density such as specific gravity, mineral: volume and ash:volume ratios and mineral contents of entire bones are simple, reliable indices of mineralization in all species, including chicks (Cheng *et al*., 2004) and pigs (Table 6.3). Non-invasive methods such as dual-photon absorptiometry have been used in chicks (Shastak *et al*., 2012) and pigs (Schlegel and Gutzwiller, 2020). Toe ash is reduced in the P-deprived fowl (e.g. Cheng *et al*., 2004) and correlates well with tibia ash and carcase P (Shastak *et al*., 2012). However, lack of dietary Ca (compare Table 6.2 with Table 4.1) and protein can

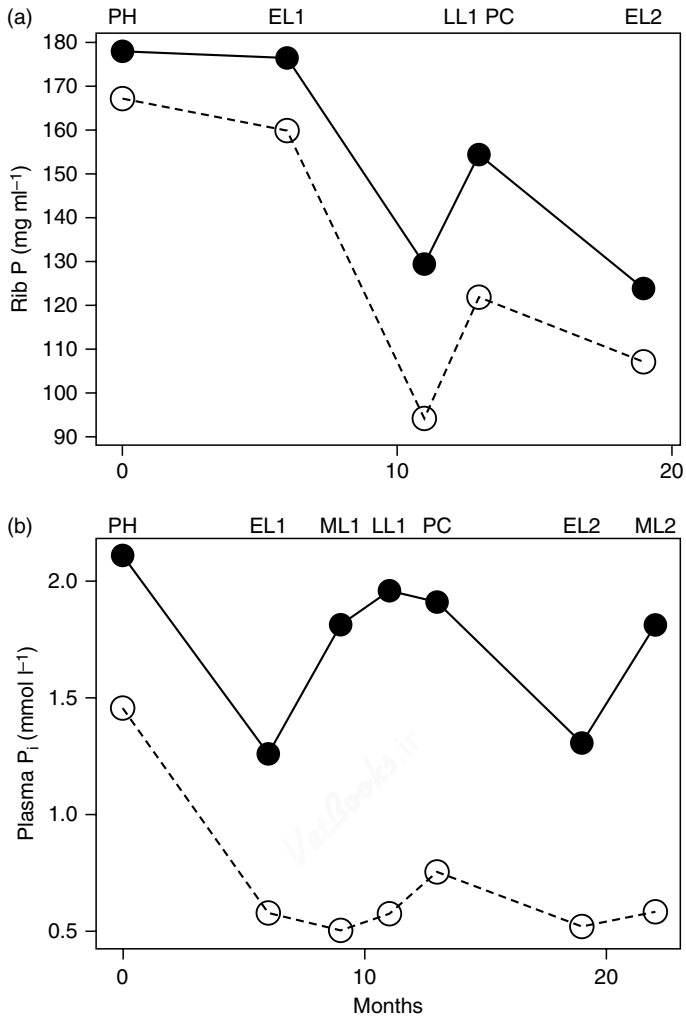

Fig. 6.4. Effects of lactation and phosphorus status on (a) P concentrations in rib biopsy and (b) plasma inorganic P(P_i) in two groups of beef cows, one receiving supplementary P (●) and one not (○), grazing P-deficient pasture: consistently low plasma P_i from month 6 did not reflect the skeletal changes (data from Engels, 1981). (PH or PC, pregnant heifer or cow; EL, ML and LL, early, mid- or late lactations)

reduce all bone density criteria. Furthermore, bone samples are a minute portion of a heterogeneous reserve and a sensitive site and parameter must be chosen from a bewildering array of possibilities. Guideline values for bone criteria are given in Table 6.3 and footnotes add diagnostic insights, but none is specific. Choice can affect verdict (e.g. Shastak *et al.*, 2012). Whether the index is radiographic, morphometric or biochemical, interpretation is complicated by variations between species, ages and bones at a given time. Values for most indices increase with age, irrespective of mineral nutrition (e.g. Fig. 6.5). In young calves, ends of long bones show greater reductions than shafts, and ribs show no change after short-term (six-week) depletion (Fig. 6.6). In steers, long bones showed greater demineralization than metatarsals (Little, 1984).

- Mineral composition of bone is not greatly changed by P deprivation in growing animals (Qian *et al.*, 1996a) but varies in the laying hen.
- Insufficiency can be confirmed by improvements in health and performance, particularly a recovery of appetite, when specific P supplements are offered.

Table 6.3. Marginal bands[a] for interpreting indices of phosphorus (or calcium) status in skeleton and bloodstream (P only): deprivation may be underestimated if bone growth is restricted by mineral deprivation.

		Bone ash[b] (% dry weight)	Plasma inorganic P[e] (mmol l^{-1})
Cattle	Calf – milk-fed	60 (R,d)[c]	2.0–2.5
	Calf – weaned	48–56 (R,c)	1.3–1.9
	Cow	50–60 (R,c)	1.0–1.5
Pigs	Starter (10–25 kg)	45–48 (d)	2.6–3.2
	Grower (25–65 kg)	52–55 (d)	2.3–2.6
	Finisher (65–80 kg)	56–58 (d,M)	2.3–2.6
Sheep	Lamb – suckling	–	–
	Lamb – weaned	20–30 (V)	1.3–1.9
	Ewe – lactating	30–36 (T)	1.0–1.5
Broiler	Chick – <4 weeks	40–45 (T,d)[d]	2.2–2.4
	Chick – >6 weeks	45–50 (T,d)	2.0–2.2
		50–55 (T,c,d)	
Hen	Laying	40–50 (T,d)	1.0–1.6
Turkey	Poult	38–40 (T)	–

[a]Mean values within bands or individual values < lower limits indicate possible impaired health.
[b]Bone ash can be converted approximately to specific mineral concentration, assuming that ash contains 0.18g P, 0.36g Ca and 0.09g Mg kg^{-1}. Ash $unit^{-1}$ volume is similar to ash $unit^{-1}$ dry fat-free weight.
[c]Source and pre-treatment: c, cortical bone; d, defatted bone; M, metacarpal/tarsal; R, rib; T, tibia; V, lumbar vertebra.
[d]In broiler chicks, marginal range for the fatty tibia is 39–42% ash. Toe ash <13% indicates P (or Ca) deprivation.
[e]Multiply by 31 to obtain units in mg l^{-1}.

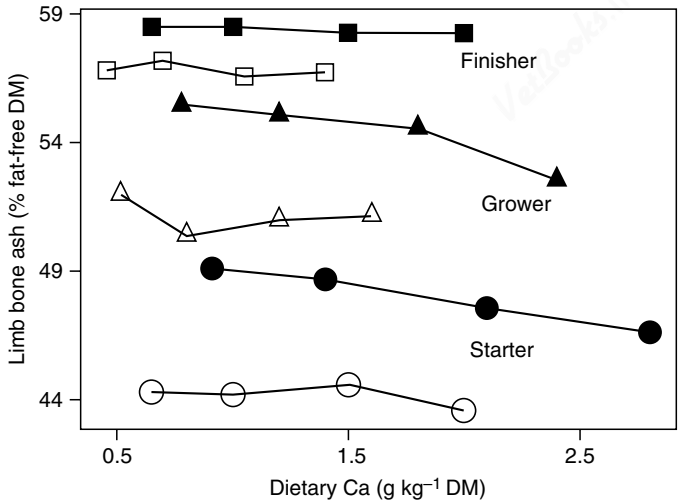

Fig. 6.5. Effects of dietary phosphorus and age on mineralization of limb bones in young pigs: bone ash increased between starter and finisher stages, regardless of whether dietary P was low (open symbols: 0.5, 0.4 and 0.3g P kg^{-1}DM) or high (closed symbols: 0.7, 0.6 and 0.5g P kg^{-1}DM) at successive stages. (From Reinhart and Mahan, 1986)

However, older beef cattle on dry-season pastures of low nutritive value can remain healthy with serum P_i around 1.0 mmol l^{-1} and may not benefit from P supplementation because dietary protein or sulfur are limiting performance (Engels, 1981; Wadsworth *et al.*, 1990; Coates and Ternouth, 1992). Although faeces contains endogenously lost as well as unabsorbed dietary P, faecal P has been used to assess status on pastures deficient in both N and P (Holechek *et al.*, 1985; Grant *et al.*, 1996; Karn, 1997). A 'critical threshold' value of <2g P kg^{-1} faecal DM has been advocated and was a significant but weak predictor of milk yield in rangeland goats (Mellado *et al.*, 2006). If a qualitative 'stick' test for urine P could be devised, a 'negative' result for

a grazing animal would strengthen the possibility that the diet lacks P since urine contains little or no P when P intakes are marginal.

Phosphorus in Feeds

Milk and milk by-products

Milk is an important source of phosphorus in all species, generally containing 1.0–1.6g P l^{-1} but less variable on a dry matter basis (around 8g P kg^{-1}DM) Mares' milk is exceptionally low in P (0.54 g l^{-1}) (see Table 2.4). Ratios of Ca:P in milk, though varying from 1.2 in the cow to 1.6 in the sow, are much lower than in bone. All sucklings receive plentiful supplies of P from their mothers, particularly from colostrum (Table 2.5). Milk P is mostly present as casein and lower in bovine milk by-products from processes that remove casein (e.g. whey from cheese-making) than from processes that do not (e.g. dried skim-milk from butter-making).

Cereals

The summary of feed P presented in Table 6.4 shows that grains are rich in P. Some 70–80% of cereal P is present in seed coats as phytate (*Py*P) in inositol tetra-, penta- and hexaphosphate (InP6), complexed with Ca, Mg and K, respectively (Reddy and Sathe, 2001; Selle *et al.*, 2003; Jondreville *et al.*, 2007; Rodehutscord *et al.*, 2016a). Structurally, the polydentate InP6 molecule resembles a turtle, with an inositol ring as 'body', esterified PO_4 molecules as four 'paws' at four corners and two more at either end, one pointing up ('head'), the other down ('tail') (Fig. 6.7). Each PO_4^{2-} is capable of binding minerals with varying affinity *in vitro* (copper > zinc > nickel > cobalt > manganese > iron > calcium) (Bohn *et al.*, 2008). Cereal genotypes differ in P and phytate concentrations (Fig. 6.8). Cereals also differ in intrinsic phytase activities, which is highest in rye, followed by triticale, wheat, barley and maize: no activity was detectable in oats (Rodehutscord *et al.*, 2016a). Genetic modifications have produced lines of barley and wheat low in phytate (Veum *et al.*, 2007) and high in phytase activity (Scholey *et al.*, 2017), with P that is highly available to pigs and poultry. Addition of exogenous phytases during mixing of rations can lead to degradation of phytate during storage (e.g. Poulsen *et al.*, 2019).

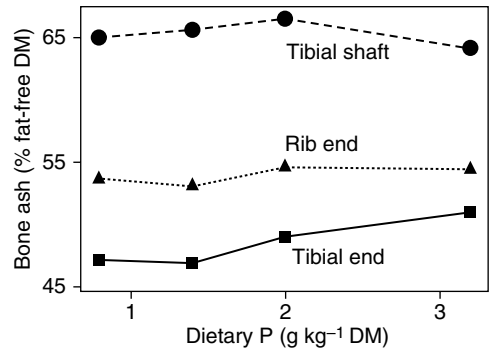

Fig. 6.6. Effects of sample site on degree of bone demineralization after short-term phosphorus depletion in calves (Miller *et al.*, 1987).

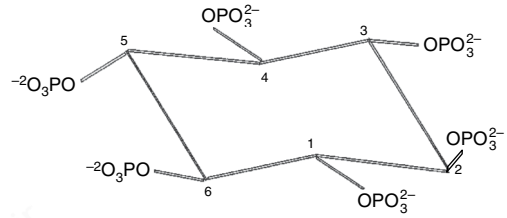

Fig. 6.7. Structure of phytic acid (myoinositol hexakis phosphate). (From Bedford and Partridge, 2001)

Legume seed, oil seed and other by-products

Vegetable protein sources are richer in P than cereals but generally contain smaller proportions as phytate, cottonseed and sunflower meals being exceptions (Fig. 6.8) (Viveros *et al.*, 2000; NRC, 2012; Table 6.4). Mean values for feeds from different countries and decades are reasonably consistent (Table 6.4), including salutary variation within sources. In 25 soybean meal (SBM) samples obtained across the USA, phytate and non-phytate provided 3.87 (0.23) and 3.16g (0.64) P kg^{-1}DM, respectively: the non-phytate fraction increased from 0.40 to 0.53 as total P increased from 5.8 to 8.8g kg^{-1}DM (Manangi and Coon, 2006) and is predictable from total P (Gao *et al.*, 2007). The large difference between maize by-products in UK and USA data is probably due to real differences between meal and feed, which also differ widely with respect to other minerals (Sauvant *et al.*, 2004). Little is known about phytate in other feeds, but alfalfa hay, bagasse and citrus pulp contained 0.82, 0.32 and 0.54g *Py*P

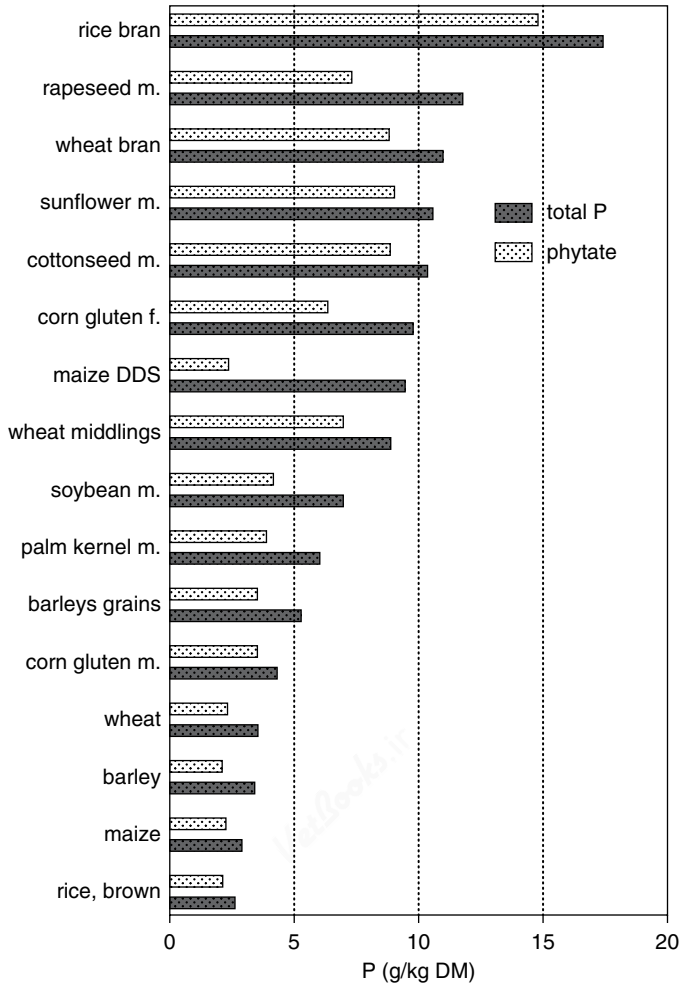

Fig. 6.8. Contributions of phytate phosphorus to total concentration in phosphorus-rich foodstuffs: together with endogenous phytase activity, these determine nutritional value to pigs and poultry (Sauvant *et al*., 2004).

kg^{-1}DM, respectively, equivalent to 21%, 64% and 45% of total P (Dias *et al*., 2006). Analytical techniques were improved. The 'standard' ferric chloride ($FeCl_3$) extraction method did not extract all *Py*P from certain feeds, including lupin seeds and field beans (Selle *et al*., 2003) but was modified (Gao *et al*., 2007). Use of genetic engineering to produce crop varieties with less phytate (e.g. Bretziger and Raboy, 2006) is discussed in more detail on p. 530. By-products from the fermentation of cereals for brewing, distillation and ethanol production are much lower in phytate than the original grains (Fig. 6.8) because phytase activity is stimulated during fermentation.

Phosphorus Availability

Accurate assessments of availability allow precision feeding to P requirement and minimize pollution of the environment with excess P, voided largely as unabsorbed *Py*P in faeces. Faecal P increases linearly and urinary P increases curvilinearly as P intake increases in pigs (Fig. 6.9) and broilers (Manangi *et al*., 2018). Absorbed but unneeded P is excreted in urine and little P is lost in urine at P intakes close to requirement. Inorganic P (P_i) is almost completely absorbed. When added as mono-sodium phosphate (MSP) to a phytate-free diet, 89–90% was absorbed (Peterson and Stein, 2006; Pettey

Table 6.4. Mean phosphorus concentrations (g kg^{-1}DM) in common foodstuffs from the UK (MAFF, 1990) and USA (*italicised*: see Appendix 2) are broadly similar and sufficiently describe nutritional value to ruminants but variation within feeds, indicated by standard deviations in parentheses, affects value to all species.

Roughages	P	Cereals	P	Protein sources	P
Barley straw	1.1 (1.10)	Barley	4.0 (0.46)	Citrus pulp	1.1 (0.09)
Oat straw	0.9 (0.24)	Maize	3.0 (0.32)	Sugarbeet pulp	0.8 (0.15)
			3.0 (0.43)		*0.9 (0.11)*
Grass	3.0 (0.68)	Wheat	3.3 (0.42)	Cassava meal	0.9 (0.14)
			4.5 (0.55)		
Kale	4.2 (0.71)	Rye		Cottonseed meal	8.9 (1.1)
White clover	3.8 (0.28)	Sorghum		Groundnut meal	6.0 (0.20)
Grass silage	3.2 (0.62)	Triticale		Linseed meal	8.7 (0.80)
					6.9 (0.45)
Maize silage	2.6 (1.20)	Rice bran	17.4 (1.70)	Soybean meal (ext)	7.4 (0.44)
					8.0 (2.25)
Lucerne silage	3.3 (0.35)	Wheat bran	12.6 (3.0)	Sunflower meal	
		middlings	*14.9 (1.67)*		
Grass hay	2.6 (0.77)	Brewers' grains	5.1 (1.00)	Rapeseed meal	
Fodder beet	1.8 (0.27)	Distillers' grains	7.9 (0.75)	Feather meal	3.1 (0.60)
			8.9 (1.14)		
Swedes	2.6 (0.28)	Maize gluten meal	2.8 (1.20)	Fish meal, white	38.0 (14.5)
		feed	*12.1(0.41)*		

et al., 2006). Pigs absorb P as efficiently from milk as from its by-products (Petty *et al.*, 2006). All livestock suckled or reared on milk or milk substitutes absorb P with almost complete efficiency (e.g. calves; Challa and Braithwaite, 1989).

Degradability of phytate phosphorus

By contrast, P is poorly but variably absorbed from phytate-rich diets by non-ruminants (Dersjant-Li *et al.*, 2015). To be absorbed from phytate, P must be freed (dephosphorylation) and hydrolysed by phytases (conversion) during digestion to free PO_4 (Zyla *et al.*, 2004). Endogenous phytase activity is very low in the intestinal mucosa of pigs and was not detected in the lumen (Pointillart *et al.*, 1987) but both mucosal and bacterial phytases can degrade >70% of phytate added to semi-purified diets for chicks. However, phytate is rendered undegradable on diets high in Ca. Upregulation of PO_4 transport in pigs deprived of P did not improve P availability (Pokharel *et al.*, 2017), presumably because *Py*P availability was not improved. Calcium phytate, present in grains, may also be wholly unabsorbable. Exogenous phytases, including those variably present in grains, can be partially degraded during food processing but residual activity aids digestion of *Py*P. In Fig. 6.9, AA_p, for an unsupplemented diet containing mostly *Py*P, was only 0.42. The availability of *Py*P was be estimated to be 0.29 from the slope of the relationship between AA_p and dietary *Py*P in Fig. 6.10. Meta-analysis of data for pigs (Létourneau-Montminy *et al.*, 2012) predicted a fourfold difference in AA_p between *Py*P (0.21), P_i (0.80) and remaining P (P_o, 0.73): the former are consistent with the slope and intercept, respectively, in Fig. 6.10.[v] Applying these coefficients to SBM, as sampled by Mangani and Coon (2006), indicates that AA_p would increase from 0.49 to 0.58 over the full range in total P. An increase in AA_p with body weight in the meta-analysis may reflect an increased capacity of older pigs to degrade phytate.

Unabsorbable calcium phytates may be synthesized *de novo* in the gut on diets rich in Ca and *PyP*. Insoluble calcium phosphates can also form when diets are rich in Ca and Pi. The increment in faecal P excretion in Fig. 6.9 indicates that only 0.57 of P, added as dicalcium phosphate (DCP) to a phytate-rich diet was apparently absorbed (*AAP*). Inhibitory effects of dietary Ca and enhancing effects of exogenous phytase on AA_p are illustrated in Fig. 6.11. Soaking diets increases AA_p by increasing phytase activity (Liu *et al.*, 1997; Larsen *et al.*,1999; Lyberg *et al.*, 2005) but pelleting (Larsen *et al.*,1999)

has the opposite effect. Blending maize with phytase-rich cereals such as triticale (Pointillart *et al.*, 1987) or by-products such as rice bran (Pointillart, 1991) and wheat middlings (Han *et al.*, 1998) will raise AA_P. Adding Ca to preserve Ca:P ratios when measuring P value (e.g. in rapeseed meal; Bournazel *et al.*, 2018) may underestimate AA_P. Feeds high in neutral detergent fibre, such as

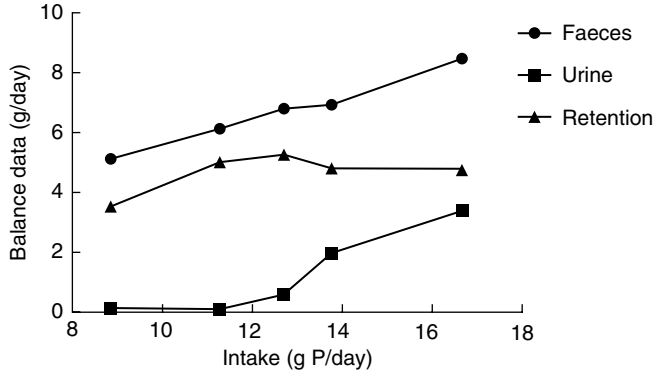

Fig. 6.9. Partition of phosphorus between body, faeces and urine changed as phosphorus intake increased in young pigs, when dicalcium phosphate* was added to the corn/barley/soybean meal (SBM) diet of 5–6 week-old pigs. (From Ekpe *et al*., 2002; *formula given in Appendix 5)

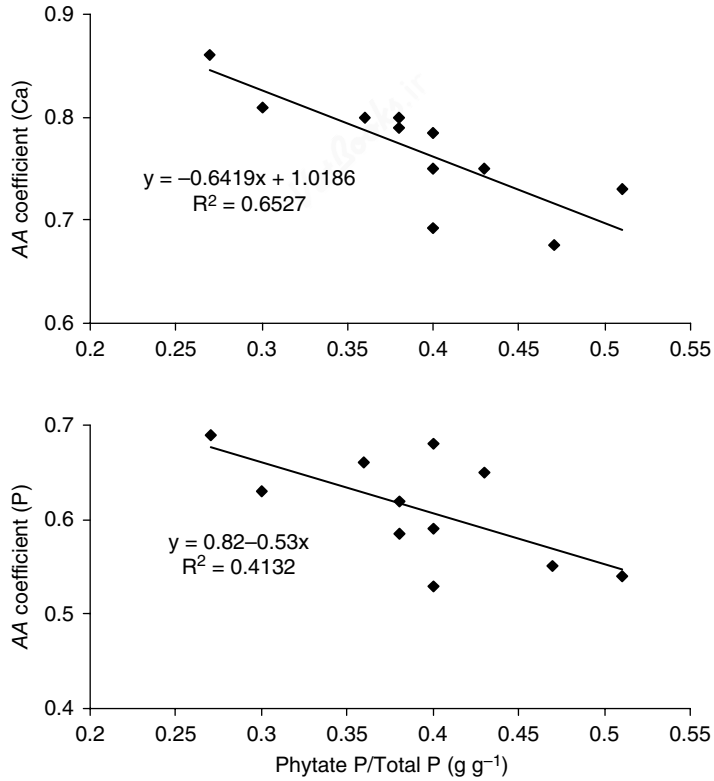

Fig. 6.10. Decreasing the phytate contribution to total P in barley/soybean meal diets (by genetic manipulation, dehulling barley and adding inorganic P) increased apparent absorption (*AA*) of phosphorus and calcium in young pigs. (Data from Veum *et al.*, 2002, 2007)

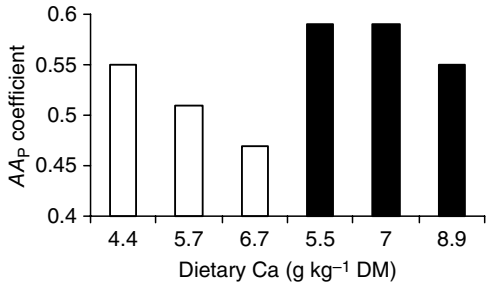

Fig. 6.11. Raising dietary calcium lowered apparent absorption of phosphorus (AA_P) from phytase-supplemented diets, but the effect was less when dietary P was raised from 'low' (3.6g, open columns) to 'high' (4.5g P kg^{-1}DM, solid columns) with inorganic P in weanling pigs. (From Qian *et al*., 1996a, pooling results for two phytase treatments)

lupin hulls and almond meal, contain P that is only half as absorbable as that in cereals and vegetable proteins (Park *et al*., 2019).

Availability of phosphorus in phytate-rich feeds

Common feeds and blends can be evaluated in terms of AA_P under appropriate conditions (Jondreville and Dourmad, 2005) and Table 6.5 presents published values for common foodstuffs, with and without added phytase: comparable data for poultry are also given. The huge problem that low availability of *Py*P creates for ration formulators is plain to see, alongside a possible solution:

- AA_P varies widely within and between feeds, due to low *Py*P degradability but is higher and less variable when phytase is added.

Although phytate has less impact on availability in poultry than pigs, the factors that determine AA_P (measured as PCD_P) are the same, hinging upon degradability of phytate. Microbiota in the gizzard and proventriculus secrete phytases, allowing *Py*P to be more extensively absorbed by chicks than pigs, though not by turkey poults (Ingelmann *et al*., 2018a).[vi] Feeds low in phytate, such as wheat-dried distillers solubles (Fig. 6.8), have high AA_P (Table 6.5), even in turkeys (Adebiyi and Olukosi, 2015), which degrade phytate less efficiently than broilers (Ingelmann *et al*., 2019).[vii] Broilers also absorb more Ca from a given feed than turkey poults and that difference was only marginally reduced by phytase supplementation. Antagonistic effects of dietary Ca on P retention in broilers are shown in Fig. 6.12 and also indicated by reduced phytate degradation by phytases *in vitro* (Zyla *et al*., 2004). Antagonism from Ca was pronounced in laying hens, given a corn/SBM diet with either 30 or 40g Ca kg^{-1}DM: phytate degradability was 0.36–0.48 and 0.10–0.21, respectively (Van der Klis *et al*., 1997). Without added P_i, hens adsorbed less P from maize than barley (PCD_P 0.25 versus 0.34) but with added phytase, values increased to 0.51 and 0.58, respectively (Francesch *et al*., 2005). Blending a phytase-rich cereal, triticale, rather than corn with SBM (45:30) in P-deficient diets for chicks was equivalent to adding up to 0.81g P_i kg^{-1}DM to the diet (Jondreville *et al*., 2007). Assessments of AA_P can be heavily method-dependent in broilers (Mutucumarana *et al*., 2015) and large differences between sources can fail to reach significance (Mutucumarana *et al*., 2013).

- Mismatches between phytate degradation and AA_P may be due to increases in FEL_P following increased absorption of released P.

'Standardized Digestibilities' for Pigs

In an attempt to improve feed P evaluation and reduce pollution of the environment with P, NRC (2012) characterized individual feeds for pigs in terms of standardized total tract digestibility of P ($STTD_P$, p.26). Standardization required $^mFEL_P$ to be determined in cohorts fed artificial, P-free, diets (She *et al*., 2017). Together, P- (and/or Ca-) free basal diets and very young, newly weaned animals constitute an inherently unstable model for assessing availability.[viii] Upward adjustments of $ATTD_P$ for $^mFEL_P$ become more important as P concentration in the source declines but the effect remains trivial compared with differences between feeds and effects of phytase addition to phytate-rich feeds (Fig. 6.13). Correcting the availability of P in SBM for phytate content (p. 138) has a greater impact than that of NRC (2012) standardization. When the above sources of error in deriving $STTD_P$ from $ATTD_P$ are gathered together,

- it seems unlikely that the precision of feeding P to pigs will be improved by NRC (2012) standardization;
- the single $STTD_P$ values listed for feeds by NRC (2012) create false certainty.

Table 6.5. Coefficients for apparent absorption of phosphorus (AA_P), determined incrementally in common feeds and blends, fed without inorganic P supplements but with (+) or without (o) added phytase in pigs and poultry.

Food or Blend	Pigs		Broilers	
	0	+	0	+
Barley, normal	0.39[1],0.40[2]		0.28	
Maize, normal	0.17[1],0.37[2], 0.36[18]		0.43[15].0.68[16],0.56[18],	0.38[21]
Wheat	0.41[1],0.15[17]		0.38[21], 0.36[23],0.45[23T]	
SBM, normal phytate	0.37-0.49[1,3,4,5,6,7,8]	0.71	0.52[15]0.6[18],0.53[21],0.36[23T]	0.48
Rapeseed (canola) meal	0.34[5]	0.61[5]	0.46[16]	0.43[19]
Wheat dried distillers solubles	0.75		0.90[20]	0.90[20]
Maize/SBM[a] (average 74:20)	0.16-0.59[9,10,11,20]	0,68[20]	0.50[22]	0.60-0.70[22]
Maize/barley/SBM (40:35:11)	0.34-0.54[12.13.14]		0.15-0.20[n]	
Barley/SBM (54:23)	0.56,[15]0.72[18]	0.79[18]	0.20	
Wheat.barley/SBM /RSM	0.53-0.56[17]		0.90[20]	0.90[20]
Corn distillers dried solubles (DDS)	0.31[19]	0.50[19]	0.78[21]	
Wheat distillers dried solubles (DDS)	0.75[16]			
Triticale DDS	0.71[18]	0.78[18]		
Corn gluten meal	0.81[18]	0.83[18]		
Corn gluten feed	0.59[18]	0.64[18]		
Corn germ meal	0.81[18]	0.83[18]		
	0.49[18]	0.64[18]		

[a]SBM, soybean meal.

[b]Using the relative availability of feed P compared to monosodium phosphate, normally given a value of 1.0 (or 100%), adjusted to a probable AA_P of 0.9 (90%).

Pig references: [1]Jongbloed and Kemme (1990); [2]Veum *et al.* (2002); [3]Jongbloed and Henkens (1996); [4]Rodehutscord *et al.* (1998); [5]Akinmusire and Adeola (2009); [6]Fan *et al.*, 2001; [7]Dilger and Adeola (2006); [8]Bohlke *et al.* (2005) [9]Spencer *et al.* (2000); [10]Adeola *et al.* (2004); [11]Brana *et al.* (2006)'; [12]Kies *et al.* (2006); [13]Veum *et al.* (2007); [14]Ekpe *et al.* (2002); [15] Baker *et al.* (2013); [16]Xue and Adeola (2015); [17]Widyaratne and Zijlstra (2007); [18]Rojas *et al.* (2013); [19]Poulsen *et al.* (2010); [20]Dersjant-Li and Kwakernaak (2019).

Poultry references: [15,16,17]Mutucumarana *et al.* (2013, 2014, 2015); [18]Adeola and Applegate (2010); [19]Olokusi *et al.* (2015); [20]Adebiyi and Olokusi (2015); [21]Ghazaghi *et al.* (2019); [22]Wealleans *et al.* (2016).

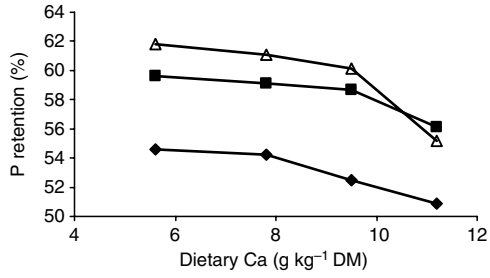

Fig. 6.12. Effects of dietary calcium alone (♦) and with added phytase, without (■) or with vitamin D (Δ) on phosphorus retention in chicks. (Data from Qian *et al.*, 1997)

Tables of mean P availabilities without an indication of the size and sources of variation (e.g. Sauvant *et al.*, 2004; NRC, 2012) do not facilitate precise P nutrition.[ix] The vagaries of NRC (2012) standardization are demonstrated in Fig. 6.14 where predicted $STTD_P$ and observed AA_P values are compared in the same treatment groups. Although the two parameters were highly correlated and a slope close to 1.0 indicated accurate prediction of AA_P for P_i (added as DCP), the significant intercept indicated that AA_P in the unsupplemented corn/wheat/SBM blend was underestimated by 50%. High AA_P in the basal diet may have been attributable to a relatively low dietary Ca (4.5g kg^{-1}DM). Advocates of the $STTD_P$ system rarely allow such comparisons to be made in their publications.

Standardized digestibilities for poultry

Indigestible markers and/or ileal cannulae must be used to measure FEL_P as PCD_P) in avian species to discount endogenous urinary excretion into the gut at the cloaca. A scheme similar to that proposed for pigs (NRC,

2012) was advocated for poultry, specifying graded increments of P source and availability calculated by linear regression of faecal P excretion on P intake (WPSA, 2013). However, $STTD_p$ coefficients ranged from 0.19 to 0. 51 for the same batch of SBM in broiler chickens in an international ring study, using common methodology (three-point linear regression) at 17 sites (Rodeshutscord *et al*., 2016b). It was suggested that:

- 'pre-experimental procedures should also be standardized and comparisons of P digestibility measurements between studies made only with great caution until the protocol is more refined'.

Protocols remain, arguably, flawed for some of the reasons outlined for pigs (p. 140)[x] but, additionally, because responses are essentially non-linear, as illustrated in Fig. 6.15. Pre-caecal P output increased as P intake from SBM increased but responses appear to become increasingly non-linear as Ca:P ratio decreased, though impossible to prove with only three SBM inclusion rates. A positive intercept of the order of 300mg P kg^{-1}DMI, indicative of $^{m}FEL_p$, and inhibitory effect of Ca on PCD_p make more physiological sense than much larger negative intercepts inferred in the original paper.[xi]

- At least four, appropriately spaced treatments should be used before curvilinearity is ruled out in assessing the availability of feed P.

Dietary Requirements of Pigs

Requirements for maintenance

Minimum $(^{m})FEL_p$ (p.6; the hypothetical losses when no P is consumed) have been widely measured to improve the assessment of P value of feeds, arguably without success (p. 140): estimates varied from 100 to 300mg kg^{-1}DMI in starter pigs and broilers on P-free diets. Determination of $^{m}FEL_p$ by linear regression and extrapolation to zero intake may introduce errors, due to curvature

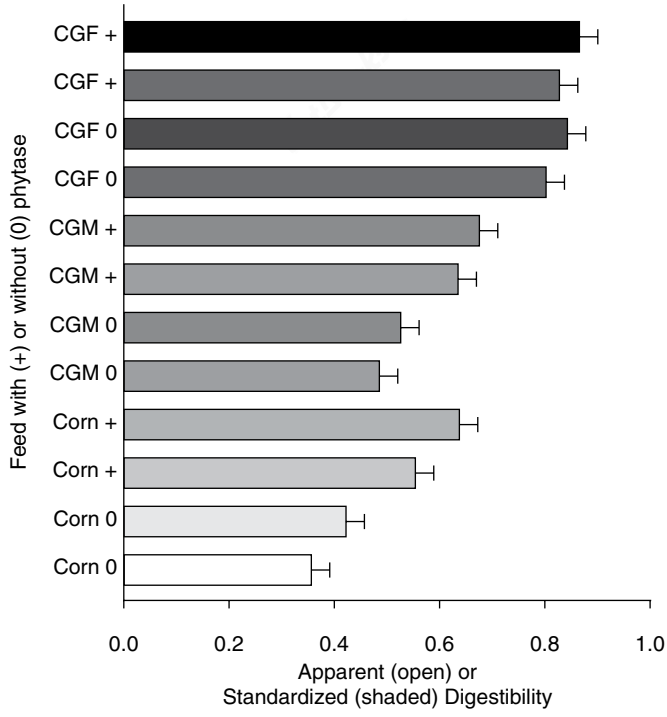

Fig. 6.13. Effect of 'standardizing' or correcting apparent total tract digestibility of phosphorus (P) in P-rich feeds, corn and corn gluten feed (CGF), corn germ meal (CGM), fed with or without phytase supplements to weanling pigs. (Data from Rojas *et al*., 2013)

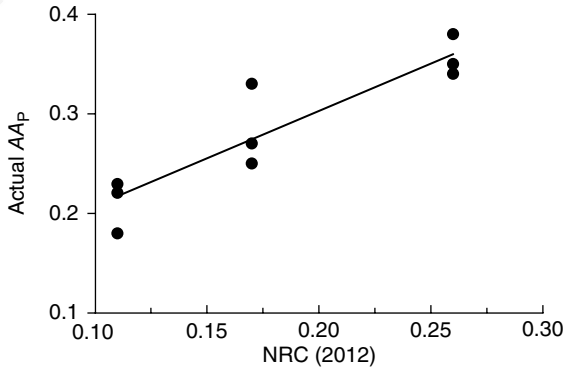

Fig. 6.14. The relationship between observed and predicted (NRC, 2012) apparent absorption of phosphorus (AA_P) in an experiment with starter pigs given a corn/hard wheat/soybean meal blend with or without dicalcium phosphate (Pokharel *et al*., 2017). (The linear regression equation was y = 0.96 ± 0.16 + 0.11 ± 0.031x; r^2 = 0.82; p = 0.0005.)

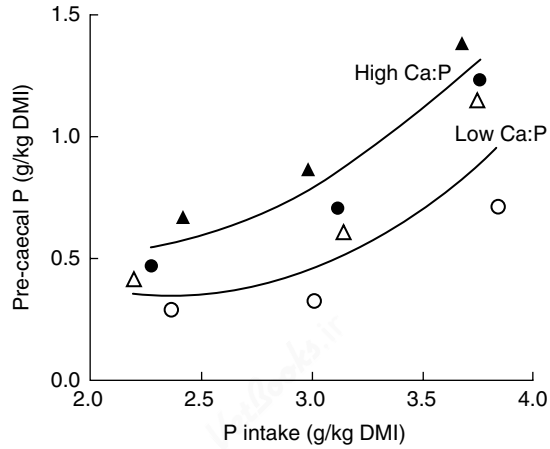

Fig. 6.15. Effects of dietary Ca:P ratio on the quadratic relationship between intake and pre-caecal faecal output of phosphorus in broiler chicks: four Ca:P ratios, 0.8, 1.2. 1.6 and 2.0, represented by different symbols, were compared; two quadratic curves are plotted for the lower and higher Ca:P ratios and their similarity indicates little effect of Ca:P ratio per se on availability of P. (Data from Liu *et al.*, 2013)

of responses at low P intakes, as in pigs (Fig. 6.9) and poultry (Fig. 6.15). Obligatory losses ($^oFEL_P$) are higher and better estimates of maintenance needs in all species (p. 6). Low AA_P of 0.11–0.22 in cereal-based diets, with or without added Pi in confined lactating sows (Wealleans *et al*., 2015), may indicate high obligatory and/or confinement-induced FEL_P due to bone resorption (p. 75). Such losses, like those in $^oFEL_{Ca}$, may not be wholly reversible by dietary supplementation.

Requirements for growth

Phosphorus accretion rates vary with planes of P and Ca nutrition, as described earlier, and vary between studies (Ruiz-Ascabar *et al*., 2019). Traditionally, optimum bone density set the standard for net growth requirement (G_P) but interpretation is complicated by variation with age (Fig. 6.5) and environmental considerations demand that it should only be used on welfare or survival grounds (p. 529). Gross requirements indicated by early dietary trials varied according to diet type, composition and measure of P status. Baby pigs required 4, 5 and 6g P kg^{-1}DM in a synthetic milk for normal growth and FCE, for normal serum P_i, alkaline phosphatase and bone development and for maximum bone density and breaking strength, respectively (Table 6.2). Antagonism of AA_P by Ca (p. 138) increases dietary needs for P (Reinhart and Mahan, 1986) but there is no specific effect of Ca:P ratio (see Fig 6.16).[xii] Complex formulae have been derived to describe performance, Ca and P retentions and bone mineralization at proscribed $STTD_P$ and $STTD_{Ca}$ concentrations

for starter (Gonzáles-Vega *et al*., 2016a), grower (Gonzáles-Vega *et al*., 2016b) and fattening (Merriman *et al*., 2017) pigs. Some data for fattening pigs are presented in Fig. 6.16 but plotted against intended total rather than predicted $STTD_P$ and $STTD_{Ca}$ because non-linear responses reflect, in part, probable effects of treatments on both parameters.[xiii] In particular, the basal corn/SBM blend probably provided twice as much $STTD_P$ as assumed at low Ca intakes (Fig. 6.12). With no P_i added, a diet deemed to provide less than the P requirement (NRC, 2012) was optimal for performance traits, as Liu *et al*. (2000) also showed on diets relatively low in Ca. Femur ash was increased by raising dietary P and Ca without reaching a plateau (Fig. 6.12) but what degree of bone mineralization should be required? Although added P was required for optimal performance at lower weights (Gonzáles-Vega *et al*., 2016a, b), problems relating performance to $STTD_P$ and $STTD_{Ca}$ remain.

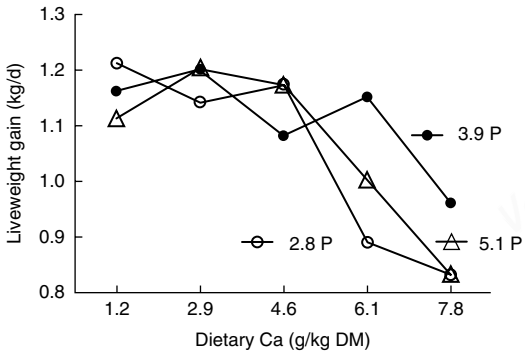

Fig. 6.16. Effects of dietary calcium on responses in growth rate (femur ash, not shown) in fattening pigs change with increases in dietary phosphorus. (Data from Merriman *et al*., 2017)

- High dietary Ca increases needs for P and should be avoided (Schlegel and Gutzwiller *et al*., 2017, 2020), especially at the fattening stage.
- Use of Ca:P ratios is unnecessary and merely encourages the overfeeding of both minerals.

Factorial estimates have particular advantages over estimates from feeding trials (p. 11) and the model here can be simplified by assuming G_P is constant between weaning and marketing (Ruiz-Ascacibar *et al*., 2019; Bikker and Blok, 2017) and using an AA_P rather than A_P coefficient to predict gross requirements above maintenance. Dietary influences can be minimized and reliance on dubious $STTD_P$ values avoided by assuming that phytases will be present and sufficiently active to maximize P availability. A set of requirements, thus generated, are shown in Table 6.6. The approach does not allow for dietary additives that increase FCE, other than phytase. A recent study (Wu *et al*., 2019) found that supra-supplementation with phytase (2000 FU kg^{-1} DM) improved performance in starter pigs (5.9 kg LW) through an 'extraphosphoric effect' and raised requirements for $STTD_P$ above those of NRC (2012).

- Such effects might require needs to be expressed on a metabolizable energy basis.

The above estimates are supported by a recent study with starter pigs in which replacement of P_i by phytase in a diet containing 4.7 g inherent P kg^{-1}DM (mostly *Py*P) resulted in neither loss of performance nor bone strength (Dersjant-Li *et al*., 2020a, b).[xiv]

- Responses to phytase in newly weaned pigs may be particularly variable (p. 140).
- Routine addition of phytase to rations will result in excess P being provided to fattening pigs and would require replacement of cereals with feeds low in P, such as cassava meal, to minimize pollution.

Table 6.6. Factorial estimates[a] of dietary phosphorus requirements of growing pigs, in rations with plentiful endogenous or exogenous phytase activity, decrease as pigs approach market weight (use in conjunction with calcium needs, given in Table 4.8).

Liveweight (kg)	DMI (kg day^{-1})	LWG (kg day^{-1})	Net need[a] (g P day^{-1})	Gross need (g P kg^{-1}DM)
20	1.0	0.60	3.56	5.1
40	1.6	0.80	4.88	4.4
60	2.3	0.90	5.74	3.6
80	2.7	0.93	6.09	3.2
100	3.0	0.88	6.00	2.9

[a]Components of the model are: net needs of 5.1g P kg^{-1}LWG for growth (Bikker and Blok, 2017), 0.5g P kg^{-1}DM for maintenance (zero balance; p.6) and an AA_P of 0.70 to calculate gross needs.

A deterministic model has been produced that integrates the major determinants of P requirement in growing pigs and a good fit was obtained with a range of commercial ration formulations, except those including dried distillers solubles and canola meals (Symeou *et al*., 2014). Phosphorus accretion was based on an assumed relationship with protein accretion, which some workers have found unhelpful, and carcase data probably came from animals given excess dietary P and Ca, overestimating P requirement.

Requirements for boars, gilts and sows

Boars should receive sufficient P to maximize bone strength but that need may be met on phytase-supplemented diets. Requirements for sows during late gestation and lactation could be as low as 3.4 and 3.8 g total P kg^{-1}DM, respectively, based on the recommendation of Bikker and Blok (2017) but assuming the use of phytase and a high AA_p of 0.70. Gilts should be fed generous amounts of P (and Ca) during the first reproductive cycle: a high proportion (30%) became lame when fed 5.5 g P with 7.2g Ca kg^{-1}DM during gestation but offspring did not benefit from higher supplementation (8.3 g P and 10.7g Ca kg^{-1}DM), dietary Ca was excessive and phytase was not added (Nimmo *et al*., 1981). Addition of 250 FU was estimated to make 1.6 g P available of 4.5–5.1g kg^{-1}DM in a cereal-based diet for lactating sows, reducing sow weight loss in the process (Wealleans *et al*., 2015).

Requirements for poultry

Growth

Carcase P increased linearly from 2.2 to 4.0g kg^{-1}LW in 21 d.o. broilers as dietary P increased from 3.5 to 5.9g kg^{-1}DM with addition of MSP, but by 35 d.o. the slope was less and intercept higher (Fig. 6.3). Minimum G_P, when performance was optimal, can be placed at 3.5 and 4.0g P_i kg^{-1}LW at 21 and 35 d.o., respectively. Required dietary P decreases as food is less efficiently converted to weight gain and given on a sliding scale for 'phase feeding' (Table 6.7). Leghorn chicks could be given <50% of NRC (1994) recommendations without impairing performance or bone quality (Keshavarz, 2000a) and 2g added P_i kg^{-1}DM was close to an optimal supply for broiler chicks (Augsburger and Baker, 2004). With the latest generation of phytases, 1000 FU kg^{-1}DM can generate 1.8 g digestible P from phytate and avoid the need to add P_i to broiler rations (Dersjant-Li *et al*., 2020b; Kriseldi *et al*., 2021).

- *Py*P need no longer be distinguished;
- Ca:*Py* and Ca:non-*Py*P ratios can be ignored if each is fed at the minimum needed for optimum performance (Akter *et al*., 2016).

Temporary underfeeding is tolerable because the 18-day-old broiler chick can compensate for previous phosphorus (and calcium) deprivation (Yan *et al*., 2005; Valable *et al*., 2018).

Table 6.7. Approximate dietary requirements of non-phytate phosphorus for optimum performance in Leghorn chicks, optimum tibia ash in broiler chicks and turkey poults given diets with 500–1500 FU added phytase kg^{-1}DM.

		Growth stage*					
	Bird	Early			Middle		Late
	Leghorn chick[a]	2.0 (4.0)			1.5 (3.5)		1.0 (3.2)
(g kg^{-1}DM)	Broiler chick[b]	2.4 (4.5)			1.8 (3.5)		1.5 (3.0)
	Poult[c]	2.6 (6.0)			2.0 (4.2)		1.8 (2.8)
		Age (weeks)			Dietary energy density (kcal me kg^{-1}DM)		
	Leghorn	Broiler	Poult		Leghorn	Broiler	Poult
*Early	0–6	0–3	0–4		2850	3200	2800
Middle	6–18	3–6	8–12		2850	3200	3000
Late	>18	6–8	20–24		2900	3200	3300

Loosely interpreted and extrapolated values from
[a]Kesharvarz (2000a); [b]Li *et al*. (2017); [c]Wealleans *et al*. (2016).
Values in parentheses were NRC (1994) recommendations.

- Minimum needs for phytase-supplemented diets are much lower than the recommendations of NRC (1994).

Requirements for turkey poults are higher than those of broiler chicks because degradability of phytate is lower (Adeyibi and Olokusi, 2015; Ingelmann *et al*., 2018a) and food converted to LWG more efficiently than in broilers. In poults up to 21 d.o., 5.5g non-*Py*P kg^{-1}DM was sufficient for growth but not maximal bone mineralization but addition of phytase may halve that value (Applegate *et al*., 2003, Wealleans *et al*., 2016). Differences between species may also be partly due to differences in rate of food passage through the gut and in phytase activity in host and/or microbiota (Ingelmann *et al*., 2018b). However,

- poults and broilers may have closer capacities to degrade phytate when fed commercial diets that meet minimum Ca and P requirements rather than basal semi-synthetic diets, deficient in both elements.

Egg production

Onset of lay increases need for P more than would be expected from the small amount of P in each egg (about 120mg) because P turnover and FEL_P increase. Early studies suggested that 50% more P_i was needed to prevent 'cage layer fatigue' than to maintain egg production (3 versus 2g P_i kg^{-1}DM) (Owings *et al*., 1977) and NRC (1994) recommended 2.5g P_i kg^{-1}DM for all stages of lay. However, phased reductions in provision, to 0.5g P_i kg^{-1}DM, did not harm health or performance (Keshavarz, 2000b): further decrements (-0.5g P at each stage) impaired egg production (Fig. 6.17). With 0.5g P_i kg^{-1}DM added throughout lay to a similar diet, egg production and quality were sustained but LW and tibia ash were suboptimal by the end of lay (Boling *et al*., 2000a). Replacement of P_i with phytase in Fig. 6.17 sustained egg weight, but egg weight was improved by phytase supplementation at each P_i level, suggesting an 'extra phosphoric' benefit.

- So little degradable P is required that the pragmatic and environmentally sensitive approach is to add phytase rather than P_i to layer rations.

Use of phytase to replace P_i (3.5g kg^{-1}DM) is supported by a recent study (Shet *et al*., 2018), which

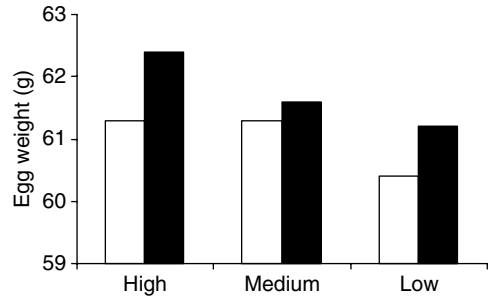

Fig. 6.17. Effects of phased reductions in inorganic phosphorus (P_i) supplementation and addition of phytase (solid columns) on mean egg weight in the final phase (age 54–66 weeks) of lay (Keshavarz, 2000b). Supplementation of a corn/soybean meal diet was either high (3.0–2.5–2.0g), medium (1.5–1.0–0.5g) or low (1.0, 0.5 and 0g P_i kg^{-1}DM) for weeks 30–42, 42–54 and 54–66, respectively: low P_i reduced egg weight, whereas adding phytase increased egg weight at all P_i levels.

reported increased expression of osteopontin and downregulation of calbindin genes in the egg gland as phytase replaced P_i. Excess provision of P_i in the control group may have downregulated osteopontin and upregulated calbindin. Dietary Ca was held constant in all three studies (3.5–3.8g Ca kg^{-1}DM) throughout lay and phased changes may limit reductions in bone and eggshell Ca at the end of lay. Requirements for P are affected by environmental conditions. High temperatures reduce feed consumption and hence raise the required dietary P concentration (NRC, 1994). Floor-housed birds recycle P through coprophagy and require less P than caged hens (Singsen *et al*., 1962).

Optimal Supplementation for Pigs and Poultry

Inorganic supplements

The challenge of meeting P requirements without polluting the environment (p. 529) cannot be met with supplementary P_i alone, whichever of five P_i sources is used (see Appendix 4). Biological availability was uniformly high in poultry, relative to MSP (0.90–0.95; Soares, 1995). In pigs, bone meal (0.8) and DFP (0.85) were of slightly lower relative value but sources of MSP of different purity varied in AA_P from 0.88 to 0.98: DCP had a relative value of 0.82 compared with the purest MSP (Peterson

and Stein, 2006). A similar disadvantage for DCP was reported in broiler chicks (Shastak *et al*., 2012). Choice of supplement depended largely on cost/kg P, the most expensive mineral to add, but also on whether extra Ca was required, accessibility and freedom from toxic impurities and dust hazards. This simple picture was disrupted by the introduction of dubious methods to standardize digestibility (pp. 140 and 141). Assessing feeds by such techniques was hard enough but problems are far greater for dense, particulate mineral sources in poultry. Figure 6.18 shows the variation in availabilities of MCP and DCP in three recent studies that all tried to follow WPSA (2013) guidelines, though only one succeeded (Bikker *et al*., 2016).[xv] Assessments of meat and bone meal bring an added complication, that of concomitant increments in P and protein. A comparison of three products gave PCD_P values ranging from 0.40 to 0.69 but corrections for $^{m}FE_{LP}$ varied between products (Mutucumarana *et al*., 2015). Retention of coarse particles in the gizzard, a known complication in assessing AA_{Ca} (p. 76), probably affects AA_P in short-term broiler studies.

Phosphorus supplements for pigs have generally varied less in value (Kim and Stein, 2014), with DCP having a high AA_P of 0.86 (Baker *et al*., 2013), but eight meat and bone meals, added to a semi-purified diet at a single level, had standardized values ranging from 0.55 to 0.84 (Sulabo and Stein, 2013): these may not represent real differences between sources. Rendered animal by-products such as meat and bone meal are valuable but variable sources of P, depending on bone content, and must contain >4% P in the USA. Use of animal by-products in compound feeds is prohibited in the European Community.

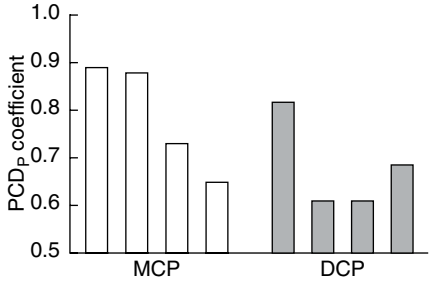

Fig. 6.18. Variation between studies in pre-caecal digestibilities of mono- (MCP) and di- (DCP) calcium phosphates in broiler chicks: from left to right, sources are: Van Harn *et al*. (2018); Bikker *et al*. (2016), experiments 1 and 2; Trairatapiwan *et al*., (2018).

- Current standardized PCD_P values for Pi sources do not allow precise formulation of available P in rations for pigs or poultry.

Phytase supplementation

The twin challenges (p. 146) can be met by using phytases to release abundant supplies of P in grains and vegetable protein sources. Phytase supplementation obviated the need to add P_i to diets for finishing pigs and laying hens (Augspurger *et al*., 2007), 500 FU kg^{-1}DM allowing removal of 0.84g P_i kg^{-1}DM from the average pig ration (Knowlton *et al*., 2004), and releasing 20–29% of *Py*P present in corn/SBM diets. The 'P_i equivalence' of phytase supplements in broiler rations was broadly similar (e.g. Augspurger *et al*., 2003) but in laying hens, 600 FU as fungal phytase releases 1.4g Pi kg^{-1}DM (Saleh *et al*., 2021), probably sufficient to meet requirement (Fig. 6.17). A burgeoning literature was reviewed by Dersjant-Li *et al*. (2015). Phytases are divided into three categories by preferred sites of dephosphorylation and pH optima (acid, neutral and alkaline). At least ten different phytases are commercially available and new products regularly appear, but differences in efficacy are relatively small.[xvi] The feed industry uses matrices to incorporate phytases into a system for predicting P availability, but cumulative errors probably frustrate the ambition. Once the preferred site has been dephosphorylated, the enzyme becomes progressively less effective at removing further PO_4 groups. Responses to *E. coli* 6-phytase in pigs (Brana *et al*., 2006) and broiler chicks (Fig. 6.19) followed the law of diminishing returns and left 50% ingested *Py*P in the excreta. Genetic modification improved heat stability and resistance to digestion (e.g. Augspurger *et al*., 2003; Gentile *et al*., 2003; Timmons *et al*., 2008). *Buttiaauxella* phytase is more effective at acid pH than *E. coli* phytase and increased PCD_P linearly in broilers (Dersjant-Li and Kwakernaak, 2019). Efficacy of phytase in chicks increases sharply with age (Fig. 4.13), with decreases in dietary Ca in chicks (Fritts and Waldroup, 2006), hens (Keshavarz, 2000b) and pigs (Lei *et al*., 1994): efficacy also decreases with increases in dietary P_i. Experiments with very young chicks (<3 weeks old) and excess dietary Ca (e.g Wealleans *et al*., 2019) may overestimate general phytase efficacy.

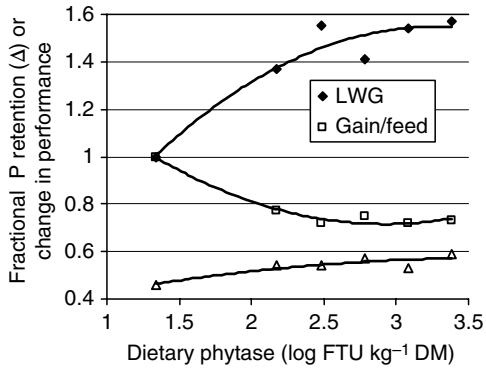

Fig. 6.19. Effects of *Escherichia coli* 6-phytase on fractional retention of phytate phosphorus, relative growth rate and feed conversion rate of broiler chicks in the first 14 days of life. (Data from Cowieson *et al.*, 2006; LWG, liveweight gain)

Phytase supplementation does far more than improve the availability of *Py*P. The anti-nutrient, phyate, is degraded and the essential nutrient, myo-inositol, generated as the end-product of complete *Py*P digestion (Zyla *et al.*, 2004). The phytate molecule is negatively charged at acid pH and can impair absorption of Na and amino acids, as well as form unabsorbable complexes with Mg and trace elements: phytase supplementation raises amino acid and trace element availabilities. Benefits in performance and bone mineralization from phytase supplementation (Lu *et al.*, 2019a, b), including those in Figs 6.17 and 6.19, often exceed those attributable to improved A_p alone and supra-supplementation with phytase has been advocated (Bedford and Rousseau, 2017). Phytase supplements can act synergistically with other enzymes in pigs (Kiarie *et al.*, 2020) and poultry, and increase transit rate through the gut in chicks (Watson *et al.*, 2006), thus increasing food intake. Phytase supplementation may affect sodium metabolism and retention (p. 189).

- Endogenous plant phytases should not be forgotten.

Already high, phytase activity in buckwheat was greatly enhanced by germination, prior to feeding (Chowdhury *et al.*, 2017). Phytases from genetically modified wheat were more effective than synthetic phytases in raising AA_p in broilers (Scholey *et al.*, 2017). The role of exogenous and endogenous plant phytases in reducing pollution of watercourses with P is discussed in Chapter 20.

Other supplements

Adding citrate (up to 6%) to marginally P-deficient diets linearly increased chick growth rate and tibia ash, probably by chelating calcium and improving utilization of *Py*P, acting additively with phytase supplements (Boling *et al.*, 2000b). Pigs showed much smaller responses to citrate in diets containing less Ca. Supplementation with vitamin D_3 (Qian *et al.*, 1997) and synthetic analogues (Biehl and Baker, 1997a, b; Snow *et al.*, 2001; Cheng *et al.*, 2004) also enhances *Py*P utilization in broiler chicks, probably by stimulating secretion of endogenous alkaline phosphatases (Edwards, 1993). *Solanum malacoxylon* (or *glaucophyllum* SG) may provide an inexpensive natural source of vitamin D_3 and simultaneously improve Ca utilization (Cheng *et al.*, 2004) but causes anorexia in broiler chicks when used without reducing dietary Ca. Combined supplements probably act synergistically at different stages of phytate degradation (Fig. 6.20). A combination of citrate (15 g), wheat phytase (461 U as middlings) and microbial phytase (300 U kg^{-1}DM) replaced P_i in rations for growing pigs (Han *et al.*, 1998). Inconsistent additive effects (Cheng *et al.*, 2004) may have been due to choice of extreme treatments (1200 FTU phytase) and a Ca-deficient basal diet. Xylanase and glucuronidase supplements may act syngergistically with phytase by enhancing accessibility to substrate.

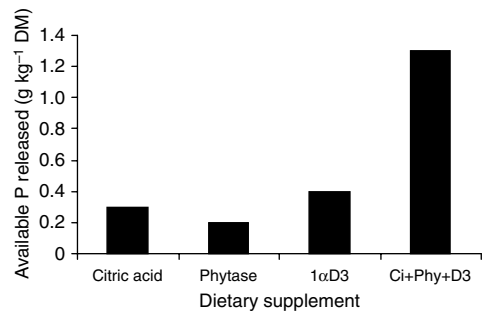

Fig. 6.20. Dietary supplements of citrate (Ci; 30–40 g), 3-phytase (300 FTU) and 1α(OH) D_3 (5 µg kg^{-1}DM) each released significant amounts of available phosphorus from a corn/soybean meal diet for broiler chicks. Combining supplements produced synergistic responses when each was used at a suboptimal level. (Data from Snow *et al.*, 2004)

Phosphorus in Feeds for Ruminants

Herbage

On a global scale, herbage P varies more widely than is shown by UK data (Table 6.4), being greatly influenced by P status of soils, plant maturity and climate. On average, herbage P increases by 0.03–0.05g kg^{-1}DM mg^{-1}extractable soil P (Minson, 1990; Jumba *et al.*, 1995). Temperate forages generally contain more P than tropical forages (3.5 > 2.3g kg^{-1}DM) but this may be due to greater use of fertilizer P. Herbage P declines as most swards mature, particularly during any dry season. Alfalfa generally contains more P than grasses and tropical legumes more P than tropical grasses (3.2 v 2.7g kg^{-1}DM) (Minson, 1990) but there are exceptions. Some *Stylosanthes* species can grow vigorously on soils low in P but contain little (often <1.0g P kg^{-1}DM). Tolerance of low soil P may be shared by other legumes. Four contrasting legume species from Costa Rica were consistently low in P compared with grasses (Table 6.8) but were much richer in Ca, giving Ca:P ratios almost fivefold higher than those in grasses.

Selective grazing

Grazing animals prefer phosphorus-fertilized pasture when given a choice (Jones and Betteridge, 1994) and select pasture components that are richer in P than the whole sward. Workers at Armoedsvlakte noted two- to threefold higher herbage P in grass selected by cattle (i.e. sampled via oesophageal fistulae) than in hand-plucked samples (Engels, 1981) and ceased using herbage samples to monitor P supply (Read *et al.*, 1986a, b; De Waal *et al.*, 1996), as have others (Karn, 1997). Samples from fistulated animals need correction for contamination by salivary P (e.g. using parenteral doses of ^{32}P: Coates *et al.*, 1987; Langlands, 1987; Coates and Ternouth, 1992). Selectivity is most important when the sward contains a mixture of young, mature and senescent material and/or species that vary in herbage P and palatability (McClean *et al.*, 1990). Selectivity is unimportant in single-species swards at a given stage of maturity because distribution within plants is relatively uniform. If an entire herbage mass of mixed species is removed during a grazing period, early selection of P-rich herbage will have little effect on cumulative P intake. Hand-plucked samples can be standardized to give samples of known maturity for particular species (Kerridge *et al.*, 1990) and herbage P should thus be measured whenever possible to record changes in available P supply from the soil from year to year.

Roughages and forage crops

Grass silages contain more P than grass hays (Table 6.4), probably reflecting the earlier growth stage at which silage is harvested. Immature, whole-crop cereal silages became widely used as feeds for ruminants in autumn and winter but can be low in P because the immature grain lacks a P-rich seedcoat. By contrast, cereals grazed at the vegetative stage, increasingly used as winter feeds in Australia, are good sources, containing 4.0–5.4g P kg^{-1}DM (Masters *et al.*, 2019). Brans have high, straws low

Table 6.8. Mean (standard deviation) concentrations of phosphorus (P) and calcium (Ca) (g kg^{-1}DM) and Ca:P ratios in some tropical grasses and legumes from Costa Rica.[a]

	n	P	Ca	Ca:P
Grass species				
Kikuyu (*Pennisetum clandestinum*)	5	3.1 (0.41)	2.5 (0.25)	0.8
Ratana (*Ischaemum ciliare*)	5	1.8 (0.70)	3.0 (0.57)	1.7
Signal grass (*Brachiaria decumbens*)	5	1.4 (0.27)	3.8 (0.72)	2.7
Star grass (*Cynodon nlemfuensis*)	4	1.5 (0.72)	2.4 (0.09)	1.6
Guinea (*Panicum maximum*)	3	2.4 (1.35)	3.1 (0.40)	1.3
Jaragua (*Hyparrhenia rufa*)	4	1.2 (0.55)	2.4 (0.20)	2.0
Legume species				
Stylo (*Stylosanthes guianensis*)	4	1.4 (0.19)	9.0 (0.39)	6.4
Centro (*Centrosema pubescens*)	3	1.3 (0.19)	9.1 (2.96)	7.0
Arachis (*Arachis glabrata*)	4	1.5 (0.16)	11.2 (0.77)	7.5
Naked Indian (*Bursera simaruba*)	5	1.1 (0.30)	10.4 (2.68)	9.5

[a]O. Adedeji and N.F. Suttle, unpublished data.

(1–2g P kg^{-1}DM) and root crops intermediate P concentrations (Table 6.4). Selection between dietary constituents that vary widely in P can affect actual P intakes from 'complete' diets offered to cows.

Availability

In ruminants, faecal endogenous losses (FEL_P) consist mostly of unabsorbed salivary P secretions and increase as P intake increases. The predominant source of faecal P is of endogenous origin (e.g. 70–90%; Breves *et al.*, 1985) and dwarfs urinary P output, unless requirement is greatly exceeded (AFRC, 1991): differences between P intake and faecal P excretion often underestimate availability as an attribute of feed P. In weaned sheep (Braithwaite, 1986; Portilho *et al.*, 2006), calves (Challa *et al.*, 1989), steers (Zanetti *et al.*, 2017) and goats (Vitti *et al.*, 2000), faecal P is linearly related to P intake over wide ranges, but indicates uniformly high efficiencies of 0.68–0.80, whether the source is fresh or dry forage (0.74 ± 0.09 s.d.; AFRC, 1991), wheat, barley, protein supplements (Field *et al.*, 1984) or blends of cereal and roughage (Zanetti *et al.*, 2017). Wheat straw (Ternouth, 1989) and brans (Knowlton *et al.*, 2001) can clearly provide well-absorbed P but rice bran provided less available P, when included in pelleted diet for sheep (A_P 0.63; Field *et al.*, 1984). Phytate-rich feeds provide readily absorbed P because phytate is extensively dephosphorylated and hydrolysed by microbial phytases and phosphatases, releasing PO_4 that is incorporated into microbial protein (Reid *et al.*, 1947; Nelson *et al.*, 1976; Morse *et al.*, 1992). Microbial P is only marginally less well absorbed than PO_4^{2-}, which – like other anions in all species – is readily absorbed in the small intestine (Bown *et al.*, 1989; Yano *et al.*, 1991). Efficiency of microbial protein synthesis may influence A_P (Bravo *et al.*, 2003b) but well-formulated diets will vary little in this respect. Phytate that escapes the rumen intact may interact with Ca in the small intestine but otherwise is degraded in the caecum (Ray *et al.*, 2012), from where PO_4 might be absorbable.

- Phytate is no longer held to be completely degraded in the rumen.

Adding phytase to dairy cow diets containing 38% grain in processed forms raised plasma P_i (Kincaid *et al.*, 2005). Surveys in US dairy cows found 18% of faecal P present as phytate (Toor *et al.*, 2005) and positively correlated with dietary Ca (Chapuis-Lardy *et al.*, 2004). In lambs given P mostly in finely ground corn and SBM, 53% was excreted in faeces (Dias *et al.*, 2006). Treatment with formaldehyde to reduce rumen degradability of protein reduced phytate degradability (Park *et al.*, 1999) and may reduce A_P from sources such as SBM. With slowly degradable rapeseed meal, formaldehyde treatment did not affect repletion rate in sheep (Bravo *et al.*, 2003a). Supplementary P_i is usually given as DCP, and AA_{Pi} can be measured in simple balance studies from fractional increments in faecal P, when small P_i supplements are added to low P diets (Table 6.9). Phosphorus was as well absorbed from DCP as in pigs when dietary Ca was low but highly variable when dietary Ca was raised slightly: Diet 4, without supplement, probably provided so little P that appetite was reduced and AA_{Pi}

Table 6.9. Apparent availability coefficients of phosphorus (AA_P) in feed and dicalcium phosphate supplement (DCP) in different diets, those for DCP calculated as the reciprocal of increase in faecal P/increase in P intake. (From Zanetti *et al.*, 2017)

Main dietary constituents (fraction of ration)	Dietary Ca (g kg^{-1}DM)	Dietary P (g kg^{-1}DM)	Feed AA_P	DCP AA_P
1. Sugarcane, ground corn, SB hulls (0.4:0.3:0.21)	2.0	1.54	0.60	0.55
2. Sugarcane, ground corn (0.4:0.5)	2.7	1.80	0.61	0.63
3. Corn silage, ground corn, SB meal (0.58:0.26:0.13)	2.5	1.98	0.50	0.63
4. Sugarcane, ground corn, SB hulls (0.69:0.13:0.14)	3.8	1.00	0.08	0.80
5. Sugarcane, ground corn, SB hulls (0.40:0.27:0.28)	3.7	1.40	0.45	0.25

for the supplement became enhanced by compensatory mechanisms. Undegraded phytate may form insoluble, unabsorbable complexes with calcium[xvii] (Goff, 2018) and may have explained low feed AA_P in Diet 4. Excess dietary Ca may also form precipitates with phosphates and should be avoided in rations for ruminants.

Availability in Feeds for Herbivores

Hind-gut fermenters such as the horse excrete excess absorbed P via faeces rather than urine, presumably facilitated by relatively high salivary P outputs. Mature trotters, given hay and concentrate in equal measure and containing 2.9g P kg^{-1}DM, absorbed only 0.024 of ingested P and excreted none in urine (van Doorn *et al.*, 2004). Supplements of either MSP or bran that raised daily intake by 23–25 g P raised urinary excretion by only 2 g P day^{-1} and AA_P to only 0.15 or 0.11, respectively. Studies with rabbits indicate that hind-gut fermenters can degrade *Py*P in the caecum (Marounek *et al.*, 2003) but this may not be fully detected by measuring AA_P. Lack of effects of Ca (Grace *et al.*, 2003), phytase and replacing P_i with bran on AA_P or urinary P (van Doorn *et al.*, 2004) does not rule out possible changes in A_P in hind-gut fermenters on high P intakes. Urinary P excretion will only reflect availability once capacity to excrete P via saliva and faeces has been saturated. In foals (Grace *et al.*, 2003) and lactating mares (Grace *et al.*, 2002) given similar forages, AA_P was far higher in mares (0.47 versus 0.07–0.14). This presumably reflected diversion of absorbed P from salivary to mammary secretion in mares and illustrates the limitations of faecal P as an indicator of potential value. Herbivores may be able to absorb P from feeds with a similar efficiency to ruminants.

Requirements for Ruminants

Factorial estimates of requirements varied between national authorities and research groups because different values were used for the *M*, *G* and *A* components of models (p. 9) (sheep and cattle, AFRC, 1991; dairy cows, NRC, 2001; small ruminants, NRC, 2007; beef cattle, NRC, 2017). Different outcomes are rarely important in terms of production because P is rarely the most limiting nutrient but are important in the environmental pollution context, because P has been consistently over-prescribed.

Net requirements for maintenance

The majority of sheep and cattle fed roughage diets excrete very little urinary P, even with plasma P_i is as high as 2 mmol l^{-1} (AFRC, 1991), but faecal endogenous losses (FEL_P) are relatively large. Obligatory losses ($^oFEL_P$) were predicted to be 0.63g kg^{-1}DMI for diets that were mostly pelleted, but 1.6 times higher (i.e 1 g) for loose, roughage-based diets, which stimulate salivary flow. Such high losses would explain the low AA_P coefficient (0.08) reported in dry cows on a low-P diet, consisting mostly of wheat straw (Valk *et al.*, 2002). NRC (2001) used the roughage factor of 1.6 to predict requirements for all dietary circumstances, but without validation.

- New studies suggest that maintenance needs vary between diets.

With extrapolation error minimized by providing a dry, roughage-based diet with P close to requirement (1.3–1.8g P kg^{-1}DM), $^mFEL_P$ was 0.8–0.9g kg^{-1}DMI in castrated goats (equivalent to 28 mg P kg LW; Tayo *et al.*, 2009; Yan *et al.*, 2011). A similar approach in cattle, receiving succulent forages, estimated $^mFEL_P$ to be much lower (8–10 mg P kg^{-1}LW; Zanetti *et al.*, 2017). With a diet containing 40% concentrates and ground roughage, $^mFEL_P$ was only 2.7 mg P kg^{-1}LW in goats, measured by radioisotope dilution (p. 27) (Vitti *et al.*, 2000). In beef cattle grazing tropical pasture in Queensland, FEL_P was extremely low (Ternouth *et al.*, 1996; Ternouth and Coates, 1997).

- Ground roughage may not raise FEL_P.[xviii]
- Low concentrate:succulent forage ratio may lower $^oFEL_P$[xix];
- A roughage effect may not be valid for lush pasture.
- The AFRC (1991) roughage factor (1.6) should only be used for sheep and cattle fed predominantly on chopped or long, dry roughages.

Net requirements for production

Many new and varied estimates have appeared of net needs for growth (G_P) without realizing that P accretion is affected by plane of P and Ca nutrition (e.g. in early-weaned goat kids and

in milk-fed lambs (Fig. 1.4)). It has also been suggested from comparative fractional retention data that G_P is higher for Zebu than crossbred Holstein cattle (De Silva *et al.*, 2015). Any inhibition of bone accretion and mineralization in confined growing animals (p. 74) would lower G_P. Similar effects in confined lactating animals complement the dietary supply of P with an endogenous supply that provides excess P, since the respective ratios of Ca:P in bone and milk are 2.0 and 1.3:1.

Gross requirements for cattle

The literature reviewed above (p. 151) supported a small differentiation in maintenance components for diets known or likely to stimulate low (grass, silage or roughage-free diets) or high (dry roughages) salivation rates and differ slightly in respective A_P (0.8 and 0.7) (Table 6.10). The 'low roughage' model for beef cattle matches findings on P metabolism in grazing beef cattle in Queensland (Hendricksen *et al.*, 1994; Ternouth *et al.*, 1996; Ternouth and Coates, 1997) and lack of responses to P supplements in either growth rate or bone quality with >1.4g P kg^{-1}DM, even when dietary Ca was raised, in two studies: 'finishing' steers on a roughage-free diet (Erickson *et al.*, 1999) and Nellore steers on succulent roughages (Zanetti *et al.*, 2017). With a diet probably falling between the two models, 2.4g P kg^{-1}DM was inadequate for lactating dairy cows, while 2.8g P kg^{-1}DM sustained both health and yield (30 kg day^{-1}) (Valk and Sebek, 1999). Feeding 3.1g P kg^{-1}DM for 2–3 years to cows yielding 40 kg milk day^{-1} had no adverse effects on health or milk yield compared with 3.9 or 4.7g P kg^{-1}DM: rib bone ash fell from 56.2% to 53.9% DM, but without affecting bone strength (Wu *et al.*, 2001).

- Most mixed diets for high-yielding cows do not need P_i supplementation.

Dietary tests of requirement usually hold dietary P constant but P requirements decline during growth and lactation. Faecal P increased steadily throughout lactation from approximately 5 to 6.5g kg^{-1}DM with dietary P at 3.1g kg^{-1}DM (Wu *et al.*, 2001), indicating an increasing excess of supply over need. With less dietary P (2.4g kg^{-1}DM), cows retained 3.2g P d^{-1} as lactation progressed and milk yield declined (Valk *et al.* 2002). Feeding more P in early

Table 6.10. Requirements of cattle for dietary phosphorus, modified from AFRC (1991)[a] by using different factorial models for diets promoting low (L) or high (H) salivary flow rates (e.g. total mixed rations including succulent forage and green pasture qualify for L; diets based on straw, long hay, and dry season pasture would qualify as H).

			DMI (kg day^{-1})	Need for P (g kg^{-1}DM)	
	Liveweight (kg)	Production level	L–H	H	L
Growing cattle	100	0.5 kg day^{-1}	1.7–2.8	3.8	3.3
		1.0 kg day^{-1}	2.4–4.5	4.3	4.4
	300	0.5 kg day^{-1}	3.4–5.7	2.4	1.7
		1.0 kg day^{-1}	4.7–8.3	2.7	2.3
	500	0.5 kg day^{-1}	6.1–10.9	1.6	1.0
		1.0 kg day^{-1}	6.5–11.6	2.3	1.8
Pregnant cow (dairy/beef)	600	23 weeks	4.0–6.3	1.7	0.8
		31 weeks	4.7–7.2	1.9	1.0
		39 weeks	6.1–9.1	2.1	1.3
Calf 40 kg at birth		Term	7.5–11.2	2.2	1.5
Lactating cow	600	10 kg day^{-1}	12.0 (m)	2.6	–
			9.9 (bm)	2.8	–
		20 kg day^{-1}	11.4 (m)	n.a.	2.3
			10.1 (bm)	n.a.	2.5
		40 kg day^{-1}	19.3 (m)	n.a.	2.6
			17.8 (bm)	n.a.	2.8

bm, fed below maintenance; m, fed to maintain body weight; n.a., unattainable performance.
[a]In the factorial model (see p. 9), where need = (M + P + L) / A, the absorption coefficients (A) are 0.8 and 0.7, M is 0.6 and 1.0g P kg^{-1}DMI for L for H diets, respectively and L = 1.4g P/kg milk.

lactation does not prevent a negative balance in either cows (Knowlton and Herbein, 2002; Elizondo Salazar *et al*., 2013) or ewes (Braithwaite, 1983) because P is being unavoidably mobilized from the skeleton (p. 58). Requirements for P need *not* be met on a day-to-day basis.

- Instead, P should be briefly and moderately overfed, along with Ca, during and after late lactation to replenish skeletal reserves.
- In practice, decreases in concentrate allowance (or energy density) as lactation progresses will conveniently reduce P supply.

Phosphorus requirements for small ruminants

Similar distinctions between diet types are made in the recommendations for sheep (Table 6.11) on the two different diet types, following the arguments made by Scott *et al.* (1984, 1985). Since A_p is subject to genetic variation in sheep and marked hereditable differences in ${}^{o}FEL_p$ are found both within and between breeds (Field *et al.*, 1984, 1986), an allowance might be needed to meet G_p in all sheep. Feeding to minimum requirements rather than exceeding generous NRC recommendations, a common practice, will greatly reduce environmental phosphorus pollution (see Chapter 20).

Phosphorus requirements for horses

Experiments with young growing ponies (Schryver *et al.*, 1974) and mature horses (Schryver *et al.*, 1970, 1971) indicated relatively high requirements for younger animals. Net requirement for growth increased over the years because growth rates increased (Underwood and Suttle, 1999). An estimated need for 0.7g P kg^{-1}DM in grazing Thoroughbred foals (200 kg LW and gaining 1 kg day^{-1}) was derived factorially, assuming a growth increment (8.3g P kg^{-1} LWG), an M_p of 10mg kg^{-1}LW (ARC, 1980) and an A_p coefficient of 0.5 (Grace *et al.*, 1999), appropriate for mixed diets. However, A_p may be closer to 0.65 for grass, giving a lower requirement at pasture of 0.54g P kg^{-1}DM. Either way, growing horses are unlikely to receive less P than they need from natural feeds, including grains.

Preventing Insufficiency at Pasture

Agronomic methods

Overcoming insufficiency by raising herbage P through fertilizer application requires both agronomic and animal husbandry skills. Phosphate applications can raise herbage yields as well as

Table 6.11. Requirements of sheep for dietary phosphorus, modified from AFRC (1991)[a] by using two different factorial models for diets that promote low (L) or high (H) salivary flow rates, as delineated in the caption to Table 6.10.

	Liveweight (kg)	Production level/stage	DMI (kg day^{-1})	Need for P (g kg^{-1} DM)	
			L–H	H	L
Growing lambs	20	100 g day^{-1}	0.40–0.67	2.4	3.0
		200 g day^{-1}	0.57	n.a	4.1
	40	100 g day^{-1}	0.66–1.11	1.9	2.1
		200 g day^{-1}	0.93–1.77	2.1	2.5
Pregnant ewe carrying twins[b]	75	9 weeks	0.71–1.10	1.5[d]	1.1[d]
		13 weeks	0.85–1.28	1.9	1.7
		17 weeks	1.13–1.68	2.1	2.1
		Term	1.62–2.37	2.0	1.9
Lactating ewe	75	2–3 kg milk day^{-1}	1.8 (m)[c]	n.a.	2.8
			1.5 (bm)	n.a.	3.2

bm, fed below maintenance; m, fed to maintain body weight; n.a., unattainable performance

[a]Predictions from two models, as described in Table 6.7.

[b]Requirements for small ewes carrying single lambs are similar, assuming that they will eat proportionately less DM.

[c]Requirements are influenced by the ability of the diet to meet energy need and prevent loss in body weight (m); diets that allow loss of body weight at 0.1 kg day^{-1} (bm) are associated with higher requirements.

[d]Sufficient for dry ewes.

increase herbage P: however, responses varied widely (Minson, 1990), whatever the source (rock phosphate, single or triple superphosphate, complete fertilizer) and rate of application (Jones, 1990). Plant uptake of fertilizer P is poor on acid, iron-rich soils, due to high sorption capacity of soils for P: benefits may not outweigh application costs on sparse, extensive, native pastures, where productivity is limited by rainfall. Responses can also depend on interactions with other elements. Superphosphate applications were popular in New Zealand because contaminant Cu and Co sometimes boosted responses but continuous use caused substantial accumulation of potentially harmful Cd in soils (p. 487). Milk yields were only increased in dairy cows on tropical grass pasture when P was applied with 300kg N ha^{-1} (see Fig. 2.7) (Davison *et al.*, 1997). Responses were attributed to increases in herbage mass rather than herbage P, although the latter increased from 0.6 to 1.3g kg^{-1}DM. Fertilizer treatments that maximize herbage yield do not invariably meet animal requirements for P or maximize animal performance. A retrospective survey of herbage N and P in 250 samples of Campos grassland in Uruguay showed that roughly half contained <1.5g P kg^{-1}DM but P fertilizers were rarely worth applying, because nearly all N:P ratios were <14 and lack of N would have limited growth responses in beef cattle (Cardozo *et al.*, 2017). Lack of N can be corrected by introducing legumes, species able to tolerate low soil P availability, such as *Stylosanthes*, being particularly useful (p. 149). Ability to absorb P from phytate (Du *et al.*, 2009), an important source of soil P, tolerance towards Al toxicity, changes in root morphology and root metabolism (Luo *et al.*, 2020) all contribute to *Stylosanthes*' tolerance of P-deficient soils. However,

- only an increase in plant P will avert P insufficiency: indeed improving herbage mass and N without P would exacerbate insufficiency.

When *Lotus cornicalatus* and *Trifolium repens* were oversown on native Campos pasture, without or with an increased fertilizer P application from 13 to 26 kg ha^{-1}, post-weaning liveweight gain was increased from 0.319 to 0.478 and 0.586 kg/day in a five-year study (del Pino *et al.*, 2016). Responses varied between years, being low if rainfall was low and the legume contribution fell, and attributed to increase in herbage P rather than herbage mass. A delicate balance that has to be struck between herbage and animal productivity by adjusting stocking rate (p. 19) and is never more critical than in times of drought. Use of fertilizer P on swards of tolerant species was restricted to that required to maximize herbage yield (up to 10 kg P ha^{-1} $year^{-1}$) and any additional P required was given directly (Miller *et al.*, 1990).

Nutritional supplements

Most sources of P_i have uniformly high availabilities for sheep, growing beef cattle and horses (Soares, 1995). In sheep given coarsely ground rock phosphate (RP), particles can be trapped in the alimentary tract and give a falsely high estimate (0.66) of A_P (and presumably A_{Ca}) (Suttle *et al.*, 1989): a value two thirds that of DCP (0.55 versus 0.80) may be more appropriate (Dayrell and Ivan, 1989). Ground RP is relatively unpalatable and can contain enough fluoride to cause toxicity (p. 493). *In vitro* solubility in citric acid differentiated availability of RP and DCP as well as an *in vivo* measurement of A_P (Suttle *et al.*, 1989): neutral ammonium citrate can also be used as an extractant. Assessments of availability can be affected by choice of basal diet (Table 6.9). Supplements for grazing animals provided in loose or block form, strategically placed (p. 47) and protected from rain, could improve performance (Miller *et al.*, 1990) but occasionally retarded growth (Grant *et al.*, 1996). Blocks containing a 1:1 mixture of DCP and salt are generally well consumed, particularly when sweetened with molasses, but not by every individual nor throughout the grazing season. Organic P is a cheap alternative and can be provided by including 20–30% wheat bran or rice bran in a molasses–urea block or loose supplement. Supplementation of piped drinking water with water-soluble phosphate was a popular and successful early method of prevention, where there were no natural water sources[xx] but sometimes corroded dispensers (Miller *et al.*, 1990). Water-soluble MCP stimulated liquid-associated rumen microbes and increased intake of a low P diet more than DCP (Ramirez-Perez *et al.*, 2008). Where MCP and DCP are used in free-access mixtures, supplementary Ca is automatically supplied but rarely needed.

Phosphorus Toxicity

The PO_4^{2-} ion is intrinsically well-tolerated, readily excreted in urine and plasma P_i is allowed to

fluctuate widely. Very high plasma P_i triggers the FGF23/klotho pathway and flow of P_i from bone to plasma is reduced (p. 128). All livestock species tolerate excess dietary P well. Problems arise through adverse interactions with other minerals and cumulative effects. Cereal-based diets are naturally high in P and, when supplemented with magnesium, predispose recipients to phosphatic calculi, crystals of magnesium phosphate commonly nucleating a calculus (Suttle and Hay, 1986). Sheep with high, inherited efficiencies for phosphorus (and magnesium) absorption have high urinary P and Mg and are particularly vulnerable (Field *et al.*, 1984). Excess dietary P predisposes broilers to tibial dyschondroplasia (TD; p. 70) unless excess Ca is also fed (Edwards and Veltmann, 1983). Incidences of TD and 'calcium rickets' increased linearly as dietary P increased, a small increment from 4.8 to 5.9g P kg^{-1}DM sufficient to increase incidences without a similar increase in dietary Ca (Driver *et al.*, 2005). Excesses of acidogenic sources such as MCP disturb acid–base balance in laying hens (Keshavarz, 1994). In the horse, feeding excess phosphorus in diets low in calcium can cause secondary hyperparathyroidism.

Notes

[i] Valk and Sebek's (1999) transitional diet contained 63% wheat straw, only 1.6g P, 1.3g Ca kg^{-1}DM and allowed only 0.8g P $day^{-1}$to be retained, despite recovery of plasma P_i to 2.0 mmol l^{-1}.

[ii] Specific transporters for PO_4 have been identified in avian jejunal mucosa but were not upregulated by P deprivation (Huber *et al.*, 2006).

[iii] Indices include increases in plasma PTH and Ca, hydroxyproline, deoxypyridinoline and type 1 collagen (Leisegang *et al.*, 2000; Peterson *et al.*, 2005), bone-specific alkaline phosphatase (Wu *et al.*, 2019) in serum or urine, all of which indicate bone resorption: values fluctuate widely around parturition. Low serum osteocalcin can confirm lack of bone growth due to lack of P but values can decrease without bone resorption and may be a secondary response (Scott *et al.*, 1994). Increases in renal 1α hydroxylase activity, plasma calcitriol and intestinal calbindin occur in cattle deprived of P (Littledike and Goff, 1987) but not in sheep (Breves *et al.*, 1985) or goats (Yano *et al.*, 1991).

[iv] Invasive methods such as rib (Little and Minson, 1977; Little and Radcliff, 1979; Beighle *et al.*, 1993) and caudal vertebrae biopsy in cattle (Keene *et al.*, 2004) have been superseded by radiographic photometry, ultrasound (Ternouth, 1990; Williams *et al.*, 1991b; Keene *et al.*, 2004), computer-assisted tomography (Grace *et al.*, 2003) and dual-photon absorptiometry.

[v] The Létourneau-Montminy *et al.*, (2012) meta-analysis also discerned positive linear effects of body weight, indicating possible increases in AA_P with age, and negative effects of dietary Ca, evident in earlier studies (e.g. Fig. 6.16) but failed to detect interactions of calcium with other factors, evident in many experiments.

[vi] Phytase activity is induced by P deprivation in chick kidney but not in intestinal mucosa (Cho *et al.*, 2006). Conversion produces a family of partially degraded phytate molecules whose spectrum changes during passage through acid and alkaline regions of the gut, allowing different phytases to act synergistically (Zyla *et al.*, 2004).

[vii] Far less phytate in corn was degraded by 28 d.o. turkeys (0.06–0.15) than by broilers (0.64–0.76) but the difference in PCD_P was only twofold (0.22–0.28 and 0.51–0.60, respectively): phytase marginally increased PCD_P in both species by <17% in diets containing <6g Ca kg^{-1}DM (Ingelmann *et al.*, 2019).

[viii] Neither model is robust. Early weaned pigs have unstable digestive systems and are prone to diarrhoea. Pharmacological dietary Zn supplementation, used to control post-weaning diarrhoea, decreased $STTD_P$ (and $STTD_{Ca}$). $ATTD_P$ varied from 0.5 to 0.8 between individual piglets, given a corn/SBM diet with little or no added phytase (Arredondo *et al.*, 2019). The chosen basal diet can also affect assessments. Cassava meal provided 24% less $STTD_P$ than copra expeller meal but not significantly so because of high variability (Son *et al.*, 2013). Fishmeal had a higher $STTD_P$ when included in a diet with maize and corn germ than in a semi-purified diet, low in fibre (González-Vega *et al.*, 2015).

[ix] With dietary Ca in blends of SBM with cereal providing 4g P kg^{-1}DM, half as *Py*P, as either 4.5 or 6.6g Ca kg^{-1}DM, AA_P was 0.66 ± 0.041 and 0.57 ± 0.036, respectively (Dersjant-Li *et al.*, 2017). Varying phytase as well as dietary Ca caused AA_P in canola meal to vary from 0.49 to 0.80 (González-Vega *et al.*, 2013).

[x] In quail, 28 d.o. and given a semi-purified diet, high in Ca (21.5g kg^{-1}DM), growth varied enormously, depending on the feedstuff added; $^{m}FEL_P$ was much higher at 382mg kg^{-1}DMI in younger than older birds (Ghazaghi *et al.*, 2019) and $STTD_P$ much higher than $ATTD_P$ (ref 21 in Table 6.5).

[xi] Liu *et al.* (2013) wished to know whether the $STTD_P$ value attributed to SBM was affected by Ca:P ratio, which varied from 1.2 to 2.0 and concluded that it was not, using three-point linear regressions that accounted for only 64–79% of variation and predicted high FEL_P 448–864mg P kg^{-1} DMI .

[xii] Underwood and Suttle (1999) concluded from the results of Reinhart and Mahan (1986) that the similar gradients for growth and plasma Pi at the two dietary P levels indicated minimal influence of Ca:P ratio: different gradients for FCE may

involve interactions with phytate. The lower P level (4g P kg^{-1}DM) failed to meet requirements and the smallest addition of Ca increased the deficit.

[xiii] Data for AA_P are not presented for starter, grower or finishing pigs in the set of three papers and are impossible to calculate from the data presented.

[xiv] It is possible that responses to P_i were muted by concomitant additions of limestone to preserve a Ca:P ratio of 1.2–1.3, increasing dietary Ca from 5 to 7g kg^{-1}DM. Bone ash, bone Ca and bone strength increased quadratically with additions of 250, 500 and 1000 FU of each of two phytases, with no dietary Ca.

[xv] The third and most deviant study added single increments to a diet of marginal P concentration (1.7g PCD_P kg^{-1}DM), used indigestible marker technology (TiO_3; p. 26) and calculated PCD_P by difference (van Harn *et al*., 2018). High availability was attributed to relatively low dietary Ca (5.0–5.6g kg^{-1}DM) in a diet containing 0.6g *Py*P kg^{-1}DM, which had a high PCD_{Ca} of 0.70. Even bone meal had a relatively high PCD_P of 0.68, while a product derived from hydrolysed bone was almost completely available (0.95).

[xvi] A wide range of exogenous phytases of microbial and fungal origin were tested for P_i-sparing effect, beginning in pigs (e.g. Kornegay and Qian, 1996; Murry *et al.*, 1997), and possessed similar efficacies within studies (Paditz *et al.*, 2004; Poulsen *et al*., 2010). However, the amount of P_i spared by 'first-generation' phytases varied widely between studies (Knowlton *et al.*, 2004; Adeola and Applegate, 2010), for reasons that are now more obvious (see p. 138).

[xvii] High dietary Ca (>8g kg^{-1}DM) reduced P status in sheep (Young *et al.*, 1966; Field *et al.*, 1975; Wan Zahari *et al.*, 1990): smaller increments from 3.4 to 5.4g kg^{-1}DM decreased A_{Pi} in sheep by 18%, but had no such effect in the low-P basal diet (1.5g P kg^{-1}DM) (Field *et al.*, 1983).

[xviii] Replacing unchopped alfalfa hay (3%) with ground soy hulls (10%) in a dairy ration consisting mainly of silage and providing 3.3g P kg^{-1}DM, increased AA_P from 0.34 to 0.44 (Wu, 2005), despite the small increase in total dietary fibre intake.

[xix] Increases in dietary alfalfa silage content (from 48% to 58%) of lactating cows tended to reduce mean AA_P from 0.37 to 0.31 (Wu *et al.*, 2003) but might partly reflect an associated reduction in milk yield and milk P export rather than a 'succulent forage' effect, surplus P having been excreted via the faeces. A 42:58 (concentrate:corn silage) diet had a lower AA_P coefficient (0.52) than two 60:40 (concentrate:sugarcane bagasse diets (0.6 ± 0.020) (Zanetti *et al*., 2017).

[xx] Supplementation of the water supply with soluble superphosphate was effective (Du Toit *et al*., 1940; Snook, 1949; Scharp, 1979) but water consumption fluctuates: more expensive water-soluble sources, such as MSP, can be used (Hemingway and Fishwick, 1975).

References

Adebiyi, A. and Olokusi, O. (2015) Determination in broilers and turkeys of true phosphorus digestibility and retention in wheat distillers dried grains. *Animal Feed Science and Technology* 207, 112–119.

Adeola, O. and Applegate, T.J. (2010) Phosphorus and calcium equivalency of phytase. *Proceedings of the 1st International Phytase Summit,* Washington, DC, pp, 45–64.

Adeola, O., Sands, J.S., Simmins, P.H. and Schulze, H. (2004) The efficacy of an *Escherichia coli*-derived phytase preparation. *Journal of Animal Science* 82, 2657–2666.

AFRC (1991) Technical Committee on Responses to Nutrients. Report No. 6. A reappraisal of the calcium and phosphorus requirements of sheep and cattle. *Nutrition Abstracts and Reviews* 61, 573–612.

Akinmusire, A.S. and Adeola, O. (2009) True digestibility of phosphorus in canola and soybean meals for growing pigs: influence of microbial phytase. *Journal of Animal Science* 87, 977–983.

Akter, M., Graham, H. and Iji, P.A. (2016) Response of broiler chickens to different levels of calcium, non-phytate phosphorus and phytase. *British Poultry Science* 57, 799–809.

Applegate, T.J., Webel, D.M. and Lei, X.G. (2003) Efficacy of phytase derived from *Escherichia coli* and expressed in yeast on phosphorus utilisation and bone mineralisation in turkey poults. *Poultry Science* 82, 1726–1732.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

Arredondo, M.A., Casas, G.A. and Stein, H.H. (2019) Increasing levels of microbial phytase increases digestibility of energy and minerals in diets fed to pigs. *Animal Feed Science and Technology* 248, 27–36.

Augspurger, N.R. and Baker, D.H. (2004) High dietary phytase levels maximise phytate-phosphorus utilisation but do not affect protein utilisation in chicks fed phosphorus- or amino acid deficient diets. *Journal of Animal Science* 82, 1100–1107.

Augspurger, N.R., Webel, D.M., Lei, X.G. and Baker, D.H. (2003) Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *Journal of Animal Science* 81, 474–483.

Augspurger, N.R., Webel, D.M. and Baker, D.H. (2007) An *Escherichia coli* phytase expressed in yeast effectively replaces inorganic phosphorus for finishing pigs and laying hens. *Journal of Animal Science* 85, 1192–1198.

Baker, S.R., Kim, B.G. and Stein, H.H. (2013) Comparison of values for standardized total tract digestibility and relative bioavailability of phosphorus in dicalcium phosphate and distillers dried grains with solubles fed

to growing pigs. *Journal of Animal Science* 91, *203–210.*

Bar, A. and Hurwitz, S. (1984) Egg shell quality, medullary bone ash, intestinal calcium and phosphorus absorption and calcium-binding protein in phosphorus-deficient hens. *Poultry Science* 63, 1975–1980.

Barkley, G.R., Miller, H.M. and Forbes, J.M. (2004) The ability of laying hens to regulate phosphorus intake when offered two feeds containing different levels of phosphorus. *British Journal of Nutrition* 92, 233–240.

Bedford, M.R. and Partridge, G.G. (2001) *Enzymes in Farm Animal Nutrition*. CAB International, Wallingford, UK.

Bedford, M.R. and Rousseau X. (2017) Recent findings regarding calcium and phytase in poultry nutrition. *Animal Production Science* 57, 2311–2316.

Beeson, W.M., Johnson, R.F., Bolin, D.W and Hickman, C.W. (1944) The phosphorus requirement for fattening lambs. *Journal of Animal Science* 3, 63–70.

Beighle, D.E., Boyazoglu, P.A. and Hemken, R.W. (1993) Use of bovine rib bone in serial sampling for mineral analysis. *Journal of Dairy Science* 76, 1047–1072.

Biehl, R.R. and Baker, D.H. (1997a) 1α-Hydroxy cholecalciferol does not increase the specific activity of intestinal phytase but does improve phosphorus utilisation in both caectomised and sham-operated chicks fed cholecalciferol-adequate diets. *Journal of Nutrition* 127, 2054–2059.

Biehl, R.R. and Baker, D.H. (1997b) Utilisation of phytate and non-phytate phosphorus in chicks as affected by source and amount of vitamin D_3. *Journal of Animal Science* 75, 2986–2993.

Bikker, P. and Blok, M.C. (2017) Phosphorus and calcium requirements of growing pigs and sows. CVB Documentation Report no. 59. Wageningen, The Netherlands.

Bikker, P., Spek, J.W., Van Emous, R.A. and Van Krimpen, M.M. (2016) Precaecal phosphorus digestibility of inorganic phosphate sources in male broilers. *British Poultry Science* 57, 810–817.

Bohlke, R.A., Thaler, R.C. and Stein H.H. (2005) Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. *Journal of Animal Science* 83, 2396–2403.

Bohn, L., Meyer, A.S. and Rasmussen, S.K. (2008) Phytate: impact on environment and human nutrition: a challenge for molecular breeding. *Journal of Zhejiang University Science B* 9, 165–191.

Boling, S.D., Douglas, M.W., Johnson, M.L., Wang, X., Parsons, C.M., Koelkebeck, K.W. and Zimmerman, R.A. (2000a) The effects of available phosphorus levels and phytase on performance of young and older laying hens. *Poultry Science* 79, 224–230.

Boling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C.M. and Baker, D.H. (2000b) The effects of citric acid on phytate-phosphorus utilisation in young chicks and pigs. *Journal of Animal Science* 78, 682–689.

Bournazel, M., Lessire, M., Duclos, M., Magnin, M., Même, N., Peyronnet, C., Recoules, E., Quinsac, A., Labussière, E. and Narcy, A. (2018) Effects of rapeseed meal fiber content on phosphorus and calcium digestibility in growing pigs fed diets without or with microbial phytase. *Animal* 12, 34–42.

Bown, M.D., Poppi, D.P. and Sykes, A.R. (1989) The effect of a concurrent infection of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* on calcium, phosphorus and magnesium transactions along the digestive tract of lambs. *Journal of Comparative Pathology* 101, 11–20.

Braithwaite, G.D. (1983) Calcium and phosphorus requirements of the ewe during pregnancy and lactation. 2. Phosphorus. *Journal of Agricultural Science, Cambridge* 50, 723–730.

Braithwaite, G.D. (1984) Changes in phosphorus metabolism in sheep in response to the increased demands for phosphorus associated with an intravenous infusion of calcium. *Journal of Agricultural Science, Cambridge* 102, 135–139.

Braithwaite, G.D. (1986) Phosphorus requirements of ewes in pregnancy and lactation. *Journal of Agricultural Science, Cambridge* 106, 271–278.

Brana, D.V., Elis, M., Casteneda, E.O., Sands, J.S. and Baker, D.H. (2006) Effect of novel phytase on growth performance, bone ash, and mineral availability in nursery and grower-finisher pigs. *Journal of Animal Science* 84, 1839–1849.

Bravo, D., Bogaert, C., Meschy, F. and Sauvant, D. (2003a) Plasma phosphorus content and dietary phosphorus availability in adult sheep. *Animal Research* 52, 427–435.

Bravo, D., Sauvant, D., Bogaert, C. and Meschy, F. (2003b) Quantitative aspects of phosphorus excretion in ruminants. *Reproductive and Nutritional Development* 43, 285–300.

Bretziger, P. and Raboy, V. (2006) Effect of four independent low-phytate mutations on barley agronomic performance. *Crop Science* 46, 1318–1322.

Breves, G., Ross, R. and Holler, H. (1985) Dietary phosphorus depletion in sheep: effects on plasma inorganic phosphorus, calcium, 1,25-$(OH)_2$-Vit. D and alkaline phosphatase on gastrointestinal P and Ca balances. *Journal of Agricultural Science, Cambridge* 105, 623–629.

Call, J.W., Butcher, J.E., Blake, J.T., Smart, R.A. and Shupe, J.L. (1978) Phosphorus influence on growth and reproduction of beef cattle. *Journal of Animal Science* 47, 216–225.

Call, J.W., Butcher, J.E., Shupe, J.L., Blake, J.K. and Olsen, A.E. (1986) Dietary phosphorus for beef

cows. *American Journal of Veterinary Research* 47, 475–481.

Call, J.W., Butcher, J.E., Shupe, J.L., Lamb, R.C., Boman, R.L. and Olsen, A.E. (1987) Clinical effects of low dietary phosphorus concentrations in feed given to lactating cows. *American Journal of Veterinary Research* 48, 133–136.

Cardozo, G., Reyno, R., Ayala, W., Cuadro, R., Fernandez, P. *et al*. (2017) Is phosphorus a limiting factor for the productivity of Campos grasslands. *Proceedings of 19th Symposium of the European Grassland Federation*, Alghero, Italy.

Challa, J. and Braithwaite, G.D. (1988) Phosphorus and calcium metabolism in growing calves with special emphasis on phosphorus homeostasis. 2. Studies of the effect of different levels of phosphorus, infused abomasally, on phosphorus metabolism. *Journal of Agricultural Science, Cambridge* 110, 583–589.

Challa, J. and Braithwaite, G.D. (1989) Phosphorus and calcium metabolism in growing calves with special emphasis on phosphorus homeostasis. 4. Studies on milk-fed calves given different amounts of dietary phosphorus but a constant intake of calcium. *Journal of Agricultural Science, Cambridge* 113, 285–289.

Challa, J., Braithwaite, G.D. and Dhanoa, M.S. (1989) Phosphorus homeostasis in growing calves. *Journal of Agricultural Science, Cambridge* 112, 217–226.

Chapuis-Lardy, L., Fiorini, J., Toth, J. and Dou, Z. (2004) Phosphorus concentration and solubility in dairy faeces: variability and affecting factors. *Journal of Dairy Science* 87, 4334–4341.

Cheng, Y.H., Goff, J.P., Sell, J.L., Dalloroso, M.E., Gil, S., Pawlak, S.E. and Horst, R.L. (2004) Utilizing *Solanum glaucophyllum* alone or with phytase to improve phosphorus utilisation in broilers. *Poultry Science* 83, 406–413.

Cho, J., Choi, K., Darden, T., Reynolds, P.R., Petitte, J.N. and Shears, S.B. (2006) Avian multiple inositol polyphosphate phosphatase is an active phytase that can be engineered to help ameliorate the planet's 'phosphate crisis'. *Journal of Biotechnology* 126, 248–259.

Chowdhury, R., Rahman, M. and Koh, K. (2017) Evaluation of buckwheat (Fagopyrum esculentum): intrinsic phytase activity to improve phosphorus availability in broilers. *Journal of Advanced Agricultural Technologies* 4, 82–86.

Coates, D.B. and Ternouth, J.H. (1992) Phosphorus kinetics of cattle grazing tropical pastures and implications for the estimation of their phosphorus requirements. *Journal of Agricultural Science, Cambridge* 119, 401–409.

Coates, D.B., Schachenmann, P. and Jones, R.J. (1987) Reliability of extrusa samples collected from steers fistulated at the oesophagus to estimate the diet of resident animals in grazing experiments. *Australian Journal of Experimental Agriculture* 27, 739–745.

Combs, N.R., Kornegay, E.T., Lindemann, M.D., Notter, D.R., Wilson, J.H. and Mason, J.P. (1991) Calcium and phosphorus requirements of swine from weaning to market weight. II. Development of response curves for bone criteria and comparison of bending and shear bone testing. *Journal of Animal Science* 69, 682–693.

Cowieson, A.J., Acamovic, T. and Bedford, M.R. (2006) Supplementation of corn–soy-based diets with an *Escherichia coli*-derived phytase: effects on broiler chick performance and the digestibility of amino acids and metabolizability of minerals and energy. *Poultry Science* 85, 1389–1397.

Cowieson, A.J., Ruckebusch, J.P., Knap, I., Guggenbuhl, P. and Fru-Nji, F. (2016) Phytate-free nutrition: a new paradigm in monogastric animal production. *Animal Feed Science and Technology* 222, 180–189.

Davison, T.M., Orr, W.N., Silver, B.A., Walker, R.G. and Duncalfe, F. (1997) Phosphorus fertilizer for nitrogen-fertilized dairy pastures. 1. Long-term effects on pasture, soil and diet. *Journal of Agricultural Science, Cambridge* 129, 205–215.

Dayrell, M. de S. and Ivan, M. (1989) True absorption of phosphorus in sheep fed corn silage and corn silage supplemented with dicalcium or rock phosphate. *Canadian Journal of Animal Science* 69, 181–186.

de Brouwer, C.H.M., Cilliers, J.W., Vermaak, L.M., van der Merwe, H.J. and Groenewald, P.C.N. (2000) Phosphorus supplementation to natural pasture grazing for beef cows in the Western Highveld region of South Africa. *South African Journal of Animal Science* 30, 43–51.

De Waal, H.O., Randall, J.H. and Keokemoer, G.J. (1996) The effect of phosphorus supplementation on body mass and reproduction of grazing beef cows supplemented with different levels of phosphorus at Armoedsvlakte. *South African Journal of Animal Science* 26, 29–36.

del Pino, A., Rodríguez, T. and Andión, J. (2016) Production improvement through phosphorus fertilization and legume introduction in grazed native pastures of Uruguay. *Journal of Agricultural Science* 154, 347–358.

Dersjant-Li, Y. and Kwakernaak, C. (2019) Comparative effects of two phytases versus increasing the inorganic phosphorus content of the diet, on nutrient and amino acid digestibility in boilers. *Animal Feed Science and Technology* 253, 166–180.

Dersjant-Li, Y., Awati, A., Schulze, H. and Partridge, G. (2015) Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *Journal of Science in Food and Agriculture* 95, 878–896.

Dersjant-Li, Y., Wealleans, A.L., Barnarda, L.P. and Lane S. (2017) Effect of increasing Buttiauxella phytase dose on nutrient digestibility and performance

in weaned piglets fed corn- or wheat-based diets. *Animal Feed Science and Technology* 234, 101–109.

Dersjant-Li, Y., Villca, B., Sewalt,V., de Kreij, A., Marchal, L. *et al.* (2020a) Functionality of a next generation biosynthetic bacterial 6-phytase in enhancing phosphorus availability to weaned piglets fed a corn-soybean meal-based diet without added inorganic phosphate. *Animal Nutrition* 6, 24–30.

Dersjant-Li, Y. Archer, G., Stiewert, A.M., Brown, A.A. Sobotik, E.B. *et al.* (2020b) Functionality of a next generation biosynthetic bacterial 6-phytase in enhancing phosphorus availability to broilers fed a corn-soybean meal-based diet. *Animal Feed Science and Technology* 264, 114481.

Dias, R.S., Kebreab, E., Vitti, D.M.S.S., Roque, A.P., Bueno, I.C.S. and France, J. (2006) A revised model for studying phosphorus and calcium kinetics in growing sheep. *Journal of Animal Science* 84, 2787–2794.

Dilger, R.N. and Adeola, O. (2006) Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing pigs fed conventional and low-phytate soybean meals. *Journal of Animal Science* 84, 627–634.

Dittmer, K.E., Morley, R.E. and Smith, R.L. (2017) Skeletal deformities associated with nutritional congenital rickets in newborn lambs. *New Zealand Veterinary Journal* 65, 51–55.

Driver, J.P., Pesti, G.M., Bakalli, R.L. and Edwards, H.M. Jr (2005) Effects of calcium and nonphytate phosphorus on phytase efficacy in broiler chicks. *Poultry Science* 84, 1406–1417.

Du, Y.M., Tian, J., Liao, H., Bai, C.J., Yan, X.L. and Liu, G.D. (2009) Aluminium tolerance and high phosphorus efficiency helps Stylosanthes better adapt to low-P acid soils. *Annals of Botany* 103, 1239–1247.

DuToit, P.J., Malan, A.I., Van der Merwe, P.K. and Louw, J.G. (1940) Mineral supplements for stock: the composition of licks. *Farming in South Africa* 15, 233–248.

Edwards, H.M. (1993) Dietary 1,25-dihydroxycholecalciferol supplementation increases natural phytate phosphorus utilisation in chickens. *Journal of Nutrition* 123, 567–577.

Edwards, H.M. and Veltmann, J.R. (1983) The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. *Journal of Nutrition* 113, 1568–1575.

Eeckhout, W., de Paepe, M., Warnants, N. and Bekaert, H. (1995) An estimation of the minimal P requirements for growing-finishing pigs, as influenced by the Ca level of the diet. *Animal Feed Science and Technology* 52, 29–40.

Ekpe, E.D., Zijlstra, R.T. and Patience. J.F. (2002) Digestible phosphorus requirement of grower pigs. *Canadian Journal of Animal Science* 82, 541–549.

Elizondo-Salazar, J.A., Ferguson, J.D., Beegle, D.B., Remsburg, D.W. and Wu, Z. (2013) Body phosphorus mobilization and deposition during lactation in dairy cows. *Journal of Animal Physiology and Animal Nutrition* 97, 502–514.

Engels, E.A.N. (1981) Mineral status and profiles (blood, bone and milk) of the grazing ruminant with special reference to calcium, phosphorus and magnesium. *South African Journal of Animal Science* 11, 171–182.

Erickson, G.E., Klopfenstein, T.J., Milton, C.T., Hanson, D. and Calkins, C. (1999) Effect of dietary phosphorus on finishing steer performance, bone status, and carcass maturity. *Journal of Animal Science* 77, 2832–2836.

Fan, M.Z., Archbold, T., Sauer, W.C., Lackeyram, D., Rideout, T., Gao, Y., de Lange C.F. and Hacker, R.R. (2001) Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. *Journal of Nutrition* 131, 2388–2396.

Field, A.C., Suttle, N.F. and Nisbet, D.I. (1975) Effects of diets low in calcium and phosphorus on the development of growing lambs. *Journal of Agricultural Science, Cambridge* 85, 435–442.

Field, A.C., Kamphues, J. and Woolliams, J.A. (1983) The effect of dietary intake of calcium and phosphorus on the absorption and excretion of phosphorus in Chimaera-derived sheep. *Journal of Agricultural Science, Cambridge* 101, 597–602.

Field, A.C., Woolliams, J.A., Dingwall, R.A. and Munro, C.S. (1984) Animal and dietary variation in the absorption and metabolism of phosphorus by sheep. *Journal of Agricultural Science, Cambridge* 103, 283–291.

Field, A.C., Woolliams, J.A. and Dingwall, R.A. (1985) The effect of dietary intake of calcium and dry matter on the absorption and excretion of calcium and phosphorus by growing lambs. *Journal of Agricultural Science, Cambridge* 105, 237–243.

Field, A.C., Woolliams, J.A. and Woolliams, C. (1986) The effect of breed of sire on the urinary excretion of phosphorus and magnesium in lambs. *Animal Production* 42, 349–354.

Fishwick, G., Fraser, J., Hemingway, R.G., Parkins, J.J. and Ritchie, N.S. (1977) The effects of dietary phosphorus inadequacy during pregnancy and lactation on the voluntary intake and digestibility of oat straw by beef cows and the performance of their calves. *Journal of Agricultural Science, Cambridge* 88, 143–150.

Francesch, M., Broz, J. and Brufau, J. (2005) Effects of an experimental phytase on performance, egg quality, tibia ash content and phosphorus availability in laying hens fed on maize- or barley-based diets. *British Poultry Science* 46, 340–348.

Fritts, C.A. and Waldroup, P.W. (2006) Modified phosphorus program for broilers based on commercial feeding

intervals to sustain live performance and reduce total and water-soluble phosphorus in litter. *Journal of Applied Poultry Research* 15, 207–218.

Gao, Y., Shang, C., Saghai Maroof, M.A., Biyashev, R.M., Grabau, E.A. *et al*. (2007) A modified colorimetric method for phytic acid analysis. *Crop Science* 47, 1797–1803.

Gartner, R.J.W., Murphy, G.M. and Hoey, W.A. (1982) Effects of induced, subclinical phosphorus deficiency on feed intake and growth in heifers. *Journal of Agricultural Science, Cambridge* 98, 23–29.

Gentile, J.M., Roneker, K.R., Crowe, S.E., Pond, W.G. and Lei, X.G. (2003) Effectiveness of an experimental consensus phytase in improving phytate-phosphorus utilisation by weanling pigs. *Journal of Animal Science* 81, 2751–2757.

Ghazaghi, M., Hassanabadi, A. and Mehri, M. (2019) Pre-cecal phosphorus digestibility for corn, wheat, soybean meal, and corn gluten meal in growing Japanese quails from 28 to 32 d of age. *Animal Nutrition* 5, 148–151.

Goff, J.P. (2018) *Invited review:* Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *Journal of Dairy Science* 101, 2763–2813.

González-Vega, J.C., Walk, C.L., Liu, Y. and Stein, H.H. (2013) Determination of endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs *Journal of Animal Science* 91*, 4807–4816.*

González-Vega, J.C., Walk, C.L. and Stein, H.H. (2015) Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. *Journal of Animal Science* 93, 4808–4818.

González-Vega, J.C., Liu, Y., McCann, J.C., Walk, C.L., Loor, J.J. and Stein, H.H. (2016a) Requirement for digestible calcium by eleven- to twenty-five-kilogram pigs as determined by growth performance, bone ash concentration, calcium and phosphorus balances, and expression of genes involved in transport of calcium in intestinal and kidney cells. *Journal of Animal Science* 94, 3321–3334.

González-Vega, J.C., Walk, C.L., Murphy, M.R. and Stein, H.H. (2016b) Requirement for digestible calcium by 25 to 50 kg pigs at different dietary concentrations of phosphorus as indicated by growth performance, bone ash concentration, and calcium and phosphorus balances. *Journal of Animal Science* 94, 5272–5285.

Goretti, M., Penido, M.G. and Alon, U.S. (2017) Phosphate homeostasis and its role in bone health**.** *Pediatric Nephrology* 27, 2039–2044.

Grace, N.D., Pearce, S., Firth, E. and Fennessy, P. (1999) Content and distribution of macro- and trace-elements in the body of young pasture-fed horses. *Australian Veterinary Journal* 77, 172–176.

Grace, N.D., Shaw, H.L., Firth, E.C. and Gee, E.K. (2002) Determination of digestible energy intake, and apparent absorption of macroelements of grazing, lactating Thoroughbred mares. *New Zealand Veterinary Journal* 50, 182–185.

Grace, N.D., Rogers, C.W., Firth, E.C., Faram, T.L. and Shaw, H.L. (2003) Digestible energy intake, dry matter digestibility and effect of calcium intake on bone parameters of Thoroughbred weanlings in New Zealand. *New Zealand Veterinary Journal* 51, 165–173.

Grant, C.C., Biggs, H.C., Meisnner, H.H. and Basson, P.A. (1996) The usefulness of faecal phosphorus and nitrogen in interpreting differences in live-mass gain and the response to phosphorus supplementation in grazing cattle in arid regions. *Onderstepoort Journal of Veterinary Research* 63, 121–126.

Han, Y.N., Roneker, K.R., Pond, W.G. and Lei, X.G. (1998) Adding wheat middlings, microbial phytase and citric acid to corn–soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. *Journal of Animal Science* 76, 2649–2656.

Hemingway, R.G. and Fishwick, G. (1975) Ammonium polyphosphate in the drinking-water as a source of phosphorus for growing sheep. *Proceedings of the Nutrition Society* 34, 78A–79A.

Hendricksen, R.E., Ternouth, J.D. and Punter, L.D. (1994) Seasonal nutrient intake and phosphorus kinetics of grazing steers in northern Australia. *Australian Journal of Agricultural Research* 45, 1817–1829.

Holechek, J.L., Galyean, M.L., Wallace, J.D. and Wofford, H. (1985) Evaluation of faecal indices for predicting phosphorus status of cattle. *Grass and Forage Science* 40, 489–492.

Huber, K., Hempel, R. and Rodehutscord, M. (2006) Adaptation of epithelial sodium-dependent phosphate transport in jejunum and kidney of hens to variations in dietary phosphorus intake. *Poultry Science* 85, 1980–1986.

Ingelmann, C.-J., Witzig, M., Mohring, J., Schollenberger, M., Kuhn, I. and Rodehutscord, M. (2018a) Phytate degradation and phosphorus digestibility in broilers and turkeys fed different corn sources with or without added phytase. *Poultry Science* 98*,* 1–11.

Ingelmann, C.-J., Witzig, M., Mohring, J., Schollenberger, M., Kuhn, I. and Rodehutscord, M. (2018b) Effect of supplemental phytase and xylanase in wheat-based diets on prececal phosphorus digestibility and phytate degradation in young turkeys. *Poultry Science* 97, 2011–2020.

Ingelmann, C.-J., Witzig, M., Mohring, J., Schollenberger, M., Kuhn, I. and Rodehutscord, M. (2019) Phytate degradation and phosphorus digestibility in 912–922.

Jondreville, C. and Dourmad, J-Y. (2005) Phosphorus in pig nutrition. *Productions Animales. Paris Institut National de la Recherche Agronomique* 18, 183–192.

Jondreville, C., Genthon, C., Bouguennec, A., Carre, B. and Nys, Y. (2007) Characterisation of European varieties of triticale with special emphasis on the ability of plant phytases to improve phytate phosphorus utilisation. *British Poultry Science* 48, 678–689.

Jones, R.J. (1990) Phosphorus and beef production in northern Australia. 1. Phosphorus and pasture productivity – a review. *Tropical Grasslands* 24, 131–139.

Jones, R.J. and Betteridge, K. (1994) Effect of superphosphate on its component elements (phosphorus, sulfur and calcium) on the grazing preference of steers on a tropical grass–legume pasture grown on a low phosphorus soil. *Australian Journal of Experimental Agriculture* 34, 349–353.

Jongbloed, A.W. and Henkens, C.H. (1996) Environmental concerns of using animal manure – the Dutch case. In: Kornegay, E.T. (ed.) *Nutrient Management of Food Animals to Enhance and Protect the Environment*. CRC Press, Boca Raton, Florida, pp. 315–333.

Jongbloed, A.W. and Kemme, P.A. (1990) Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. *Netherlands Journal of Agricultural Science* 38, 567–575.

Jumba, I.O., Suttle, N.F., Hunter, E.A. and Wandiga, S.O. (1995) Effects of soil origin and composition and herbage species on the mineral composition of forages in the Mount Elgan region of Kenya. 1. Calcium, phosphorus, magnesium and sulfur. *Tropical Grasslands* 29, 40–46.

Karn, J.F. (1997) Phosphorus supplementation of range cows in the Northern Great Plains. *Journal of Range Management* 50, 2–9.

Karn, J.F. (2001) Phosphorus nutrition of grazing cattle: a review. *Animal Feed Science and Technology* 89, 133–153.

Keene, B.E., Knowlton, K.F., McGilliard, M.L., Lawrence, L.A., Nickols-Richardson, S.M. *et al.* (2004) Measures of bone mineral content in mature dairy cows. *Journal of Dairy Science* 87, 3816–3825.

Kerridge, P.L., Gilbert, M.A. and Coates, D.B. (1990) Phosphorus and beef production in northern Australia. 8. The status and management of soil phosphorus in relation to beef production. *Tropical Grasslands* 24, 221–230.

Keshavarz, K. (1994) Laying hens respond differently to high dietary levels of phosphorus in monobasic and dibasic calcium phosphate. *Poultry Science* 73, 687–703.

Keshavarz, K. (2000a) A reevaluation of the non-phytate phosphorus requirement of growing pullets with and without phytase. *Poultry Science* 79, 748–763.

Keshavarz, K. (2000b) Non-phytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. *Poultry Science* 79, 748–763.

Kiarie, E.G., Parenteau, I.A., Zhu, C., Ward, N.E. and Cowieson, A.J. (2020) Digestibility of amino acids, energy, and minerals in roasted full-fat soybean and expelled-extruded soybean meal fed to growing pigs without or with multienzyme supplement containing fiber-degrading enzymes, protease, and phytase. *Journal of Animal Science* 98, skaa174.

Kies, A.K., Kemme, P.A., Sebek, L.B.J., van Diepen J.Th.M., and Jongbloed, A.W. (2006) Effect of graded doses and a high dose of microbial phytase on the digestibility of various minerals in weaner pigs. *Journal of Animal Science* 84, 1169–1175.

Kincaid, R.L., Garikipati, D.K., Nennich, T.D. and Harrison, J.H. (2005) Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. *Journal of Dairy Science* 88, 2893–2902.

Knowlton, K.F. and Herbein, J.H. (2002) Phosphorus partitioning during early lactation in dairy cows fed diets varying in phosphorus content. *Journal of Dairy Science* 85, 1227–1236.

Knowlton, K.F., Herbein, J.H., Meister-Werisbarth, M.A. and Wark, W.A. (2001) Nitrogen and phosphorus partitioning in lactating Holstein cows fed different sources of dietary protein and phosphorus. *Journal of Dairy Science* 85, 1210–1217.

Knowlton, K.F., Radcliffe, J.S., Novak, C.L. and Emmerson, D.A. (2004) Animal waste management to reduce phosphorus losses to the environment. *Journal of Animal Science* 82 (E Suppl.), E173–E195.

Koch, M.E. and Mahan, D.C. (1985) Biological characteristics for assessing low phosphorus intake in growing swine. *Journal of Animal Science* 60, 699–708.

Kornegay, E.T. and Qian, H. (1996) Replacement of inorganic phosphorus by microbial phytase for young pigs fed a maize–soya bean meal diet. *British Journal of Nutrition* 76, 563–578.

Kriseldi, R., Walk, C.L., Bedford, M.R. and Dozier, W.A. (2021) Inositol and gradient phytase supplementation in broiler diets during a 6-week production period: 1. Effects on growth performance and meat yield. *Poultry Science* 100, 9.

Langlands, J.P. (1987) Assessing the nutrient status of herbivores. In: Hacker, J.B. and Ternouth, J.H. (eds) *The Nutrition of Herbivores*. Academic Press, Sydney, Australia, pp. 363–390.

Larsen, T., Skoglund, E., Sandberg, A.S. and Engberg, R.M. (1999) Soaking and pelleting of pig diets alters the apparent absorption and retention of minerals. *Canadian Journal of Animal Science* 79, 477–483.

Lee, S.A., Lagos, L.V., Walk, C.L. and Stein, H.H. (2019) Basal endogenous loss, standardized total tract digestibility of calcium in calcium carbonate, and retention of calcium in gestating sows change during gestation, but microbial phytase reduces basal

endogenous loss of calcium. *Journal of Animal Science* 97, 1712–1721.
Lei, X.G., Ku, P.K., Miller, E.R., Yokoyama, M.T. and Ullrey, D.E. (1994) Calcium level effects the efficacy of supplemental microbial phytase in corn–soya bean meal diets for pigs. *Journal of Animal Science* 72, 139–143.
Létourneau-Montminy, M.P., Jondreville, C., Sauvant, D. and Narcy, A. (2012) Meta- analysis of phosphorus utilization by growing pigs: effect of dietary phosphorus, calcium and exogenous phytase. *Animal* 6, 1590–1600.
Li, X., Zhang, D. and Bryden, W.L. (2017) Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess? *Animal Production Science* 57, 2304–2310.
Liesegang, A., Eicher, R., Sassi, M.-L., Risteli, J., Kraenzlin, M. and Riond, J.-L. (2000) Biochemical markers of bone formation and resorption around parturition and during lactation in dairy cows with high and low milk yields. *Journal of Dairy Science* 83, 1773–1781.
Lin, Y., Berger, L. and Sun, Z. (2018) Regulation of insulin sensitivity by phosphorus. *Diabetes* 67 (Supplement 1), 1772-P.
Little, D.A. (1968) Effect of dietary phosphate on the voluntary consumption of Townsville lucerne (*Stylosanthes humilis*) by cattle. *Proceedings of the Australian Society of Animal Production* 7, 376–380.
Little, D.A. (1984) Definition of objective criterion of body phosphorus reserves in cattle and its evaluation *in vivo*. *Canadian Journal of Animal Science* 64 (Suppl.), 229–231.
Little, D.A. and Minson, D.J. (1977) Variation in the phosphorus content of bone samples obtained from the last three ribs of cattle. *Research in Veterinary Science* 23, 393–394.
Little, D.A. and Radcliff, D. (1979) Phosphorus content of bovine rib: influence of earlier biopsy of the same rib. *Research in Veterinary Science* 27, 239–241.
Littledike, E.T. and Goff, J.P. (1987) Interactions of calcium and phosphorus, magnesium and vitamin D that influence the status of domestic meat animals. *Journal of Dairy Science* 70, 1727–1743.
Liu, J.B., Bollinger, D.W., Ledoux, D.R., Ellersieck, M.R. and Veum, T.L. (1997) Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn–soybean meal diet for growing pigs. *Journal of Animal Science* 75, 1292–1298.
Liu, J.B., Bollinger, D.W., Ledoux, D.R. and Veum, T.L. (1998) Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilisation in low-phosphorus corn–soybean meal diets supplemented with microbial phytase for growing-finishing pigs. *Journal of Animal Science* 76, 808–813.
Liu, J.B., Bollinger, D.W., Ledoux, D.R. and Veum, T.L. (2000) Effects of calcium:phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, caecum and colon of pigs. *Journal of Animal Science* 78, 106–109.
Liu, J.B., Chen, D.W. and Adeola, O. (2013) Phosphorus digestibility response of broiler chickens to dietary calcium-to-phosphorus ratios. *Poultry Science* 92, 1572–1578.
Long, T.A., Tillman, A.D., Nelson, A.B., Gallup, W.D. and Davis, W. (1957) Availability of phosphorus in mineral supplements. *Journal of Animal Science* 16, 444–450.
Lu, H., Kühn, I., Bedford, M.R., Whitfield, H., Brearley, C., Adeola, O. and Ajuwon, K.M. (2019a) Effect of phytase on intestinal phytate breakdown, plasma inositol concentrations, and glucose transporter type 4 abundance in muscle membranes of weanling pigs. *Journal of Animal Science* 97, 3907–3919.
Lu, H., Cowieson, A.J., Wilson, J.W., Ajuwon, K.M. and Adeola, O. (2019b) Extra- phosphoric effects of super dosing phytase on growth performance of pigs is not solely due to release of *myo*-inositol. *Journal of Animal Science* 97, 3898–3906.
Luo, J., Liu, Y., Zhang, H. Wang, J., Chen, Z. *et al*. (2020) Metabolic alterations provide insights into *Stylosanthes* roots responding to phosphorus deficiency. *BMC Plant Biology* 20, 85.
Lyberg, K., Simonsson, A. and Lindberg, J. (2005) Influence of phosphorus level and soaking of food on phosphorus availability and performance in growing-finishing pigs. *Animal Science* 81, 375–381.
MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.
Manangi, M.K. and Coon, C.N. (2006) Evaluation of phytase enzyme with chicks fed basal diets containing different soybean samples. *Journal of Applied Poultry Research* 15, 292–306.
Manangi, M.K., Maharjan, P. and Coon, C.N. (2018) Effect of different concentrations of dietary P and Ca on plasma inorganic P and urinary P excretion using noncolostomized and colostomized broilers. *Poultry Science* 97, 522–530.
Marounek, M., Duskova, D. and Skivanova, V. (2003) Hydrolysis of phytic acid and its availability to rabbits. *British Journal of Nutrition* 89, 287–294.
Masters, D.G., Norman, H.C. and Thomas, D.T. (2019) Minerals in pastures: are we meeting the needs of livestock? *Crop and Pasture Science* 70, 1184–1195.
Mellado, M., Rodriguez, S., Lopez, R. and Rodriguez, A. (2006) Relation among milk production and composition and blood profiles and faecal P and nitrogen in goats on rangeland. *Small Ruminant Research* 65, 230–236.
Merriman, L.A., Walk, C.L., Murphy, M.R., Parsons, C.M. and Stein, H.H. (2017) Inclusion of excess dietary calcium in diets for 100- to 130-kg growing pigs reduces

feed intake and daily gain if dietary phosphorus is at or below the requirement. *Journal of Animal Science* 95, 5439–5446.

Miller, E.R., Ullrey, D.C., Zutaut, C.L., Baltzer, B.V., Schmidt, D.A., Hoefer, J.A. and Luecke, R.W. (1964) Phosphorus requirement of the baby pig. *Journal of Nutrition* 82, 34–40.

Miller, W.J., Neathery, M.W., Gentry, R.P., Blackmon, D.M., Crowe, C.I., Ware, G.C. and Fielding, A.J. (1987) Bioavailability of phosphorus from defluorinated and dicalcium phosphates and phosphorus requirement of calves. *Journal of Dairy Science* 70, 1885–1892.

Miller, C.P., Winter, W.H., Coates, D.B. and Kerridge, P.C. (1990) Phosphorus and beef production in northern Australia. 10. Strategies for phosphorus use. *Tropical Grasslands* 24, 239–249.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Morse, D., Head, H.H. and Wilcox, C.J. (1992) Disappearance of phosphorus in phytase from concentrates *in vitro* and from rations fed to lactating dairy cows. *Journal of Dairy Science* 75, 1979–1986.

Murry, A.C., Lewis, R.D. and Amos, H.E. (1997) The effect of microbial phytase in a pearl millet–soybean meal diet on apparent digestibility and retention of nutrients, serum mineral concentration and bone mineral density of nursery pigs. *Journal of Animal Science* 75, 1284–1291.

Muschen, H., Petri, A., Breves, G. and Pfeffer, E. (1988) Response of lactating goats to low phosphorus intake. 1. Milk yield and faecal excretion of P and Ca. *Journal of Agricultural Science, Cambridge* 111, 255–263.

Mutucumarana, R.K., Ravindran, V., Ravindran, G. and Cowieson A.J. (2013) Measurement of phosphorus digestibility in maize and canola meal for broiler chickens. *Poultry Science* 93, 412–419.

Mutucumarana, R.K., Ravindran, V., Ravindran, G. and Cowieson A.J. (2014) Measurement of true ileal digestibility of phosphorus in some feed ingredients for broiler chickens. *Journal of Animal Science* 92, 5520–5529.

Mutucumarana, R.K., Ravindran, V., Ravindran, G. and Cowieson A.J. (2015) Measurement of true ileal phosphorus digestibility in maize and soybean meal for broiler chickens: comparison of two methodologies. *Animal Feed Science and Technology* 206, 76–86.

Narayanan, D., Adebiyi, A. and Jaggar, J.H. (2012) Inositol trisphosphate receptors in smooth muscle cells. *American* Journal of *Physiology: Heart and Circulatory Physiology* 302, H2190–2210.

Nelson, T.S., Shieh, T.R., Wodzinski, R.J. and Ware, J.H. (1971) Effect of supplemental phytase on the utilisation of phytate phosphorus by chicks. *Journal of Nutrition* 101, 1289–1293.

Nelson, T.S., Daniels, L.B., Hall, J.R. and Shields, L.G. (1976) Hydrolysis of natural phytate phosphorus in the digestive tract of calves. *Journal of Animal Science* 42, 1509–1512.

Nimmo, R.D., Peo, E.R., Crenshaw, D.D., Maser, B.D. and Lewis, A.J. (1981) Effect of level of dietary calcium and phosphorus during growth and gestation on calcium–phosphorus balance and reproductive performance of first litter sows. *Journal of Animal Science* 52, 1343–1349.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2001) *Nutrient Requirements of Dairy Cows*, 7th edn. National Academy of Sciences, Washington, DC.

NRC (2007) *Nutrient Requirements of Small Ruminants, Sheep, Goats, Cervids, and New World Camelids*. National Academy of Sciences, Washington, DC.

NRC (2012) *Nutrient Requirements of Swine*, 11th edn. National Academy of Sciences, Washington, DC.

Olukosi, O.A., Combémorel, C., Kightley, S., Wiseman, J. and Houdijk, J.G.M. (2015) True digestibility of phosphorus determined by regression method for double zero rapeseed meal. *Livestock Science* 182, 8–10.

Owings, W.J., Sell, J.L. and Balloun, S.L. (1977) Dietary phosphorus needs of laying hens. *Poultry Science* 56, 2056–2060.

Paditz, K., Kluth, H. and Rodehutscord, M. (2004) Relationship between graded doses of three microbial phytases and digestible phosphorus in pigs. *Animal Science* 78, 429–438.

Park, W.-Y., Matsui, T., Konishi, C., Sung-Won, K., Yano, F. and Yano, H. (1999) Formaldehyde treatment suppresses ruminal degradation of phytate in soyabean meal and rapeseed meal. *British Journal of Nutrition* 81, 467–471.

Park, S., Cho, E., Chung, H., Cho, K., Sa, S., Balasubramanian, B., Choi, T. and Jeong, Y. (2019) Digestibility of phosphorous in cereals and co-products for animal feed. *Saudi Journal of Biological Sciences* 26, 373–377.

Petersen, G. and Stein, N.H. (2006) Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. *Journal of Animal Science* 84, 2126–2132.

Peterson, A.B., Orth, M.W., Goff, J.P. and Beede, D.K. (2005) Periparturient responses of multiparous Holstein cows fed different dietary phosphorus concentrations prepartum. *Journal of Dairy Science* 88, 3582–3594.

Petri, A., Muschen, H., Breves, G., Richter, O. and Pfeiffer, E. (1989) Response of lactating goats to low phosphorus intake. 2. Nitrogen transfer from rumen ammonia to rumen microbes and proportion of milk protein derived from microbial amino-acids. *Journal of Agricultural Science, Cambridge* 111, 265–271.

Pettey, L.A., Cromwell, G.L. and Lindemann, M.D. (2006) Estimation of endogenous phosphorus loss in growing-finishing pigs fed semi-purified diets. *Journal of Animal Science* 84, 618–626.

Pointillart, A.L. (1991) Enhancement of phosphorus utilisation in growing pigs fed phytate-rich diets by using rye bran. *Journal of Animal Science* 69, 1109–1115.

Pointillart, A.L., Fourdin, A. and Fontaine, N. (1987) Importance of cereal phytase activity for phytate phosphorus utilisation by growing pigs fed triticale or corn. *Journal of Nutrition* 117, 907–913.

Pokharel, B.B., Regassa, A., Nyachoti, C.M. and Kim, W.K. (2017) Effect of low levels of dietary available phosphorus on phosphorus utilization, bone mineralization, phosphorus transporter mRNA expression and performance in growing pigs. *Journal of Environmental Science and Health, Part B* 52, 395–401.

Portilho, F.P., Vitti, D.M.S.S., Abdalla, A.L., McManus, C.M., Rezende, M.J.M. and Louvandini, H. (2006) Minimum phosphorus requirement for Santa Ines lambs reared under tropical conditions. *Small Ruminant Research* 63, 170–176.

Poulsen, H.D. Blaabjerg, K., Strathe, A., Ader, P. and Feuerstein, D. (2010) Evaluation of different microbial phytases on phosphorus digestibility in pigs fed a wheat and barley based diet. *Livestock Science* 134, 97–99.

Poulsen, H.D., Voergaard, A.L., Strathe, A.B. and Blaabjerg, K. (2019) Wheat and barley increase phytate degradation and phosphorus digestibility in corn fed to pigs. *Animal Feed Science and Technology* 248, 77–84.

Qian, H., Kornegay, E.T. and Connor, D.E. (1996a) Adverse effects of wide calcium:phosphorus ratios on supplemental phytase efficacy for weanling pigs fed phosphorus at two levels. *Journal of Animal Science* 74, 1288–1297.

Qian, H., Kornegay, E.T. and Veit, H.P. (1996b) Effects of supplemental phytase and phosphorus on histological, mechanical and chemical traits of tibia and performance of turkeys fed on soyabean meal-based semi-purified diets high in phytate phosphorus. *British Journal of Nutrition* 76, 263–272.

Qian, H., Kornegay, E.T. and Denbow, D.M. (1997) Utilisation of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol and the calcium:total phosphorus ratio in broiler diets. *Poultry Science* 76, 37–46.

Rajaratne, A.A.J., Scott, D. and Buchan, W. (1994) Effects of a change in phosphorus requirement on phosphorus kinetics in the sheep. *Research in Veterinary Science* 56, 262–264.

Ramirez-Perez, A.H., Sauvant, D. and Meschy, F. (2008) Effect of phosphate solubility on phosphorus kinetics and rumenal fermentation activity in dairy goats. *Animal Feed Science and Technology* 149, 209–227.

Ray, P.P., Shang, C., Pearson, R.E. and Knowlton, K.F. (2012) Disappearance of infused phytate from the large intestine of dairy heifers. *Journal of Dairy Science* 95, 5927–5935.

Read, M.P., Engels, E.A.N. and Smith, W.A. (1986a) Phosphorus and the grazing ruminant: the effect of supplementary P on sheep at Armoedsvlakte. *South African Journal of Animal Science* 16, 1–6.

Read, M.P., Engels, E.A.N. and Smith, W.A. (1986b) Phosphorus and the grazing ruminant: the effect of supplementary P on cattle at Glen and Armoedsvlakte. *South African Journal of Animal Science* 16, 7–12.

Reddy, N.R. and Sathe, S.K. (2001) *Feed Phytates*. CRC Press Ltd, Boca Raton, Florida.

Reid, R.L., Franklin, M.C. and Hallsworth, E.G. (1947) The utilization of phytate phosphorus by sheep. *Australian Veterinary Journal* 23, 136–140.

Reinhart, G.A. and Mahan, D.C. (1986) Effects of various calcium:phosphorus ratios at low and high dietary phosphorus for starter, grower and finishing swine. *Journal of Animal Science* 63, 457–466.

Rodehutscord, M., Haverkamp, R. and Pfeffer, E. (1998) Inevitable losses of phosphorus in pigs, estimated from balance data using diets deficient in phosphorus. *Archiv fur Tierernahrung* 51, 27–38.

Rodehutscord, M., Adeola, O., Angel, R., Bikker, P., Delezie, E. *et al.* (2016a) Results of an international phosphorus digestibility ring test with broiler chickens. *Poultry Science* 96*, 1679–1687.*

Rodehutscord, M., Ruckert, C., Maurer H.P., Schenkel, H., Schipprack, W. *et al.* (2016b) Variation in chemical composition and physical characteristics of cereal grains from different genotypes. *Archives of Animal Nutrition* 70, 87–107.

Rojas, O.J., Liu, Y. and Stein, H.H. (2013) Phosphorus digestibility and concentration of digestible and metabolizable energy in corn, corn coproducts, and bakery meal fed to growing pigs. *Journal of Animal Science* 91*, 5326.*

Ruiz-Ascacibar, I., Stoll, P., Bee, G. and Schlegel, P. (2019) Dynamics of the mineral composition and deposition rates in the empty body of entire males, castrates and female pigs. *Animal* 13, 950–958.

Saleh, A.A., Elsawee, M., Soliman, M.M., Elkon, R.Y.N., Alzawqari, M.H., Shukry, M., Abdel-Moneim, A.-M.E. and Eltahan, H. (2021) Effect of bacterial or fungal phytase supplementation on the performance, egg quality, plasma biochemical parameters, and reproductive morphology of laying hens. *Animal* 11, 540–551.

Sauvant, D., Perez, J.M. and Tran, G. (2004) Tables INRA-AFZ de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage: 2nd édition. INRA Editions, Versailles, France.

Scharp, D.W. (1979) Effect of adding superphosphate to the drinking water on the fertility of dairy cows. *Australian Veterinary Journal* 55, 240–243.

Schlegel, P. and Gutzwiller, A. (2017) Effect of dietary calcium level and source on mineral utilisation by piglets fed diets containing exogenous phytase. *Journal of Animal Physiology and Animal Nutrition* 101, e165–e174.

Schlegel, P. and Gutzwiller, A. (2020) Dietary calcium to digestible phosphorus ratio for optimal growth performance and bone mineralization in growing and finishing pigs. *Animal* 10, 178.

Scholey, D., Burton, E., Morgan, N., Sanni, C., Madsen, C., Dionisio, G. and Brinch-Pedersen, H. (2017) P and Ca digestibility is increased in broiler diets supplemented with the high-phytase HIGHPHY wheat. *Animal* 11, 1457–1463.

Schryver, H.F., Craig, P.H. and Hintz, H.F. (1970) Calcium metabolism in ponies fed varying levels of calcium. *Journal of Nutrition* 100, 955–964.

Schryver, H.F., Hintz, H.F. and Craig P.H. (1971) Phosphorus metabolism in ponies fed varying levels of phosphorus. *Journal of Nutrition* 101, 1257–1263.

Schryver, H.F., Hintz, H.F., Lowe, J.E., Hintz, R.L., Harper, R.B. and Reid, J.T. (1974) Mineral composition of the whole body, liver and bone of young horses. *Journal of Nutrition* 104, 126–132.

Scott, D., McLean, A.F. and Buchan, W. (1984) The effect of variation in phosphorus intake on net intestinal phosphorus absorption, salivary phosphorus secretion and pathway of excretion in sheep fed roughage diets. *Quarterly Journal of Experimental Physiology* 69, 439–452.

Scott, D., Whitelaw, F.C., Buchan, W. and Bruce, L.A. (1985) The effect of variation in phosphorus secretion, net intestinal phosphorus absorption and faecal endogenous phosphorus excretion in sheep. *Journal of Agricultural Science, Cambridge* 105, 271–277.

Scott, D., Robins, S.P., Nicol, P., Chen, X.B. and Buchan, W. (1994) Effects of low phosphorus diets on bone and mineral metabolism and microbial protein synthesis in lambs. *Experimental Physiology* 79, 183–187.

Selle, P.H., Walker, A.R. and Bryden, W.L. (2003) Total and phytate-phosphorus contents and phytase activity of Australian-sourced ingredients for pigs and poultry. *Australian Journal of Agricultural Research* 43, 475–479.

Sharifi, K., Mohri, M., Abedi, V., Shahinfar, R., Farzaneh, M. and Shalchi, M.H. (2005) Serum and blood inorganic phosphorus in lambs from birth to 400th day of life: effect of weaning as a cut-off point between neonatal and adult levels. *Comparative Clinical Pathology* 14, 160–167.

Shastak, Y., Witzig, M. and Rodehutscord, M. (2013) Whole body phosphorus to tibia phosphorus ratio in broilers. *Arciv fur Geflügelkunde* 76, S.217–222.

She, Y., Li, D. and Zhang, S. (2017) Methodological aspects of determining phosphorus digestibility in swine: A review. *Animal Nutrition* 3, 97–102.

Shet, D., Ghosh, J., Ajith, S., Awachat, V.B. and Elangovan, A.V. (2018) Efficacy of dietary phytase supplementation on laying performance and expression of osteopontin and calbindin genes in eggshell gland. *Animal Nutrition* 4, 52–58.

Simons, P.C.M., Versteegh, H.A.J., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Walters, M.G.E., Beudeker, R.F. and Verschoar, G.J. (1990) Improvement of phosphorus availability by microbial phytase in broilers and pigs. *British Journal of Nutrition* 64, 525–540.

Singsen, E.P., Spandorf, A.H., Matterson. L.D., Serafin, J.A. and Tlustohowicz, J.J. (1962) Phosphorus in the nutrition of the adult hen. 1. Minimum phosphorus requirements. *Poultry Science* 41, 1401–1414.

Snook, L.C. (1949) Phosphorus deficiency in dairy cows: its prevalence in South-Western Australia and possible methods of correction. *Journal of the Department of Agriculture for Western Australia* 26, 169–177.

Snook, L.C. (1958) Phosphorus deficiency as a cause of bovine infertility. *Proceedings of the Australia and New Zealand Association for the Advancement of Science, Adelaide*.

Snow, J.L., Baker, D.H. and Parsons, C.M. (2004) Phytase, citric acid and 1α hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn–soybean meal diet. *Poultry Science* 83, 1187–1192.

Soares, J.H. (1995) Phosphorus bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 257–294.

Son, A.R., Shin, S.Y. and Kim, B.G. (2013) Standardized total tract digestibility of phosphorus in copra expellers, palm kernel expellers, and cassava root fed to growing pigs. *Asian-Australasian* Journal of Animal Science 26, 1609–1613.

Spencer, J.D., Allee, G.L. and Sauber, T.E. (2000) Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. *Journal of Animal Science* 78, 675–681.

Stewart, J. (1934–35) The effect of phosphorus deficient diets on the metabolism, blood and bones of sheep. *University of Cambridge Institute of Animal Pathology Fourth Report*, pp. 179–205.

Sulabo, R.C. and Stein, H.H. (2013) Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs. *Journal of Animal Science* 91, 1285–1294.

Suttle, N.F. and Hay, L. (1986) Urolithiasis. In: Martin, W.B. and Aitken, I.D. (eds) *Diseases of Sheep*, 2nd edn. Blackwell Scientific Publications, Oxford, UK, pp. 250–254.

Suttle, N.F., Dingwall, R.A. and Munro, C.S. (1989) Assessing the availability of dietary phosphorus for sheep. In: Southgate, D.A.T., Johnson, I.T. and Fenwick, G.R. (eds) *Nutrient Availability: Chemical and Biological Aspects*. Royal Society of Chemistry

Special Publication No. 72, Cambridge, UK, pp. 268–270.
Symeou, V., Leinonen, I. and Kyriazakis, I. (2014) Modelling phosphorus intake, digestion, retention and excretion in growing and finishing pig: model evaluation. *Animal* 8, 1622–1631.
Tayo, T.O, Tang, S.X., Tan, Z.L., Sun, Z.H., Wang, M., Zhou, C.S. and Han, X.F. (2009) Determination of endogenous faecal phosphorus loss in goats. *Archives of Animal Nutrition* 63, 104–111.
Ternouth, J.H. (1989) Endogenous losses of phosphorus in sheep. *Journal of Agricultural Science, Cambridge* 113, 291–297.
Ternouth, J.H. (1990) Phosphorus and beef production in Northern Australia. 3. Phosphorus in cattle – a review. *Tropical Grasslands* 24, 159–169.
Ternouth, J.H. and Coates, D.B. (1997) Phosphorus homeostasis in grazing breeder cattle. *Journal of Agricultural Science, Cambridge* 128, 331–337.
Ternouth, J.H. and Sevilla, C.L. (1990a) The effect of low levels of dietary phosphorus upon dry matter intake and metabolism in lambs. *Australian Journal of Agricultural Research* 41, 175–184.
Ternouth, J.H. and Sevilla, C.L. (1990b) Dietary calcium and phosphorus repletion in lambs. *Australian Journal of Agricultural Research* 41, 413–420.
Ternouth, J.H., Bortolussi, G., Coates, D.B., Hendricksen, R.E. and McLean, R.W. (1996) The phosphorus requirements of growing cattle consuming forage diets. *Journal of Agricultural Science, Cambridge* 126, 503–510.
Theiler, A. (1912) Facts and theories about styfziekte and lamziekte. In: *Second Report of the Directorate of Veterinary Research*. Onderstepoort Veterinary Institute, Pretoria, South Africa.
Theiler, A. and Green, H.H. (1932) Aphosphorosis in ruminants. *Nutrition Abstracts and Reviews* 1, 359–385.
Thorp, B.H. and Waddington, D. (1997) Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. *Research in Veterinary Science* 62, 67–73.
Timmons, J.R., Angel, R., Harter-Dennis, J.M., Saylor, W.W. and Ward, N.E. (2008) Evaluation of heat-stable phytases in pelleted diets fed to broilers from day zero to thirty-five during the summer months. *Journal of Applied Poultry Research* 17, 482–489.
Toor, G.S., Cade-Menun, B.J. and Sims, J.T. (2005) Establishing a linkage between phosphorus forms in dairy diets, feces and manures. *Journal of Environmental Quality* 34, 1380–1391.
Trairatapiwan, T., Ruangpanit, Y., Songserm, O. and Attamangkune, S. (2018) True ileal phosphorus digestibility of monocalcium phosphate, monodicalcium phosphate and dicalcium phosphate for broiler chickens. *Animal Feed Science and Technology* 241, 1–7.
Underwood, E.J. and Suttle, F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.
Underwood, E.J., Beck, A.B. and Shier, F.L. (1939) Further experiments on the incidence and control of pica in sheep in the botulism areas of Western Australia. *Australian Journal of Experimental Biology and Medical Science* 17, 249–255.
Underwood, E.J., Shier, F.L. and Beck, A.B. (1940) Experiments in the feeding of phosphorus supplements to sheep in Western Australia. *Journal of the Department of Agriculture for Western Australia* 17, 388–405.
Valable, A.S., Narcy, A., Duclos, M.J., Poma, C., Page, G., Nasir, Z., Magnin, M. and Létourneau-Montminy, M.P. (2018) Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. *Animal* 12, 1555–1563.
Valk, H. and Sebek, L.B.J. (1999) Influence of long-term feeding of limited amounts of phosphorus on dry matter intake, milk production and bodyweight change of dairy cows. *Journal of Dairy Science* 82, 2157–2163.
Valk, H., Sebek, L.B.J. and Beynen, A.C. (2002) Influence of phosphorus intake on excretion and blood plasma in dairy cows. *Journal of Dairy Science* 85, 2642–2649.
Van der Klis, J.D., Versteegh, H.A., Simons, P.C. and Kies, A.K. (1997) The efficacy of phytase in corn–soybean-based diets for laying hens. *Poultry Science* 76, 1535–1542.
Van Doorn, D.A., Everts, H., Wouterse, H. and Beynen, A.C. (2004) The apparent digestibility of phytate phosphorus and the influence of supplemental phytase in horses. *Journal of Animal Science* 82, 1756–1763.
van Harn, J., Spek, J.W., van Vuure, C.A. and van Krimpen, M.M. (2018) Determination of pre-cecal phosphorus digestibility of inorganic phosphates and bone meal products in broilers. *Poultry Science* 96, *1334–1340.*
Veum, T.L., Ledoux, D.R., Bollinger, D.W., Raboy, V. and Cook, A. (2002) Low-phytic acid barley improves calcium and phosphorus utilisation and growth performance in growing pigs. *Journal of Animal Science* 80, 2663–2670.
Veum, T.L., Ledoux, D.R. and Raboy, V. (2007) Low-phytate barley cultivars improve the utilisation of phosphorus, calcium, nitrogen, energy and dry matter in diets fed to young swine. *Journal of Animal Science* 85, 961–971.
Vitti, D.M.S.S., Kebreab, E., Lopes, J.B., Abdalla, A.L., De Carvalho, F.F.R. *et al*. (2000) A kinetic model of phosphorus metabolism in growing goats. *Journal of Animal Science* 78, 2706–2712.
Viveros, A., Centeno, C., Brenes, A., Canales, R. and Lozano, A. (2000) Phytase and acid phosphatases in

plant feedstuffs. *Journal of Agricultural and Food Chemistry* 48, 4009–4013.
Vucenik, I., Passaniti, A., Vitolo, M.I., Tantivejkul, K., Eggleton, P. and Shamsuddin, A.M. (2004) Anti-angionetic activity of inositol hexaphosphate (IP6). *Carcinogenesis* 25, 2115–2123.
Wadsworth, J.C., McClean, R.W., Coates, D.B. and Winter, J.H. (1990) Phosphorus and beef production in northern Australia. 5. Animal phosphorus status and diagnosis. *Tropical Grasslands* 24, 185–196.
Waldroup, P.W., Simpson, C.F., Damron, B.L. and Harms, R.H. (1967) The effectiveness of plant and inorganic phosphorus in supporting egg production in hens and hatchability and bone development in chick embryos. *Poultry Science* 46, 659–664.
Wan Zahari, M., Thompson, J.K., Scott, D. and Buchan, W. (1990) The dietary requirements of calcium and phosphorus for growing lambs. *Animal Production* 50, 301–308.
Wang, R.M., Zhao, J.P., Wang, X.J., Jiao, H.C., Wu, J.M. and Lin, H. (2018) Fibroblast growth factor 23 mRNA expression profile in chickens and its response to dietary phosphorus. *Poultry Science* 97, 2258–2266.
Wang, X.-L., Gallagher, C.H., McClure, T.J., Reeve, V.E. and Canfield, P.J. (1985) Bovine post-parturient haemoglobinuria: effect of inorganic phosphate on red cell metabolism. *Research in Veterinary Science* 39, 333–339.
Watson, B.C., Matthews, J.O., Southern, L.L. and Shelton, J.L. (2006) The interactive effects of *Eimeria acervulina* and phytase for broiler chicks. *Poultry Science* 84, 910–913.
Wealleans, A.L., Bold, R.M., Dersjant-Li, Y. and Awati, A. (2015) The addition of a *Buttiauxella* sp. phytase to lactating sow diets deficient in phosphorus and calcium reduces weight loss and improves nutrient digestibility. *Journal of Animal Science* 93, 5283–5290.
Wealleans, A.L., Barnard, L.P., Romero, L.F. and Kwakernaak, C. (2016) A value based approach to determine optimal phytase dose: a case study in turkey poults. *Animal Feed Science and Technology* 21_288–295.
Widyaratne, G.P. and Zijlstra, R.T. (2007) Nutritional value of wheat and corn distiller's dried grain with solubles: digestibility and digestible contents of energy, amino acids and phosphorus, nutrient excretion and growth performance of grower-finisher pigs. *Canadian Journal of Animal Science* 87, 103–114.
Williams, S.W., Lawrence, L.A., McDowell, L.R., Wilkinson, N.S., Ferguson, P.W. and Warmick, A.C. (1991a) Criteria to evaluate bone mineralisation in cattle. I. Effect of dietary phosphorus on chemical, physical and mechanical properties. *Journal of Animal Science* 69, 1232–1242.
Williams, S.W., McDowell, L.R., Lawrence, L.A., Wilkinson, N.S., Ferguson, P.W and Warmick, A.C. (1991b) Criteria to evaluate bone mineralisation in cattle. II. Non-invasive techniques. *Journal of Animal Science* 69, 1243–1254.
Winks, L. (1990) Phosphorus and beef production in northern Australia. 2. Responses to phosphorus by ruminants – a review. *Tropical Grasslands* 24, 140–158.
WPSA (Working Group No 2: Nutrition of the European Federation of Branches of World Poultry Science Association) (2013) Determination of phosphorus availability in poultry. *World's Poultry Science Journal* 69, 687-698.
Wright, R.D., Blair-West, J.R., Nelson, J.F. and Tregear, G.W. (1984) Handling of phosphate by the parotid gland (ovine). *American Journal of Physiology* 246, F916–F926.
Wu, Z. (2005) Utilisation of phosphorus in lactating cows fed varying amounts of phosphorus and sources of fibre. *Journal of Dairy Science* 88, 2850–2859.
Wu, Z., Satter, L.H., Blohowiak, A.J., Stauffacher, R.H. and Wilson, J.H. (2001) Milk production, estimated phosphorus excretion and bone characteristics of dairy cows fed different amounts of phosphorus for two or three years. *Journal of Dairy Science* 84, 1738–1748.
Wu, Z., Tallam, S.K., Ishler, V.A. and Archibald, D.D. (2003) Utilisation of phosphorus in lactating cows fed varying amounts of phosphorus and forage. *Journal of Dairy Science* 86, 3300–3308.
Wu, F., Woodworth, J., Tokach, M., Dritz, S., DeRouchey, J., Goodband, R. and Bergstrom, J. (2019) Standardized total tract digestible phosphorus requirement of 6 to 13 kg pigs fed diets without or with phytase. *Animal* 132473–132482.
Xue, P.C. and Adeola, O. (2015) Phosphorus digestibility response of growing pigs to phytase supplementation of triticale distillers' dried grains with solubles. *Journal of Animal Science* 93, 646–651.
Yan, F., Angel, R., Ashwell, C., Mitchell, A. and Christman, M. (2005) Evaluation of a broiler's ability to adapt to an early deficiency of phosphorus and calcium. *Poultry Science* 84,1232–1241.
Yan, Q., Tang, S., Bamikole, M.A., Han, X., Zhou, C., Wang, M., Sun, Z. and Tan, Z. (2011) Influences of dietary phosphorus variation on nutrient digestion, fecal endogenous phosphorus output and plasma parameters of goats. *Livestock Science* 142, 63–69.
Yano, F., Yano, H. and Breves, G. (1991) Calcium and phosphorus metabolism in ruminants. In: *Proceedings of the Seventh International Symposium of Ruminant Physiology*. Academic Press, New York, pp. 277–295.
Young, V.R., Richards, W.P.C., Lofgreen, G.P. and Luick, J.R. (1966) Phosphorus depletion in sheep and the ratio of calcium to phosphorus in the diet

with reference to calcium and phosphorus absorption. *British Journal of Nutrition* 20, 783–794.

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle, T.E., Silva, B.C. *et al*. (2017) Estimating mineral requirements of Nellore beef bulls fed with or without inorganic mineral supplementation and the influence on mineral balance. *Journal of Animal Science* 95, 1696–1706.

Zyla, K., Mika, M., Stodolak, B., Wikiera, A., Koreleski, J. and Swiatkiewicz, S. (2004) Towards complete dephosphorylation and total conversion of phytates in poultry feeds. *Poultry Science* 83, 1175–1186.

7 Potassium

The essentiality of potassium in animal diets was demonstrated experimentally early in the 19th century. Potassium sustains nerve and muscle excitability, osmolality and acid–base balance (Ward, 1966). Under extreme circumstances, such as cattle grazing senescent pasture (Preston and Linser, 1985) and sheep forced to subsist on Na-rich halophytes, dietary K may be lacking. However, common rations and pastures are so rich in K that excess is a greater hazard. Repeated, large inputs of fertilizer K have raised soil and herbage K to potentially harmful levels. Sudden increases in K intake in ruminants grazing lush vegetation can perturb sodium, magnesium and calcium homeostasis. Mineral imbalances have been suspected to reduce productivity in ewes rearing lambs and grazing either cereals in the vegetative state or dual-purpose rape (Masters *et al*., 2017). The intricacies of potassium metabolism at the cellular level continue to excite physiologists and biochemists because, in humans, sudden failure to preserve the massive gradient between extra- and intracellular K can have lethal consequences (Hunter and Bailey, 2019). Excessive K inputs are matched by high K outputs, increasing K in run-offs from fields and open feedlots. While excesses of N and P are more important than K as causes of eutrophication in inland waters and high soil K may lower P export, the flow of K from farms is a waste of resource and a threat to the environment.

Functions of Potassium

Potassium is the major intracellular ion in tissues and usually presents at concentrations of 100–160 mmol l^{-1} that are 25–30 times greater than those in plasma (Ward, 1966). The gradients create an electrical potential across cell membranes that is integral to responsiveness to stimuli, muscle tone and contractility. All soft tissues are much richer in K than in Na, making K the third most abundant mineral in the body at about 3.0g kg^{-1} LW, ahead of Na at 1.2g kg^{-1} LW (Fig. 7.1). The highest concentrations are found in muscle (e.g. 3.2g K kg^{-1} in sheep, Bellof *et al*., 2006) and so constant that lean body mass can be estimated by measuring activity of the naturally occurring radioisotope, ^{40}K, in a whole-body monitor (Ward, 1966). Studies with growing rats indicated that lack of dietary K specifically reduces protein synthesis in skeletal muscle (Dorup and Clausen, 1989). Potassium affects acid–base balance and participates in respiration via the chloride shift (p. 184). Many enzymes have specific or facilitative requirements for K and the element influences many intracellular reactions requiring energy release from adenosine triphosphate (ATP) (Ussing, 1960; Thompson, 1972).

Clinical Consequences of Deprivation

Appetite was soon reduced when growing pigs (Hughes and Ittner, 1942) and lambs (Campbell and Roberts, 1965) were given semi-purified diets, low in K: poor growth, muscular weakness and stiffness, paralysis and intracellular acidosis followed. Poor growth derived primarily from inappetence but partly from impaired protein metabolism. Lactating dairy cows, given diets based on beet pulp, brewers' grains and maize, containing only 0.6 or 1.5g K kg^{-1}DM developed severe anorexia within four weeks (Pradhan and Hemken, 1969). Milk yield fell and pica, loss of coat condition and decreased pliability of the hide were evident. Potassium deprivation led to a greater reduction in milk K than in plasma K but milk Na and haematocrit increased, the latter from 35.3% to 38.1%: diets containing either 6.6 or 4.5g K kg^{-1}DM allowed recovery of appetite and body weight after peak lactation (+92.9 and +15.7 kg, respectively) but not milk yield (Dennis *et al*., 1976).

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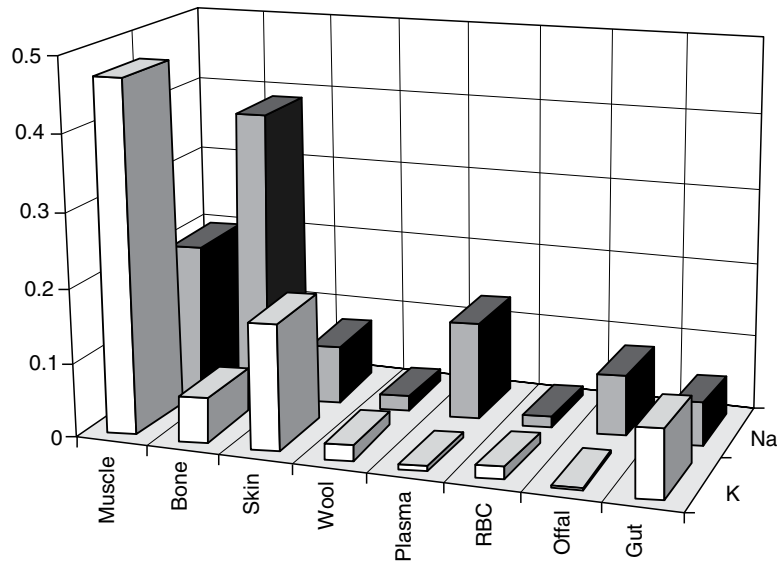

Fig. 7.1. Contrasting distributions of potassium and sodium (%) in the unshorn, empty body of young sheep. (Data from Grace, 1983). RBC, red blood cell.

Natural Occurrence of Insufficiency

Herbage K declines in dry swards due to senescence and leaching: values of 0.9–5.7g K kg^{-1}DM were found in winter-range pasture (Preston and Linser, 1985). However, such pastures support little or no growth and requirements for K will be low. Growth responses to K supplements were obtained in cattle when urea was also given but varied in magnitude from year to year (Karn and Clanton, 1977).

- Responses to K are more likely if supplements are given to alleviate concurrent deficits in digestible energy and rumen-degradable N.

Dietary K may also be lacking if cereals alone are relied upon as supplements during droughts. Supplements of K in corn-based diets improved gains and lowered mortality in finishing beef cattle, improved milk production in dairy cows and alleviated stiffness and excessive irritability in swine (Preston and Linser, 1985) but such responses have not been confirmed by others. Acidosis, stress and diarrhoea may exacerbate lack of dietary K (Preston and Linser, 1985). Raising K in the 'receiving' diet of recently transported calves from 7–9 to 13–14g kg^{-1}DM improved weight gain in the first 4–7 weeks and reduced calf mortality (Hutcheson *et al.*, 1984) but a response to the accompanying anion (chloride) cannot be ruled out. Compensatory growth after food deprivation may raise the needs for K and Cl.

Hypokalaemia syndrome

Dairy cows occasionally develop a syndrome characterized by low plasma K, anorexia, weakness, recumbency, gastrointestinal stasis and cardiac arrhythmia, sometimes associated with infections (Sattler and Fectau, 2014) or calving (Yogeshpriya *et al.*, 2010). Administration of glucogenic and corticosteriod drugs may have lowered plasma K but daily administration of 60 mg K as KCl in oral fluids can aid recovery (Peek, 2012). The condition is a variant of the 'Downer Cow' syndrome (p. 60). Fifty downer cows in a dried-up delta in south-east India had a mean plasma K of 3.9 ± 1.10 mmol/l^{-1} compared with 5.1 ± 0.69 mmol/l^{-1} in healthy cows; corresponding Ca^{2+} values were 0.9 ± 0.20 and 1.2 ± 0.07 s.e. mmol l^{-1}. In Wisconsin, plasma K < 2.2 mmol/l^{-1} was associated with recumbency, against a normal range of 3.6–5.4 mmol K /l^{-1} (Peek, 2012).

Biochemical Changes on Diets Low in K

Depletion

The capacity to store K has always been regarded as limited, since the major depot is muscle and intracellular K is kept within narrow bounds. Severe deprivation in rats immediately halted growth and moderate deprivation reduced growth without reducing muscle K (Fig. 7.2). Comparative slaughter studies in the new millennium suggest that this perspective must be changed. Accretion of K in the goat carcase, like Na (Fig. 8.3) has varied widely between studies (Fig. 7.3) and may have been attributable to colossal (>40-fold) differences in dietary K:Na ratio between studies. Carcase K has varied threefold in sheep (from 1.1 to 3.6g K kg^{-1}FW, respectively, in Teixeira *et al.* (2013) and Ji *et al.* (2015)) and twofold in pigs (1.2 and 2.3 g K kg^{-1} EBW in Wiseman *et al.*, 2009, and Ruiz-Ascacibar *et al.*, 2019, respectively).

- The growing skeleton must be able to store K, since muscle K varies little.

Bone K is affected by gender, being significantly lower in confined heifers than in bulls or steers (84, 103 and 111 ± 5.3 s.d. mg kg; Chizzotti *et al.*, 2009) and decreased from 2.6 to 1.6g kg^{-1}DM in sheep, given a ration providing 16g K kg^{-1}DM as they grew from 18 kg to 50 kg LW (Teixeira et al., 2015). The effect of maturity would have been partly due to increases in fat content. Dairy calves, marginally deprived of K, lost appetite and grew poorly with plasma K unaffected at 6.1–6.6 mmol l^{-1} (Weil *et al.*, 1988). Transition to states of deficiency and dysfunction (p. 40) may be delayed by flows of K from the skeleton and depletion of the K pool.

Deficiency

- There is no endocrine regulation of K status when intakes of K and/or food are low (Peek, 2012).

Absorption is normally almost complete and occurs principally from the small intestine by unregulated processes in non-ruminants. In ruminants, over 50% of K entering the rumen is passively absorbed from the rumen (Fig. 7.4). Lack of scope for increased absorption contributes to the early fall in plasma K. However, there are more mechanisms for transporting K across internal membranes than there are for any other element,

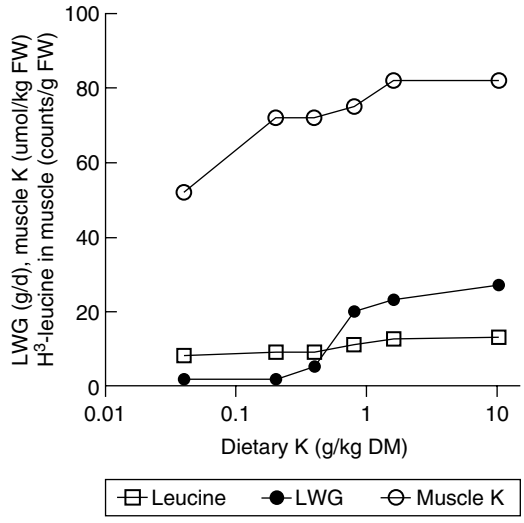

Fig. 7.2. Effect of dietary potassium (K) concentration on growth, muscle K and protein synthesis in rats: protein synthesis was measured by the rate of tritiated leucine (H^3-leucine) into muscle protein. (Data from Dorup and Clausen, 1989)

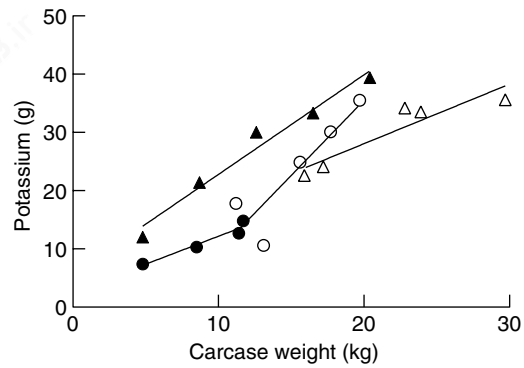

Fig. 7.3. Contrasting relationships between potassium content of the carcase and liveweight in four comparative slaughter studies with goat kids (see Fig. 1.4 and accompanying text on p. 113 for fuller details of the studies).

bar zinc, reflecting priority for maintaining high intracellular K concentrations. In addition to the ubiquitous Na/K ATPase pump and co-transporters (see Fig. 8.1), there are hydrogen/K ATPases and at least six types of voltage-gated, K^+ channel, each acting independently (Peterson, 1997). The hypokalaemia syndrome may largely reflect the consequences of abrupt decreases in K intake, such as those associated with abomasal displacement

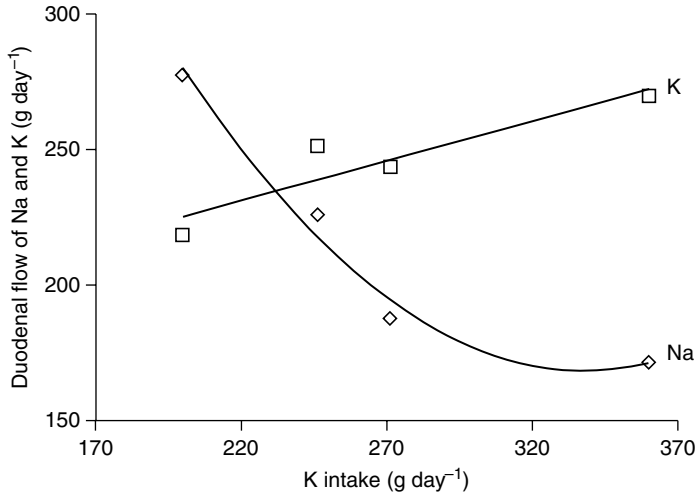

Fig. 7.4. Effect of dietary potassium (K) intake on duodenal flows of sodium (Na) and K in lactating cows given four different silages (containing 15–26 g K kg^{-1}DM), together with a Na-rich concentrate (data from Khorasani *et al.*, 1997). Up to 100 g ingested K was absorbed prior to the duodenum but flow of Na to the duodenum decreased as K intake increased: a similar K × Na antagonism is shown in Fig. 8.4.

(Schneider *et al.*, 2016). Excretory losses of K in calves may be increased by transport stress and increased aldosterone activity (Hutcheson and Cole, 1986). Simulation of stress by the intravenous administration of cortisol lowered plasma K in steers from 4.7 to 3.3 mmol l^{-1} (Parker *et al.*, 2004).

Dysfunction

There have been no detailed studies of the metabolic consequences of K deprivation in livestock but studies with rats suggest that muscle protein synthesis is soon impaired (Fig. 7.2). Although food intake was reduced, pair-fed control rats showed no reduction in protein synthesis or intracellular K (Dorup and Clausen, 1989). Rats deprived of K grew poorly because protein synthesis was impaired, but recovered completely when repleted. Prolonged depletion in more mature individuals can eventually decrease muscle tone and contractility, impair gut motility and cause cardiac arrhythmia.

Diagnosis of Potassium Insufficiency

Choice of plasma or serum sample for analysis rarely makes a difference to results of mineral analysis but it does for K. In humans, serum K can be up to 0.8 mmol K l^{-1} higher than plasma K, due to leakage of K from erythrocytes (Hartland and Neary, 1999): the difference is likely to increase with interval between sampling and separation of plasma from erythrocytes.

- Serum K should be measured, not plasma K.
- Use of marginal bands (Table 7.1) lessens the impact of sampling protocol.

In beef cattle, urinary creatinine: K ratios (molar) below 2.1 indicated likelihood of negative K balances (Karn and Clanton, 1977). Depletion of intracellular K may be partially offset by uptake of hydrogen ions, but at the expense of intracellular acidosis. Milk K declines slightly during severe potassium deprivation (Pradhan and Hemken, 1969).

Potassium Requirements

Potassium availability

Dietary sources of K are highly soluble and, unlike Ca, Mg and P, there is no difficulty in measuring 'potassium value' and little systematic variation in availability between feeds. In sheep, apparent absorption (AA_K, p. 26) from four tropical grasses cut at three stages of regrowth varied little about a high mean of 0.86 (Perdomo *et al.*, 1977). In cattle, AA_K increased marginally from 0.95 to 0.98 in steers as dietary K increased from 6 to 48g kg^{-1}DM.

Table 7.1. Suggested marginal bands[a] for potassium in plasma, urine and diet as a guide to the diagnosis of dietary deprivation (D) or excess (E) in livestock.

		Serum (mmol l^{-1})	Urine (mmol l^{-1})	Diet (g kg^{-1}DM)
Cattle	D	**2.5**–3.6	<19	5–8
	E	6–**10**	>120	30–40
Sheep	D	**2.4**–4.0	–	4–8
	E	5–**10**	–	30–40
Pigs	D	**2.5**–3.5	3–5	3–6
	E	5–**10**	–	>18
Poultry	D	**3.0**–5.5	–	1.0–3.5
	E	>10	–	>9

[a]Mean values below (for D) or above (for E) the given bands indicate probability of ill-health from deprivation or toxicity. Values within bands indicate a possibility of future disorder.

Sugarcane bagasse may provide poorly absorbed K in certain feed blends: AA_K was <0.7 unless fed with less corn and more soybean hulls (Zanetti *et al.*, 2019). Apparent availability from acetate or bicarbonate salts to young pigs was estimated to be 0.9 from increments in urinary K (Combs *et al.*, 1985).

Ruminant requirements

Early feeding trials in growing cattle suggested a requirement of between 3.4 and 5.8g K kg^{-1}DM (Weil *et al.*, 1988; Brink *et al.*, 1984) and feedlot cattle grow adequately at such concentrations (Costa e Silva *et al.*, 2015). Early factorial estimates used maintenance requirements ranging from 30 (Karn and Clanton, 1977) to 37.8 mg K kg^{-1} LW (ARC, 1980), which embrace the value derived by comparative slaughter methodology (p. 6) (33mg; Costa e Silva *et al.*, 2015). However, the regression equation relating K retention to K intake accounted for <50% of variation: breed differences may have contributed to variation because similar techniques gave a 60% lower maintenance need in the tropical Nellore breed (Zanetti *et al.*, 2019). Net requirement for growth was 1.4g K kg^{-1}EBWG, irrespective of growth stage, on diets relatively low in K but an AA_K of only 0.67 used to convert net to gross requirements probably overestimated needs (Costa e Silva *et al.*, 2015). Early experiments with feedlot lambs (Telle *et al.*, 1964; Campbell and Roberts, 1965) used dietary treatments that were too widely spaced to define need (ARC, 1980).

- Reliable factorial estimates of requirements for K cannot be derived, given uncertainty surrounding each component of the model, but are of academic interest and rarely unmet by conventional rations.
- Cereal grains, the feeds lowest in K, meet most needs.

Improvements in growth rate following addition of 15g K, as KCl, to a basal diet containing 16g K and 1.7g Na kg^{-1}DM, were attributed to increased food intake and digestibility (Masters *et al.*, 2005): improved FCE with 20g Na kg^{-1}DM may have reflected improved utilization of protein because efficiency of wool growth improved (p. 197). A positive relationship between plasma K and residual FCE in heifer calves has been reported and may have indicated lower activity of energy-consuming Na^+/K^+ pumps (Cônsolo *et al.*, 2018) but the difference within the normal range between 'efficient' and 'inefficient' groups, though significant, was small (+ 0.12 mmol Kl^{-1}).

Pig and poultry requirements

The minimum net requirement for growth in pigs is probably 1.2g K kg^{-1} EBWG (from Wiseman *et al.*, 2009). Weanling pigs on semi-purified diet required 2.6–3.3g K kg^{-1}DM for optimal growth (Meyer *et al.*, 1950; Combs *et al.*, 1985). Corresponding requirements for poultry appeared to vary according to dietary conditions, being increased by dietary P (Gillis, 1948, 1950), chloride (Nesheim *et al.*, 1964), carbohydrate (Leach *et al.*, 1959) and protein supplements: such effects may have reflected improved performance when feeding such supplements. NRC (1994) distinguished between different types and ages of growing bird, with particularly high needs for the young turkey poult (7g K kg^{-1}DM) (Table 7.2). Requirement declines to 2.5g kg^{-1}DM as pullets approach lay and 1g K

kg^{-1}DM may suffice for the hen, although it may fail to optimize shell thickness (Sauveur and Mongin, 1978). Optimum dietary K will be influenced by interactions with sodium and chloride.

- Feeding poultry to minimum standards is important because water intake increases with K (and Na) intake, increasing moisture content of faeces and risks of contamination of carcases and eggs with pathogens.

Dietary Sources of Potassium

Forages

Forage K is highly variable, ranging from 10–45 g about a mean of 24g K kg^{-1}DM for fresh herbage in the UK (Table 7.3) and equally variable in New Zealand, where most samples contained >30g K (Knowles and Grace, 2014). Similar variation was found in forages from Victoria and Queensland (McNeill *et al.*, 2002) and grass silages in the Netherlands (Schonewille *et al.*, 1997) and is due largely to effects of season and fertilizer policy. High pasture K is attributable to large farm inputs of K in organic and artificial fertilizers and recycling via slurry application. The relationship between slurry application rate and pasture K is shown in Fig. 7.5. The effect varied slightly with soil type (Soder and Stout, 2003). At a given soil K status, herbage K is influenced by plant species, state of maturity and sward management. Cool-season grass species (e.g. *Lolium perenne*) maintain higher herbage K than warm-season species (e.g. Robinson, 1985) and temperate legumes have higher levels than tropical

Table 7.2. Dietary potassium requirements (g kg^{-1}DM) of growing broiler and Leghorn chicks, turkey poults and laying birds (from NRC, 1994).

	Growth stage[a]			Lay stage[b]		
Bird type	Early	Middle	Late	High	Medium	Low
Broiler	3.0 (5.0)[c]	3.0	3.0	–	–	–
Leghorn	2.5 (4.0)[c]	2.5	2.5	1.9	1.5	1.3
Poults	7.0	5.0	4.0	–	6.0	–

[a]For broilers, Leghorn chicks and poults, respectively: early = 0–3, 0–6 or 0–8 weeks; middle = 3–6, 6–12, or 8–12 weeks; late = 6–18, >18 weeks to first lay and 20–24 weeks of age.
[b]High, medium and low represent declining energy densities and food intakes of 80, 100 and 120 g day^{-1}, respectively.
[c]A factorial model would predict higher potassium requirements for early growth in chicks and suggested values are given in parentheses, matching a corresponding increase in sodium requirement (Table 8.4).

Table 7.3. Mean (standard deviation) potassium concentrations (g kg^{-1}DM) in pastures and foodstuffs in the UK (data mostly from MAFF, 1990).

Forages	K	Concentrates	K	Roots and by-products	K
Herbage	24.3 (6.6)	Fish meal	10.2 (1.3)	Fodder beet	17.5 (4.8)
Clover silage	27.4 (7.6)	Safflower meal	17.1 (1.8)	Turnips	37.8[a]
Lucerne silage	24.6 (4.0)	Palm-kernel cake	6.9 (1.2)	Meat and bone meal	5.2 (0.6)
Maize silage	12.3 (4.1)	Maize gluten	12.5 (2.7)	Molasses (beet)	49.1 (5.4)
Grass hay	20.7 (5.3)	Rapeseed meal	14.3 (2.2)	Molasses (cane)	38.6 (15.7)
Lucerne hay	27.5 (5.0)	Cottonseed meal	15.8 (0.8)	Molassed beet pulp	18.2 (1.9)
Dried grass	26.0 (8.0)	Linseed meal	11.2 (0.13)	Beet pulp	11.7 (7.6)
Dried lucerne	25.4 (8.3)	Soyabean meal	25.0 (0.99)	Brewers' grains	0.6 (1.0)
		Maize	3.5 (0.22)		
Straws-barley	16.0 (6.5)	Barley	5.0 (0.7)	Wheat feed	13.0 (2.2)
-wheat	10.2 (3.7)	Wheat	4.6 (0.4)	DDG : barley	10.2 (0.8)
-alkali-treated barley	11.6 (5.3)	Oats	5.0 (0.9)	Cassava meal	8.1 (1.5)
		Sorghum	3.2 (0.1)		

[a]Data from New Zealand (Cornforth *et al.*, 1978). DDG-Distillers dark grains

legumes (Table 7.4; Lanyon and Smith, 1985). For a given sward, pasture K can decrease markedly as the season progresses (Reid *et al.*, 1984; Pelletier *et al.*, 2006), although the effect varies between species (Fig. 7.6) (Cherney and Cherney, 2005; Pelletier *et al.*, 2008). The effect of season is illustrated by data for pasture K in Australia, which can vary fivefold during the year (Fig. 7.7) and occurs in all pasture species.

Other feeds

Most feeds show less variation than forage K but there is considerable overlap between types (Table 7.3). Carbohydrate sources are generally low in K (3–5g kg^{-1}DM) but protein sources generally contain 10–20g K kg^{-1}DM and it is hard to compound a balanced ration from the ingredients given in Table 7.3 that does not meet the requirements of any class of livestock. Over-reliance on cereals and non-protein (also non-K) nitrogen sources might, however, create a K-deficient diet. Treatment with ammonia or NaOH appears to reduce straw K by about 25%. At the other extreme, root crops, molasses, molasses by-products (beet pulp, bagasse and distillers grains), cottonseed meal and soybean meal can add significant amounts of K to supplements for dairy cows. As always, reliance should not be placed on book values. A more recent source gives lower values of 19.3 ± 1.9g K kg^{-1}DM for SBM but less variable values for distillers grains (10.5 ± 1.6; Kerr *et al.*, 2008), but:

- high K feeds should be avoided in transition diets for dairy cows (p. 177) and where there is difficulty in restricting K accumulation in pasture.

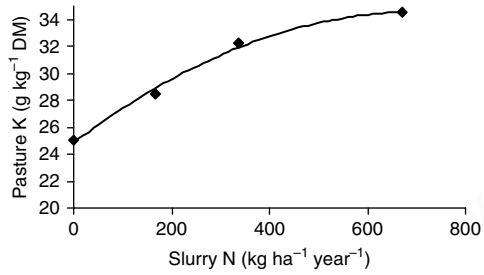

Fig. 7.5. Dispersal of dairy slurry on *Dactylis glomerata* pasture to supply nitrogen also recycled potassium, greatly increasing pasture K (slurry K:N was 0.75) (Data from Soder and Stout, 2003)

Primary Potassium Toxicity

Accretion

Accretion is not a feature of responses to excesses of Type 2 minerals, like K (p. 40) and is avoided by regulation in the kidneys, where tubular reabsorption is restricted during overload under the influence of aldosterone (Kem and Trachewsky, 1983; Bie, 2018; Hunter and Bailey, 2019). Aldosterone is induced by small increases in plasma K and binds to kidney receptors that stimulate transcription of specific regulatory genes. However, adaptation begins in the gut, where splanchnic sensors provide early warning of potentially lethal amounts ingested (Rabinowitz, 1988). Calves discriminated against diets containing 20g K as KCl kg^{-1}DM when given a choice and appetite was depressed when dietary K reached 60g kg^{-1}DM (Neathery *et al.*, 1979), a

Table 7.4. Critical concentrations for potassium (g kg^{-1}DM) below which plant yield may be reduced in forage species. (From Lanyon and Smith, 1985; Robinson, 1985)

Climate	Grass	K	Legume	K
Cool temperate	*Poa pratensis*	16–20	*Trifolium repens*	10
	Dactylis glomerata	23–35	*Medicago sativa*	12
	Lolium multiflorum	26–30	*Trifolium fragiferum*	10
	Lolium perenne	26–30		
	Festuca elatior	25–28		
Tropical	*Digitaria decumbens*	12–14	*Stylosanthes humilis*	6.0
	Sorghum sudanese	15–18	*Centrosema brasilianum*	11.2[a]
	Cynodon dactylon	15–17	*Lotononis bainesii*	9.0
			Lespedeza capitata	9.8–12.2[a]
			Capsicum pubescens	7.5[a]

[a]In the dry season critical values are <7 g kg^{-1}DM

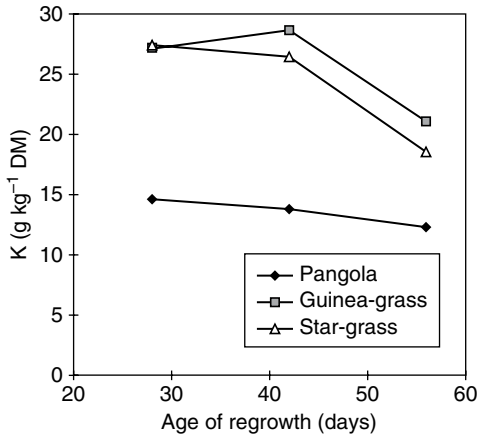

Fig. 7.6. The seasonal decline in potassium concentrations in pasture differs between pasture species. (Data from Perdomo *et al.*, 1977)

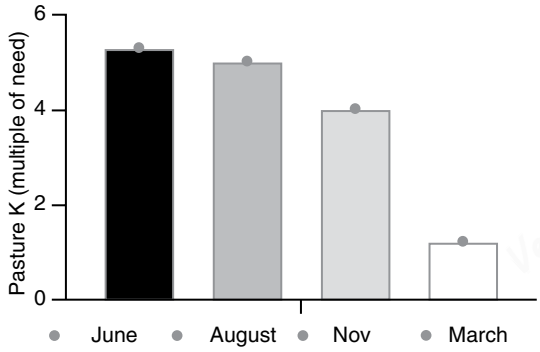

Fig. 7.7. Seasonal differences in pasture K in Australian pastures, relative to the potassium requirement for maintenance. (From Masters *et al.*, 2017)

level sometimes encountered in intensively managed grassland and molasses (Table 7.3). Increases in Na/K ion ATPase activity and the number of pumps in the basolateral membrane of both the colon and renal tubule increase K excretion by both faecal and urinary routes (Hayslett and Binder, 1982) but reabsorption of Na is increased (Bie, 2018). Aldosterone may also reduce the net flux of K into cells in the short term (Bie, 2018). On the whole, regulation is less effective than for excess Na and plasma K rises in response to increases in dietary K in pigs (Combs *et al.*, 1985) as well as steers (Greene *et al.*, 1983). Accumulation of K in growing bone (Fig. 8.3) would decrease regulatory pressure on the kidney.

Dysfunction

Cardiac arrhythmia and renal tubular acidosis supervene when plasma K exceeds 5.5 mol l^{-1} in calves (Neathery *et al.*, 1979) and humans (Hunter and Bailey, 2019) because cell membranes become depolarized and intracellular signalling is disrupted. Non-specific, ligand-gated ion channels (ionotropic receptors) allow K+ to pass through membranes in response to neurotransmitters and other chemical messengers.

Disorder

Potassium was poorly tolerated by calves, under acute challenge, and deaths were attributed to cardiac arrest (Neathery *et al.*, 1979). Use of inotropes such as adrenalin to stabilize fluxes of K across muscle membranes is an active area of research in intensive care, where trauma patients can present with hyperkalaemia.

Secondary Toxicity in Ruminants

Antagonism of sodium and magnesium

As K intakes rise, duodenal flow of Na is markedly reduced (Fig. 7.4) and Na becomes largely displaced by K in rumen liquor. The range of dietary K over which this 'aldosterone-like' response occurs (10–46g K kg^{-1}DM; Humphery *et al.*, 1984) is commonly found in forages in New Zealand, accompanied by low dietary Na (<1.5g Na kg^{-1}DM; Knowles and Grace, 2014). Similar distributions of K and Na were found in spring cereals, grazed by lambs in southern Australia, and correction of K-induced lack of dietary Na may have contributed to asymptomatic growth responses to mixed supplements providing Na, Mg and Ca (Dove and McMullen, 2009; Dove *et al.*, 2016; Masters *et al.*, 2017). Dietary Ca supply was adequate but Mg was generally marginal (2–3g kg^{-1}DM), giving abnormally high K:Na+Mg ratios in the grazed cereals. High K in rumen liquor inhibits absorption of Mg from the rumen (p. 108). Intensity of such antagonisms varies with feed type but can double Mg requirement over the ranges in herbage K reported (Table 5.4, p. 114). The K/Mg antagonism was exacerbated by lack of dietary Na and by induction of aldosterone (Charlton and Armstrong, 1989). High K intakes can thus increase risk of hypomagnesaemic tetany

through a triple interaction (K × Na × Mg) but the antagonism may be weak on corn silage diets for reasons still not understood (p. 111).

Acid–base Imbalance in Ruminants

Optimal production requires the avoidance of acid–base imbalance (i.e. of acidosis and alkalosis) and K is the major determinant of equilibrium on forage-based diets (Tremblay *et al.*, 2006). Imbalance has historically been measured by estimating dietary cation–anion differences (DCAD) in milli-equivalents (me) by a variety of formulae:

- using fixed, monovalent ions, $DCAD_1 = [Na^+ + K^+] - [Cl^-]$
- with divalent, metabolized ions, $DCAD_2 = Na^+ + K^+ + Ca^{2+} + Mg^{2+}] - [Cl^- + S^{2-}]$

The relationship between $DCAD_1$ and urine pH is negatively curvilinear in dairy cows (see Fig 4.5). Differences in ion absorption or urine-acidifying capacity can be incorporated (Lean *et al.*, 2006) but the chosen absorption coefficients for Ca (0.4) and S (0.65) are questionable. The importance of DCAD led NRC (2001) to quote values for feeds: forages were given high (>100 me kg^{-1}DM), cereals low (<20 me kg^{-1}DM) and protein supplements high (e.g. soybean meal) or low (e.g. fish meal) $DCAD_1$ values. However, DCAD in Australian grasses, hays and silages ranged from –2 to 470 me kg^{-1}DM (McNeill *et al.*, 2002). Between spring and summer, DCAD declined and became much less variable but was twofold higher in leguminous than in gramineous species (Pelletier *et al.*, 2008). $DCAD_2$ ranged from –14 to +726 me kg^{-1}DM in Swedish pastures. Increasing DCAD has had inconsistent effects on production, possibly due to a number of confounded effects: excluded ions (e.g. Ca^{2+} from $CaCl_2$, NH_4^+ from NH_4Cl and HCO_3^- from Na and K salts); buffering effects of saliva; volatile fatty acid synthesis in the rumen; and interactions between ions. As $DCAD_1$ increased from –71 to +290 me kg^{-1}DM, digestibility of neural detergent fibre, blood pH and urinary excretion of K and Na all increased linearly in lactating cows but serum K, Na and Ca^{2+} all decreased: corresponding decreases in milk K, Na and Ca^{2+} were anticipated (Martins *et al.*, 2016). In steers, a DCAD of +50 me kg^{-1}DM was sufficient to induce calciuria and raise urine pH above 8.0 (McGrath *et al.*, 2013). In feedlot lambs and steers, raising DCAD from –32 to +3 me kg^{-1}DM was sufficient to raise DMI and urine pH (Luebbe *et al.*, 2011). Using $KHCO_3$ and $NaHCO_3$ to raise DCAD from 200 to 400 me kg^{-1}DM raised milk yield (Razzhagi *et al.*, 2012). Species differ in responses to acidification of the diet, bone resorption increasing in dairy goats and cows but not in sheep (Liesegang, 2008).

Potassium, DCAD and Hypocalcaemia

Research on DCAD in transition diets for dairy cows has intensified since the last edition, driven by negative correlations with incidence of sub-acute clinical hypocalcaemia (SCH) as well as milk fever (p. 63; Table 7.5). The discovery that either $KHCO_3$ or $NaHCO_3$ supplementation prior to calving raised incidence of milk fever suggested that the effect was attributable to DCAD and not to K *per se* (Goff and Horst, 1997) but incidences may have been amplified by use of HCO_3 salts. The hypocalcaemic effect of K fertilizers occurred without affecting $DCAD_2$ in pasture (Roche *et al.*, 2002). Furthermore, provision of Mg as sulfate or chloride rather than MgO alleviated SCH without affecting DCAD, suggesting that the anions independently affected calcium status. An acidogenic diet reduced placental expulsion time (Razzhagi *et al.*, 2012). Since retained foetal membranes and other disorders associated with SCH (p. 61) can reduce subsequent milk yield, improved yields following addition of anions to transition diets may be neither wholly nor partially attributable to changes in DCAD *per se*. Meta-analysis showed that K, S, Mg and Ca each had more significant effects than any of the four DCAD formulae evaluated (Lean *et al.*, 2006) and K was the least influential mineral. Metaanalysis of

Table 7.5. Influence of dietary potassium on incidence of milk fever (C, %) and number of treatments per case (T) in cows given two dietary calcium levels (from Goff and Horst, 1997).

Dietary K (g kg^{-1} DM)		Dietary Ca (g kg^{-1}DM)	
		5	15
11	C	0	20
	T	0	1.0
21	C	36	66
	T	2.25	1.5
31	C	80	23
	T	2.0	1.25

a restricted data set confirmed that a high DCAD increased milk fever incidence (Charbonneau *et al.*, 2006). Decreases in $DCAD_2$ may be less useful in preventing milk fever in grazing than in housed herds (McNeill *et al.*, 2002) and may reduce milk yield (Roche *et al.*, 2003).

Heat stress in ruminants

Heat stress can induce respiratory alkalosis, associated with panting, and supplementation with either KCl or $NaHCO_3$ but not KCO_3 can increase milk yield in heat-stressed dairy cows (West *et al.*, 1991). The specific role of potassium is questionable because renal conservation allows plasma K to remain stable during heat stress, even in the relatively susceptible *B. taurus* (Beatty *et al.*, 2006).

Prevention of secondary toxicity in ruminants

Reductions in forage K should minimize incidence of metabolic diseases in ruminants. Applications of fertilizer K should be the minimum needed to attain maximum plant yields (Cherney and Cherney, 2005), making allowances for substantial recycling of K via slurry dispersal (Fig 7.5). Whenever possible, mixtures of N and P *without* K should be used for the first fertilizer application in spring, when herbage K is maximal. Advantage should also be taken of variation in plant K and DCAD between species (Table 7.4), values being 30–50% higher in white clover than in annual ryegrass (Pelletier *et al.*, 2008). Replacing alfalfa with timothy hay in transition diets greatly reduces K intake prior to calving and lambing. The quest for pasture species (Cherney and Cherney, 2005; Tremblay *et al.*, 2006) and husbandry practices (Pelletier *et al.*, 2006; Swift *et al.*, 2007) that gave low DCAD forages as a strategy for improving herd health was driven by an exaggerated view of the importance of DCAD as opposed to K *per se*. Selection for strains with low K requirements may be more effective, lessening risks of hypomagnesaemia and sodium deprivation as well as hypocalcaemia in cattle. In sheep, risks of induced Na and Mg deficits have been assessed from historic K/Mg and K/Ca+Mg ratios derived for lactating cows on grass pastures (Masters *et al.*, 2017).

- Customized relationships based on meta-analysis of sheep data, using new formula (e.g. K/Na × Mg) might improve prediction of responses.

Secondary Toxicity in Pigs and Poultry

Acid–base imbalance

Acid–base balance is also important in monogastric species but a single formula, equivalent to $DCAD_1$ but with different name and acronym (dietary electrolyte balance [DEB]) is used (Mongin, 1981). Diets formulated to contain 250 me kg^{-1}DM were recommended for optimum growth in chicks and similar recommendations made for pigs (Lei *et al.*, 2017). Where dietary K and/or Na supplementation improved production on diets apparently adequate in both elements, responses were widely attributed to optimization of DEB by default. However, replacing bitter $CaCl_2$ with KCl or NaCl may simply have improved palatability. In heat-stressed broiler chicks, adding either KCl or NH_4Cl to drinking water (1.5 and 2.0g l^{-1}, respectively), with a diet adequate in K (7.3g kg^{-1}DM), improved growth and FCE: higher dietary K, with or without Na, was detrimental (Teeter and Smith, 1986). Benefits of supplementing drinking water with 0.067 mol l^{-1}, as either KCl or NaCl, on growth and water consumption were confirmed without alleviating osmotic stress: plasma Na and K remained low and plasma aldosterone high (Deyhim and Teeter, 1994). Imbalance between K, Na and Cl intakes has been implicated in 'round heart', a disorder found in rapidly growing turkey poults, reared at high altitude (p. 187). Studies with heat-stressed chicks indicate a protective role for anions rather than cations. Cold stress did not affect adrenal responses in broiler chicks (Hangalapura *et al.*, 2004).

References

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

Beatty, D.T., Barnes, A., Taylor, E., Pethick, D., McCarthy, M. and Maloney, S.K. (2006) Physiological responses of *Bos taurus* and *Bos indicus* to prolonged continuous heat and humidity. *Journal of Animal Science* 84, 972–985.

Bellof, G., Most, E. and Pallauf, J. (2006) Concentration of Ca, P, Mg, Na and K in muscle, fat and bone tissue of lambs of the breed German Merino landsheep in the course of the growing period. *Journal of Animal Physiology and Animal Nutrition* 90, 385–393.

Bie, P. (2018) Mechanisms of sodium balance: total body sodium, surrogate variables, and renal sodium excretion. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 315, R945–R962.

Brink, D.R., Turgeon, O.A. Jr, Harmon, D.L., Steele, R.T., Mader, T.L. and Britton, R.A. (1984) Effects of additional limestone of various types on feedlot performance of beef cattle fed high corn diets differing in processing method and potassium level. *Journal of Animal Science* 59, 791–798.

Campbell, L.D. and Roberts, W.K. (1965) The requirements and role of potassium in ovine nutrition. *Canadian Journal of Animal Science* 45, 147–156.

Charbonneau, E., Pellerin, D. and Oetzel, G.R. (2006) Impact of lowering dietary cation–anion balance in non-lactating cows: a meta-analysis. *Journal of Dairy Science* 89, 537–548.

Charlton, J.A. and Armstrong, D.G. (1989) The effect of an intravenous infusion of aldosterone upon magnesium metabolism in sheep. *Quarterly Journal of Experimental Physiology* 74, 329–337.

Cherney, J.H. and Cherney, D.J.R. (2005) Agronomic response of cool season grasses to low-intensity harvest management and low potassium fertility. *Agronomy Journal* 97, 1216–1221.

Chizzotti, M.L., Valadares Filho, S.-C., Tedeschi, L.O., Paulino, P.V.R., Paulino, M.F. *et al.* (2009) Net requirements of calcium, magnesium, sodium, phosphorus, and potassium for growth of Nellore×Red Angus bulls, steers, and heifers. *Livestock Science* 124, 242–247.

Combs, N.R., Miller, E.R. and Ku, P.K. (1985) Development of an assay to determine the bioavailability of potassium in feedstuffs for the young pig. *Journal of Animal Science* 60, 709–714.

Cônsolo, N.R.B., Munro, J.C., Bourgon, S.L., Karrow, N.A., Fredeen, A.H., Martell, J.E. and Montanholi, Y.R. (2018) Associations of blood analysis with feed efficiency and developmental stage in grass-fed beef heifers. *Animals* 8, 133.

Cornforth, I.S., Stephen, R.C., Barry, T.N. and Baird, G.A. (1978) Mineral content of swedes, turnips and kale. *New Zealand Journal of Experimental Agriculture* 6, 151–156.

Costa e Silva, L.F., de Campos Valadares Filho, S., Engle, T.E., Rotta, P.P., Marcondes, M.I. *et al*. (2015) Macrominerals and trace element requirements for beef cattle. *PLOS ONE* 10, e0144464.

Dennis, R.J., Hemken, R.W. and Jacobson, D.R. (1976) Effect of dietary potassium percent for lactating cows. *Journal of Dairy Science* 59, 324–328.

Deyhim, F. and Teeter, R.G. (1994) Effect of heat stress and drinking water salt supplements on plasma electrolytes and aldosterone concentrations in broiler chickens. *International Journal of Biometeorology* 38, 219–217.

Dôrup, I. and Clausen, T. (1989) Effects of potassium deficiency on growth and protein synthesis in skeletal muscle and the heart of rats. *British Journal of Nutrition* 62, 269–284.

Dove, H.R. and McMullen, K.G. (2009) Diet selection, herbage intake and liveweight gain in young sheep grazing dual-purpose wheats and sheep responses to mineral supplements. *Animal Production Science* 49, 749–758.

Dove, H., Masters, D.G. and Thompson, A.N. (2016) New perspectives on the mineral nutrition of livestock grazing cereal and canola crops. *Animal Production Science* 56, 1350–1360.

Gillis, M.B. (1948) Potassium requirement of the chick. *Journal of Nutrition* 36, 351–357.

Gillis, M.B. (1950) Further studies on the role of potassium in growth and bone formation. *Journal of Nutrition* 42, 45–57.

Goff, J.P. and Horst, R.L. (1997) The effect of dietary potassium and sodium but not calcium on the incidence of milk fever in dairy cows. *Journal of Dairy Science* 80, 176–186.

Grace, N.D. (1983) Amounts and distribution of mineral elements associated with fleece-free empty body weight gains in the grazing sheep. *New Zealand Journal of Agricultural Research* 26, 59–70.

Greene, L.W., Webb, K.E. and Fontenot, J.P. (1983) Effect of potassium level on site of absorption of magnesium and other macroelements in sheep. *Journal of Animal Science* 56, 1214–1221.

Hangalapura, B.N., Nieuwland, M.G.B., Buyse, J., Kemp, B. and Parmentier, H.K. (2004) Effect of duration of cold stress on plasma adrenal and thyroid hormone levels and immune responses in chicken lines divergently selected for antibody responses. *Poultry Science* 83, 1644–1649.

Hartland, A.J. and Neary, R.H. (1999) Serum potassium is unreliable as an estimate of in vivo plasma potassium. *Clinical Chemistry* 45, 1091–1092.

Hayslett, J.P. and Binder, H.J. (1982) Mechanism of potassium adaptation. *American Journal of Physiology* 243, F103–F112.

Hughes, E.H. and Ittner, N.R. (1942) The potassium requirement of growing pigs. *Journal of Agricultural Research* 64, 189–192.

Humphery, T.J., Coghlan, J.P., Denton, D.A., Fan, J.S., Scoggins, B.B., Stewart, K.W. and Whitworth, J.A. (1984) Effect of potassium, angiotensin II on blood aldosterone and cortisol in sheep on different dietary potassium and sodium intakes. *Clinical and Experimental Pharmacology and Physiology* 11, 97–100.

Hunter, R.W. and Bailey, M.A. (2019) Hyperkalemia: pathophysiology, risk factors and consequences. *Nephrology Dialysis Transplantation* 34, Supplement 3, iii2–iii11.

Hutcheson, D.P. and Cole, A.J. (1986) Management of transit stress syndrome in cattle: nutritional and environmental factors. *Journal of Animal Science* 58, 700–707.

Hutcheson, D.P., Cole, A.J. and McLaren, J.B. (1984) Effects of pretransit diets and post-transit potassium levels for feeder calves. *Journal of Animal Science* 58, 700–707.

Ji, S., Xu, G., Diao, Q., Deng, K., Jiang, C., Tu, Y. and Zhang, N. (2015) Macromineral requirements of Dorper×thin-tailed Han crossbred female lambs. *Journal of Integrative Agriculture* 14, 1617–1626,

Karn, J.F. and Clanton, D.C. (1977) Potassium in range supplements. *Journal of Animal Science* 45, 1426–1434.

Kem, D.C. and Trachewsky, D. (1983) Potassium metabolism. In: Whang, R. (ed.) *Potassium: Its Biological Significance*. CRC Press, Boca Raton, Florida, pp. 25–35.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S.L., Bearson, B.L., Shurson, G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Khorasani, G.R., Janzen, R.A., McGill, W.B. and Kennelly, J.J. (1997) Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage or alfalfa silage. *Journal of Animal Science* 75, 239–248.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–310.

Lanyon, L.E. and Smith, F.W. (1985) Potassium nutrition of alfalfa and other forage legumes: temperate and tropical. In: Munson, R.D. (ed.) *Potassium in Agriculture*. American Society of Agronomy, Madison, Wisconsin, pp. 861–894.

Leach, R.M. Jr., Dam, R., Zeigler, T.R. and Norris, L.C. (1959) The effect of protein and energy on the potassium requirement of the chick. *Journal of Nutrition* 68, 89–100.

Lean, I.J., DeGaris, P.J., McNeill, D.M. and Block, E. (2006) Hypocalcaemia in dairy cows: meta-analysis and dietary cation–anion difference theory revisited. *Journal of Dairy Science* 89, 669–684.

Lei, X.J., Chung, J.Y., Park, J.H. and Kim, I.H. (2017) Evaluation of different dietary electrolyte balance in weanling pigs diets. *Animal Feed Science and Technology* 226, 98–102.

Liesegang, A. (2008) Influence of anionic salts on bone metabolism in dairy goats and sheep. *Journal of Dairy Science* 91, 2449–2460.

Luebbe, M.K., Erickson, G.E., Klopfenstein, T.J., Greenquist, M.A. and Benton, J.R. (2011) Effect of dietary cation-anion difference on urinary pH, feedlot performance, nitrogen mass balance, and manure pH in open feedlot pens. *Journal of Animal Science*, 89, 489–500.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Martins, C., Arcari, M., Welter, K., Gonçalves, J. and Santos, M. (2016) Effect of dietary cation–anion difference on ruminal metabolism, total apparent digestibility, blood and renal acid–base regulation in lactating dairy cows. *Animal* 10, 64–74.

Masters, D.G., Rintoul, A.J., Dynes, R.A. and Norman, H.C. (2005) Feed intake and production in sheep fed diets high in sodium and potassium. *Australian Journal of Agricultural Research* 56, 427–434.

Masters, D.G., Hancock, S., Refshauge, G., Robertson, S., Bhanugopan, M., Friend, M. and Thompson, A.N. (2017) Mineral status of reproducing ewes grazing vegetative cereal crops. *Animal Production Science* 58, 2049–2060.

McGrath, J., Savage, D., Nolan, J., Rodgers, N. and Elliott, R. (2013) Anionic salts and dietary 25-hydroxyvitamin D stimulate calcium availability in steers. *Animal* 7, 404–40

McNeill, D.M., Roche, J.R., McLachlan, B.P. and Stockdale, C.R. (2002) Nutritional strategies for the prevention of hypocalcaemia at calving for dairy cows in pasture-based systems. *Australian Journal of Agricultural Research* 53, 755–770.

Meyer, J.H., Grummer, R.H., Phillips, R.H. and Bohstedt. G. (1950) Sodium, chlorine, and potassium requirements of growing pigs. *Journal of Animal Science* 9, 300–306.

Mongin, P. (1981) Recent advances in dietary anion–cation balance applications in poultry. *Proceedings of the Nutrition Society* 40, 285–294.

Neathery, M.W., Pugh, D.G., Miller, W.J., Whitlock, R.H., Gentry, R.F. and Allen, J.C. (1979) Potassium toxicity and acid:base balance from large oral doses of potassium to young calves. *Journal of Dairy Science* 62, 1758–1765.

Nesheim, M.C., Leach, R.M. Jr, Zeigler, T.R. and Serafin, J.A. (1964) Interrelationships between dietary levels of sodium, chlorine and potassium. *Journal of Nutrition* 84, 361–366.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2001) *Nutrient Requirements of Dairy Cows*, 7th edn. National Academy of Sciences, Washington, DC.

Parker, A.J., Hamlin, G.P., Colemen, C.J. and Fitzpatrick, L.A. (2004) Excess cortisol interferes with a principal mechanism of resistance to dehydration in *Bos indicus* steers. *Journal of Animal Science* 82, 1037–1045.

Peek, S. (2012) Managing hypokalaemia. *Bovine Veterinarian,* 23 January.

Pelletier, S., Belanger, G., Tremblay, G.F., Bregard, A. and Allard, G. (2006) Dietary cation–anion difference of Timothy as affected by development stage and nitrogen and phosphorus fertilisation. *Agronomy Journal* 98, 774–780.

Pelletier, S., Simpson, R.J., Culvenor, R., Belanger, G. , Tremblay G., Allard, G., Braschkat, J. and Randall, P.J. (2008) Dietary cation–anion differences in some pasture species, changes during the season and effects of soil acidity and lime amendment. *Australian Journal of Experimental Agriculture* 48, 1143–1153.

Perdomo, J.T., Shirley, R.L. and Chicco, C.F. (1977) Availability of nutrient minerals in four tropical forages fed freshly chopped to sheep. *Journal of Animal Science* 45, 1114–1119.

Peterson, L.N. (1997) Potassium in nutrition. In: O'Dell, B.L. and Sunde, R.A. (eds) *Handbook of Nutritionally Essential Mineral Elements*. Marcel Dekker Inc., New York, pp. 153–183.

Pradhan, K. and Hemken, R.W. (1969) Potassium depletion in lactating dairy cows. *Journal of Dairy Science* 51, 1377–1381.

Preston, R.L. and Linser, J.R. (1985) Potassium in animal nutrition. In: Munson, R.D. (ed.) *Potassium in Agriculture*. American Society of Agronomy, Madison, Wisconsin, pp. 595–617.

Rabinowitz, L. (1988) Model of homeostatic regulation of potassium excretion in sheep. *American Journal of Physiology* 254, R381–R388.

Razzaghi, A., Aliarabi, H., Tabatabaei, M.M., Saki, A.A., Valizadeh, R. and Zamani, P. (2012) Effect of dietary cation-anion difference during prepartum and postpartum periods on performance, blood and urine minerals status of Holstein dairy cow. *Asian-Australasian Journal of Animal Science* 25, 486–495.

Reid, R.L., Baker, B.S. and Vona, L.C. (1984) Effects of magnesium sulphate supplementation and fertilization on quality and mineral utilisation of timothy hays by sheep. *Journal of Animal Science* 59, 1403–1410.

Robinson, D.L. (1985) Potassium nutrition of forage grasses. In: Munson, R.D. (ed.) *Potassium in Agriculture*. American Society of Agronomy, Madison, Wisconsin, pp. 895–903.

Roche, J.R., Morton, J. and Kolver, E.S. (2002) Sulfur and chlorine play a non-acid base role in periparturient calcium homeostasis. *Journal of Dairy Science* 85, 3444–3453.

Roche, J.R., Dalley, D., Moate, P., Grainger, C., Rath, M. and O'Mara, F. (2003) Dietary cation–anion difference and the health and production of pasture-fed dairy cows. 2. Nonlactating periparturient cows. *Journal of Dairy Science* 86, 979–987.

Ruiz-Ascacibar, I., Stoll, P., Bee, G. and Schlegel, P. (2019) Dynamics of the mineral composition and deposition rates in the empty body of entire males, castrates and female pigs. *Animal* 13, 950–958.

Sattler, N. and Fecteau, G. (2014) Hypokalemia syndrome in cattle. *Veterinary Clinics North American Food Animal Practitioners* 30, 351–356.

Sauveur, B. and Mongin, P. (1978) Interrelationships between dietary concentrations of sodium, potassium and chloride in laying hens. *British Poultry Science* 19, 475–485.

Schneider, S., Müller, A. and Wittek, T. (2016) Concentration of potassium in plasma, erythrocytes, and muscle tissue in cows with decreased feed intake and gastrointestinal ileus. *Journal of Veterinary Internal Medicine* 30, 679–685.

Schonewille, J. Th., Ram, L., Van't Klooster, A. Th., Wonterse, H. and Beynen, A.C. (1997) Intrinsic potassium in grass silage and magnesium absorption in dry cows. *Livestock Production Science* 48, 99–110.

Soder, K.J. and Stout, W.L. (2003) The effect of soil type and fertilisation level on mineral concentration in pasture: potential relationships to ruminant performance and health. *Journal of Animal Science* 81, 1603–1610.

Swift, M.L., Bittman, S., Hunt, D.E. and Kowalenko, C.G. (2007) The effect of formulation and amount of potassium fertiliser on macromineral concentration and cation–anion difference in tall fescue. *Journal of Dairy Science* 90, 1063–1072.

Teeter, R.G. and Smith, M.O. (1986) High chronic ambient temperature stress effects on acid–base balance and the response to supplemental ammonium chloride and potassium carbonate. *Poultry Science* 65, 1777–1781.

Teixeira, I.A.M.A. Resende, K.T., Silva, A.M.A., Sobrinho, A.G.S., Härter, C.J. and Sader, A.P.O. (2013) Mineral requirements for growth of wool and hair lambs. *Brazilian Journal of Animal Science* 42, 347–353.

Teixeira, I.A.M.A., Härter, C.I., Pereira Filho, J.M., da Silva Sobrinho, A.G. and Resende, K.T. (2015) Macromineral requirements for the maintenance and growth of F1 Boer × Saanen kids. *Journal of Animal Science* 93, 2349–2356.

Telle, P.P., Preston, R.L., Kintner, L.D. and Pfander, W.H. (1964) Definition of the ovine potassium requirement. *Journal of Animal Science* 23, 59–66.

Thompson, D.J. (1972) *Potassium in Animal Nutrition*. International Minerals and Chemical Corporation, Libertyville, Illinois.

Tremblay, G.F., Brassard, H., Belanger, G., Seguin, P., Drapeau, R. *et al.* (2006) Dietary cation anion differences in five cool-season grasses. *Agronomy Journal* 98, 339–348.

Ussing, H.H. (1960) The biochemistry of potassium. In: *Proceedings of the 6th Congress*. International Potash Institute, Amsterdam.

Ward, G.M. (1966) Potassium metabolism of domestic ruminants: a review. *Journal of Dairy Science* 49, 268–276.

Weil, A.B., Tucker, W.B. and Hemken, R.W. (1988) Potassium requirement of dairy calves. *Journal of Dairy Science* 71, 1868–1872.

West, J.W., Mullinix, B.G. and Sandifer, T.G. (1991) Changing dietary electrolyte balance for dairy cows

in cool and hot environments. *Journal of Dairy Science* 74, 1662–1674.

Wiseman, T.G., Mahan, D.C. and St-Pierre, N.R. (2009) Mineral composition of two genetic lines of barrows and gilts from twenty to one hundred and twenty-five kilograms of body weight. *Journal of Animal Science* 87, 2306–2314.

Yogeshpriya, S., Selveraj, P., Ramkumar, P.K., Jayalakshmi, K. and Arulkumar, T. (2010) Ionized calcium and electrolyte profile in Downer cows: a retrospective study of 50 cases (2016–2018). *International Journal of Livestock Research.* DOI:10.5455/ijlr.20180623060819

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle T.E., Pacheco, M.V.C., Pereira, J.M.V., Detmann, E., Paulino, M.F. and Valadares Filho, S.C. (2019) Influence of a mineral supplement containing calcium, phosphorus and micronutrients on intake, digestibility, performance and mineral status of young Nellore bulls in a feedlot. *Animal Production Science* 60, 277–287.

8 Sodium and Chloride

Sodium and chloride are considered together because of interrelated functions and shared presence in the ubiquitous supplement, common salt. Although plants use sodium and potassium interchangeably, the distinctive metabolism of potassium in animals merited a specific chapter (p. 169) but some interactions with sodium and chloride will be dealt with here. The need for salt was recognized long before reasons and extent were established. A craving for salt by grazing animals was recorded by early settlers and provision of salt became synonymous with good husbandry. Indeed, transition from a nomadic to an agricultural way of life, with dependence on cereals or vegetables rather than meat and milk, was only sustainable with dietary supplements of salt (Denton, 1982). There are fears that the right sodium balance has still to be found under certain conditions in Australia (Masters, 2018). Long after the first experimental induction of salt deprivation in livestock in the 19th century (p. 1), Australian researchers began to unravel the complex hormonal control of sodium metabolism by ingenious surgical interventions that prevented recycling of Na via salivary secretion in sheep, some of which had adrenal glands removed (Denton, 1982). A need for supplemental Na in pigs and poultry, fed on cereal-based rations, was also established (Aines and Smith, 1957). By contrast, chloride deprivation rarely occurred on farms (Summers *et al.*, 1967), although it has been induced experimentally in poultry (Leach and Nesheim, 1963) and calves (Neathery *et al.*, 1981). Welfare problems associated with dehydration during prolonged transportation refocused attention on the hormonal control of thirst and Na metabolism (Hogan *et al.*, 2007) and the findings had a bearing on controversy surrounding Na requirements of dairy cows (Edmeades and O'Connor, 2003). Attention has now switched to the management of salt excess. Approximately one third of the earth's land surface is affected by salinity, sodicity and aridity (Chiy and Phillips, 1995). Exploitation of halophytic browse species, salt-tolerant grasses and seawater irrigation require the successful handling of sodium excesses by livestock (Pasternak *et al.*, 1985; Masters *et al.*, 2006; Norman *et al.*, 2013).

Functions

Sodium and chloride are highly labile and maintain osmotic pressure, regulate acid–base equilibrium and control water metabolism in the body, Na^+ being the major cation in extracellular fluid (ECF) and Cl^- the major anion, at concentrations of 140 and 105 mmol l^{-1}, respectively. There are marked contrasts between sodium and potassium (Fig. 8.1), with Na providing >90% of the bases in plasma but contributing little to blood cells, where K is the dominant cation (Fig 7.1). Sodium has been described as an 'osmotic skeleton' that is 'clothed' with an appropriate volume of water (Michell, 1995). When ion intakes increase, water intakes also increase (Wilson, 1966; Suttle and Field, 1967), protecing the gut, facilitating excretion and 'clothing' the enlarged 'skeleton'. The 'osmotic skeleton' is sustained by Na^+/K^+ ATPase pumps in cell membranes, which actively transport sodium and convert energy of ATP into osmotic gradients along which water can flow (Fig. 8.1), while fuelling other cation-transporting mechanisms. Thus, transmembrane potential differences are established that influence uptake of other cations and create excitability (i.e. responsiveness to stimuli). Amino acid uptake and metabolism, like those of glucose, are dependent on Na^+/K^+ ATPase pumps and other Na-linked co-transporters (Fig. 8.1).[i] High intracellular K concentrations are maintained but account for 50% of the cell's maintenance need for energy (Milligan and Summers, 1986). Production increases Na^+/K^+ transport, increasing energy expenditure by 18–23% as lamb growth increases from 90 to 230 g day^{-1}, increased metabolite transport (e.g. amino acids) across the gut mucosa being

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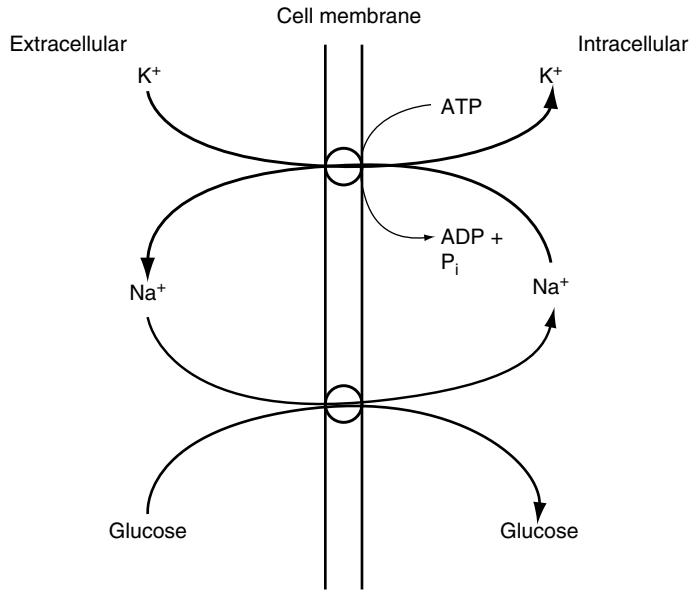

Fig. 8.1. 'Sodium pumps' maintain electrochemical differences across cell membranes and promote cellular uptake of glucose through the activity of sodium/potassium ion-dependent ATPase but at a significant energy cost.

primarily responsible (Gill *et al*., 1989). Exchange of Na^+ with H^+ influences pH regulation in gut and tissues, while exchange with Ca^{2+} influences vascular tone. The contribution of Na to acid–base balance is covered elsewhere (p. 177).

Chloride is found both within cells, including gastric goblet cells where it occurs as HCl, and in body fluids as Cl^- ions. Respiration is based on the 'chloride shift', whereby the K salt of oxyhaemoglobin exchanges O_2 for CO_2 via the bicarbonate ion (HCO_3^-) in tissues and reverses that process in the lung, where reciprocal Cl^- exchanges maintain anion balance (Block, 1994). Absorption of chloride from dietary and endogenous (gastric secretions) sources is also achieved by exchange for HCO_3^-. Chloride transport is complex, with both voltage-gated and mechanically ('stretch') driven, Ca-activated channels and Cl^-- HCO_3^- exchangers contributing to fluxes (Harper *et al*., 1997; Burnier, 2007).

Clinical Signs of Lack of Dietary Sodium

After only 2–3 weeks of sodium deprivation, milking cows exhibit pica, a craving for salt, and avidly lick wood, soil, urine and sweat: water consumption can become excessive (polydipsia) and urine output greatly increased. After several weeks, appetite and milk yield begin to decline, weight is lost and roughened coats contribute to a haggard appearance. Milk fat content decreases and high-producing cows can die suddenly but intervention with supplementary salt can produce a dramatic recovery. Impaired conception rate was linked to lack of dietary Na in dairy cows in Victoria, where pastures were rich in K and urinary Na outputs were low (Harris *et al*., 1986). In growing pigs, poultry, sheep and goats, sodium deprivation is also manifested within a few weeks by inappetence, growth retardation and inefficient feed use, due to impairment of protein and energy metabolism, but digestibility is not affected. Mortality in pregnant ewes, grazing wheat in the vegetative state, was thought to be due in part to lack of dietary Na (McGrath *et al*., 2013); such forage commonly contains <0.9g Na kg^{-1}DM (Masters *et al*., 2017). Laying hens on low-salt rations lose weight, are prone to cannibalism and reduce both egg production and egg size (Sherwood and Marion, 1975). Marginal lack of dietary Na induced moult and reduced egg production from 60% to 20% over 15 days (Ross and Herrick, 1981). In sodium-depleted, lactating sows, interval between weaning and oestrus increased from 6.2 days to 12.6 days, although sow liveweight and piglet growth were unaffected (Seynaeve *et al*., 1996).

Occurrence of Sodium Insufficiency

Sodium insufficiency is attributable to a number of dietary, climatic and disease factors, acting alone or in combination.

Low dietary sodium

Forages lack Na in the centres of many continents, including Saharan (Little, 1987), southern (Walker, 1957; De Waal *et al*., 1989) and tropical Africa and Latin America (McDowell *et al*., 1993). In arid inland areas of Australia (Murphy and Plasto, 1972; Denton, 1982) and Africa (Howard *et al*., 1962) where the water table is low, grass pastures commonly contained 0.1–0.8g Na kg^{-1}DM and even grains and seeds were low in Na (Chamberlain, 1955; Morris and Gartner, 1971). Temperate continents can also produce forage low in Na (Chiy and Phillips, 1995). Islands benefit from the deposition of sodium from the atmosphere. In New Zealand (NZ), 95% of pastures contained 1.9–2.1g Na kg^{-1} DM: only 8% and 17%, respectively, failed to meet the requirements of sheep and cattle (Knowles and Grace, 2014) (Table 8.1). Sodium is readily leached from soils with a low cation exchange capacity, such as the pumices of NZ (Edmeades and O'Connor, 2003) and such soils support pastures low in Na. Plant genotype affects herbage Na and lucerne contained insufficient Na to sustain milk yield in a NZ dairy herd (Joyce and Brunswick, 1975). Cereal crops are increasingly grazed in the vegetative state in southerly regions of Australia but may contain as little as 2 g Na kg^{-1}DM and salt supplements, given with Ca and Mg, have improved lamb growth (Dove and McMullen, 2009; Masters *et al*., 2017).

High pasture potassium

Potassic fertilizer applications accentuate lack of dietary Na by reducing pasture Na and by impairing Na retention in the animal. Applications of KCl (80 kg ha^{-1}) reduced pasture Na in the Netherlands (Lehr *et al*., 1963): effects are greatest in natrophilic species (p. 17) such as *Dactylis glomerata*, in which herbage Na was reduced to 0.4g Na kg^{-1}DM (McNaught, 1959). Raising dietary K in sheep (Humphery *et al*., 1984) and cattle (Fig. 8.2) reduced flows of Na into the abomasum, which normally exceed dietary Na intake: the effect is rapid, due to a reduction in salivary Na secretion (Scott, 1974), and accompanied by a large increase in urinary Na,

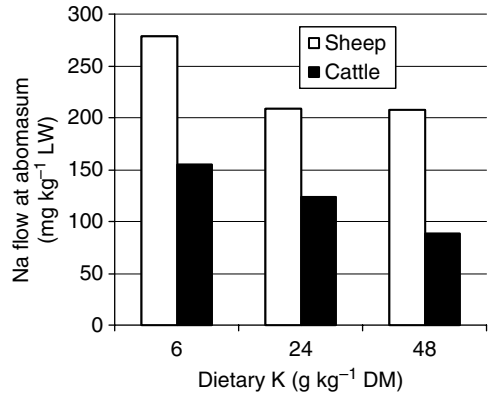

Fig. 8.2. High potassium intakes reduce sodium flow from the rumen in sheep and cattle: since respective intakes were only 40 and 55 mg Na kg^{-1} LW, respectively, flow of salivary Na from the rumen must have been reduced by K supplementation. (Data from Greene *et al.*, 1983a, b)

displaced from the rumen (Suttle and Field, 1967). Natriuresis can still be evident 10–17 days after K exposure (Greene *et al*., 1983a, b) and may be mediated by aldosterone, following the increase in renal tubular load of Na. Similar interactions may occur in ewes and lambs grazing forage cereals and explain asymptomatic responses to mixed mineral supplements that include Na (Dove *et al*., 2016; Masters, 2018; Masters *et al*., 2019). Effects of K/Na imbalance on magnesium metabolism in ruminants are considered elsewhere (p. 185).

Heat stress

Heat stress disturbs electrolyte metabolism in ruminants, pigs and poultry (Gonzalez-Rivas *et al*., 2020). In ruminants, sodium losses in sweat increase as temperature and humidity increase. In unacclimatized cattle, dribbling of saliva resulted in additional daily losses of up to 14mg Na and 9mg Cl kg^{-1}LW (Aitken, 1976). Plasma Na and K decreased in heat-stressed steers that had free access to water but effects are less in species accustomed to such conditions (e.g. *Bos indicus* versus *B. taurus*) (Beatty *et al*., 2006). Heat stress can induce respiratory alkalosis, associated with panting, and supplementation with Na or K can increase milk yield in heat-stressed cows (West *et al*., 1991). In heat-stressed broiler chicks receiving adequate dietary K, adding KCl or NH_4Cl to the drinking water (1.5 and 2 g l^{-1}, respectively) improved growth rate and food

conversion efficacy (FCE) (Teeter and Smith, 1986), without restoring low plasma Na and K, and restored aldosterone to normal (Dayhim and Teeter, 1994). However, mitigation of heat stress in poultry requires far more than attendance to mineral nutrition (Nawab *et al*., 2018).

Exercise in tropical climates

In hot, humid climates, working animals lose large amounts of Na in sweat and become vulnerable to sodium insufficiency. Equine species are particularly vulnerable because profuse volumes of hypertonic sweat are produced. Endurance horses produced sweat with 159 mmol Na and 165 mmol Cl l^{-1} in one study (Snow *et al*., 1982). Plasma Cl is normally kept within narrow limits but declined after endurance races, even in the cool Scandinavian climate and despite dehydration, which increased plasma Na (Larsson *et al*., 2013). Low availabilities of Na from rations in beef cattle in tropical conditions (e.g. 0.63–0.73; Zanetti *et al*., 2017) may indicate contamination of faeces with sweat. Increased use of lactating cows for draught purposes in developing countries magnifies such risks. Unproductive livestock are unlikely to lack dietary Na. Supplementation with seawater improved the health of sheep that became starved and diarrhoeic during prolonged, crowded transportation by sea, through tropical zones: lack of dietary Na may have hindered water absorption from the gut (Black, 1997).

Gut infections

Infections of the small and large intestine perturb mechanisms that normally maintain sodium status. Acute bacterial infections of the gut that cause severe diarrhoea in calves require the provision of Na, together with glucose, bicarbonate and even K in a carefully balanced rehydration therapy (Michell, 1989). Parasitic infections of the abomasum in lambs cause efflux of Na into the lumen but compensatory reabsorption at uninfected distal sites can conserve Na, so that salivary Na:K, is unaffected in infected lambs on a diet marginal in Na (Ortolani, 2000). When the small intestine is also parasitized, as is often the case in naive lambs, calves and immune-suppressed females that have recently given birth at pasture, sodium insufficiency may be induced, despite mineral supplementation (Suttle *et al*., 1996) and adequate pasture Na (Suttle and Brebner, 1995): an appetite for salt may be induced.

Lactation

Sufficient sodium is secreted in all milks to make lactation a period of maximum vulnerability but concentrations differ between species (see Table 2.4). Mares' milk contains only 5 mmol l^{-1}, a possible line of defence against deprivation, but donkeys produce milk containing as much Na as ruminants (19 mmol kg^{-1}; Manuelian *et al*., 2018). The long lactation in donkeys makes milk production an important source of income for small farmers but salt intakes must be maintained.

Acid–Base Balance

The role of Na and Cl in determining acid–base balance has been overshadowed by that of K (p. 177) but supplementing the diet of lambs with either 2% $NaHCO_3$ or 1% NH_4Cl produced alkalosis and acidosis, respectively. Retention of Ca and P was reduced on the acidic diet, and changes in acid and alkaline phosphatase activities suggested increases in osteoclast and decreases in osteoblast activities in bone (Abu Damir *et al*., 1991). In a comparable study with pigs (Budde and Crenshaw, 2003), no such effects were found, despite apparent retention of Cl, but $CaCl_2$ was used to provide chloride. Replacing 3g NaCl with $NaHCO_3$ in boar diets increased blood pH and reduced lameness, despite increasing osteochondrosis (van der Wal *et al*., 1986). Providing working horses with 100g NaCl d^{-1} lowered blood pH, without raising plasma Na or Cl (Zeyner *et al*., 2017). Abomasal parasitism inhibits the normal secretion of HCl into the gut lumen (Coop, 1971) but the consequences in terms of acid–base balance and pathogenesis of infection have not been explored. Acute challenge infections with *Haemonchus contortus* raised salivary Cl (and presumably serum Cl) and increased salivary Na:K by increasing salivary K, indicating possible acidosis and loss of intracellular K (N.F. Suttle, unpublished data). Formulae used to calculate dietary cation-anion difference (DCAD) contain Na and Cl and optimal DCAD values have been set, based on performance criteria (p. 178).

- However, DCAD-dependent effects are impossible to separate from direct effects on palatability and food intake (p. 195).

Electrolyte imbalance may involve Cl rather than Na or K in heat-stressed poultry and additions of

$NaHCO_3$ rather than NaCl, with a target DCAD of 250 me kg^{-1}DM, have been recommended (Mushtaq *et al.*, 2013).

Spontaneous turkey cardiomyopathy (STC)

Sudden deaths in turkeys, associated with swollen hearts, ascites and hydropericardium, have been linked to electrolyte imbalance (Frame *et al.*, 2001). The disease occurs at a young age (11–21 days), particularly in females, with an increased frequency in birds kept at high altitude. Relatively high K intakes, provided by high-protein diets, may raise an already high haematocrit and raise cardiac workload by increasing blood viscosity. Incidence of 'round heart' (STC) is usually low (<3%), but was increased by raising dietary Na (from 1.4 to 2.4g kg^{-1}DM) and decreased by raising dietary Cl from 1.6 to 4.0g kg^{-1}DM) in experiments conducted at high altitude (Frame *et al.*, 2001). Chloride supplementation may have increased K excretion. Incidence of STC is reduced by restricting food intake and growth rate.

Physiochemical Changes on Diets Lacking Salt

Sodium depletion in ruminants

The existence and physiological significance of an initial depletion phase, as shown in the general model (Fig. 3.2) presupposes a capacity to store mineral in times of plenty. Like potassium (p. 171), capacity to store Na in the skeleton has probably been underestimated. Comparative slaughter studies with goat kids, described in detail earlier (Fig 1.4), showed wide variation in Na accretion (Fig. 8.3). In similar studies with lambs, accretion rate varied from 1.1 (Teixeira *et al.*, 2015) to 2.3g Na kg^{-1}EBW (Jiang *et al.*, 2019): differences were probably attributable to diverse dietary conditions, including large differences in dietary Na:K, rather than differences between animal genotypes. In sheep, muscle contained 0.6–0.75g Na kg^{-1}FW, values decreasing slightly with approach to maturity (Bellof *et al.*, 2006) but varying little between studies. Sodium concentrations in the equine body are almost twice as high as in the average lamb and almost half the total body Na was found in the skeleton (Grace *et al.*, 1999).

- Large pools of Na in rumen and skeleton may act as short-term 'stores' when dietary Na falls, depletion of skeletal Na affording particular resilience to horses.

Sodium deficiency in ruminants

Plasma Na must be maintained to preserve osmotic balance and does not decline until the deprived animal is *in extremis*.[iii] Regulation in all species is principally achieved in the kidneys by hormonal control of urinary Na excretion. Reabsorption from the distal tubule is enhanced by aldosterone and urinary losses become negligible when Na (and K) intakes are low (Harper *et al.*, 1997). Urinary Na rapidly declines to extremely low values and urinary K also declines (Singh and Rani, 1999). However, mechanisms and outcomes vary between ruminants and non-ruminants. In ruminants, adaptation also involves secretion of saliva, which is produced at a rate of around 0.3 l kg^{-1}LW day^{-1} and contains 150 mmol Na l^{-1} as the major cation. Most of the Na entering the rumen comes from saliva. In early experiments with sheep, acutely deprived of Na, salivary Na was replaced on a molar basis by K in an adaptation modulated by aldosterone (Blair-West *et al.*, 1963). The relationship between plasma aldosterone and salivary Na:K ratio is curvilinear and salivary Na:K decreases rapidly to <5.0 in sheep and goats with early increases in hormone concentrations, indicating a state of deficiency (Fig. 8.4). In bullocks grazing pasture low in Na, salivary Na and K were 40 and 90 mmol l^{-1}, respectively, compared with 145 and 7 mmol l^{-1} in normal animals (Murphy and Plasto, 1972). Reciprocal changes in Na and K also occur

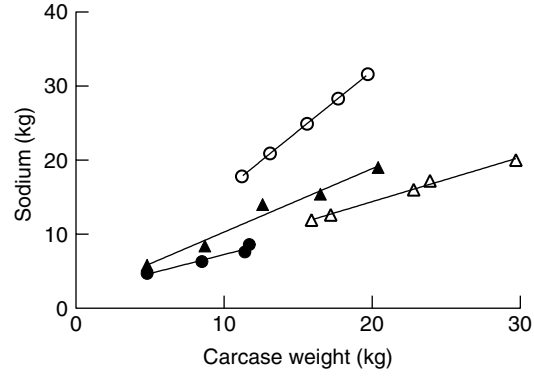

Fig. 8.3. Relationships between carcase sodium and empty body weight (EBW) in goat kids are linear but vary widely between studies (see text associated with Fig. 1.4).

in sweat but they are less marked than those in saliva (Fig. 8.4). Dietary supplements of ionophores interact with dietary Na to affect ruminal metabolism (Spears and Harvey, 1987), increasing Mg absorption and decreasing Na retention by increasing urinary Na loss (Kirk *et al.*, 1994).

Ruminants also differ from non-ruminants in other respects. The interaction between aldosterone and angiotensin does not affect centrally mediated sodium appetite in sheep (Shade *et al.*, 2002). Plasma Na is normally kept within narrow limits but can vary from 138 to 160 mmol l^{-1} through the oestrus cycle in lactating cows, reaching a peak between days 5–10 and a trough on day 20. The pattern is less striking and accompanied by changes in plasma aldosterone (Rouffet *et al.*, 1983) and more pronounced than those induced by sodium loading in pregnant cows (Rouffet *et al.*, 1990). The mammary gland is unresponsive to aldosterone and milk Na declines only slightly on diets lacking Na in cows (Schellner, 1972/1973) and ewes (Morris and Petersen, 1975). Faecal Na is reduced (Jones *et al.*, 1967; Michell *et al.*, 1988) through increased reabsorption against a concentration gradient in the lower intestine (Bott *et al.*, 1964) and may involve changes reported in non-ruminants.

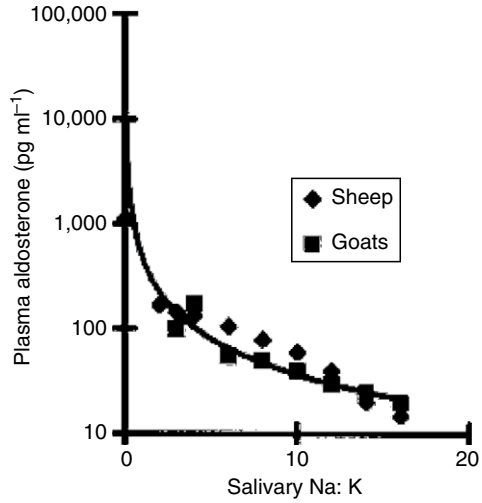

Fig. 8.4. Sodium is conserved through the action of the adrenal hormone aldosterone, which facilitates replacement of Na by K in saliva and urine and Na:K ratios in saliva are inversely related to plasma aldosterone and a useful indicator of deprivation. (Data from McSweeney *et al.,* 1988)

Chloride deprivation in ruminants

Lactating cows given a diet low in Cl developed pica, lowered milk yield, constipation and cardiovascular depression (Fettman *et al.*, 1984). Without the demand of lactation, however, extreme measures are needed to induce ill-health. Chloride deprivation has been produced experimentally by giving young calves a diet low in Cl while removing Cl-rich abomasal contents twice daily (Neathery *et al.*, 1981). The calves became anorexic and lethargic after seven days, with mild polydipsia and polyuria, and severe eye defects developed after 24–46 days of depletion: calves given supplementary Cl grew normally despite the removal of digesta. Chloride-deprived animals craved KCl as well as NaCl and plasma Cl fell from 96 to 31 mmol l^{-1} and salivary Cl from 25 to 16 mmol l^{-1}. There was secondary alkalosis, with reductions in plasma Na and K (Neathery *et al.*, 1981). A similar deprivation technique had less severe clinical effects in lactating cows but similar biochemical consequences (Fettman *et al.*, 1984).

Chloride insufficiency may not occur naturally but diets based on maize (as grain, gluten feed and silage) are the most likely to lack Cl (Coppock, 1986): working, lactating animals in tropical climates are relatively vulnerable.[ii] Increases in muscle Cl have been reported in 'downer cows' (p. 60) and may reflect a perturbed 'chloride shift' but serum Cl is unaffected (p. 170).

Sodium and chloride deficiencies in non-ruminants

Pigs and poultry do not sweat and cannot conserve Na and Cl by reducing secretory losses, other than in saliva, but absorption is regulated. Sodium is absorbed from the intestinal lumen by coupling to glucose and amino acid uptake via co-transporters and also by exchange with hydrogen ions (H^+) (Harper *et al.*, 1997). Animals can adapt to low dietary Na by increasing the number or activity of transporters, thus increasing absorptive efficiency. Expression of Na-dependent glucose transporter 1 (SGLT1) is increased in deficient piglets. Phytate decreases jejunal absorption of Na in pigs (Woyengo *et al.*, 2012) and increases faecal endogenous losses of Na (FEL_{Na}) in chicks (Cowieson *et al.*, 2004) and early-weaned pigs (Woyengo et al., 2009): addition of phytase counters the phytate effect in broilers (Moss *et al.*, 2018). Ingested phytate may increase HCl secretion and draw compensatory Na^+

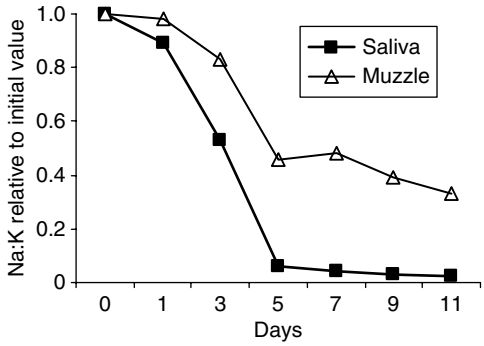

Fig. 8.5. Sodium deprivation of buffalo heifers decreases the Na:K ratio in muzzle secretions as well as in saliva (data from Singh and Rani, 1999): initial Na:K ratios were 30 and 0.46 for saliva and muzzle secretions, respectively.

into the gut; and also regulate activity of endogenous, intestinal phytase. In chicks given a diet containing only 0.2g Cl kg^{-1}DM, mortality increased and nervous symptoms were reported (Leach and Neisheim, 1963).

Dysfunction in all species

Transition from a state of deficiency to one of dysfunction may be similar in all species and marked by multiple system failures. Reducing dietary Na from 1.8 to 0.3g kg^{-1}DM in young pigs: increased plasma K from 5.6 to 7.4 ± 0.17 (s.e.) mmol l^{-1}; disturbed amino acid metabolism, indicated by changes in plasma lysine:arginine ratio; caused haemoconcentration, blood haemoglobin increasing from 124 to 159 ± 8g l^{-1} (Honeyfield *et al.*, 1985). Sodium is absorbed principally from the large intestine, which can show striking morphological evidence of adaptation (Elbrond *et al.*, 1991). After 3–4 weeks on a low-Na diet, microvillus surface area in the lower intestine of hens more than doubled, due to increases in epithelial cell number and surface area per cell. A greater density of open Na^+ channels and an increase in short-circuit current contributed to greatly increased net Na transport. Width of the zona glomerulosa doubled in adrenals of sodium-deprived cattle (Morris, 1980).

Diagnosis of Sodium Insufficiency in Ruminants

The earliest and most obvious abnormality, a craving for salt, is not definitive because some healthy individuals eat salt avidly (Denton, 1982) but salt craving is a feature of other disorders, such as lack of dietary P. Polydipsia and polyuria (Whitlock *et al.*, 1975) are also non-specific and reliance must be placed on biochemical criteria, particularly salivary Na:K ratios, but values increase prior to disorder and use of a marginal band is advocated (see Table 8.5).

- Salivary Na:K can vary widely within groups and repeatedly sampled individuals, but reciprocal variations in plasma aldosterone indicate extreme physiological sensitivity of salivary Na:K rather than random changes (N.F. Suttle, unpublished data).
- *Median* salivary Na:K ratio and *proportion* of values <2.0 rather than mean Na:K ratio should be used to assess risk of sodium deprivation in ruminants.

Parotid saliva can be easily drawn into a syringe from the hollow between cheek and teeth, using a 'trombone slide' gag to open the mouth (Murphy and Connell, 1970). In bovines, Na:K ratios in muzzle secretions decrease and droplets can be conveniently procured by syringe but the critical ratio is much lower (<0.2) than in saliva (Singh and Rani, 1999). Faeces containing <1g Na kg^{-1}DM was held to indicate deprivation (Little, 1987) but may be influenced by factors such as DM intake and digestibility (Morris, 1980): faecal Na:K ratio <1.0 may be more reliable. Urine Na <3mmol Na l^{-1} indicates insufficiency in *B. taurus* (Morris, 1980) and *B. indicus* (Singh and Rani, 1999) but values are influenced by urine output, which is much higher on lush pasture than dry diets: creatinine should be used as a marker. Low plasma Na indicates terminal deprivation and values given in Table 8.1 should be carefully interpreted.

- The surest indication of deprivation is a positive response in appetite, appearance and productivity to salt supplementation.

Dietary Sources of Sodium and Chloride

Sodium in forages

The distribution of pasture Na worldwide is skewed towards low values, with 50% of samples containing <1.5g kg^{-1}DM. Low values were more common in tropical than in temperate pastures: 50% of tropical legume samples contained <0.4g Na kg^{-1}DM (Minson, 1990). Distributions in New Zealand (NZ) pastures were similarly skewed, with

Table 8.1. Marginal bands[a] for sodium and chloride in serum, urine and diet as a guide to diagnose dietary deprivation (D) or excesses (E) of each element.

			Serum[b] (mmol l^{-1})	Urine (mmol l^{-1})	Diet (g kg^{-1}DM)
Na	Cattle[c]	D	130–140	<3	0.5–1.0
		E	140–145	40–60	30–60
	Sheep[c]	D	140–145	–	0.5–1.0
		E	150–160	40–60	30–60
	Pigs	D	137–140	1–3	0.3–1.0
		E	150–180	>11	20–40
	Poultry	D	130–145	–	0.8–1.5
		E	150–160	–	50–80
Cl	Cattle	D	70–85	2–5	1–3
		E	>150	–	–
	Sheep	D	70–85	–	1–3
		E	>150	>100	>50
	Pigs	D	88–100	–	0.9–2.4
		E	>110	–	20–49
	Poultry	D	90–115	–	0.8–2.0
		E	>174	>100	6.0–12.0

[a]Mean values below (for D) or above (for E) the given bands indicate probability of ill-health from deprivation or toxicity. Values within bands indicate the possibility of future disorder.
[b]If serum is unobtainable, similar interpretations can be applied to concentrations in vitreous humour.
[c]Salivary ratios of Na to K are more useful for diagnosing insufficiency in cattle and sheep. Median values of 4.0–10.0 are regarded as 'marginal'; the equivalent range for bovine muzzle secretions is 0.2–0.3.

a median value of 2g Na kg^{-1}DM; Knowles and Grace, 2014). Soils and pastures within 25 km of NZ east coast are enriched by deposition of sea spray and coastal pastures can be three times richer in Na than those growing inland (Ledgard and Upsdell, 1991; Edmeades and O'Connor, 2003). Plant genotype influences leaf Na (p. 17).[iv] Natrophilic species, whether grasses (e.g. *Phalaris* species) or legumes (e.g. white clover, subterranean clover and *Lotus*), commonly contained 3–4g Na kg^{-1}DM, while natrophobic species, including *Pennisetum clandestinum* (kikuyu), lucerne and red clover, commonly contained <0.5g kg^{-1}DM (Edmeades and O'Connor, 2003). Grass species show a seasonal decline: values in Californian rangeland pasture decreased from 0.5 to 0.1–0.2g Na kg^{-1}DM between spring and September (Morris *et al.*, 1980). Applications of N in fertilizers increased pasture Na in a dose-dependent manner but concurrent application of K limited that response, particularly at high rates (Fig. 8.6) (Kemp, 1971). Maize silage, like some other cereal silages (Khorasani *et al.*, 1997), is a poor source of sodium.

Chloride in forages

Most pastures are appreciably richer in Cl than Na, irrespective of species or state of maturity. For example, respective mean values for Na and Cl of 0.8 and 4.0g kg^{-1}DM in legumes and 1.4 and 5.0g kg^{-1}DM were reported in grasses (Thomas *et al.*, 1952). Pastures invariably provide sufficient Cl for grazing animals. Applications of chloride fertilizer (Chiy and Phillips, 1993) raise pasture Cl, which may benefit acid–base balance. Reductions in DCAD were relatively small in a USA study (Goff *et al.*, 2007) but much larger and barely positive in a recent Norwegian study (Fig. 8.7). Application rates were similar but K fertilizer was restricted to 50 kg ha^{-1} and pastures were dominated by timothy and meadow fescue, species naturally low in DCAD, in Norway (Nesheim *et al.*, 2020): timing of application (early or late spring) and chloride source ($CaCl_2$ or $MgCl_2$) were without influence.

- Combining pasture type and chloride fertilization may afford protection against milk fever and the more prevalent sub-acute clinical hypocalcaemia (p. 177).

Sodium and chloride in concentrates

A USA survey showed that many foodstuffs contained less Na than widely quoted NRC data (Berger, 1990): mean value for maize (0.07g kg^{-1}DM) was

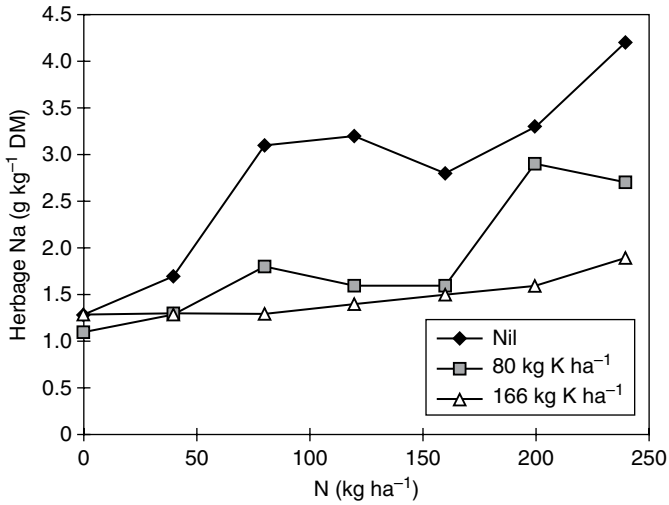

Fig. 8.6. Herbage sodium concentrations are increased by applications of nitrogenous fertilizers unless potassium is simultaneously applied. (Data from Kemp, 1971)

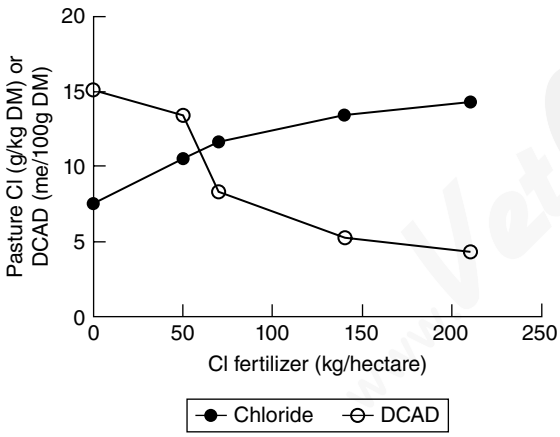

Fig. 8.7. Applying either magnesium or calcium chloride fertilizers increased chloride (Cl) concentrations and reduced dietary cation–anion difference (DCAD) in Norwegian pasture: results are averages for three sites, which differed significantly; extra potassium, 150 kg ha^{-1}, was given with the lowest level of chloride, 50 kg ha^{-1}, raising DCAD. (Data from Nesheim *et al.*, 2020)

77% lower and a more recent value was even lower (Table 8.2). Data for Na and Cl in common foodstuffs from the UK in 1990, given in Table 8.2, are several-fold higher than those recorded in the new millennium in the USA (Kerr *et al.*, 2008) for nearly all feeds and may reflect the difference between islands and continents, mentioned earlier (p. 185). For example, 29 soybean meals from the USA contained 0.05 ± 0.08g Na kg^{-1}DM.

- Median values would be more representative than means for such abnormally distributed data.

Most grain and plant protein sources (with the exception of sunflower meal) are low in Na, but values are often abnormally distributed. By-products, sugar as sugarbeet pulp and distiller grain solubles, are particularly variable (Table 8.2). Maize gluten feed in Europe contained 0.9g Na kg^{-1}DM, 75% less than protein-rich gluten meal (Sauvant *et al.*, 2004) but limited confidence can be placed in mean values without indication of variability. In the USA, the difference between gluten meal and feed was far wider (Kerr *et al.*, 2008). Root crops, root by-products and animal products are relatively rich in Na.

- Feed blend Na cannot be predicted from book values for feed constituents.

Cereal grains, like grasses, generally provide more Cl than Na, with maize providing the least and barley the most (0.5 and 1.8g Cl^- kg^{-1}DM, respectively). Cereal straws contain three- to sixfold more Cl than their grains. Vegetable protein supplements are consistently low in chloride (0.3–0.7g kg^{-1}DM) and it is possible to produce blends with cereals that do not meet Na and Cl requirements of pigs and poultry but inclusion of animal by-products and grass meal would correct any chloride deficit.

Table 8.2. Mean (standard deviation) sodium concentrations (g kg^{-1}DM) in pastures and foodstuffs in the UK (from MAFF, 1990) and USA (Kerr *et al*., 2008: chloride (Cl) concentrations for the USA are from NRC, as quoted by McDowell, 2003).

Forages	Na	Cl	Concentrates	Na	Cl	Roots and by-products	Na	Cl
Herbage	2.5 (2.1)	4–6	Fish meal	11.2 (1.5)	–	Fodder beet	3.0 (1.6)	–
Grass silage	2.6 (1.6)	–	Soybean meal (ext)	0.2 (0.1)	0.4	Swedes	0.7[a]	–
Clover silage (mix)	0.8 (0.5)	–	Safflower meal	1.0 (1.2)	1.3	Turnips	2.0[a]	–
Lucerne silage	1.3 (1.0)	4.1	Palm-kernel cake	0.2 (0.1)	–	Cassava meal	0.6 (0.3)	–
Maize silage	0.3 (0.2)	1.8	Maize gluten meal	2.6 (1.4)	0.8	Meat and bone meal	8.0 (1.0)	8–12
Grass hay	2.1 (1.7)	4–5	Rapeseed meal	0.4 (0.3)	–	Molasses (beet)	25.0 (8.2)	16.4
Lucerne hay	0.6 (0.1)	3.0	Cottonseed meal	0.2 (0.2)	0.5	Molasses (cane)	1.2 (0.9)	31.0
Dried grass	2.8 (1.6)	–	Linseed meal	0.7 (0.0)	0.4	Molassed beet pulp	4.4 (0.3)	–
Dried lucerne	1.3 (0.8)	4.8	Maize	< 0.1[b]	0.5	Beet pulp	3.2 (2.1)	0.4
Barley straw	1.3 (1.4)	6.7	Barley	0.3 (0.4)	1.8	Brewers' grains	0.3 (0.3)	1.7
Wheat straw	0.6 (1.0)	3.2	Wheat	0.1 (0.1)	0.5	Wheat feed	0.1 (0.1)	0.5
Alkali-treated barley straw	32.0 (10.5)	–	Oats	0.2 (0.1)	1.0	Distillers' grains	1.4 (1.3)[b]	
			Sorghum	0.5 (0.0)	1.1	Wheat	0.02 (0.01)[b]	–
						Barley	0.3 (0.3)	–

[a]New Zealand data from Cornforth *et al.* (1978).
[b]USA data from Kerr *et al*., (2008): corn had a mean value of 0.012 ± 0.013g kg^{-1}DM for 23 samples.

Drinking water

Water sources vary enormously in Na and Cl (and other minerals), from virtually none in some streams and reticulation sources to highly saline supplies, usually from deep wells or bores (Shirley, 1978; Wilkes *et al*., 2019). Sodium was the major cation, averaging 250 mg l^{-1}, and chloride the most abundant anion amongst total solids that reached 5 g l^{-1} (Wilkes *et al*. 2019). Drinking water can therefore constitute a significant source of Na, Cl and other minerals. Sodium-deprived animals show a marked preference for salty water (Blair-West *et al*., 1968) and will even consume $NaHCO_3$, which is usually avoided (Bell, 1995). When the diet provides sufficient Na, cattle will discriminate against water containing 12.5g NaCl l^{-1} in favour of pure water; pygmy goats showed a similar preference, while both sheep and normal goats remained indifferent (Goatcher and Church, 1970). Wide individual and seasonal variation in water consumption (p. 22) affects the contribution to total salt intake. Cows yielding 20 kg milk and consuming 50 l water each day would meet their sodium requirement from drinking water alone if it contained 1g NaCl l^{-1}. Supplementary NaCl can be given to poultry via drinking water (Ross, 1979).

Requirements for Sodium and Chloride

Absorption of sodium and chloride is usually almost complete and sources can be ranked by Na or Cl concentration but the two elements are mutually inhibitory (Henry, 1995). In young pigs on a cereal-based diet containing 2g Na kg^{-1}DM, apparent absorption (AA_{Na}) was 0.85 ± 0.015 and increased linearly with phytase additions to 0.91 (Dersjent-Li and Dusel, 2019). Low ileal AA_{Na} (0.49 ± 0.07) in young, early weaned pigs on a semi-purified diet (Woyengo *et al*., 2009) may reflect instability in developing of digestive processes. Safety factors may be needed for livestock unsuited to hot, humid environments and for young, grazing livestock that have yet to acquire resistance to infectious nematode larvae that contaminate most temperate pastures. Feeding to minimum sodium requirements will reduce water and salt accumulation in excreta and associated environmental problems. Donkeys' milk contained 0.44g Na l-1 throughout a long lactation (Malacarne *et al*., 2019), more than most species (Table 2.4); this may translate into a high requirement for females that work through lactation.

Sodium needs of sheep

Early feeding trials with lambs given high-grain diets, suggested needs 0.6–1.0 g Na (McClymont

et al., 1957; Hagsten *et al.*, 1975). Factorial estimates of requirements were higher, ranging from 0.8 to 2.7g Na kg^{-1}DM, but assumed faecal endogenous losses (FEL_{Na}) of 20mg Na kg^{-1}LW (ARC, 1980). Costa e Siva *et al.* (2015) used a model based on linear regression of retention against intake to generate a low retention coefficient of 0.37 and extrapolated maintenance need of 6.3mg kg^{-1}LW. Obligatory ($^{o}FEL_{Na}$) losses on diets low in K may be <6 mg Na kg^{-1}LW (Michell *et al.*, 1988) and would reduce estimated need. However, increasing dietary K from 6 to 24g kg^{-1}DM increased urinary Na losses from 30 to 40 mg kg^{-1}LW in wether lambs and induced a negative Na balance (Greene *et al.*, 1983a) with dietary Na at 1.8g kg^{-1}DM, clearly a marginal supply. Since most forage-based diets are relatively rich in K, higher obligatory urinary losses and $^{o}FEL_{Na}$ are allowed for in Table 8.3. In contrast to the constant 1.1g Na kg^{-1} EBW assumed by ARC (1980), recent carcase analysis studies suggest that accretion can vary from 0.7 (Bellof *et al.*, 2006) to 1.7 g (Ji *et al.*, 2015) in lambs and goat kids (Fig. 8.3).

- Accreted Na may not be wholly obligatory.

Field studies with grazing lambs and ewes indicated needs of 0.5 and 1.0g Na kg^{-1}DM (Edmeades and O'Connor, 2003). With salivary Na:K ratios used to assess requirements for lactating ewes (Fig. 8.8), maximal status was achieved with 0.80–0.87g Na kg^{-1}DM (Morris and Peterson, 1975). Furthermore, appetite, ewe body weight and lamb growth rate did not deteriorate on an unsupplemented diet, containing only 0.2g Na kg^{-1}DM. Tolerance of sodium depletion, commencing in pregnancy, has been confirmed (Michell *et al.*, 1988). Minimum chloride requirements have not been defined.

Sodium needs of cattle

Early field experiments indicated that 0.5–0.8g Na kg^{-1}DM was sufficient for growth under normal climatic conditions (Morris and Gartner, 1971; Morris and Murphy, 1972) but lactating cows needed slightly more (1.0g Na kg^{-1}DM) (Morris, 1980; Edmeades

Table 8.3. Estimates of minimum dietary sodium concentrations required by sheep[a] and cattle on forage-based diets containing >2gK kg^{-1}DM*.

				Gross[b] Na requirement	
	Liveweight (kg)	Growth or product	DMI (kg day^{-1})	(g day^{-1})	(g kg^{-1}DM)
Lamb	20	0.1 kg day^{-1}	0.5	0.34	0.7
		0.2 kg day^{-1}	0.8	0.42	0.5
	40	0.1 kg day^{-1}	0.83	0.61	0.7
		0.2 kg day^{-1}	1.23	0.68	0.6
Ewe	75	0	0.8	1.0	1.25
Ewe, pregnant	75	1 fetus, week 12	1.03	1.0	1.0
		Term	1.51	1.0	0.7
Ewe, lactating	75	1 kg day^{-1}	1.48	1.6	1.1
		2 kg day^{-1}	2.18	2.1	1.0
		3 kg day^{-1}	2.90	2.7	0.9
Steer	200	0.5 kg day^{-1}	3.3	2.3	0.70
		1.0 kg day^{-1}	4.7	3.1	0.65
	400	0.5 kg day^{-1}	5.2	3.9	0.75
		1.0 kg day^{-1}	7.3	4.7	0.65
Cow, dry	600	0	5.0	4.5	0.9
Cow, pregnant	600	Week 12	5.8	4.3	0.9
		Term	9.0	7.2	0.8
Cow, milking	600	10 kg day^{-1}	9.4	10.3	1.1
		20 kg day^{-1}	14.0	16.8	1.2
		30 kg day^{-1}	18.8	22.6	1.2

[a]Values for sheep assume faecal+ urinary endogenous loss (*F+U EL*) of 12 mg Na kg^{-1} LW, 0.7g Na kg^{-1}LWG, milk contains 0.51g Na kg^{-1} and absorption coefficient is 0.91.
*Requirements are probably lower on diets containing much less K, due to lower $F+U\ EL_{Na}$
Assuming a net growth requirement of 1.5g Na kg^{-1} LWG, maintenance needs of 20mg Na kg^{-1}LW and an AA_{Ca} coefficient of 0.91, ARC (1980) calculated this need.

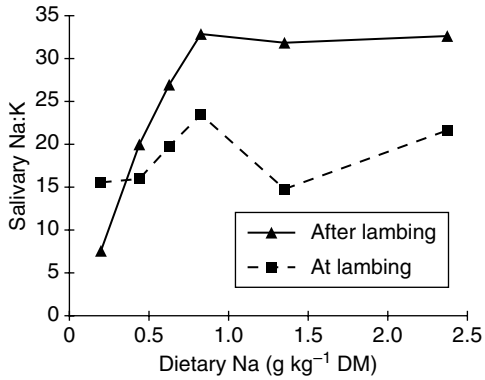

Fig. 8.8. Salivary sodium to potassium ratios reflect dietary Na supply after lambing because of the higher Na requirement for lactation than gestation and possible increase in the set point for Na:K around parturition. (Data from Morris and Peterson, 1975)

and O'Connor, 2003): these remain the recommendations in USA for dairy (NRC, 2001) and beef cattle (NRC, 2016). Reductions in salivary Na:K after parturition in grazing beef cows confirmed the increased demand associated with lactation (Morris *et al.*, 1980) but supplementation did not improve calf weaning weight on pasture containing only 0.15–0.50g Na kg^{-1}DM and drinking water only 10–25mg Na l^{-1}. Using salivary Na:K as an indicator of need, housed buffalo heifers required between 0.6 and 1.0g Na kg^{-1}DM (Singh and Rani, 1999). Needs from 4.9 to 13g Na d^{-1} (equivalent to 0.6–1.9g kg^{-1}DM) were estimated for tropical beef breeds by dubious comparative slaughter methods (Zanetti *et al.*, 2017): needs were inflated by adding substantial urinary losses to the net requirement. The requirements given in Table 8.3 allow for similar effects of K on the need for maintenance to those allowed for in sheep and increase slightly as milk yield rises. Applications of NaCl that increased herbage Na from 2 to 5g kg^{-1}DM also improved milk fat yield (Chiy and Phillips, 1993) but the improvement was confined to low-yielding cows in a subsequent study (Chiy and Phillips, 2000) and not confirmed in NZ (Edmeades and O'Connor, 2003). Such responses may be attributable to short-term effects on palatability and herbage intake (Chiy *et al.*, 1993) and might not be seen in longer-term studies, due to diminishing amounts of herbage to graze. Increased provision of Na was suggested at onset of lactation to offset massive losses (1.0–2.6g Na d^{-1}) and large negative balances, (Silanikove *et al.*, 1997) but such losses may be irreversible consequences of obligatory bone resorption (p. 58).

Need for chloride in cattle

Beef cattle gaining 1.0 kg day^{-1} were estimated to require the equivalent of 0.7g Cl kg^{-1}DM (ARC, 1980), close to the need for Na. Requirements for lactating dairy cows should be substantially higher than those for Na because cow's milk contains more than twice as much Cl as Na. Milk Cl rises as lactation progresses, values of 1.16, 1.29 and 1.90g Cl l^{-1} being found in months 1, 5 and 10 of lactation, respectively, in Holstein cows (Lengemann *et al.*, 1952): this contrasts with lactation changes for most minerals. However, milk yield concurrently diminishes and may provide a route for excretion of excess Cl: such losses may not be obligatory. A supplement of 0.6g Cl prevented symptoms of deprivation on a diet providing 1g Cl kg^{-1}DM (Fettman *et al.*, 1984) and NRC (2001) recommended 2.0g Cl kg^{-1}DM.

Goats and herbivores

Growth, reproduction and lactation were impaired in goats given 0.3 rather than 1.7 g Na kg^{-1}DM (Schellner, 1972/1973) and the higher level was apparently sufficient. Goat's milk contained less Na than ewe's or cow's milk (Table 2.4) and carcase Na in three out of four studies in Fig. 8.3 is similar to values in sheep. Requirements for goats will be met or exceeded by those given for sheep. Chloride requirements of goats are not available. From carcase analysis of the foal carcase and assumed values for endogenous loss and absorptive efficiency, it was estimated that growing horses needed 1g Na kg^{-1}DM (Grace *et al.*, 1999).

Sodium and chloride needs of pigs

Early studies with semi-purified (Meyer *et al.*, 1950) and conventional diets (Hagsten and Perry, 1976; Honeyfield *et al.*, 1985) placed needs at 0.8–1.1g Na and 1.2–1.3g Cl kg^{-1}DM for optimum growth but very young pigs required three times as much (Mahan *et al.*, 1996). Needs may be high in the young because phytate greatly reduces AA_{Na}. NRC (1998) recommended 2g Na kg^{-1}DM for the youngest pigs, decreasing to 0.8 Na kg^{-1}DM for fattening pigs: needs for Cl were of the same order. However, raising dietary Cl in starter diets containing

milk by-products to 3.2g Cl by adding HCl improved growth in the first two weeks after weaning at 22 days old: similar responses were found to added NaCl (Mahan *et al.*, 1999). NRC (2012) duly raised recommendations for Na and Cl from those made in 1998 (Table 8.4). High needs of 1.7g Cl kg^{-1}DM for finisher pigs had been indicated for optimal food conversion efficiency, particularly if diets were low in Na (Honeyfield *et al.*, 1985). A recent study confirmed NRC (2012) recommendations for the youngest pigs and showed no advantage in raising dietary Na and Cl above 1 and 2.3g kg^{-1}DM, respectively, for older pigs (Shawk *et al.*, 2018). A factorial model (Table 8.4) predicted marked decreases in need between 20 and 90 kg LW: extrapolation to the lactating sow gives requirements far below those of NRC (2012) of 1.0 and 1.9 Na kg^{-1}DM. Generous provision during pregnancy (1.5g Na kg^{-1}DM; NRC, 2012) would establish a store to meet any lactational deficit.

Poultry

NRC (1994) requirements are summarized in Table 8.5 and were considerably greater than those indicated by early studies with less productive birds. More sodium may be needed for very young birds. Broiler chicks needed 4–5g Na kg^{-1}DM in the first week, declining by 30% at 3 weeks of age (Edwards, 1984; Britton, 1990). Chloride requirements are also much higher (4–5g kg^{-1} DM) early in life. Requirements for Na, K and Cl probably decline with stage of growth in all types of bird, as is the case with pigs. The average hen's egg contains 73 mg Na, 88 mg Cl and 82 mg K but increases in daily requirement with onset of lay are more than met by increases in food intake. Requirements remain low when stated as dietary concentrations, although they are higher for high-energy diets (Table 8.4). The NRC standards suggested and recent studies (Jiang *et al.*, 2019) confirm that supplementation of conventional poultry rations with Na is essential. However, turkey poults were reared for

Table 8.4. Sodium requirements of pigs predicted from a factorial model compared with those of NRC (2012)[a].

Liveweight (kg)	Growth rate (kg day^{-1})	Net need (g day^{-1})	Total need (g day^{-1})	DM intake (kg day^{-1})	Dietary Na (g kg^{-1}DM)[b]	NRC (2012) (g kg^{-1}DM)
5*	0.27	0.416	0.462	0.37	2.50	3.5
25	0.55	0.659	0.732	1.04	0.71	2.8
45	0.78	0.851	0.946	1.78	0.53	1.0
90	0.79	0.833	0.926	2.78	0.33	1,0
150	5.5[c]	2.08	2.14	5.00	0.43	2.9

[a]Model components were 1.0–1.5g Na kg^{-1} liveweight (LW) gain for growth, 56g Na total need for pregnancy, 0.3–0.4 g Na kg^{-1} for milk yield, 1mg kg^{-1} LW for maintenance and an absorption coefficient of 0.9, except for the youngest pigs*, for which it is 0.45.
[b]Corresponding requirements for NaCl are 2.62-fold greater.
[c]Milk yield in sow.

Table 8.5. Dietary requirements (g kg^{-1}DM) of growing broiler (B) and Leghorn (L) chicks, turkey poults (P) and laying birds for sodium and chloride (NRC, 1994).

		Growth stage[a]			Lay stage[b]		
	Bird type	Early	Middle	Late	High	Medium	Low
Na	B	3.0[c]	1.5	1.2	–	–	–
	L	3.0[c]	1.5	1.5	1.9	1.5	1.3
	P	1.7	1.2	1.2	–	1.2	–
Cl	B	3.0[c]	1.5	1.2	–	–	–
	L	3.0[c]	1.2	1.5	1.6	1.3	1.1
	P	1.5	1.4	1.2	–	1.2	–

[a] For broilers, Leghorn chicks and poults, respectively: early = 0–3, 0–6 or 0–4 weeks; middle = 3–6, 6–12, or 8–12 weeks; late = 6–8, 18 weeks to first lay and 20–24 weeks of age.
[b] High, medium and low represent declining energy densities and food intakes of 80, 100 and 120g day^{-1}, respectively.
[c] For the first week of life, 8–10 g NaCl kg^{-1}DM is indicated by some studies (see text).

35 days on diets containing only 0.9g Na kg^{-1}DM, well below NRC (1994) requirement (Frame *et al.*, 2001). Chloride requirements for poultry are similar to those for Na (Table 8.5) (NRC, 1994).

Prevention of Sodium Deprivation

Supplementary Na is invariably supplied commercially as common salt and enhances the palatability of feeds (Grovum and Chapman, 1988). Salt is one of several dietary components that sheep can show preferences for, given a choice, and thereby be led to select a feed blend that optimizes performance, while exceeding minimum requirements for Na and Cl (Thomas *et al.*, 2007). Salt became more sparingly used as environmental and energy costs of metabolizing excess Na were revealed (Arieli *et al.*, 1989) and costs to small farmers became significant. For housed, growing and fattening beef cattle, salt supplements need not add >0.5g Na kg^{-1}DM to total mixed rations but housed lactating dairy cows should be allowed twice as much. In pig and poultry feeding it was common to add 5g NaCl kg^{-1}DM to rations, but 2.5 g should suffice, even for the youngest pigs (see Table 8.4) and chicks. Sodium deficiency in laying hens, given 0.5g Na kg^{-1}DM, was aggravated by restriction of dietary Cl to 0.8g kg^{-1}DM, but partly alleviated by increasing K from 7 to 12g kg^{-1}DM (Sauveur and Mongin, 1978).

Supplementation of grazing livestock

At pasture, it is customary to rely on continuous, voluntary consumption of loose or block salt. However, individual consumption can be highly variable and greatly exceed need (Fig. 3.5, p. 47) (Suttle and Brebner, 1995). Physical form can affect salt consumption. Lactating cows and heifers at pasture consumed significantly more loose than block salt (Smith *et al.*, 1953) but when salt is included in blocks containing molasses, the preference is reversed. Application of NaCl to pasture rarely increased pasture yields in New Zealand, even on soils low in Na and high in K, but herbage Na increases linearly with application rate: 50kg NaCl ha^{-1} and was sufficient to raise herbage Na to levels required by grazing livestock, even in natrophobic species (p. 17) (Edmeades and O'Connor, 2003). Where the problem is one of K-induced sodium insufficiency, K inputs should be restricted and seasonal applications delayed (p. 178). Use of low-grade kainite (KCl), containing 22% subsidiary Na, should maintain herbage Na. In sheep grazing cereal crops in the vegetative state, provision of MgO as well as NaCl is recommended (Dove and McMullen, 2009).

Salt Toxicity in Ruminants

In many respects, chloride toxicity is synonymous with salt toxicity. Dietary excesses of Na and/or Cl cause osmotic imbalance, and trigger complex physiological control mechanisms, some mediated by the central nervous system and influenced by cortisol (Parker *et al.*, 2004). Dietary excesses of Cl, like those of Na, are predominantly excreted through the kidneys via a K co-transporter in the renal tubules: although Cl can be reabsorbed, the process is passive. Addition of salt to feeds replete with Na can simply lower feed intake (Wilson, 1966; Moseley, 1980; Masters *et al.*, 2005) and may be used to restrict intake of supplementary foods in ruminants (De Waal *et al.*, 1989). Rate of outflow of digesta from the rumen is increased, with adverse consequences for digestibility (Arieli *et al.*, 1989) but possible benefits in terms of undegraded protein outflow (Hemsley *et al.*, 1975). Natural excesses can occur through consumption of saline drinking water and/or saline plants, growing on saline soils. Excesses can also arise from accidental or intentional human interventions (e.g. access to NaCl-containing fertilizers or mineral mixtures, failed irrigation schemes and alkali treatment of grain). In a recent episode, a sheep flock just turned out onto pasture with access to water, also had access to salt blocks that had been badly weathered by heavy overnight rainfall: 51 sheep of all ages (about 17%) died within 24 hours without specific abnormalities but with cyanosed, visible mucous membranes, inflammation and hyperaemia of the gut, localized oedema and frothy discharges: liver Na was high (Siroka *et al.*, 2017). Guidelines for the assessment of excesses in diets and animals were given in Table 8.5. Water deprivation causes a rise in plasma Na in all species of livestock and should be ruled out.

Tolerance of dietary sodium

Tolerance of dietary Na is relatively high, provided salt-free drinking water is freely available. Water intake increased from 3.9 to 12.3l d^{-1} when up to

80g Na kg^{-1}DM was added as NaCl to the pelleted diet of weaner Merino lambs. Food intake decreased curvilinearly (Fig. 8.9) and growth eventually ceased (Masters *et al.*, 2005). Concurrent increases in dietary K, as KCl, providing 17-38 g K kg^{-1} DM had lesser, additive effects on water intake but more complex effects on DMI (Fig. 8.9), stimulating DMI at the lowest Na intake but decreasing DMI at the highest Na intake. High rumen osmolality may have depressed food intake via vagus nerve signals. There was no sign of adaptation during the six-week experiment. Total body water content was increased to about 68% at the highest salt intake compared to about 63% in other groups but included gut fill. Manipulation of Na:K ratios was considered unlikely to improve tolerance of NaCl. Although wool growth was depressed by high Na, efficiency of wool growth increased from 10.0 to 15.4 g/kg organic matter intake, possibly because protein was protected from rumen degradation by increased rate of digesta flow from the rumen (Masters *et al.*, 2005). High salt intakes may therefore be advantageous where the major output is fine wool, although sulfur intakes may also need to be increased (p. 216). Feeding salt-rich diets (130g Na kg^{-1}DM) to ewes during pregnancy 'programmed' the subsequent responses of lambs to saline drinking water (15g NaCl l^{-1}); aldosterone responses and appetites were lower than in lambs from ewes given 5g Na kg^{-1} DM (Digby *et al.*, 2010). Calves were continuously supplemented with 1.83 g NaCl kg^{-1} LW day^{-1}, without disturbance of electrolyte status or growth (Wamberg *et al.*, 1985). However, an increase in dietary Na from 1 (low) to 4 (modest excess) g kg^{-1}DM increased the water intake of lactating sows from 12.4 to 13.9 l day^{-1} (Seynaeve et al., 1996).

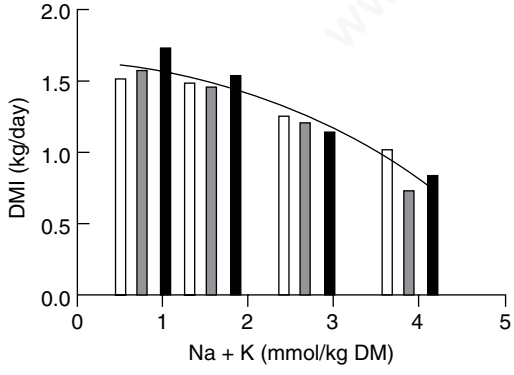

Fig. 8.9. Effects of increasing intakes of sodium and potassium (added as chlorides) on dry matter intake (DMI) in lambs: the contribution of K at each of four Na concentrations was 0.42 (clear), 0.67 (grey) or 0.95 (black columns). (From Masters *et al.*, 2005)

- Individual intakes of salt from free-access mixtures are unlikely to be sufficient to impair ruminant production, provided they are not rich in Mg (p. 119) and there is free access to fresh drinking water.

Tolerance of saline drinking water

Recommendations that 5g NaCl l^{-1} was safe for lactating cattle and up to 7g l^{-1} for non-lactating cattle and sheep (Shirley, 1978) have been revised downwards. Limits are now defined in terms of total dissolved solids (TDS) and should not exceed 3g S l^{-1} (Wilkes *et al.*, 2019). Stock can adapt to higher concentrations, at least in temperate climates. In a mild, cool winter and on lush pasture, sheep tolerated water containing 13g NaCl l^{-1} but with 20g NaCl l^{-1}, feed consumption and body weight declined and some animals became weak and emaciated (Peirce, 1957, 1965): goats in Greece tolerated that concentration for two weeks but food intake decreased; biochemical changes occurred that were not simply due to haemo-concentration: urinary excretion of K as well as Na increased (Table 8.6).

Sodium hydroxide-treated feeds

The treatment of grains, straws and mature or whole-crop silages with sodium hydroxide (NaOH) can improve nutrient utilization by ruminants (e.g. Leaver and Hill, 1995; Chaudhry *et al.*, 2001) but the amounts of Na added are substantial. In the former study, wheat and wheat straw containing 18g Na kg^{-1} were fed with silage (40:60) to dairy cows without mishap for 12 weeks from day 54 of

Table 8.6. Significant effects of consuming water containing 20g NaCl l^{-1} for 14 days in goats (Zoidis and Hadjigeorgiou, 2017).

Parameter	0	+NaCl
Water intake (l d^{-1})	2.0	2.5
Dry food intake (kg d^{-1})	1.4	1.1
Plasma Na (mmol l^{-1})	143	150
glucose (mg l^{-1})	675	808
urea (mg l^{-1})	265	470
osmolality (mosm kg^{-1})	284	289
protein (g l^{-1})	63	83
Urine Na (mmol l^{-1})	55	377
K (mmol l^{-1})	144	329

lactation. Calves can tolerate 1.2g NaOH kg^{-1} LW day^{-1} and show no benefits in terms of acid–base status from neutralization of the alkali with HCl (Wamberg *et al.*, 1985). However, the preferred alkali is ammonium hydroxide because it provides nitrogen and may be less likely than NaOH to cause kidney lesions (nephritis) (D. Rice, personal communication, 1985). Alkali-treated feeds should not be used for transition cows because they may increase the incidence of milk fever (see p. 60).

Ruminant production in saline environments

Vast areas of cultivable land on all continents support no livestock production because soils are saline and the natural vegetation is either unpalatable, poorly digestible or harmful: in Australia, 5.7 Mha were thus categorized (Masters *et al.*, 2006). Furthermore, water scarcity and excessive extraction is extending affected areas and creating new ones as freshwater sources become saline ('brackish'). The flora on saline soils is made up of species that tolerate sodium by avoidance (deep roots), exclusion (salt glands) and dilution (bladder cells, succulence) and there is no correlation between soil salinity and plant Na (Gorham, 1995; Ventura *et al.*, 2015). Cultivated Old Man saltbush (*Atriplex nummularia*) contains 200–300g ash kg^{-1}DM, consisting mostly of salt but also 20–40g K kg^{-1}DM (Ben Salem *et al.*, 2010; Masters and Norman, 2016). The scope for crop and animal production in saline environments is great (Ashraf *et al.*, 2006; Ventura *et al.*, 2015; Masters and Norman, 2016). Establishment of the International Centre for Biosaline Agriculture in 1999 contributed to a six-fold increase in agricultural production in an arid climate (Rao and Shahid, 2006). Grasses such as *Distichlis spicata*, *Paspalum vaginatum*, *Sporobolus virginicus* and *S. arabicus* have produced biomass yields of 30–40 tonnes DM ha^{-1} y^{-1} under irrigation with saline water containing 14–17g TDS l^{-1}, harvesting three times a year.

Constraints and prospects

Salt-tolerant species differ widely in the animal productivity that they can sustain and this is partly due to extreme but diverging concentrations of Na and other minerals (Table 8.7). Monocotyledenous grasses contain less Na than chenopods like *Atriplex sps*. when grown on the same saline soils. In some Mediterranean climates (e.g. S. Australia) native halophytes are grazed when sown pasture has senesced. Halophytes contain Ca, P and Mg that are less available than the same elements in a pelleted concentrate (Mayberry *et al.*, 2010). Livestock performance is often less than expected on saline feeds, due to low food intake and organic matter digestibility (Ben Salem *et al.*, 2010). High energy losses associated with increased sodium turnover (Arieli *et al.*, 1989) and high rates of digesta flow from the rumen may be responsible (Attia-Ismail, 2018). High osmolality of rumen liquor will inhibit microbial protein synthesis, particularly that of the resident protozoal population. One solution is partial substitution of saline forage for conventional roughage (Ahmed *et al.*, 2015). Sustainable animal production in saline environments will require a variety of approaches, including: intercropping with deep-rooted species such as sunflower (Rao and Shahid, 2006) and salt-tolerant perennial grasses (Khan *et al.*, 2009); irrigation systems that limit the upward migration of salt and use of complementary feeds, low in Na (e.g. maize and lucerne) (Masters *et al.*, 2006; Masters and Norman, 2016); selection of salt-tolerant varieties of traditional crops, such as sorghum (Bafeel, 2014). Vegetation can be ensiled and with addition of fibrolytic and proteolytic

Table 8.7. Concentrations of macro (g kg^{-1}DM) and trace elements (mg kg^{-1}DM) in the whole plant of some halophytic species. (From Attiah-Ismail, 2018)

Species	Na	K	Mg	Ca	P	S	Cu	Zn
Acacia anuera	0.1	–	2.6	13.9	0.7	1.7	–	76
salighna	11.5	10.5	1.4	37.5	–	–	–	141
Atrpiplex kalimia	39.1	5.7	3.3	14.9	3.2	1.7	10	64
mummularia	49.9	29.9	156.3	20.8	11.7	–	16	134
Halocyclon	50.1	17.4	3.3	40.0	1.5	0.7	18	73
Numania saligna	53.5	6.6	3.1	19.6	2.2	1.4	31	32

enzymes, digestibilities can reach 64% when fed with barley (Alsersy *et al*., 2015).

Salt toxicity in pigs and poultry

Toxicity of both sodium and chloride is indicated clinically by anorexia and water retention and physiologically by intracellular dehydration due to hypertonicity of the ECF. Excessive intakes of Na increase water consumption in pigs and poultry (Mongin, 1981), increasing volumes and moisture contents of excreta. Low tolerance limits of 15g NaCl kg^{-1}DM have been set for pigs and poultry (NRC, 2006) but are highly dependent on free access to water low in salt (for review, see Marai *et al*., 1995). Young and old pigs tolerated drinking water containing 10g NaCl l^{-1}, but 15g l^{-1} was toxic (Heller, 1932). Laying hens are much less tolerant of saline water than pigs and adverse effects are of particular concern because egg-shell quality is rapidly reduced by relatively low concentrations (2 days exposure to 0.2g NaCl l^{-1}) and residual effects are prolonged (at least 15 weeks) (Balnave and Yoselewitz, 1985; Balnave and Zhang, 1998). The percentage of damaged shells increased linearly to a maximum of 8.9% as sodium concentrations increased up to 0.6 g l^{-1}, a level found in some local underground sources in New South Wales. Excess chloride has been specifically incriminated as a factor contributing to tibial dyschondroplasia in broilers (Shirley *et al*., 2003).

Notes

[i] Insulin regulates glucose uptake by changing the number of Na+/K+glucose transporters in enterocytes, for example (Serhan and Kreydiyyeh, 2010).

[ii] Cattle exposed to 40°C for 7 h were estimated to lose slightly more Cl than Na in sweat (1g day^{-1} for a 200 kg steer and 1.7 g^{-1} day for a 500kg cow; ARC, 1980).

[iii] Activation of renin to form angiotensins (I, II and III), with vasopressin, can modulate responses to aldosterone, changing extracellular fluid (ECF) volume and blood pressure through appropriate adjustments in thirst and water balance (Bell, 1995; Michell, 1995; Burnier, 2007). The hormonal control axes are active in the bovine fetus, which responds to variations in maternal sodium intake (Rouffet *et al*., 1990), and in the newborn calf (Safwate, 1985).

[iv] Among the more widely grazed native range species, herbage Na ranks in the order *Holcus lanatus* > *Dactylis glomerata* > *Festuca pratensis* > *Phleum pratense,* a natrophobic species. Varietal differences are most marked in the richest (natrophilic) species, such as *Lolium perenne* (Chiy and Phillips, 1995).

References

Abu Damir, H., Scott, D., Loveridge, N., Buchan, W. and Milne, J. (1991) The effects of feeding diets containing either $NaHCO_3$ or NH_4Cl on indices of bone formation and resorption and on mineral balance in the lamb. *Journal of Experimental Physiology* 76, 725–732.

Ahmed, M.H., Salemb, A.Z.M., Zeweila, H.S., Sunc, X.Z., Kholifd, A.E., Elghandourb, M.M.Y. and Bahar, M.S.I. (2015) Growth performance and carcass characteristics of lambs fed halophytes as a partial or whole replacement of berseem hay. *Small Ruminant Research* 128, 1–9.

Aines, P.D. and Smith, S.E. (1957) Sodium versus chloride for the therapy of salt-deficient dairy cows. *Journal of Dairy Science* 40, 682–688.

Aitken, F.C. (1976) Technical communication of the Commonwealth Bureaux of Nutrition No. 26. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Alsersy, H., Salem, A.Z.M., Borhami, B.E., Olivares, J., Gado, H.M. *et al*. (2015) Effect of Mediterranean saltbush (Atriplex halimus) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep. *Animal Science Journal* 86, 51–58.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 213–216.

Arieli, A., Naim, E., Benjamin, R.W. and Pasternak, D. (1989) The effect of feeding saltbush and sodium chloride on energy metabolism in sheep. *Animal Production* 49, 451–457.

Ashraf, M.Y., Sarwar, G., Ashraf, M., Hussain, F., Wahed, R.A. and Iqbal, M.M. (2006) Growth performance and nutritional value of salt tolerant plants growing under saline environments. In: Öztürk, M., Waisel, Y., Khan, M.A. and Görk, G. (eds) *Biosaline Agriculture and Salinity Tolerance in Plants.* Birkhäuser, Basel, Switzerland, pp. 237–245.

Attia-Ismail, S.A. (2018) Halophytes as forages. In: Edvan, R.L. and Bezerra, L. (eds) *New Perspectives in Forage Crops*. DOI:10.5772/intechopen.69616

Bafeel, S.O. (2014) Physiological parameters of salt tolerance during germination and seedling growth of Sorghum bicolor cultivars of the same subtropical origin. *Saudi Journal of Biological Sciences* 21, 300–304.

Balnave, D. and Yoselewitz, I. (1985) The relation between sodium chloride concentration in drinking water and egg-shell damage. *British Journal of Nutrition* 58, 503–509.

Balnave, D. and Zhang, D. (1998) Adverse responses in egg shell quality in late-lay resulting from short-term use of saline drinking water in early- or mid-lay. *Australian Journal of Agricultural Research* 49, 1161–1165.

Beatty, D.T., Barnes, A., Taylor, E., Pethick, D., McCarthy, M. and Maloney, S.K. (2006) Physiological responses of *Bos taurus* and *Bos indicus* to prolonged continuous heat and humidity. *Journal of Animal Science* 84, 972–985.

Bell, F.R. (1995) Perception of sodium and sodium appetite in farm animals. In: Phillips, C.J.C. and Chiy, P.C. (eds) *Sodium in Agriculture*. Chalcombe Publications, Canterbury, UK, pp. 82–90.

Bellof, G., Most, E. and Pallauf, J. (2006) Concentration of Ca, P, Mg, Na and K in muscle, fat and bone tissue of lambs of the breed German Merino landsheep in the course of the growing period. *Journal of Animal Physiology and Animal Nutrition* 90, 385–393.

Ben Salem, H., Norman, H.C., Nefzaouic, A. Mayberry, D.E., Pearce, K.L. and Revell, D.K. (2010) Potential use of oldman saltbush (Atriplex nummularia Lindl.) in sheep and goat feeding. *Small Ruminant Research* 91, 13–28.

Berger, L.L. (1990) Comparison of National Research Council feedstuff mineral composition data with values from commercial laboratories. In: *Proceedings of Georgia Nutrition Conference, Atlanta, 1990*. University of Georgia, Atlanta, Georgia, pp. 54–62.

Black, H. (1997) Sea water in the treatment of inanition in sheep. *New Zealand Veterinary Journal* 45, 122.

Blair-West, J.R., Coghlan, J.P., Denton, D.A., Goding, J.R., Wintour, M. and Wright, R.D. (1963) The control of aldosterone secretion. In: Plincus, S. (ed.) *Recent Progress in Hormone Research*. Academic Press, New York, pp. 311–383.

Blair-West, J.R., Coghlan, J.P., Denton, D.A., Nelson, J.F., Orchard, E. *et al*. (1968) Physiological, morphological and behavioural adaptation to a sodium deficient environment by wild native Australian and introduced species of animals. *Nature UK* 217, 922–928.

Block, E. (1994) Manipulation of cation-difference on nutritionally related production diseases, productivity and metabolic responses in dairy cows. *Journal of Dairy Science* 77, 1437–1450.

Bott, E., Denton, D.A., Goding, J.R. and Sabine, J.R. (1964) Sodium deficiency and corticosteroid secretion in cattle. *Nature UK* 202, 461–463.

Britton, W.M. (1990) Dietary sodium and chlorine for maximum broiler growth. In: *Proceedings of Georgia Nutrition Conference*, *Atlanta*. University of Georgia, Atlanta, Georgia, pp. 152–157.

Budde, R.A. and Crenshaw, T.D. (2003) Chronic metabolic acid load induced by changes in dietary electrolyte balance increased chloride retention but did not compromise bone in growing swine. *Journal of Animal Science* 81, 197–208.

Burnier, M. (2007) *Sodium in Health and Disease*. CRC Press, Boca Raton, Florida.

Chamberlain, G.T. (1955) The major and trace element composition of some East African feeding stuffs. *East African Agricultural Journal* 21, 103–107.

Chaudhry, A.S., Cowan, R.T., Granzan, B.C. and Klieve, A.V. (2001) The nutritive value of Rhodes grass (*Chloris guyana*) when treated with CaO, NaOH or a microbial inoculant and offered to dairy heifers as big-bale silage. *Animal Science* 73, 329–340.

Chiy, P.C. and Phillips, C.J.C. (1993) Sodium fertilizer application to pasture. 1. Direct and residual effects on pasture production and composition. *Grass and Forage Science* 48, 189–202.

Chiy, P.C. and Phillips, C.J.C. (1995) Sodium in forage crops and sodium fertilization. In: Phillips, C.J.C. and Chiy, P.C. (eds) *Sodium in Agriculture*. Chalcombe Publications, Canterbury, UK, pp. 43–81.

Chiy, P.C. and Phillips, C.J.C. (2000) Sodium fertilizer application to pasture. 10. A comparison of the responses of dairy cows with high and low milk yield potential. *Grass and Forage Science* 55, 343–350.

Coop, R.L. (1971) The effect of large doses of *Haemonchus contortus* on the level of plasma pepsinogen and the concentration of electrolytes in the abomasal fluid of sheep. *Journal of Comparative Pathology* 81, 213–219.

Coppock, C.E. (1986) Mineral utilisation by the dairy cow – chlorine. *Journal of Dairy Science* 69, 595–603.

Cornforth, I.S., Stephen, R.C., Barry, T.N. and Baird, G.A. (1978) Mineral content of swedes, turnips and kale. *New Zealand Journal of Experimental Agriculture* 6, 151–156.

Costa e Siva, L.F., Valdares Filho, S.C., Rotta, P.F., Marcandes, M.I. *et al*. (2015) Mineral requirements for beef cattle. In: *Nutrient Requirements of Zebu and Crossbred Cattle*. BR-Corte, Viscondo do Rio Branco, Brazil, pp. 213–249.

Cowieson, A.J., Acamovic, T. and Bedford, M.R. (2004) The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. *British Poultry Science* 45, 101–108.

Dayhim, F. and Teeter, R.G. (1994) Effect of heat stress and drinking water salt supplements on plasma electrolytes and aldosterone concentrations in broiler chickens. *International Journal of Bacteriology* 38, 216–217.

de Waal, H.O., Baard, M.A. and. Engels, E.A.N. (1989) Effects of sodium chloride on sheep. 1. Diet composition, body mass changes and wool production of young Merino wethers grazing. *South African journal of Animal Science* 19, 27–33.

Denton, D.A. (1982) *The Hunger for Salt: An Anthropological, Physiological and Medical Analysis*. Springer-Verlag, Berlin/Heidelberg.

Dersjant-Li, Y. and Dusel, G. (2019) Increasing the dosing of a *Buttiauxella* phytase improves phytate degradation, mineral, energy, and amino acid digestibility in weaned pigs fed a complex diet based on wheat,

corn, soybean meal, barley, and rapeseed meal. *Journal of Animal Science* 97, 2524–2533.

Digby, S.N., Blache, D., Masters, D.G. and Revell, D.K. (2010) Responses to saline drinking water in offspring born to ewes fed high salt during pregnancy. *Small Ruminant Research* 91, 87–92.

Dove, H. and McMullen, K.G. (2009) Diet selection, herbage intake and liveweight gain in young sheep grazing dual-purpose wheats and sheep responses to mineral supplements. *Animal Production Science* 49, 749–758.

Dove, H., Masters, D.G. and Thompson, A.N. (2016) New perspectives on the mineral nutrition of livestock grazing cereal and canola crops. *Animal Production Science* 56, 1350–1360.

Edmeades, D.C. and O'Connor, M.B. (2003) Sodium requirements for temperate pastures in New Zealand. *New Zealand Journal of Agricultural Research* 46, 37–47.

Edwards, H.M. (1984) Studies on the aetiology of tibial dyschondroplasia in chickens. *Journal of Nutrition* 114, 1001–1013.

Elbrond, V.S., Danzer, V., Mayhew, T.M. and Skadhouge, E. (1991) Avian lower intestine adapts to dietary salt (NaCl) depletion by increasing transepithelial sodium transport and microvillus membrane surface area. *Experimental Physiology* 76, 733–744.

Fettman, M.J., Chase, L.E., Bentinck-Smith, C., Coppock, E. and Zinn, S.A. (1984) Nutritional chloride deficiency in early lactation Holstein cows. *Journal of Dairy Science* 67, 2321–2335.

Frame, D.D., Hooge, D.M. and Cutler, R. (2001) Interactive effects of dietary sodium and chloride on the incidence of spontaneous cardiomyopathy (round heart) in turkeys. *Poultry Science* 80, 1572–1577.

Gill, M., France, J., Summers, M., McBride, B.W. and Milligan, L.P. (1989) Simulation of the energy costs associated with protein turnover and Na^+K^+-transport in growing lambs. *Journal of Nutrition* 119, 1287–1299.

Goatcher, W.D. and Church, D.C. (1970) Taste responses in ruminants. III. Reactions of pigmy goats, normal goats, sheep and cattle to sucrose and sodium chloride. *Journal of Animal Science* 31, 364–372.

Goff, J.P., Brummer, E.C., Henning, S.J., Doorenbos, R.K. and Horst, R.L. (2007) Effect of application of ammonium chloride and calcium chloride on alfalfa cation–anion content and yield. *Journal of Dairy Science* 90, 5159–5164.

Gonzalez-Rivas, P.A., Chauhan, S.S., Ha, M., Fegan, N., Dunshea, F.R. and Warner, R.D. (2020) Effects of heat stress on animal physiology, metabolism, and meat quality: a review. *Meat Science* 162, 108025.

Gorham, J. (1995) Sodium content of agricultural crops. In: Phillips, C.J.C. and Chiy, P.C. (eds) *Sodium in Agriculture*. Chalcombe Publications, Canterbury, UK, pp. 17–32.

Grace, N.D., Pearce, S.G., Firth, E.C. and Fennessy, P.F. (1999) Content and distribution of macro- and trace-elements in the body of young pasture-fed horses. *Australian Veterinary Journal* 77, 172–176.

Greene, L.W., Fontenot, J.P. and Webb, K.E. Jr (1983a) Site of magnesium and other macro-mineral absorption in steers fed high levels of potassium. *Journal of Animal Science* 57, 503–513.

Greene, L.W., Webb, K.E. and Fontenot, J.P. (1983b) Effect of potassium level on site of absorption of magnesium and other macroelements in sheep. *Journal of Animal Science* 56, 1214–1221.

Grovum, W.L. and Chapman, H.W. (1988) Factors affecting the voluntary intake of food by sheep. 4. The effect of additives representing the primary tastes on sham intakes by oesophageally fistulated sheep. *British Journal of Nutrition* 59, 63–72.

Hagsten, I. and Perry, T.W. (1976) Evaluation of dietary salt levels for swine. 1. Effect on gain, water consumption and efficiency of feed conversion. *Journal of Animal Science* 42, 1187–1190.

Hagsten, I., Perry, T.W. and Outhouse, J.B. (1975) Salt requirements of lambs. *Journal of Animal Science* 42, 1187–1190.

Harper, M.-E., Willis, J.S. and Patrick, J. (1997) Sodium and chloride in nutrition. In: O'Dell, B.L. and Sunde, R.A. (eds) *Handbook of Nutritionally Essential Mineral Elements*. Marcel Dekker Inc., New York, pp. 93–116.

Harris, J., Caple, I.W. and Moate, P.J. (1986) Relationships between mineral homeostasis and fertility of dairy cows grazing improved pastures. In: *Proceedings of the Sixth International Conference on Production Disease in Farm Animals*, *Belfast*. Veterinary Research Laboratory, Stormont, UK, pp. 315–318.

Heller, V.G. (1932) Saline and alkaline drinking waters. *Journal of Nutrition* 5, 421–429.

Hemsley, J.A., Hogan, J.P. and Weston, R.H. (1975) Effect of high intake of sodium chloride on the utilisation of a protein concentrate by sheep. *Australian Journal of Agricultural Research* 26, 715–727.

Henry, P.R. (1995) Sodium and chlorine bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 337–348.

Hogan, J.P., Petherick, J.C. and Phillips, J.C. (2007) The physiological and metabolic impacts on sheep and cattle of feed and water deprivation before and during transport. *Nutrition Research Reviews* 20, 17–28.

Honeyfield, D.C., Frosseth, J.A. and Barke, R.J. (1985) Dietary sodium and chloride levels for growing-finishing pigs. *Journal of Animal Science* 60, 691–698.

Howard, D.A., Burdin, M.L. and Lampkin G.H. (1962) Variation in the mineral and crude-protein content of pastures at Muguga in the Kenya Highlands. *Journal of Agricultural Science, Cambridge* 59, 251–256.

Humphery, T.J., Coghlan, J.P., Denton, D.A., Fan, J.S., Scoggins, B.B., Stewart, K.W. and Whitworth, J.A. (1984)

Effect of potassium, angiotensin II on blood aldosterone and cortisol in sheep on different dietary potassium and sodium intakes. *Clinical and Experimental Pharmacology and Physiology* 11, 97–100.

Ji, S.-K., Gui-shan Xu, G.-S., Diao, Q.-Y., Deng, K.-D., Jiang, C.-J., Tu, Y. and Nai-feng Zhang, N.-F. (2015) Macromineral requirements of Dorper×thin-tailed Han crossbred female lambs. *Journal of Integrative Agriculture* 14, 1617–1626.

Jiang, S., Azzam, M., Yu, H., Fan, Q., Li, L., Gou, Z. and Jiang, Z. (2019) Sodium and chloride requirements of yellow-feathered chickens between 22 and 42 days of age. *Animal* 13, 2183–2189.

Jones, D.I.H., Miles, D.G. and Sinclair, K.B. (1967) Some effects of feeding sheep on low-sodium hay with or without a sodium supplement. *British Journal of Nutrition* 21, 391–397.

Joyce, J.P. and Brunswick, I.C.F. (1975) Sodium supplementation of sheep and cattle. *New Zealand Journal of Experimental Agriculture* 3, 299–304.

Kemp, A. (1971) *The Effects of K and N Dressings on the Mineral Supply of Grazing Animals*. Proceedings of the 1st Colloquium of the Potassium Institute. IBS, Wageningen, The Netherlands, pp. 1–14.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue S.L., Bearson, B.L., Shurson, G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Khan, M.A., Ansari, R., Ali, H., Gul, B. and Nielsen B.L. (2009) Panicum turgidum, a potentially sustainable cattle feed alternative to maize for saline areas. *Agriculture, Ecosystems & Environment* 129, 542–546.

Khorasani, G.R., Janzen, R.A., McGill, W.B. and Kenelly, J.J. (1997) Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage or alfalfa silage. *Journal of Animal Science* 75, 239–248.

Kirk, D.J., Fontenot, J.P. and Rahnema, S. (1994) Effects of lasalocid and monensin on digestive tract flow and partial absorption on minerals in sheep. *Journal of Animal Science* 72, 1029–1037.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–310.

Larsson, J., Pilborg, P.H., Johansen, M., Christophersen, M.T., Holte, A. *et al*. (2013) Physiological parameters of endurance horses pre- compared to post-race, correlated with performance: a two race study from scandinavia. *ISRN Veterinary Science*, 684353. DOI: 10.1155/2013/684353

Leach, R.M. and Nesheim, M.C. (1963) Studies on chloride deficiency in chicks. *Journal of Nutrition* 81, 193–199.

Leaver. J.D. and Hill, J. (1995) The performance of dairy cows offered ensiled whole-crop wheat, urea-treated whole-crop wheat or sodium hydroxide-treated wheat grain and wheat straw in a mixture with silage. *Animal Science* 61, 481–489.

Ledgard, S.F. and Upsdell, M.P. (1991) Sulfur inputs from rainfall throughout New Zealand. *New Zealand Journal of Agricultural Research* 34, 105–111.

Lehr, J.J., Grashuis, J. and Van Koetsveld, E.E. (1963) Effect of fertilization on mineral-element balance in grassland. *Netherlands Journal of Agricultural Science* 11, 23–37.

Lengemann, F.W., Aines, P.D. and Smith, S.E. (1952) The normal chloride concentration of blood plasma, milk and urine of dairy cattle. *Cornell Veterinarian* 42, 28–35.

Little, D.A. (1987) The influence of sodium supplementation on the voluntary intake and digestibility of low-sodium *Setaria sphacelatae* cv. Nandi by cattle. *Journal of Agricultural Science, Cambridge* 108, 231–236.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Mahan, D.C., Newton, E.A. and Cera, K.R. (1996) Effect of supplemental sodium chloride, sodium phosphate or hydrochloric acid in starter pig diets containing dried whey. *Journal of Animal Science* 74, 1217–1222.

Mahan, D.C., Wiseman, T.D., Weaver, E. and Russell, L. (1999) Effect of supplemental sodium chloride and hydrochloric acid added to initial starter diets containing spray-dried blood plasma and lactose on resulting performance and nitrogen digestibility of 3-week-old weaned pigs. *Journal of Animal Science* 77, 3016–3021.

Malacarne, M., Criscione, A., Franceschi, P., Bordonaro, S., Formaggioni, P. *et al*. (2019) New insights into chemical and mineral composition of donkey milk throughout nine months of lactation. *Animal* 9, 1161– 1174.

Manuelian, C.L., Penasa, M., Visentin, G., Zidi, A., Cassandro, M. *et al*. (2018) Mineral composition of cow milk from multibreed herds. *Animal Science Journal* 81, 1622–1627.

Marai, I.F.M., Habeeb, A.A. and Kamal, T.H. (1995) Response of livestock to excess sodium intake. In: Phillips, C.J.C. and Chiy, P.C. (eds) *Sodium in Agriculture*. Chalcombe Publications, Canterbury, UK, pp. 173–180.

Masters, D.G. (2018) Practical implications of mineral and vitamin imbalance in grazing Sheep. *Animal Production Science* 58(8), 1438–1450.

Masters, D.G. and Norman, H.C. (2016) Genetic and environmental management of halophytes for improved livestock production. In: Khan, M.A., Ozturk, M., Gul, B. and Ahmed, M. (eds) *Halophytes*

for Food Security in Dry Lands. Academic Press, New York, pp. 243–257.

Masters, D.G., Rintoul, A.J., Dynes, R.A. and Norman, H.C. (2005) Feed intake and production in sheep fed diets high in sodium and potassium. *Australian Journal of Agricultural Research* 56, 427–434.

Masters, D.G., Benes, S.E. and Norman, C. (2006) Biosaline agriculture. *Agriculture, Ecosystems and Environment* 119, 234–248.

Masters D.G., Hancock, S., Refshauge, G., Robertson, S., Bhanugopan, M., Friend, M. and Thompson, A.N. (2017) Mineral status of reproducing ewes grazing vegetative cereal crops. *Animal Production Science* 58, 2049–2060.

Masters, D.G., Norman, H.C. and Thomas, D.T. (2019) Minerals in pastures: Are we meeting the needs of livestock? *Crop and Pasture Science*. DOI: 10.1071/CP18546

Mayberry, D., Masters, D. and Vercoe, P. (2010) Mineral metabolism of sheep fed saltbush or a formulated high-salt diet. *Small Ruminant Research* 91, 81–86.

McClymont, G.L., Wynne, K.N., Briggs, P.K. and Franklin, M.C. (1957) Sodium chloride supplementation of high-grain diets for fattening Merino sheep. *Australian Journal of Agricultural Research* 8, 83–90.

McDowell, L.R. (2003) *Minerals in Animal and Human Nutrition*. Elsevier, Amsterdam, p. 688.

McDowell, L.R., Conrad, J.H. and Hembry, F.G. (1993) *Minerals for Grazing Ruminants in Tropical Regions*, 2nd edn. Animal Science Department, University of Florida, Gainsville, Florida.

McGrath, S.R., Lievaart, J.J., Virgona, A.J., Bhanugopan, M.S. and Friend, M.A. (2013) Factors involved in high ewe losses in winter lambing flocks grazing dual-purpose wheat in southern New South Wales: a producer survey. *Animal Production Science* 53, 458–463.

McNaught, K.J. (1959) Effect of potassium fertiliser on sodium, calcium and magnesium in plant tissues. *New Zealand Journal of Agriculture* 99, 442–448.

McSweeney, C.S., Cross, R.B., Wholohan, B.T. and Murphy, M.R. (1988) Diagnosis of sodium status in small ruminants. *Australian Journal of Agricultural Research* 39, 935–942.

Meyer, J.H., Grummer, R.H., Phillips, R.H. and Bohstedt. G. (1950) Sodium, chlorine, and potassium requirements of growing pigs. *Journal of Animal Science* 9, 300–306.

Michell, A.R. (1989) Practice tip: oral and parenteral rehydration therapy. *In Practice*, May, 96–99.

Michell, A.R. (1995) Physiological roles for sodium in mammals. In: Phillips, C.J.C. and Chiy, P.C. (eds) *Sodium in Agriculture*. Chalcombe Publications, Canterbury, UK, pp. 91–106.

Michell, A.R., Moss, P., Hill, R., Vincent, I.C. and Noakes, D.E. (1988) The effect of pregnancy and sodium intake on water and electrolyte balance in sheep. *British Veterinary Journal* 144, 147–157.

Milligan, L.P. and Summers, M. (1986) The biological basis of maintenance and its relevance to assessing responses to nutrients. *Proceedings of the Nutrition Society* 45, 185–193.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Mongin, P. (1981) Recent advances in dietary anion–cation balance applications in poultry. *Proceedings of the Nutrition Society* 40, 285–294.

Morris, J.G. (1980) Assessment of sodium requirements of grazing cattle: a review. *Journal of Animal Science* 50, 145–151.

Morris, J.G. and Gartner, R.J.W. (1971) The sodium requirements of growing steers given an all-sorghum grain ration. *British Journal of Nutrition* 25, 191–205.

Morris, J.G. and Murphy, G.W. (1972) The sodium requirements of beef calves for growth. *Journal of Agricultural Science, Cambridge* 78, 105–108.

Morris. J.G. and Peterson, R.G. (1975) Sodium requirements of lactating ewes. *Journal of Nutrition* 105, 595–598.

Morris, J.G., Delmas, R.E. and Hull, J.L. (1980) Salt supplementation of range beef cows in California. *Journal of Animal Science* 51, 71–73.

Moseley, G. (1980) Effects of variation in herbage sodium levels and salt supplementation on the nutritive value of perennial ryegrass for sheep. *Grass and Forage Science* 35, 105–113.

Moss, A.F., Chrystal, P.V., Dersjant-Li, Y., Selle, P.H. and Liu, S.Y. (2018) Responses in digestibilities of macrominerals, trace minerals and amino acids generated by exogenous phytase and xylanase in canola meal diets offered to broiler chickens. *Animal Feed Science and Technology* 240, 22–30.

Murphy, G.M. and Connell, J.A. (1970) A simple method of collecting saliva to determine the sodium status of cattle and sheep. *Australian Veterinary Journal* 46, 595–598.

Murphy, G.M. and Plasto, A.W. (1972) Sodium deficiency in a beef cattle herd. *Australian Veterinary Journal* 48, 129.

Mushtaq, M.M.H., Pasha, T.N., Mushtaq, T. and Parvin, R. (2013) Electrolytes, dietary electrolyte balance and salts in broilers: an updated review on growth performance, water intake and litter quality. *World's Poultry Science Journal* 69, 789–802.

Nawab, A., Ibtisham, F., Li, G., Kieser, B., Wu, J., Liu, W. et al. (2018) Heat stress in poultry production: mitigation strategies to overcome the future challenges facing the global poultry industry. *Journal of Thermal Biology* 78, 131–139.

Neathery, M.W., Blackmon, D.M., Miller, W.J., Heinmiller, S., McGuire, S., Tarabula, J.M., Gentry, R.F. and Allen, J.C. (1981) Chloride deficiency in Holstein calves from a low chloride diet and removal of abomasal contents. *Journal of Dairy Science* 64, 2220–2233.

Nesheim, L., Synnes, O.M., Steen, A., Langerud, A. and Bakken, A.K. (2020) Controlling dietary cation-anion differences in forages by chloride fertilisation. *Agricultural and Food Science* 29, *18–28.*

Norman, H.C., Masters, D.G. and Barrett-Lennard, E.G. (2013) Halophytes as forages in saline landscapes: interactions between plant genotype and their environment change their feeding value to ruminants. *Environmental and Experimental Botany* 92, 96–109.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (1998) *Nutrient Requirements of Swine*, 10th revised edn. NRC Press, Washington, DC.

NRC (2001) *Nutrient Requirements of Dairy Cows*, 5th edn. National Academy of Sciences, Washington, DC.

NRC (2006) *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids.* National Academy of Sciences, Washington, DC.

NRC (2012) *Nutrient Requirements of Swine*, 11th edn. National Academy of Sciences, Washington, DC.

NRC (2016) *Nutrient Requirements of Beef Cattle*, 8th edn. National Academy of Sciences, Washington, DC.

Ortolani, O (2000) Effects of *haemonchus contortus* infection on sodium status of sheep. *Ciência Rural, Santa Maria* 30, 521–523.

Parker, A.J., Hamlin, G.P., Coleman, C.J. and Fitzpatrick, L.A. (2004) Excess cortisol interferes with a principal mechanism of resistance to dehydration in *Bos indicus* steers. *Journal of Animal Science* 82, 1037–1045.

Pasternak, D., Danon, A., Arouson, J.A. and Benjamin, R.W. (1985) Developing the seawater agriculture concept. *Plant and Soil* 89, 337–348.

Peirce, A.W. (1957) Saline content of drinking water for livestock. *Veterinary Reviews and Annotations* 3, 37–43.

Peirce, A.W. (1965) Studies on salt tolerance of sheep. 5. The tolerance of sheep for mixtures of sodium chloride, sodium carbonate and sodium bicarbonate in the drinking water. *Australian Journal of Agricultural Research* 49, 815–823.

Rao, N.K. and Shahid, M. (2006) Greening the desert: quest for new crops *in Biosalinity News, ICBA*, *United Arab Emirates* 7 (December), 3–7.

Ross, E. (1979) The effect of water sodium on the chick requirement for dietary sodium. *Poultry Science* 58, 626–630.

Ross, E. and Herrick, R.B. (1981) Forced rest induced by moult or low-salt diet and subsequent hen performance. *Poultry Science* 60, 63–67.

Rouffet, J.D., Clement, T.J. and Aranas, T.J. (1983) Changes of aldosterone in blood serum of dairy cattle during estrous cycle. *Journal of Dairy Science* 66, 1734–1737.

Rouffet, J.D., Dalle, M., Tournaire, C., Barlet, J.-P. and Delost, P. (1990) Sodium intake by pregnant cows and plasma aldosterone and cortisol concentrations in the foetus during late pregnancy. *Journal of Dairy Science* 73, 1762–1765.

Safwate, A. (1985) Urinary sodium excretion and the renin–aldosterone system in the new-born calf. *Journal of Physiology* 362, 261–271.

Sauvant, D., Perez, J.M. and Tran, G. (2004) *Tables INRA-AFZ de composition et de valeur nutritive des matières premières destinées aux animaux élevage*, 2nd edn. INRA Editions, Versailles, France.

Sauveur, B. and Mongin, P. (1978) Interrelationships between dietary concentrations of sodium, potassium and chloride in laying hens. *British Poultry Science* 19, 475–485.

Schellner, G. (1972/1973) Die Wirkung von Natriummangel und Natriumbeifutterung auf Wachstum, Milch- und Milchfettleistung und Fruchtbarkeit bei Ziegen. *Jahrbuch fur Tierernahrung und Futterung* 8, 246–259.

Scott, D. (1974) Changes in water, mineral and acid–base balance associated with feeding and diet. In: McDonald, I.W. and Warner, A.C. (eds) *Digestion and Metabolism in the Ruminant.* Proceedings of the 6th International Symposium on Ruminant Physiology. University of New England Publishing Unit, Armidale, Australia, pp. 205–215.

Serhan, M.F. and Kreydiyyeh, S.I. (2010) Insulin down-regulates the Na+/K+ ATPase in enterocytes but increases intestinal glucose absorption. *General and Comparative Endocrinology* 167, 228–233.

Seynaeve, M., de Widle, R., Janssens, G. and de Smet, B. (1996) The influence of dietary salt level on water consumption, farrowing and reproductive performance of lactating sows. *Journal of Animal Science* 74, 1047–1055.

Shade, R.E., Blair-West, J.R., Carey, K.D., Madden, L.J., Weisunger, R.S. and Denton, D.A. (2002) Synergy between angiotensin and aldosterone in evoking sodium appetite in baboons. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology* 283, R1070–R1078.

Shawk, D.J., Goodband, R.D., Tokach, M.D., Dritz, G.S., DeRouchey, J.M. *et al.* (2018) Effects of added dietary salt on pig growth performance. *Translational Animal Science* 2, 396–406.

Sherwood, D.H. and Marion, J.E. (1975) Salt levels in feed and water for laying chickens. *Poultry Science* 54, 1816 (abstract).

Shirley, R.B., Davis, A.J., Compton, M.M. and Berry, W.D. (2003) The expression of calbindin in chicks that are divergently selected for low or high incidence of tibial dyschondroplasia. *Poultry Science* 82, 1965–1973.

Shirley, R.L. (1978) Water as a source of minerals. In: Conrad, J.H. and McDowell, L.R. (eds) *Latin American Symposium on Mineral Nutrition Research with Grazing*

Ruminants. Animal Science Department, University of Florida, Gainsville, Florida, pp. 40–47.

Silanikove, N., Malz, E., Halevi, A. and Shinder, D. (1997) Metabolism of water, sodium, potassium and chloride by high yielding dairy cows at the onset of lactation. *Journal of Dairy Science* 80, 949–956.

Singh, S.P. and Rani, D. (1999) Assessment of sodium status in large ruminants by measuring the sodium-to-potassium ratio in muzzle secretions. *American Journal of Veterinary Research* 60, 1074–1081.

Široká, Z., Labaj, V., Pijácek, M. and Zdeňka· Z. (2017) Accidental sodium chloride poisoning in sheep – a case study. *Acta Veterimaria. Brno* 86, 213–218.

Smith, S.E., Lengemann, F.W. and Reid, J.T. (1953) Block versus loose salt consumption by dairy cattle. *Journal of Dairy Science* 36, 762–765.

Snow, D.H., Kerr, M.G., Nimmo, M.A. and Abbott, E.M. (1982) Alterations in blood, sweat, urine and muscle composition during prolonged exercise in the horse. *Veterinary Record* 110, 377–384.

Spears, J.W. and Harvey, R.W. (1987) Lasalocid and dietary sodium and potassium effects on rumen mineral metabolism, ruminal volatile fatty acids and performance of finishing steers. *Journal of Animal Science* 65, 830–840.

Summers, J.D., Moran, E.T. and Pepper, W.F. (1967) A chloride deficiency in a practical diet encountered as a result of using common sulphate antibiotic potentiating procedure. *Poultry Science* 46, 1557–1560.

Suttle, N.F. and Brebner, J. (1995) A putative role for larval nematode infection in diarrhoeas which did not respond to anthelmintic drenches. *Veterinary Record* 137, 311–316.

Suttle, N.F. and Field, A.C. (1967) Studies on magnesium in ruminant nutrition. 8. Effect of increased intakes of potassium and water on the metabolism of magnesium, phosphorus, sodium, potassium and calcium in sheep. *British Journal of Nutrition* 21, 819–826.

Suttle, N.F., Brebner, J., McClean, K. and Hoeggel, U. (1996) Failure of mineral supplementation to avert apparent sodium deficiency in lambs with abomasal parasitism. *Animal Science* 63, 103–109.

Teeter, R.G. and Smith, M.O. (1986) High chronic ambient temperature stress effects on acid–base imbalance and their response to supplemental ammonium chloride, potassium chloride and potassium carbonate. *Poultry Science* 65, 1777–1781.

Teixeira, I.A.M.A., Hunter, C.I., Pereira Filho, J.M., Sobrinho, A.G. and Resende, K.T. (2015) Macromineral requirements for the maintenance and growth of F1 Boer × Saanen kids. *Journal of Animal Science* 93, 2349–2356.

Thomas, B., Thompson, A., Oyenuga, V.A. and Armstrong, R.H. (1952) The ash constituents of some herbage plants at different stages of maturity. *Empire Journal of Experimental Agriculture* 20, 10–22.

Thomas, D.T., Rintoul, A.J. and Masters, D.G. (2007) Sheep select combinations of high and low sodium chloride, energy and crude protein feed that improve their diet. *Applied Animal Behaviour Science* 105, 140–153.

van der Wal, P.G., Hemminga, H., Goedegebuure, S.A. and van der Valk, P.C. (1986) The effect of replacement of 0.30% sodium chloride by 0.43% sodium bicarbonate in rations of fattening pigs on leg weakness, osteochondrosis and growth. *Veterinary Quarterly* 8, 136–144.

Ventura, Y., Eshel, A., Pasternak, D. and Sagi, D. (2015) The development of halophyte- based agriculture: past and present. *Annals of Botany* 115, 529–540.

Walker, C.A. (1957) Studies of the cattle of Northern Rhodesia. 1. The growth of steers under normal veld grazing and supplemented with salt and protein. *Journal of Agricultural Science, Cambridge* 49, 394–400.

Wamberg, S., Engel, D. and Stigsen, P. (1985) Acid–base balance in ruminating calves given sodium-hydroxide treated straw. *British Journal of Nutrition* 54, 655–657.

West, J.W., Mullinix, B.G. and Sandifer, T.G. (1991) Changing dietary electrolyte balance for dairy cows in cool and hot environments. *Journal of Dairy Science* 74, 1662–1674.

Whitlock, R.H., Kessler, M.J. and Tasker, J.B. (1975) Salt (sodium) deficiency in dairy cattle: polyuria and polydypsia as prominent clinical features. *Cornell Veterinarian* 65, 512–526.

Wilkes, J., Cowley, F., Hegarty, R. and Jewell, M. (2019) *Survey of Australian Feedlot Drinking Water Quality.* Project B.FLT.1005, Meat and Livestock Australia Ltd, North Sydney, Australia.

Wilson, A.D. (1966) The tolerance of sheep to sodium chloride in food or drinking water. *Australian Journal of Agricultural Research* 17, 503–514.

Woyengo, T., Cowieson, A., Adeola, O. and Nyachoti, C. (2009) Ileal digestibility and endogenous flow of minerals and amino acids: responses to dietary phytic acid in piglets. *British Journal of Nutrition* 102, 428–433.

Woyengo, T.A., Weihrauch, D. and Nyachoti, C.M. (2012) Effect of dietary phytic acid on performance and nutrient uptake in the small intestine of piglets. *Journal of Animal Science* 90, 543–549.

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle, T.E., Silva, B.C. et al. (2017) Estimating mineral requirements of Nellore beef bulls fed with or without inorganic mineral supplementation and the influence on mineral balance. *Journal of Animal Science* 95, 1696–1706.

Zeyner, A., Romanowski, K., Vernunft, A., Harris, P., Müller, A.M., Wolf, C. and Kienzle, E. (2017) Effects of different oral doses of sodium chloride on the basal acid-base and mineral status of exercising horses fed low amounts of hay. *PlOS ONE* 12, e0168325. doi. org/10.1371/journal.pone.0168325

Zoidis, E. and Hadjigeorgiou, I. (2017) Effects of drinking saline water on food and water intake, blood and urine electrolytes and biochemical and haematological parameters in goats: a preliminary study. *Animal Production Science* 58 1822–1828.

9 Sulfur

Introduction

The high reactivity of sulfur (S) fuelled evolution in primeval ecosystems until it was replaced by oxygen. Recognition that sulfur was an important nutrient for higher animals stemmed from the discovery in the 1930s that the essential amino acid, methionine, contained one S atom per mol (McCollum, 1957). Subsequent investigations showed that sulfur was an integral part of most tissue proteins, which contained 0.5–2.0% S. Other sulfur amino acids (SAA) such as cystine, cysteine, cystathionine and taurine contribute to protein S, but each can be derived from methionine and is not an essential dietary constituent. Strictly speaking, sulfur is only an essential nutrient for plants and microbes because only they can synthesize SAA and proteins from inorganic S sources. Mammals digest plant proteins and incorporate SAA into tissue proteins. Non-ruminants require particular balances amongst dietary amino acids, and methionine is often the second most-limiting amino acid behind lysine. Manipulation of dietary S has little effect on SAA supply in non-ruminants – marginally sparing cystine (Lovett *et al.*, 1986). Microbial synthesis of SAA in the large intestine – if altered – cannot yield absorbable SAA: those interested in this aspect of monogastric nutrition should consult texts on amino acid nutrition (e.g. D'Mello, 2003) and availability (Stein *et al.*, 2007). By contrast, ruminants possess a substantial microbial population in the fore-stomach or rumen, which incorporates sulphur from degradable inorganic sources into microbial proteins (Kandylis, 1984a). Following digestion in the small intestine, microbial protein provides SAA in a balanced amino acid supply for all purposes, except growth of wool, hair and mohair. Ruminant needs for SAA can be met at far less cost, financially and environmentally, by providing inorganic S and nitrogen (N) or S-rich by-products, rather than dietary protein. The reactivity that gave us life as we know it is only now being revealed (Fig. 9.1).

Functions

The functions of sulfur were thought to stem from the diverse proteins of which it was a part. Functions were often dependent on the presence of highly reactive sulfydryl (SH) groups or disulfide bonds that maintained the spatial configuration of elaborate polypeptide chains, providing sites of attachment for prosthetic groups and substrates. Thus, sulfur was essential to the activity of many enzymes, of hormones such as insulin and oxytocin, and two vitamins, thiamine and biotin. Interconversion between the SAA, homocysteine and methionine facilitates methylation, the core process in building carbon chains. Cysteine-rich molecules such as metallothionein protect animals from excesses of copper, cadmium and zinc, while selenoproteins influence selenium transport and protect tissues from Se toxicity. Glutathione protects tissues from oxidants by interconverting between the reduced (GSH) and oxidized (GSSG) state and may thus protect erythrocytes from lead (Pb) toxicity. Glutathione may also facilitate uptake of copper by the liver. Sulfur is present in the natural anti-coagulant heparin, in connective tissue as chondroitin sulfate, and particularly abundant in keratin-rich appendages (hoof, horn, hair, feathers, wool fibre and mohair). The primary rate-limiting function of sulfur in ruminants was unrelated to the foregoing molecules but rooted in the anaerobic environment of the rumen, where primitive life forms broke down cellulose to useful energy sources and synthesized protein (Durand and Komisarczuk, 1988). The most important function of sulfur in ruminants is to facilitate microbial protein synthesis (MPS) and growth of rumen microbes (Fig. 9.2). Microbial species metabolize sulfur by different

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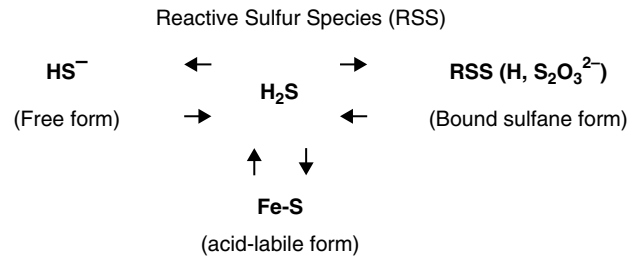

Fig. 9.1. Sulfur is highly reactive in cells by virtue of an ability, as hydrogen sulfide (H_2S), to form reactive sulfur species (RSS): by interacting with reactive oxygen species and free Fe ions, sulfur can influence the vital process of cell signalling and thus cell life and death.

pathways: a few (approx. 1%) are 'dissimilatory', degrading inorganic and organic sources to H_2S as an end-product, while the vast majority are 'assimilatory' species, using sulfite oxidase to incorporate S from H_2S into SAA (Kandylis, 1984a; Drenowski *et al*., 2014b). Rumen S metabolism is a small component of arguably the most genetically and structurally (Leng, 2014) complex metabolome known to humankind, and attempts to either simulate the rumen environment *in vitro* or draw representative samples from it are fraught with difficulty.

The life-sustaining properties of sulfur are now being linked to the ability of hydrogen sulfide (H_2S) to interact with reactive species (RSS) (Kolluru *et al*., 2020; Fig. 9.1). Though unstudied in livestock, effects of RSS on membrane permeability and inflammation are feasible.

Clinical and Subclinical Insufficiency

Clinical signs of sulfur deprivation in ruminants are not specific, being shared by any nutritional deficiency or factor that depresses rumen MPS. On fibrous diets, low in S, there is often an early depression of appetite and digestibility due to a failure to digest cellulose in sheep (Kennedy and Siebert, 1972a, b; Hegarty *et al*., 1994) and cattle (Kennedy, 1974). Sheep deprived of sulfur spend more time ruminating and have increased rumen liquor volumes (Weston *et al*., 1988). Eventually, growth is retarded, wool or hair is shed, salivation and lacrimation become profuse, the eyes cloudy and the emaciated animal dies (Thomas *et al*., 1951; Kincaid, 1988).

- Long before this stage and far more frequently, ruminants will fail to thrive.

Physiochemical Insufficiency

Rumen fluid changes in both biochemical (Whanger, 1972) and microbial (Weston *et al*., 1988; Morrison *et al*., 1990) composition on diets low in S are associated with a striking fall in rumen sulfide (S^{2-}) concentrations. Sulfur deprivation causes hypoalbuminaemia in the host (e.g. Ortolani, 2001) but blood urea will be high if intake of degradable dietary N is high. Plasma SO_4 generally reflects S intake if sulfur alone is limiting MPS but if another factor, such as N, is limiting, serum SO_4 values at a given S intake are increased (Kennedy and Siebert, 1972b). Plasma SO_4 was low (around 10 mg S l^{-1}) in sheep on poor, dry-season pasture, but values rose to 20–40 mg l^{-1} when rain fell and pasture greened (Kennedy and Siebert, 1972a; White *et al*., 1997). Plasma and liver methionine fell when calves were given a diet sufficiently low in S (0.4g kg^{-1}DM) to retard growth (Slyter *et al*., 1988). If dietary S alone is limiting MPS, additional S should lower blood urea and urinary N excretion. Diets low in S may enhance tissue accumulation of Cd by reducing rumen S^{2-} and limiting formation of insoluble and unabsorbable CdS (Smith and White, 1997).

Diagnosis of Sulfur Insufficiency

Sulfur deprivation in ruminants relates to health of the rumen microbiome and analysis of rumen fluid samples, obtained by stomach tube, can aid diagnosis. Rumen S_2^- can indicate whether sufficient sulfide is available for MPS but the critical level increased 1 mg S^{2-} l^{-1} (Bray and Till, 1975; Hegarty *et al*., 1991) to 1.6–3.8 mg S^{2-} l^{-1} (Kandylis and Bray, 1987; Weston *et al*., 1988) and may depend on relative contributions from assimilatory and dissimilatory sulfur pathways. Inorganic and organic sources are dissimilated to sulfide, which is partitioned bisulfide (HS^-) in liquor

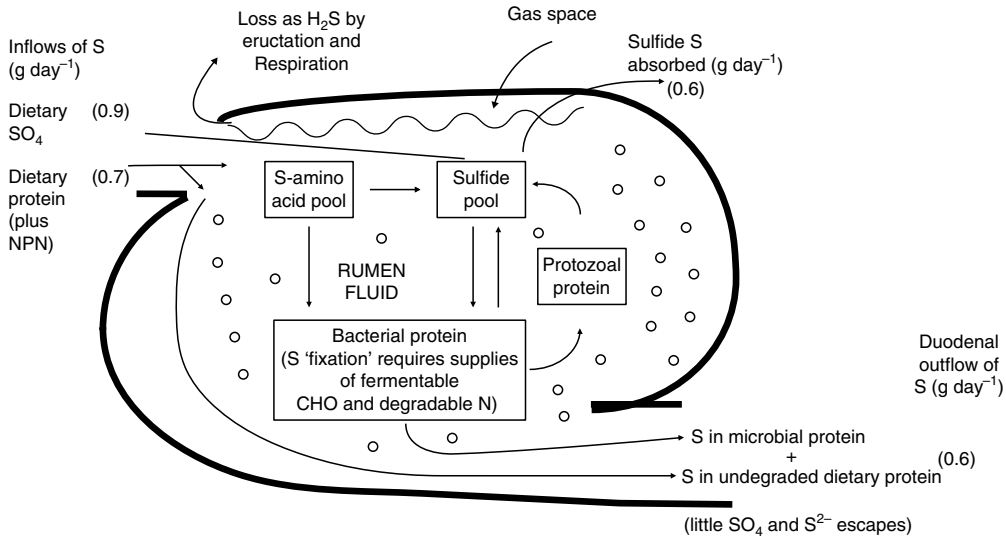

Fig. 9.2. Primary flows of sulfur in the liquid compartment of the rumen: metabolizable energy and nitrogen supplies, as well as sulfur and phosphorus, determine the most important product, microbial protein, entering the duodenum (amounts of S, g day^{-1} are from the 'low' S treatment of Kandylis and Bray, 1987): the rumen also has a gas compartment into which sulfide diffuses as H_2S: approximately 50% leaves as sulfide by absorption, eructation and exhalation, when SO_4 is the major dietary source.

and H_2S in the rumen gas cap: low pH favours H_2S production (Bradley *et al.*, 2011). Sulfide in liquor is easily determined by acid displacement of H_2S and titration (Kennedy and Siebert, 1972a)[i] and was taken to represent the entire sulfide pool (Gawthorne and Nader, 1976; Kandylis and Bray, 1987). Sulfide that escapes from the rumen as H_2S from the rumen has no nutritional value but is potentially toxic (p. 220). Low serum SO_4-S (<10 mg l^{-1}), low blood GSH and liver GSH also indicate sulfur status (Mata *et al.*, 1997; White *et al.*, 1997). Blood GSH and plasma SO_4 showed similar marked decreases during the dry season to subnormal values (Fig. 9.3): dietary SO_4 supplements did not improve values (White *et al.*, 1997) but 'protected methionine' did, suggesting that GSH synthesis was restricted by lack of protein rather than S (Mata *et al.*, 1997). Blood GSH could complement serum SO_4 as a diagnostic aid when dietary N is also lacking. The surest diagnosis is provided by responses in appetite or growth (of wool or body) to continuous sulfur supplementation, provided that no other factors are limiting MPS.

Occurrence of Sulfur Deprivation

Any area away from the coast and industrial activity, where annual rainfall is moderate (>500 mm) and soils are derived from weathered volcanic material, is likely to have leached soils, low in S and swards that respond to applications of S in fertilizers. Sulfur deficiency is rare in arid regions (<250 mm rainfall per annum) because of upward movement of SO_4 through the soil profile. Areas of deficiency were delineated in Australia, New Zealand (Edmeades *et al.*, 2005), many regions across the USA (Beaton *et al.*, 1971; Underwood and Suttle, 1999), China (Qi *et al.*, 1994) and Brazil (Ortolani, 2001). Regular S inputs were needed to maintain productivity of clover-based pastures in Australasia (Goh and Nguyen, 1997) and maize in Nigeria (Ojeniyi and Kayode, 1993). Risk of insufficiency in plants and livestock has gradually increased as atmospheric inputs declined, under stricter regulations to decrease industrial emissions (p. 22). Ruminants subsisting on halophytes digest their fibrous diet inefficiently (p. 198) and this may be partly due to a failure of MPS and imbalance between supplies of fermentable energy, S and N.

Plant factors

Sulfur should not be lacking in green swards, particularly those containing legumes, because rumen microbes need less dietary S than plants for protein

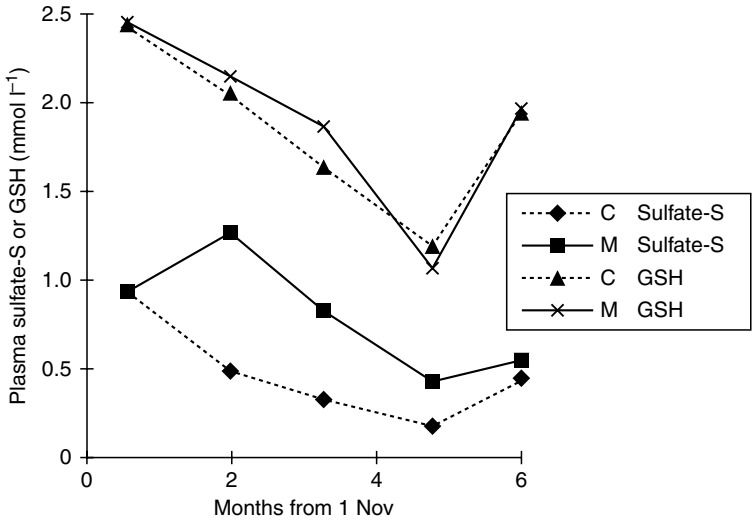

Fig. 9.3. Seasonal fluctuations in two biochemical indices of sulfur status, plasma sulfate (SO_4) and blood glutathione (GSH), in two groups of sheep grazing in Western Australia. One group (■,×) received a sulfur-rich mineral (M), while the other (◆,▲) did not (C). (Data from White *et al.*, 1997)

synthesis. However, livestock fed predominantly on brown swards, low-quality roughage or corn silage might need supplementation. Some species of tropical grasses (e.g. spear grass (*Heteropogon contortus*) (approximately 0.5g kg^{-1}DM) (Kennedy and Siebert, 1972a, b; Morrison *et al.*, 1990), Guinea grass *(Panicum*) and legumes (Stylosanthes species) are often low in S, particularly relative to N (Schmidt and Monteiro, 2015). In Brazil, subtropical savannah pastures, populated by *Brachiaria decumbens* commonly contain <1g S kg^{-1}DM (Ortolani, 2001). In all pastoral systems, risks of sulfur insufficiency in livestock increases with distance from the coast and with increasing herbage maturity. The steppes of inner Mongolia are a low-S region, judged by the high percentage of herbage samples with low S:N ratios (50% < 0.07) (Wang *et al.*, 1996). Improvements in wool production and liveweight gain are commonly found when sulfur is included in supplements for sheep grazing dry-season pastures in Western Australia (Mata *et al.*, 1997; White *et al.*, 1997). Forage harvested in a mature state, for winter use as a maintenance feed, can sustain production if rumen-degradable energy and N sources are added, but dietary S (and P) may be inadequate (Leng, 1990; Suttle, 1991). The converse is also true: responses to added S will only be obtained if energy, N and P requirements of microbes are met (Suttle, 1991). Marked improvements in production can be obtained by supplementing low-quality roughages with molasses–urea mixtures, which must contain added S (and P). The interrelationships between dietary S, P and N were illustrated by an experiment in Brazil in which calves grazed paddocks of *Brachiaria decumbens* by day for 180 days, with overnight access to a corn-based supplement with or without added S (Fig. 9.4). The pasture became increasingly low in dietary S, P and N as the study progressed and calves not given S eventually lost weight and plasma SO_4 decreased: addition of S allowed calves to gain weight and plasma SO_4 increased. Intensive animal production from cereals and non-protein N sources also requires inputs of sulfur (and P).

- The inexplicably low nutritive value of many silages (AFRC, 1993) may be related to unusually high soluble N concentrations and lack of equally degradable S, which limits microbial capture of ammonia.

Animal factors

Incidence of sulfur deprivation depends on the type of stock being reared. With mixed stocking systems, pastures that are sufficient for cattle may provide insufficient sulfur for sheep. In temperate

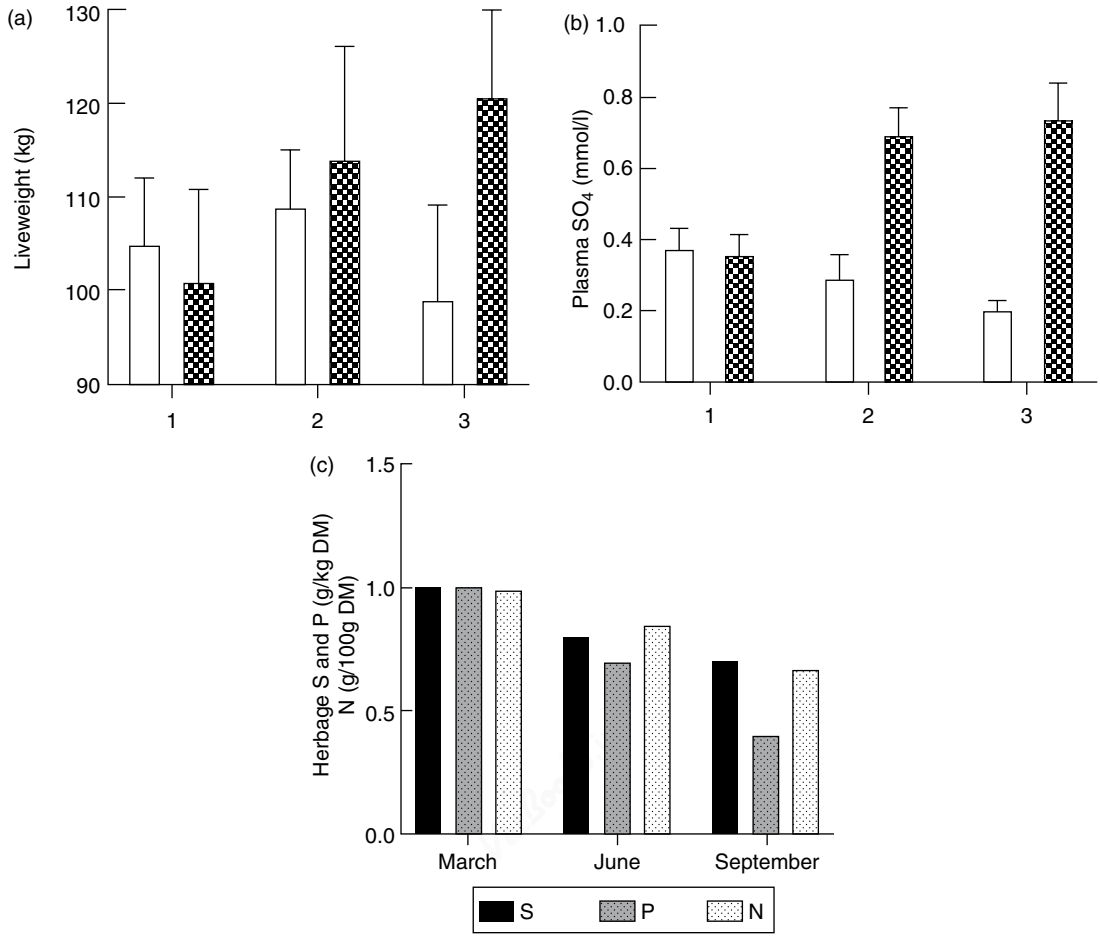

Fig. 9.4. Seasonal decreases in sulfur (S), phosphorus (P) and nitrogen (N) in *Brachiaris decumbens* (signal grass), grazed by calves (c): free access to a supplement containing ground corn, urea and dicalcium phosphate with (cross-hatched) or without (open columns) added S was allowed overnight and contained 4.5 and 1.5g S kg^{-1}DM, respectively. Correction of the deficit in dietary S improved growth (a) and plasma SO_4 (b) but is unlikely to have improved growth without concomitant addition of N and P. (Data from Ortolani, 2001.)

zones, lambing coincides with spring grass growth, rich in protein at times of maximum needs of ewe and lamb. However, factors that increase protein requirements, such as infections by gut nematodes (Coop and Sykes, 2002), will increase sulfur requirements. Widespread deficiencies of sulfur have been predicted for cattle on the basis of dietary S:N ratios of 0.1 for beef and 0.08 for dairy animals (Qi *et al.*, 1994) but remain unsubstantiated. In tropical areas of Queensland and Western Australia, seasonal supplementation with sulfur (and nitrogen) was suggested to maximize growth rate in defaunated sheep (p. 215) by raising suboptimal rumen S_2^- (Hegarty *et al.*, 1994).

Sulfur Sources for Ruminants

Pasture and forages

Sulfur concentrations in pasture and conserved forages range widely from 0.5 to >5.0g kg^{-1}DM (Table 9.1), depending on plant species, availability of soil S, N and P, sward maturity and climate. In the temperate UK, concentrations in fresh grass ranged

Table 9.1. Sulfur and total sulfur-to-nitrogen ratios (S:N) in feedstuffs for ruminants.

	Samples	S (g kg^{-1}DM)	S:N[a]	Reference
Grasses	137	2.2	0.088	MAFF (1990)
Dried grass	8	3.5	0.12	MAFF (1990)
Stylo hay	2	1.25	0.071	Bird (1974)
Alfalfa hay	2	3.6	0.095	Qi *et al.* (1994)
Spear-grass hay	2	0.5, 0.8	0.055, 0.205	Kennedy & Siebert (1972a, b)
Corn silage	73	0.6-0.8	–	Motta *et al*. (2020)
Barley straw	4	2.0	0.33	MAFF (1990)
Wheat straw	2	1.0	0.32	Kennedy (1974)
Barley	10	1.5	0.073	MAFF (1990)
Maize	5 *(23)*[b]	1.6 *(1.1)*	0.10	MAFF (1990)
Wheat	*10*	*1.8*	0.084	Kerr *et al.* (2008)
Distillers dried grains-UK	5	3.7		MAFF (1990)
Distillers dried grains-US		6–8		Drewnoski *et al*. 2014
Rape (leaf)	6	6.2	–	Evans (unpublished)
Swedes	–	4.8	0.179	Cornforth *et al.* (1978)
Turnips	–	6.1	0.233	Cornforth *et al.* (1978)
Kales	–	9.0	0.313	Cornforth *et al.* (1978)
Feather meal	5	18.1	0.127	MAFF (1990)
Corn gluten meal	*8*	*10.2*		Kerr *et al.* (2008)
Rapeseed meal	5	16.9	0.26	MAFF (1990)
Linseed meal	5 *(6)*	4.1 *(2.4)*	0.065	MAFF (1990)
Cottonseed meal	5	5.0	0.083	MAFF (1990)
Soybean meal	5	4.6 *(4.6)*	0.058	MAFF (1990)
Safflower meal	1	2.2	0.029	Qi *et al.* (1994)
Fish meal	1	4.9	0.046	Qi *et al.* (1994)
Molasses (cane)	1	7.3	0.811	Bogdanovic (1983)
Old man saltbush	3	6.0	0.169	Ben Salem *et al*. (2010)
Cassava leaf	?	3.4	0.083	Lukuyu *et al*. (2014)
Cassava root	?	0.3	0.5	Lukuyu *et al*. (2014)
Fodder beet	?	1.5	0.5	Sauvant *et al.* (2004)

[a]This ratio only improves assessment of nutritive value for ruminants if S and N have similar rumen degradabilities.
[b]all italicised values are from Kerr *et al*. (2008).

from 1.2–4.0g S kg DM^{-1} and the mean (2.2g S kg DM^{-1}) was adequate for plant growth. Applications of SO_4 fertilizer increase herbage S, even when baseline levels are high, by increasing herbage SO_4 rather than protein (Spears *et al.*, 1985). Pastures in coastal areas receive S from sea spray (3–5 kg ha^{-1}) (Ledgard and Upsdell, 1991) and those downwind of certain industrial activities up to 8kg S ha^{-1} year^{-1}) (McClaren, 1975). In temperate mixed swards, herbage S can rise with approach of summer, due to a rise in legume contribution (p. 22). The 'wilding' of pastures to promote biodiversity may increase herbage S (p. 25). Plant S is highly correlated with protein concentration, which declines at a rate of 1–3g kg^{-1}DM day^{-1} with approach of maturity, depending on species and growth conditions (Minson, 1990); this is equivalent to a decline of 0.1–0.3g S kg^{-1}DM week^{-1}. Dry-season forages often contain <1g S kg^{-1}DM, as in West Kenya (Jumba *et al.*, 1996) and Brazil (Fig. 9.4).

Other feeds

Plant species vary in S requirement: legumes show optimal growth with around 2 g S kg^{-1}DM in their tissues, while maize tolerates 1.4 g and sugarcane <1g S kg^{-1}DM. Concentrations (g S kg DM^{-1}) are generally low in cereal grains and root crops, richer in cereal straws (1.4–4.0; Suttle, 1991), richer still in protein supplements (2.2–4.9) and excessive in brassica crops (4.8–9.0) and halophytes (Table 9.1). Corn silages from Brazil were consistently low in S (Motta *et al.*, 2020). Leafy brassicas such as kale and the leaves of root crops such as swedes and turnips contain S in potentially toxic forms (p. 223). The disparity

between leaf (high) and root (low) S in root crops will cause S intake to decrease when animals are given access to a standing crop. Harvesting and mixing the cassava leaf and root can raise S intake. Among by-products, those from animals are moderate S sources: some from plants, such as corn gluten meal, and dried distillers grain solubles (DDGS) are high in S because H_2SO_4 is used in processing: DDGS can contain enough S to present risks of S toxicity, especially when the oil is stripped out (Drewnowski *et al.*, 2014b) (p. 221).

Nutritive Value of Sulfur in Feeds

Nutritive value is regarded by some to be more closely related to ratios of dietary S:N than dietary S *per se*. Dietary S and N are often well correlated within feeds but differ widely between feeds (Table 9.1), S:N ratios being up to tenfold higher in brassicas and cereal straws (0.2–0.3) than in cereal grains and vegetable protein sources (0.03–0.05). While plant S is comprised mostly of S in proteins, roughly a third is present in inorganic forms. Some S in fibrous foods may be insoluble in acid detergent and unavailable to rumen microbes. Sulfur incorporated into microbial nucleic acids may be of limited availability to the animal. Plants species like *Lotus corniculatus*, rich in tannins as well as S, contain protein and S of high nutritive value (Wang *et al.*, 1996). Tannin-rich *L. corniculatus* contains methionine and cysteine that are protected from degradation by forming tannin-complexes.

- Total dietary S:N bears no relation to nutritive value.

Rumen degradability of sulfur sources

The efficiency of utilization of S from a given source is partly dependent on degradability to S_2^- in the rumen. Using generation of H_2S gas to reflect degradability, four hours after regular feeds of silage and concentrates and when H_2S was barely detectable, cysteine was degraded much faster than SO_4, which was slightly more degradable than methionine (Fig. 9.5): the former finding confirmed earlier conclusions in sheep, with rumen microbial populations given time to adapt to dietary supplements, but the latter did not (Bird, 1974). Peak H_2S generation from cysteine varied almost twofold between experiments and widely between and within individuals on the same treatment (Dewhurst *et al.*, 2007). When cows grazing *L. perenne* pasture were brought in to compare H_2S degradation from *L. perenne* and white clover, degradability was far higher in *L. perenne*. It was postulated that H_2S may have been 'captured' as thiocyanate from the cyanogenetic legume (Dewhurst *et al.*, 2007). That hypothesis was supported by a study in Thailand, where consumption of a feed block containing 4% S raised plasma thiocyanate in cattle on a cassava root diet rich in HCN: capture of H_2S as thiocyanate was inferred (Cherdthong *et al.*, 2018).

- H_2S generation can be a poor indicator of nutritive value of a sulfur source.

Adaptation of rumen microflora to sulfur sources

Rumen microbes adapt to S source and basal diet and long-term exposure to SO_4-rich diets changes species distribution in the rumens of cattle (McCann *et al.*, 2017) and sheep (Morrison *et al.*, 1990): changes can be monitored by real-time polymerase chain-reaction (PCR) methodology (McSweeney and Denman, 2007). In feedlot cattle introduced to SO_4-rich drinking water, H_2S concentrations in the rumen gas space remained low for 7–14 days but then increased rapidly. Assessments of degradability are therefore dependent on experimental technique, which may explain the above contrasts on H_2S generation, within and between experiments. Cows on a regular maintenance diet of maize silage and concentrate were used to compare H_2S profiles in animals fasted overnight and given single feeds of maize germ feed (MGF) and soybean meal (SBM) and sunflower meals (SFM) (Fonseca *et al.*, 2013). Generation of H_2S was faster from MGF and SFM than from SBM, a source known to be slowly degraded in the rumen (Fig. 9.6). Four sources of MGF, differing in probable degree of heat denaturation, also showed a positive correlation between degradability and H_2S generation but the sources differed in S concentration, which was linearly related to mean H_2S concentration in the gas space. Comparisons were made with rumen populations that were unadapted to some sources in the above studies (white clover, SO_4 and SFM) and degradabilities may have been underestimated for the longer term.

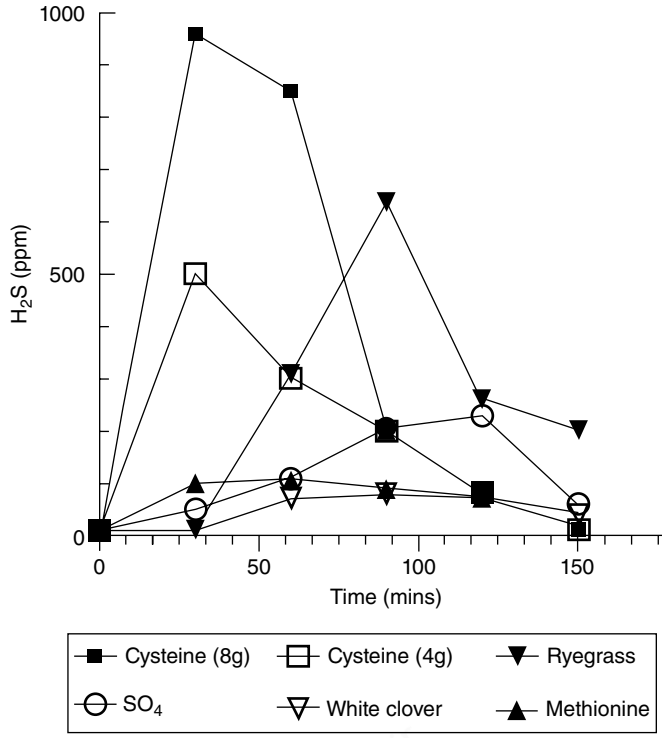

Fig. 9.5. Hydrogen sulfide (H_2S) concentrations in the rumen headspace after inoculating unadapted rumen populations, *in vivo*, with different sulfur sources: dose- and source-dependent differences in degradability were indicated. (Data for selected groups in experiments 1, 2 and 3 of Dewhurst *et al*., 2007)

- H_2S generation should be more closely related to nutritive value if adapted rumen microbial populations are used and results corrected for differences in dietary S.

Reducing rumen sulfide production

Rumen degradability lay at the heart of a new concept, that of 'rumen available sulfur (RAS)', introduced to improve the efficiency of MPS and predict risk of sulfide toxicity (Sarturi *et al*., 2013a): RAS is inversely related to H_2S generation, which can be measured *in vitro*. However, degradability is much faster *in vitro* than *in vivo* and influenced by adaptation of the microbial innoculum to the source under test. Greenhouse gas emissions will be influenced by RAS, since S-reducing bacteria compete with methanogenic bacteria for hydrogen in the rumen, leading to inverse relationships between H_2S and CH_4 emissions (Qian *et al*., 2020). Adding 8g S kg^{-1}DM to an *in vitro* rumen culture system, with cane sugar by-products supplying readily fermentable carbohydrate, reduced methane production: replacing urea with $Ca(NO_3)_2$ also reduced methane production and effects of S and N source were additive (Binh Phoung *et al*., 2011). High S intakes from DDGS reduce methane outputs from feedlot cattle units.

Capture of degraded sulfur in rumen microbial protein

The proportion of total S-inflow 'captured' by MPS varies widely and is determined by source (organic versus inorganic; methionine versus cysteine) (Bird, 1972a; Fonseca *et al*., 2013), dietary S concentration, co-availability of other substrates (chiefly degradable N), adaptation by rumen microflora and gaseous loss via eructation and inhalation. Approximately 50% of bacterial organic S was derived from S_2^- in sheep given either grass or lucerne hay (Kennedy and Milligan, 1978). Dietary supply is augmented by S secreted in saliva in both inorganic and organic forms: inputs are small in sheep and only slightly larger in cattle (Kennedy and Siebert, 1972b; Bird, 1974), so that

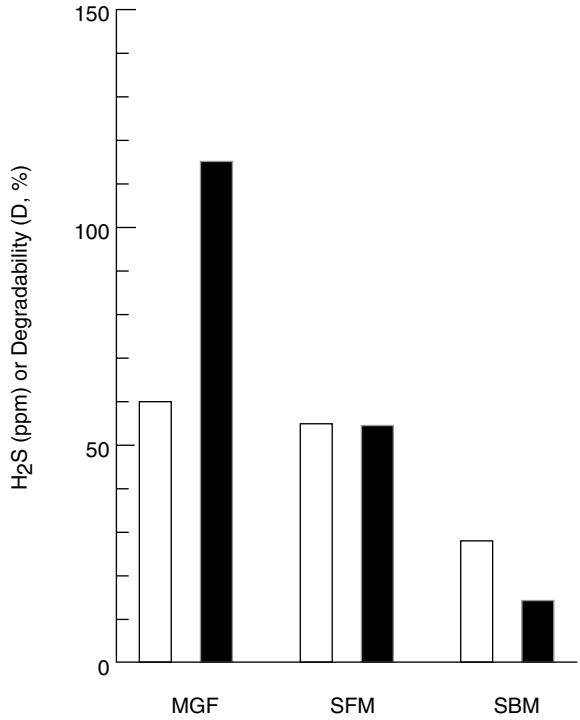

Fig. 9.6. Mean hydrogen sulfide (H_2S, solid columns) concentrations in the rumen headspace over four hours rank with sulfur amino acid degradability (D), (open columns): test feeds of maize germ feed (MGF), sunflower meal (SFM) and soybean meal (SBM) were placed in the rumen of fasted cows. (From Fonsesca *et al*., 2013, averaging similar results for cysteine and methionine degradabilities)

capacity for recycling is limited (Kandylis and Bray, 1987). Optimal capture occurs when fermentable energy, degradable S, N and P are supplied at rates that match capacity for MPS (i.e. by 'rumen synchrony') (Moir, 1970; Beever, 1996). Microbial protein is generally assumed to have an S:N ratio of 0.067 (ARC, 1980).[ii] Rumen protozoa feed on rumen bacteria, returning microbial S to the S_2^- pool as they do so (Kandylis, 1984a). Efficiency of capture can be increased by defaunating the rumen (i.e. removing the protozoa) (Bird and Leng, 1985), a process that should lower rumen S_2^- (Hegarty *et al.*, 1994). The impact of rumen S^{2-} on copper metabolism is discussed on p. 272. Anaerobic fungi require S to degrade structural fibre in the rumen and contribute significantly to MPS and the synthesis of SAA (Weston *et al.*, 1988): fungi are constrained more by low dietary S than bacteria (Morrison *et al.*, 1990) but utilize reduced forms that bacteria cannot use (e.g. mercaptans).

Post-rumenal Metabolism

Sulfur leaves the rumen in several forms in digesta, the proportions determined by dietary composition and efficiencies of S and N capture by the rumen microbiome but principally as undegraded dietary, microbial and fungal protein (Fig. 9.2) (Bray and Till, 1975). Thereafter, sulfur metabolism is essentially similar to that of monogastric species, SAA, peptides and dipeptides being liberated by proteolytic digestion in the small intestine: thus, 80–90% of S leaving the rumen in MPS is normally absorbed by sheep (Wang *et al.*, 1996). Although protein complexed with tannins can resist subsequent proteolysis, the net effect of tannins is to increase methionine and cysteine absorbtion. Further degradation of SO_4^{2-} to S^{2-} and incorporation into bacterial protein can occur in the hind gut but makes little contribution to sulfur utilization (Kandylis, 1984a). The small amounts of SO_4 that leave the

rumen in digesta are absorbed by an active transport pathway, shared by molybdate (MoO_4). Absorbed methionine is demethylated in the tissues to provide methyl groups (CH_3) for elaboration into carbon chains, leaving homocysteine: the non-essential SAA, cystathionine and cysteine, are formed from homocysteine by trans-sulfuration in the liver, viscera and skin (Liu and Masters, 2000). Tissue protein synthesis can now proceed and cysteine accumulates at sites such as muscle and mammary gland in proteins such as myosin and casein, which contain S:N in a ratio close to 0.067. Sulfur is incorporated mostly as cysteine into the keratin of wool, hair and mohair proteins, which contain 2.7–5.4% S in an S:N ratio of 0.2 (Langlands and Sutherland, 1973).

Retention

When dietary S was added incrementally as DDGS to the diet of sheep, 69% was excreted in urine, 6% in faeces and 25% appeared to be retained, the amount increasing linearly with S intake (faecal S is largely undegraded organic S and undigested microbial protein) (Kandylis and Bray, 1987). However, rumen H_2S increased exponentially with dietary S (Fig. 9.7) and insensible losses of H_2S from the rumen gas cap by eructation and volatilization during rumination probably increased.

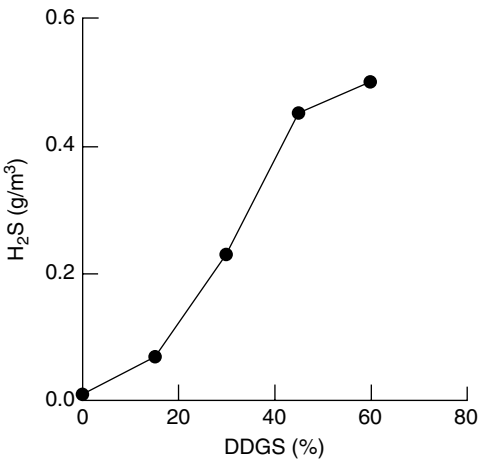

Fig. 9.7. Stepped exposure of sheep to dried distillers grain solubles (DDGS) increased hydrogen sulfide (H_2S) concentrations in the rumen gas space, measured four hours after feeding (from Neville *et al*., 2011, after seven days' exposure at each inclusion rate).

- Ingested sulfur that is not excreted in urine and faeces cannot be regarded as retained in tissues at high sulfur intakes.

The dietary S threshold above which insensible losses become significant will decrease as rumen degradability of the source increases and may be relatively high for DDGS, which contains protein of relatively low degradability. During SAA or protein deficiency, catabolism of tissue protein will allow redistribution of SAA from the more expendable protein pools (e.g. bone matrix in pregnant and lactating females) to less expendable sites and processes (e.g. fetus and mammary gland). Wool growth is more sensitive to reduced protein supply than fetal growth, maternal weight or milk production (Masters *et al*., 1996) and reductions in wool growth on diets lacking S should allow far more protein to be synthesized elsewhere because other proteins contain far fewer SAA. Urine S consists mostly of SO_4 derived from the oxidation of sulfide and SAA and catabolism of other organic molecules.[iii]

Sulfur Requirements of Ruminants

Stating S requirements for ruminants as dietary S concentrations (e.g. 1.5g S kg^{-1}DM; NRC, 2001, 2007, 2016) is of little value and a more sophisticated approach is needed (Liu and Masters, 2000), following the convention in monogastric animals of stating net S requirements as needs for SAA (D'Mello, 2003) (Fig. 9.9).

Needs for hair and wool production

Sheep and goats differ from cattle in that hair and wool growth adds significantly to the need for S (Williams *et al*., 1972). Hair and wool growth is inevitable and increases with body size and growth rate: net needs for S increase in consequence (Fig. 9.8) and historically are considered part of a maintenance need (ARC, 1980; AFRC, 1993). Unlike other components of maintenance, wool growth is affected by photoperiodic and reproductive cycles (Kendall *et al*., 2006). Wool growth is higher in intact than in castrated lambs (Iran), low in early pregnancy and winter but rises at parturition, under the influence of prolactin. In double-coated, cashmere-producing goats, there is an additional need for S during the cashmere-growing season in autumn (Souri *et al*., 1998). The

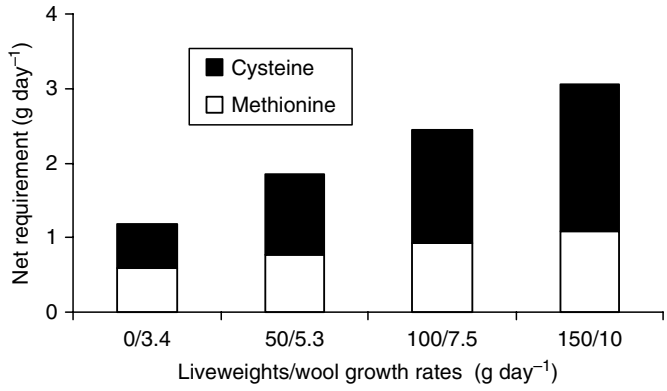

Fig. 9.8. Effects of liveweight and wool growth rates on net methionine and cysteine requirements in a 40-kg sheep. (From Liu and Masters, 2000)

closeness of S:N ratios in major animal tissues and products means that efficiencies of utilization of microbial proteins for most types of production are similar, regardless of species. However, efficiency of protein use for fleece growth is singularly low (only 0.26 compared to 0.68 for milk production and growth) (AFRC, 1993) because of high SAA concentrations in wool protein.

- S requirements are higher in sheep than cattle, whatever the season.

Rumen microbial system for estimating sulfur requirements

Gross SAA requirements cannot be derived from net requirements by applying an absorption coefficient for SAA absorption (p. 11). Flows of SAA from the rumen exceed intakes because of MPS (Fig. 9.2), which reduces dietary need for pre-formed SAA. The needs of rumen microbes for S preempts those of the host and cannot be expressed as single dietary S concentrations, the NRC (2001, 2007, 2016) convention. Sulfur requirements were first linked to MPS by harnessing estimates to the calculation of protein requirements (by ARC (1980)), who assumed that S and N, naturally present in feed proteins, would be appropriately balanced for MPS, having similar S:N ratios of 0.067, whether rumen-degradable or -undegradable. If additional non-protein N was required, S should be supplied in the same ratio. However, early studies with mature male Merino sheep indicated that when low-quality roughages were supplemented with urea and SO_4, higher dietary S:N ratios were required (approximately 0.10) (Moir, 1970; Bird, 1974). With a similar animal

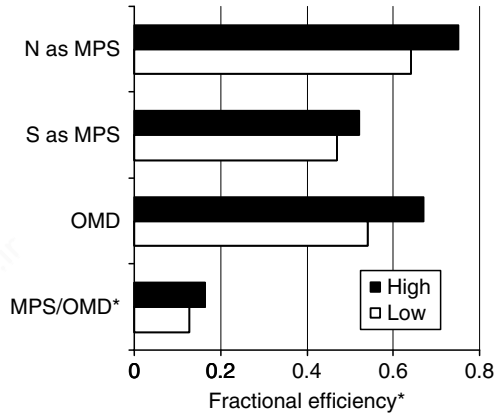

Fig. 9.9. Efficiencies of organic matter digestion (OMD) and use of dietary sulfur (S) and nitrogen (N) for microbial protein synthesis (MPS) is improved by addition of degradable S (as Na_2SO_4) and N (as urea) (compare high versus low) to a poor-quality roughage for sheep, S being used less efficiently than N. (Data from Kandylis and Bray, 1987: *results for MPS/OMD are in g kg^{-1})

model, digestibility of diet and MPS were improved by supplements that increased dietary S:N ratio from 0.11 to 0.18 (Kandylis and Bray, 1987). A major factor contributing to higher S:N requirements was probably the lower efficiency of incorporation of S than N into MPS (50% versus 70%), due to losses of S from the rumen as H_2S (Fig. 9.9). The 'metabolizable protein' system for estimating protein requirements (AFRC, 1993) divided dietary crude protein (N × 6.25) into three components – quickly degradable (e.g. fresh forages), slowly degradable and non-rumen-degradable protein (QDP, SDP and UDP, respectively) – but made no

Table 9.2. Theoretical sulfur requirements for microbial protein synthesis (MPS) in ruminants given balanced diets adequate in metabolizable energy (ME) and protein (MP)[a].

Animal	Liveweight (kg)	'Production' (kg day^{-1} r no.)	Required MPS (g day^{-1})	Required S[b] (g day^{-1})	DMI (kg day^{-1})	Dietary S (g kg^{-1}DM)
Lamb	20	0.1	61	0.97	0.6	1.6
		0.2	88	1.40	1.0	1.3
	40	0.1	72	1.15	1.1	1.0
		0.2	95	1.52	1.9	0.85
Pregnant Ewe	40	1[c]	78	1.24	0.9	1.3
		2	90	1.43	1.1	1.3
	80	1	116	1.85	1.4	1.3
		2	137	2.19	1.8	1.2
	40	1	133	2.12	1.2	1.8
		2	209	3.34	1.9	1.8
	80	2	234	3.70	2.2	2.1
		3	309	4.94	2.9	1.6
Goat (Angora)	30	0.1	95	1.52	0.9	1.6
Steer	100	0.5	220	3.51	2.1	1.6
		1.0	348	5.55	3.2	1.8
	300	0.5	299	4.78	4.3	1.0
		1.0	409	6.54	6.1	1.0
	500	0.5	373	5.96	7.0	0.9
		1.0	477	7.60	10.1	0.8
Cow	600	1[c]	420	6.70	8.8	0.8
		10[d]	762	12.16	11.8	1.0
		20	1229	19.63	14.4	1.3
		40	2165	34.58	23.9	1.5

[a]Requirements of ME and MP for MPS are those given by AFRC (1993): deduct S inputs from saline drinking water.
[b]The formula to obtain S requirements from the MPS required for a given level of production is (MPS / 6.25) × 0.1.
[c]The number of fetuses at week 20 of ovine pregnancy and week 36 of bovine pregnancy.
[d]Milk yield

provision for sulfur. That gap was filled by using a S:N ratio of 0.1 rather than 0.067 (to allow for inefficient capture of plant S) to derive estimates for different types of production, as shown in Table 9.2. Sheep generally need more dietary S than cattle for similar stages in development and younger animals need more than older growing animals. Needs for S are little affected by milk yield within species because DMI increases with yield. A similar approach has been advocated in calculating tolerable sulfur intakes, which are related to RAS rather than total dietary S (Sarturi *et al*., 2013a) (p. 222)

Application and tests of rumen microbial needs

Where ration formulation models indicate benefits from supplementation with QDP, sulfur should be added in a ratio of 0.1 to QDP. In feeding trials, cattle needed less sulfur than sheep to digest the same urea-supplemented, low-quality diet (Kennedy and Siebert, 1972a; Bird, 1974). Responses to sulfur supplementation in sheep grazing pastures containing 0.95–1.18g S kg^{-1}DM (total S:N 0.08–0.09) (Mata *et al*., 1997) support the novel estimates. Abomasal infusion of methionine and casein increased wool growth by 28% in Merino wethers on a diet containing 1.1g S kg^{-1}DM (S:N 0.091), as oaten hay and oat grain, while a 23% increase in food allowance above maintenance did not (Revell *et al*., 1999), suggesting that MPS was constrained by lack of sulfur. Responses in production and metabolic parameters in Angora goats indicated high optimum dietary S:N ratios of 0.1–0.4 (Qi *et al*., 1992, 1993) and it was argued that other ruminants may also benefit from higher S:N ratios than those recommended in Table 9.2 (Qi *et al*., 1994). However, protein and sulfur requirements are high for rapid mohair

growth and there is a limit to the extent to which higher requirements can be met by increasing *degradable* S:N, unless SAA concentrations in microbial protein can be increased. Optimal supplements in the experiments of Qi *et al.* (1992, 1993) provided degradable S:N in ratios between 0.047 and 0.088 for mature goats and between 0.061 and 0.11 for growing kids.

- For all production purposes, sulfur requirements should be calculated relative to nitrogen needs for rumen MPS (Table 9.2), with separate calculations for supplements fed separately, rather than in a total mixed ration (e.g. for concentrates fed in parlour to a grazing herd) and allowing for sulfur intake from drinking water.

Avoidance of Sulfur Insufficiency

Fertilizers

Pasture plant species require higher tissue S to maximize growth than animals require from the herbage they consume. Fertilizer policies that maximize herbage yields will prevent insufficiency in grazing animals and those recommended for pastoral conditions in New Zealand have been reviewed (Edmeades *et al.*, 2005). Phosphorus fertilizers once provided sufficient adventitious S for plant needs, but S to P ratios declined greatly with changes in manufacture: as with animals, uptake of S, P and N must all meet respective plant needs to maximize animal productivity. To achieve 95% of maximum yield, clover requires at least 2.3g S kg^{-1}DM in ratios of 0.0048 S to N and 0.7-0.8 for S to P; optimum in mixed herbage was 3.8g S kg^{-1} DM. If these criteria were achieved, there was sufficient S present not to limit the response to applied N or P. Sophisticated steady-state models of S inputs and outputs were developed, based on two soil tests that measured availabile SO_4 and available organic sulfur, from which SO_4^{2-} is 'mineralized'. Thus, it was estimated that fertilizer inputs of 20–25 kg S ha^{-1} per annum were required on soils of South Island but twice as much on the pumice soils of North Island, where leaching losses were high. Using a simpler yardstick (soil extractable SO_4 <12mg kg^{-1}), biennial applications of 10 kg S ha^{-1} were recommended for maize grown in a rainforest–savannah transition zone in Nigeria (Ojeniyi and Kayode, 1993). Sulfur is usually applied as either $CaSO_4$ or K_2SO_4 and responses in herbage S are dependent on application rate (Fig. 9.10) (Spears *et al.*, 1985). Sulfur fertilization of forage crops (Buttrey *et al.*, 1986; Ahmed *et al.*, 1995) also improves animal production. Need for S inputs can be minimized by grazing management practices that maximize the contribution of naturalized clover species, rich in S, in late-season pastures at high altitude (Thomas *et al.*, 2016).

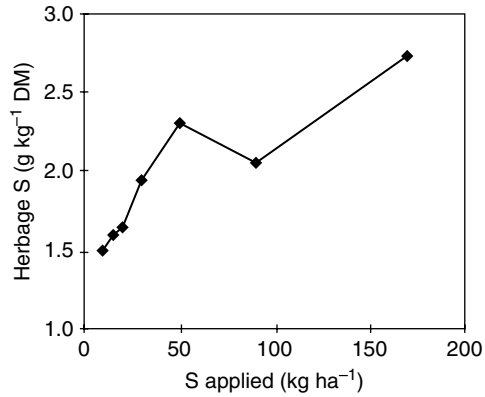

Fig. 9.10. Responses in herbage sulfur concentration to different application rates of fertilizer sulfur as calcium sulfate ($CaSO_4$) in south-east Scotland (McLaren, 1975).

Direct supplements

Whether or not fertilizer S is applied, herbage S shows a seasonal decline and may become insufficient, particularly in any dry season (Fig. 9.4). Lack of dietary S can readily be corrected by supplements of elemental S, Na_2SO_4 and $CaSO_4$, but only if other constraints upon MPS are lifted (Kennedy and Siebert, 1972b; Bird, 1974). Poor-quality pastures and forages are usually lacking in metabolizable energy, rumen degradable protein and possibly P as well as S (Fig. 9.4). Inclusion of $CaSO_4$ in a molasses–urea–bran block yields an ideal supplement for such pastures, with proportions of $CaSO_4$ and urea providing an S:N ratio of 0.1. One procedure is to heat molasses to 90°C for 20 minutes, cool to 60°C, then thoroughly mix in (kg kg^{-1}) urea (0.1), $CaSO_4$ (0.022), MgO (0.01), $Ca(OH)_2$ (0.1), NaCl (0.05), wheat bran (0.23) and trace elements as needed. The mixture is poured into moulds and allowed to solidify (modified from Habib *et al.*, 1991). For stall-fed animals, a loose dry mixture of the above ingredients can be spread over hand-fed fodder or feeds in the trough. Pastured sheep, offered 175 g $head^{-1}$ d^{-1} of a mineral mixture containing 46g S kg^{-1}, consumed 1.15g S d^{-1}, which

probably improved performance (White *et al.*, 1997). Cereals used for drought feeding should be supplemented with 10 g urea and 2.4g $CaSO_4.2H_2O$ kg^{-1}DM (Bogdanovic, 1983). Digestibility of organic matter and growth rate were improved by adding 30 g S (with P) to a diet containing 50% rice straw, ammoniated with 4% urea (Zain *et al.*, 2010), but raised S:N ratio unnecessarily to a high 0.37. Supplying N and S in slowly degradable forms (e.g. biuret and $CaSO_4$) may improve MPS by up to 25%, compared with that attained with urea and Na_2SO_4, by minimizing respective losses as ammonia and sulfide, thus improving microbial capture. Biuret is expensive and rumen microbes need time to adapt and synthesize biuretase: $Ca(NO_3)_2$ is a preferable N source to reduce CH_4 emission (Binh Phoung *et al.*, 2011).

Nutritive value of inorganic sources

Many comparisons of different S sources have been reported (Henry and Ammerman, 1995) but those based on S balance give little or no indication of ability to promote MPS and overestimate availability if insensible losses are not measured (p. 216). Techniques based on *in vitro* cultures of rumen microbes get closer to true nutritional value to the extent that they adequately simulate rumenal events *in vivo*, but they can give implausible results compared with animal responses to supplementation. For example, elemental S was accorded low nutritive values of 0–36% by *in vitro* methods, 28–69% by balance methods and 73–102% by sheep growth tests when compared to Na_2SO_4 or methionine (Henry and Ammerman, 1995). Elemental S is as good as any S source for ruminants (Slyter *et al.*, 1988; Da Silva *et al.*, 2014) but potentiates the same rumen-based antagonisms as other sources, interacting with iron, copper, and molybdenum.

- There is no evidence that inorganic S sources differ in nutritional value for MPS.
- Absolute nutritional value will depend hugely on concurrent availability of other substrates for MPS, notably fermentable carbohydrate and soluble N.

In some formulae for calculating dietary cation–anion difference (DCAD) SO_4^{2-} ions have been accorded metabolic significance in preventive strategies for milk fever (p. 177) but ignore the variable proportions of ingested SO_4^{2-} ions that leave the rumen in un-ionized microbial protein and H_2S (Fig. 9.2).

'Protected' sulfur sources

Where MPS cannot meet needs for SAA, feeding sulfur sources 'protected' from rumen degradation, such as the analogues malyl and methyl malyl methionine (^{pr}Met), can be more cost-effective than slowly degradable protein supplements, such as fish meal and lupin seeds. An additional 100 g lupin seeds day^{-1} are needed to provide the improvements in wool and body growth afforded by 3g ^{pr}Met day^{-1} (Mata *et al.*, 1997). However, the first priority must be to maximize MPS, using inorganic sulfur supplements if necessary.

- Protected and slowly degradable supplements are only justified, economically and nutritionally, if metabolizable protein requirement exceeds the capacity for MPS (AFRC, 1993).

All claims for benefits from feeding costly protected S supplements should be evaluated against the background of what was either achieved or achievable with rumen-degradable S and N sources. If supply of S *per se* is limiting MPS, provision of 'protected' forms might be disadvantageous. Methionine analogues have mostly improved forms of production with high SAA requirements, such as merino wool (Coetzee *et al.*, 1995), angora and cashmere fibre (Souri *et al.*, 1998), but, in the latter study, may have been wholly or partially obtainable with less expensive SO_4. Since there is far more cysteine than methionine in wool (Fig. 9.8) and trans-sulfuration is far from complete (Liu and Masters, 2000), it would be more logical to supply protected cysteine for such animals. Supplements of ^{pr}Met improved milk fat yield in high-yielding dairy cows (Overton *et al.*, 1996) but not in low-yielding cows on ryegrass/white clover pasture (Pacheco-Rios *et al.*, 1999) and others on a diet thought to provide insufficient methionine (Casper *et al.*, 1987). Conditions favouring improved productivity by using ^{pr}Met have been reviewed (Broderick, 2018). When fed at high levels of 12–24g day^{-1} to sheep, protected methionine reduced appetite (Kandylis, 1984a). However, ^{pr}Met may have a sparing effect on selenium by reducing deposition of the predominant S source in natural diets, selenomethionine, into a muscle protein 'sink' (Fig. 15.6).

Sulfide Toxicity in Cattle

The margin between desirable and harmful dietary S in ruminant diets can be only two- to threefold

but is dependent primarily on degradability of the source (Sarturi *et al.*, 2013a, b). Performance of steers was impaired when $(NH_4)_2SO_4$ was added to a concentrate diet, raising dietary S from 2.0 to a mere 2.5g kg^{-1}DM (Zinn *et al.*, 1997). Reductions in appetite and growth rate in cattle and sheep, given diets with 3–4g S kg^{-1}DM (Kandylis, 1984b) were attributed to sulfide toxicity (Bird, 1972b). Tolerance is influenced, interdependently, by the following determinants of rumen H_2S: rate and duration of S intake; degradability of the source; rate of S_2^- removal from the rumen by absorption, eructation and digesta flow to the omasum; rumen pH; dietary fibre and rate of S_2^- capture by rumen microbes (Kandylis, 1984b; Drewnoski *et al.*, 2014a) (see Fig. 9.2). Tolerance is far lower when S is consumed in drinking water rather than diet and intakes equivalent to 2.2g S kg^{-1}DM can reduce food intake (Loneragan *et al.*, 2001). Rumen microflora adapt to high SO_4 intakes and dissimilate S^{2-} (Cummings *et al.*, 1995b): tolerance increases as exposure time lengthens. The first site and function to be affected is rumen motility, where S^{2-} is generated and rapidly forms either HS or H_2S, the latter favoured by low pH. However, intolerance of 4.6g SO_4-S kg^{-1}DM by fattening cattle was not improved by adding $NaHCO_3$ to the diet (Spears *et al.*, 2011). Absorbed sulfide harms the central nervous system (Smart *et al.*, 1986; Raisbeck, 1982; Cummings *et al.*, 1995a) and causes a polioencephalomalacia (PEM) similar to that caused by thiamine deficiency. High S intakes do not affect rumen thiamine synthesis but lower concentrations of thiamine pyrophosphate in brain, possibly by increasing thiamine turnover (Amat *et al.*, 2013). Pulmonary absorption of H_2S from the rumen gas gap, where concentrations can reach >4g l^{-1} (Drewnoski *et al.*, 2012). is particularly dangerous because detoxification capacity in the liver is by-passed. Temporal (Fig. 9.11) and diurnal changes (Fig. 9.5) affect rumen S^{2-} in cattle. Regardless of S source, temporal changes are partly due to changes in rumen microbial populations, less than 1% of those normally present being H_2S generators. Multiple changes in microbial composition occurred in cattle given diets containing up to 27% DDGS for 19 days, including increased numbers of the dissimilatory *Desulfovibrio* spp (McCann *et al.*, 2017), but such changes were not necessarily adaptative because so much else changed, including a five-fold increase in dietary fat.

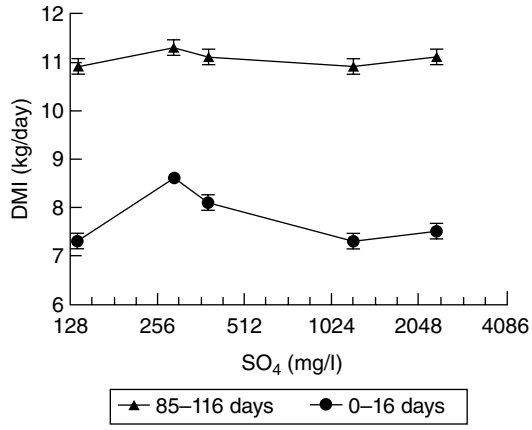

Fig. 9.11. Dry matter intake (DMI) of feedlot steers given increasing proportions of sulfate-rich (SO_4) well water to drink and a diet providing 1.8g S kg^{-1}DM: the first increment improved DMI but >586mg SO_4 l^{-1} initially decreased DMI; both effects had waned after 116 days exposure. (Data from Loneragan *et al.*, 2001)

Occurrence

Wherever ruminants have access to diets and/or drinking water, either naturally or artificially high in readily degradable S, PEM is likely to occur in cattle (NRC, 2016). Water supplies rich in SO_4 have caused outbreaks of PEM in feedlot cattle in the USA but were not considered a problem in Australia where waters contained <0.57g S l^{-1} (Wilkes *et al.*, 2019). Use of Na_2SO_4 supplements to restrict food intake by steers in feedlots caused PEM in USA (NRC, 2016). Sulfur intakes are increased to potentially toxic levels by inclusion of S-rich by-products, such as dried distillers grain solubles (DDGS) and maize gluten feed, both processed with sulfuric acid (Drewnoski *et al.*, 2014b). A large survey found that incidence of PEM increased from 0.4% to 6.1% when dietary S was >5.6g kg^{-1}DM and mostly ingested as DDGS (Vanness *et al.*, 2009). Wet grains (WDGS) are more likely to depress food intake than dried grains because H_2S concentrations in the rumen gas space are higher (Sarturi *et al.*, 2013b). Sustained high S intakes can also induce copper deficiency, with or without assistance from molybdenum (p. 261). Distillers grains supplied by the whisky distillery industry had long been used safely as a feed for ruminants but were usually rich in Cu. Increasing amounts of by-product

being generated by the biofuel industry are low in copper. Sulfur-induced copper deficiency may also be a risk factor in ruminants consuming saltbushes which can be high in S (Table 9.1) but low in copper (Ben Salem *et al.*, 2010).

Diagnosis

Since symptoms of PEM can be caused by factors other than sulfide toxicity, including thiamine deficiency, lead toxicity and salt poisoning, supportive biochemical abnormalities are needed to confirm sulfide toxicity. Urinary thiosulfate increases within days of exposure to high S diets and values >50 mg l^{-1} confirm high S intakes (Drewnoski *et al.*, 2012). High rumen S_2^- in gas or fluid samples (>4g or xg l^{-1}) confirm excessive S intakes but are best measured in unaffected cohorts, where the source is principally dietary S, because badly affected animals stop eating. Samples should be taken from fed rather than fasted animals with minimal disturbance.

Prevention

Since PEM is irreversible, emphasis must be placed on prevention. Reverse osmosis can be used to remove SO_4 from 'saline' water supplies (Smart *et al.*, 1986) but is rarely used on farms in Australia (Wilkes *et al.*, 2019). Simultaneous addition of urea can lessen the depression of appetite and digestibility caused by high dietary S (Bird, 1974). Higher, sustained post-prandial H_2S concentrations were reached with $(NH_4)_2SO_4$ and WDGS than with corn gluten meal (Sarturi *et al.*, 2013b). *In vitro* determination of RAS can help to predict H_2S generation (Sarturi *et al.*, 2013a). Provision of roughage reduces risk of toxicity. As the neutral detergent fibre concentration increased from 3.4% to 11.4% with addition of chopped brome grass hay, rumen H_2S decreased linearly from 1.00 to 0.62 ± 0.037 g/m^3, while pH decreased linearly from 5.8 to 5.4 ± 0.041, 6 h after feeding a diet based on distillery by-products, containing 45g S kg^{-1}DM (Morine *et al.*, 2014). Rumen S^{2-} can also be reduced by dietary supplements of soluble Fe, which trap H_2S as insoluble FeS (Drewnoski *et al.*, 2014a), values decreasing linearly as dietary Fe, given as ferric citrate, increased from 200 to 400 mg kg^{-1}DM. *In vitro* studies comparing the soluble iron source with Fe_2O_3 showed that the latter, though reducing H_2S generation, was the less effective (Wu *et al.*, 2016).

- Antagonism of copper absorption by soluble and insoluble Fe is also probably dependent on temporary formation of FeS in the rumen (p. 274) and additional Cu antidote should be given with Fe.
- Primary and secondary Fe toxicity (e.g. induced lack of Mn) should be monitored.
- Allowing feedlot cattle access to bare exercise paddocks may reduce incidence of PEM by allowing access to soil Fe and causing more H_2S to escape from the rumen during movement.
- Where bore water is the source of SO_4, Fe and Mn in the water may reduce risk: other sources may be made safer by metering in additional Fe and Mn.

Sulfide Toxicity in Sheep

It was the induction of PEM, by injecting H_2S into gas space in the ovine rumen, that revealed the likely cause – inhalation and pulmonary absorption of eructated H_2S. Normal detoxification of H_2S by the liver was by-passed and sulfide damaged DNA, proteins and lipids in the brain. The disorder developed in lambs as well as calves when a dietary acidifier, included to reduce risk of urolithiasis, was changed from NH_4HCO_3 to $(NH_4)_2SO_4$ (Jeffrey *et al.*, 1994). Losses were also reported in lambs given a concentrate containing 4.1g S kg^{-1}DM, *ad libitum*: brain lesions differed in fine pathological detail and lack of responsiveness to vitamin B_1 from PEM due to thiamine deficiency (Low *et al.*, 1996). No PEM was induced and food intake did not decrease during ramped exposure to diets containing up to 60% DDGS in place of rolled maize and 8.4g S kg^{-1}DM over 35 days (Neville *et al.*, 2011). Rumen H_2S rose to a maximum of 0.5g $m^{3\,-1}$ (Fig. 9.7), much lower than that reached in cattle studies. Water intake increased fourfold and over half of the additional S intake was excreted in urine. Tolerance was probably heightened by stepped increases in DDGS inclusion over 28 days, which limited H_2S accumulation from a slowly degradable source.

- Diagnostic and preventive methods will be similar to those for cattle.

Toxicity of organic sulfur compounds in ruminants

Some sulfur in crops and forages is present in potent organic forms. *S*-methylcysteine sulfoxide (SMCO), an amino acid resembling methionine, was widely studied because it caused haemolytic anaemia in ruminants consuming large quantities of brassicas (Whittle *et al.*, 1976), such as kale (Barry *et al.*, 1981; Barry and Manley, 1985) and rape (Suttle *et al.*, 1987). Conversion of SMCO to dimethyl disulfoxide in the rumen plays a key role in pathogenesis (Smith, 1978). Oral dosing with methionine provided partial protection from kale anaemia, while stimulating liveweight gain and wool growth, possibly by reducing the normally rapid and complete conversion of SMCO in the rumen (Barry and Manley, 1985).

- Plant SMCO concentrations >15g kg^{-1}DM are potentially toxic and increase with soil sulfur concentration, season and nitrogen fertilization (Fletcher *et al.*, 2010).

Brassica anaemia is rarely encountered nowadays. Reduced deposition of anthropomorphic sulfur on farms may have lowered SMCO concentrations, which can also be reduced by plant breeding. However, SMCO is present as a phytoaloxin, affording protection from infection through microbicidal activity and lines low in SMCO may be prone to infection (Edmands *et al.*, 2013). Ingestion of phytoaloxins in brassicas affects rumen microbial activity, reducing methaneogenesis, particularly from rape and swedes for example (Sun *et al.*, 2012). The unusually high availability of copper from sulfur-rich brassicas (Table 11.5) may result from suppression of the rumen protozoal population and consequent reduction in rumen S^{2-}. The toxicity of sulfur-containing glucosinolates is dealt with later (p. 312).

Sulfur Toxicity in Non-ruminants

Adverse effects of excess sulfate in non-ruminants are unrelated to H_2S. Undegraded SO_4 enters the small and large intestine and causes an osmotically driven diarrhoea, but rarely does so on farms.

Notes

[i] Samples of rumen fluid can be obtained by means of a simple vacuum source (a 50 ml disposable syringe fitted with a two-way valve) and interchangeable stomach tubes. Ease of sampling depends on consistency of digesta. Relevance of results depends upon time of sampling in relation to time of feeding, since rumen sulfide increases after a meal. Three samples should be taken, one pre-prandial, one post-prandial (1–2 h) and another after 5-8 h in discontinuously fed livestock.

[ii] Lower ratios were reported for mixed rumen bacteria and protozoa of 0.054 and 0.046, respectively, (Harrison and McAllan, 1980) but are subject to dietary influences, being increased from 0.075 to 0.11 by high SO_4 intakes (Kandylis and Bray, 1987).

[iii] SO_4 is filtered at the glomerulus at rates that exceed the tubular reabsorption rate, and is therefore rapidly excreted. SO_4 competes unequally with MoO_4 for renal tubular reabsorption (Bishara and Bray, 1978): the macronutrient (S) can greatly impede reabsorption of micronutrient (Mo), but MoO_4 can only slightly influence urinary excretion of SO_4. Urinary Mo was greatly increased and retention decreased in MoO_4-loaded sheep when SO_4 was subsequently added to the diet (Dick, 1956). When S and Mo were simultaneously added to the diet, S *decreased* urinary Mo by decreasing MoO_4 absorption and increasing Mo retention (Grace and Suttle, 1979) (Fig. 17.7).

References

AFRC (1993) *Energy and Protein Requirements of Ruminants*. An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Ahmed, M.R., Allen, V.G., Fontenot, J.P. and Hawkins, G.W. (1995) Effect of sulfur fertilization on chemical composition, ensiling characteristics and utilisation by lambs on sorghum silage. *Journal of Animal Science* 73, 1803–1810.

Amat, S., McKinnon, J.J., Olkowski, A.A., Penner, G.B., Simko, E., Shand, P.J. and Hendrick, S. (2013) Understanding the role of sulphur-thiamine interaction in the pathogenesis of sulphur-induced polioencephalomalacia in beef cattle. *Research in Veterinary Science* 95, 1081–1087.

ARC (1980) *The Nutrient Requirements of Ruminant Livestock*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 166–168.

Barry, T.N. and Manley, T.R. (1985) Responses to oral methionine supplementation in sheep fed on kale (*Brassica oleracea*) diets containing S-methyl-L-cysteine sulphoxide. *British Journal of Nutrition* 54, 753–761.

Barry, T.N., Reid, T.C., Miller, K.R. and Sadler, W.A. (1981) Nutritional evaluation of kale (*Brassica oleracea*) diets 2. *Journal of Agricultural Science, Cambridge* 96, 269–282.

Beaton, J.D., Tisdale, S.L. and Platou, J. (1971) Crop responses to sulfur in North America. *Technical Bulletin 18*. The Sulfur Institute, Washington, DC, pp. 1–10.

Beever, D.E. (1996) Meeting the protein requirements of ruminant livestock. *South African Journal of Animal Science* 26, 20–26.

Ben Salem, H., Norman, H.C., Nefzaouic, A. Mayberry, D.E., Pearce, K.L. and Revell, D.K. (2010) Potential use of oldman saltbush (Atriplex nummularia Lindl.) in sheep and goat feeding. *Small Ruminant Research* 91, 13–28.

Binh Phoung, L.T., Preston, T.R. and Leng, R.A. (2011) Mitigating methane production from ruminants: effect of supplementary sulphate and nitrate on methane production in an *in vitro* incubation using sugar cane stalk and cassava leaf meal as substrate. *Livestock Research for Rural Development 23*, Article 22.

Bird, P.R. (1972a) Sulfur metabolism and excretion studies in ruminants. V. Ruminal desulfuration of methionine and cysteine. *Australian Journal of Biological Sciences* 25, 185–193.

Bird, P.R. (1972b) Sulfur metabolism and excretion studies in ruminants. X. Sulfide toxicity in sheep. *Australian Journal of Biological Sciences* 25, 1087–1098.

Bird, P.R. (1974) Sulfur metabolism and excretion studies in ruminants. XIII. Intake and utilisation of wheat straw by sheep and cattle. *Australian Journal of Agricultural Sciences* 25, 631–642.

Bird, S.H. and Leng, R.A. (1985) Productivity responses to eliminating protozoa from the rumen of sheep. In: Leng, R.A., Barker, J.S.F., Adams, D. and Hutchinson, K. (eds) *Reviews in Rural Science 6*. University of New England Publishing Unit, Armidale, Australia, pp. 109–117.

Bishara, H.N. and Bray, A.C. (1978) Competition between molybdate and sulfate for renal tubular reabsorption in sheep. *Proceedings of the Australian Society of Animal Production* 12, 123.

Bogdanovic, B. (1983) A note on supplementing whole wheat grain with molasses, urea, minerals and vitamins. *Animal Production* 37, 459–460.

Bradley, A.S., Leavitt, W.D. and. Johnston. D.T (2011) Revisiting the dissimilatory sulfate reduction pathway. *Geobiology* 9, 446–457.

Bray, A.C. and Till, A.R. (1975) Metabolism of sulfur in the gastro-intestinal tract. In: McDonald, I.W. and Warner, A.C.I. (eds) *Digestion and Metabolism in the Ruminant*. The University of New England Publishing Unit, Armidale, Australia, pp. 243–260.

Broderick, G.A. (2018) *Review*: Optimizing ruminant conversion of feed protein to human food protein. *Animal* 12, 1722–1734.

Buttrey, S.A., Allen, V.G., Fontenot, J.P. and Renau, R.B. (1986) Effect of sulfur fertilization on chemical composition, ensiling characteristics and utilisation of corn silage by lambs. *Journal of Animal Science* 63, 1236–1245.

Casper, D.P., Schingoethe, D.J., Yang, C.-M.J. and Mueller, C.R. (1987) Protected methionine supplementation with extruded blend of soybeans and soybean meal for dairy cows. *Journal of Dairy Science* 70, 321–330.

Cherdthong, A., Khonkhaeng, B., Seankamsorn, A., Supapong, C., Wanapat, M. *et al.* (2018) Effects of feeding fresh cassava root with high-sulfur feed block on feed utilization, rumen fermentation, and blood metabolites in Thai native cattle. *Tropical Animal Health and Production* 50, 1365–1371.

Coetzee, J., de Wet, P.J. and Burger, W.J. (1995) Effects of infused methionine, lysine and rumen-protected methionine derivatives on nitrogen retention and wool growth of Merino wethers. *South African Journal of Animal Science* 25, 87–94.

Coop, R.L. and Sykes, A.R. (2002) Interactions between gastrointestinal parasites and nutrients. In: Freer, M. and Dove, H. (eds) *Sheep Nutrition*. CAB International, Wallingford, UK, pp. 313–331.

Cornforth, I.S., Stephen, R.C., Barry, T.N. and Baird, G.A. (1978) Mineral content of swedes, turnips and kale. *New Zealand Journal of Experimental Agriculture* 6, 151–156.

Cummings, B.A., Caldwell, D.R., Gould, D.H. and Hawar, D.W. (1995a) Identity and interactions of rumen microbes associated with sulfur-induced polioencephalomalacia in cattle. *American Journal of Veterinary Research* 56, 1384–1389.

Cummings, B.A., Gould, D.H., Caldwell, D.R. and Hawar, D.W. (1995b) Ruminal microbial alterations associated with sulfide generation in steers with dietary sulfate-induced polioencephalomalacia. *American Journal of Veterinary Research* 56, 1390–1395.

Da Silva, C.J., de Paula Leonel, F., Pereira, J.C., Costa, M.G., Moreira, L.M., de Oliveira, T.S. and de Abreu, C.L. (2014) Sulfur sources in protein supplements for ruminants. *Revista Brasileira de Zootecnia* 43, 537–543.

Dewhurst, R.J., Kim, E.J., Evans, R.T., Cabrita, A.R.J. and Fonseca, A.J.M. (2007) Effects of dietary sulphur sources on concentrations of hydrogen sulphide in the rumen head-space gas of dairy cows. *Animal* 1, 531–535.

Dick, H.T. (1956) Molybdenum in animal nutrition. *Soil Science* 81, 229–258.

D'Mello, J.P.F. (2003) *Amino Acids in Animal Nutrition*, 2nd edn. CAB International, Wallingford, UK.

Drewnoski, M.E., Ensley, S.M., Beitz, D.C., Schoonmaker, J.P., Loy, D.D. *et al*. (2012) Assessment of ruminal hydrogen sulfide or urine thiosulfate as diagnostic tools for sulfur induced polioencephalomalacia in cattle. *Journal of Veterinary Diagnostic Investigation* 24, 702–709.

Drewnoski, M., Doane, P. and Hansen, S. (2014a) Ferric citrate decreases ruminal hydrogen sulphide concen-

trations in feedlot cattle fed diets high in sulphate. *British Journal of Nutrition* 111, 261–269.

Drewnoski, M.E., Pogge, D.J. and Hansen, S.L. (2014b) High-sulfur in beef cattle diets: a review. *Journal of Animal Science* 92, 3763–3780.

Durand, M. and Komisarczuk, K. (1988) Influence of major minerals on rumen microbiota. *Journal of Nutrition* 118, 249–260.

Edmands, W.M.B., Gooderham, N.J., Holmes, E. and Mitchell, S.C. (2013) *S*-Methyl-l-cysteine sulphoxide: the Cinderella phytochemical? *Toxicology Research* 2, 11–22.

Edmeades, D.C., Thorrold, B.S. and Roberts, A.H.C. (2005) The diagnosis and correction of sulfur deficiency and the management of sulfur requirements in New Zealand pastures: a review. *Australian Journal of Experimental Agriculture* 45, 1205–1223.

Fletcher, A.L., Wilson, D.R., Maley, S., McCallum, J. and Shaw, M. (2010) The effect of sulphur and nitrogen fertiliser on levels of anti-nutritional compounds in kale. *Proceedings of the New Zealand Grassland Association* 72, 79–84.

Fonseca, A.J.M., Cabrita, A.R.J., Pinho, L.A.O., Kim, E.J. and Dewhurst, R.J. (2013) Effects of protein sources on concentrations of hydrogen sulphide in the rumen headspace gas of dairy cows. *Animal* 7, 75–81.

Gawthorne, J.M. and Nadar, C.J. (1976) The effect of molybdenum on the conversion of sulfate to sulfide and microbial protein sulfur in the rumen of sheep. *British Journal of Nutrition* 35, 11–23.

Goh, K.M. and Nguyen, M.L. (1997) Estimating net annual soil sulfur mineralisation in New Zealand grazed pastures using mass balance models. *Australian Journal of Agricultural Research* 48, 477–484.

Grace, N.D. and Suttle, N.F. (1979) Some effects of sulfur intake on molybdenum metabolism in sheep. *British Journal of Nutrition* 41, 125–136.

Habib, G., Basit Ali Shah, S., Jabbar, G. and Ghufranullah and Wahidullah (1991) The importance of urea-molasses blocks and bypass protein in animal production: the situation in Pakistan. In: *Proceedings of Symposium on Isotope and Related Techniques in Animal Production and Health*. International Atomic Energy Agency, Vienna, pp. 133–144.

Harrison, D.G. and McAllan, A.B. (1980) Factors affecting microbial growth yields in the reticulo-rumen. In: Ruckebusch, Y. and Thivend, P. (eds) *Digestive Physiology and Metabolism in Ruminants*. MTP Press, Lancaster, UK, pp. 205–226.

Hegarty, R.S., Nolan, J.V. and Leng, R.A. (1991) Sulfur availability and microbial fermentation in the fauna free rumen. *Archive fur Animal Nutrition, Berlin* 41, 725–736.

Hegarty, R.S., Nolan, J.V. and Leng, R.A. (1994) The effects of protozoa and of supplementation with nitrogen and sulfur on digestion and microbial metabolism in the rumen of sheep. *Australian Journal of Agricultural Research* 45, 1215–1227.

Henry, P.R. and Ammerman, C.B. (1995) Sulfur bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 349–366.

Jeffrey, M., Duff, J.P., Higgins, R.J., Simpson, V.R., Jackman, R. *et al*. (1994) Polioencephalomalacia associated with the ingestion of ammonium sulfate by sheep and cattle. *Veterinary Record* 134, 343–348.

Jumba, I.O., Suttle, N.F. and Wandiga, S.O. (1996) Mineral composition of tropical forages in the Mount Elgon region of Kenya. *Tropical Agriculture* 73, 108–112.

Kandylis, K. (1984a) The role of sulfur in ruminant nutrition: a review. *Livestock Production Science* 11, 611–624.

Kandylis, K. (1984b) Toxicity of sulfur in ruminants: a review. *Journal of Dairy Science* 67, 2179–2187.

Kandylis, K. and Bray, A.C. (1987) Effects of variation in dietary sulfur on movement of sulfur in the rumen. *Journal of Dairy Science* 70, 40–49.

Kendall, P.E., Sumner, R.M.W. and Pearson, A.J. (2006) Environmental and physiological mechanisms underlying wool growth rhythms in coarse wool sheep. *Proceedings of the New Zealand Society of Animal Production* 66, 144–148.

Kennedy, P.M. (1974) The utilisation and excretion of sulfur in cattle fed on tropical roughages. *Australian Journal of Agricultural Research* 25, 1015–1022.

Kennedy, P.M. and Milligan, L.P. (1978) Quantitative aspects of the transformations of sulfur in sheep. *British Journal of Nutrition* 39, 65–84.

Kennedy, P.M. and Siebert, B.D. (1972a) The utilisation of spear grass (*Heteropogon contortus*). *II. The influence of sulfur on energy intake and rumen and blood parameters in sheep and cattle. Australian Journal of Agricultural Research* 23, 45–46.

Kennedy, P.M. and Siebert, B.D. (1972b) The utilisation of spear grass (*Heteropogon contortus*). *III. The influence of the level of dietary sulfur on the utilisation of spear grass by sheep. Australian Journal of Agricultural Research* 24, 143–152.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S.L., Bearson, B.L., Shurson, G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Kincaid, R. (1988) Macro elements for ruminants. In: Church, D.C. (ed.) *The Ruminant Animal – Digestive Physiology and Nutrition*. Prentice-Hall, Englewood Cliffs, New Jersey, pp. 326–330.

Kolluru, G.K., Shen, X. and Kevil, C.G. (2020) Reactive sulfur species: a new redox player in cardiovascular

pathophysiology. *Artherosclerosis, Thrombosis and Vascular Biology* 40, 874–884.
Langlands, J.P. and Sutherland, H.A.M. (1973) Sulfur as a nutrient for Merino sheep. *I. Storage of sulfur in tissues and wool and its secretion in milk. British Journal of Nutrition* 30, 529–535.
Ledgard, S.F. and Upsdell, M.P. (1991) Sulfur inputs from rainfall throughout New Zealand. *New Zealand Journal of Agricultural Research* 34, 105–111.
Leng, R.A. (1990) Factors affecting the utilisation of 'poor-quality' forages by ruminants particularly under tropical conditions. *Nutrition Research Reviews* 3, 277–303.
Leng. R.A. (2014) Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. *Animal Production Science* 54, 519–543.
Liu, S.M. and Masters, D.G. (2000) Quantitative analysis of methionine and cysteine requirements for wool production of sheep. *Animal Science* 71, 175–185.
Loneragan, G.H., Wagner, J.J., Gould, D.H., Garry, F.B. and Thoren, M.A. (2001) Effects of water sulfate concentration on performance, water intake, and carcass characteristics of feedlot steers. *Journal of Animal Science* 79, 2941–2948,
Lovett, T.D., Coffey, M.T., Miles, R.D. and Combs, G.E. (1986) Methionine, choline and sulfate interrelationships in the diet of weanling pigs. *Journal of Animal Science* 63, 467–471.
Low, J.C., Scott, P.R., Howie, F., Lewis, M., Fitzsimons, J. and Spence, J.A. (1996) Sulfur-induced polioencephalomalacia in sheep. *Veterinary Record* 138, 327–329.
Lukuyu, B., Okike, I., Duncan, A., Beveridge, M. and Blümmel, M. (2014) Use of cassava in livestock and aquaculture feeding programs. *ILRI Discussion Paper 25.* International Livestock Research Institute, Nairobi.
MAFF (1990) UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.
Masters, D.G., Stewart, C.A., Mata, G. and Adams, N.R. (1996) Responses in wool and liveweight when different sources of dietary protein are given to pregnant and lactating ewes. *Animal Science* 62, 497–506.
Mata, G., Masters, D.G., Chamberlain, N.L. and Young, P. (1997) Production and glutathione responses to rumen-protected methionine in young sheep grazing dry pastures over summer and autumn. *Australian Journal of Agricultural Research* 48, 1111–1120.
McCann, J.C., Segers, J.R., Derakhshani, H., Felix, T.L, Khafipour, E. and Shike, D.W. (2017) Increasing corn distillers solubles alters the liquid fraction of the rumenal microbiome. *Journal of Animal Science* 95, *3540–3551.*
McClaren, R.G. (1975) Marginal sulfur supplies for grassland herbage in south east Scotland. *Journal of Agricultural Science, Cambridge* 85, 571–573.
McCollum, E.V. (1957) *A History of Nutrition*. Houghton Mifflin, Boston, Massachusetts.
McSweeney, C. and Denman, S. (2007) Effect of sulfur supplements on cellulolytic rumen micro-organisms and microbial protein synthesis in cattle fed a high fibre diet. *Journal of Applied Microbiology* 103, 1757–1765.
Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.
Moir, R.J. (1970) Implications of the N:S ratio and differential recycling. In: Muth, O.H. and Oldfield, J.E. (eds) *Sulfur in Nutrition – Symposium Proceedings*. AVI Publishing Company, Westport, Connecticut.
Morine, S.J., Drewnoski, M.E. and Hansen, S.L. (2014) Increasing dietary neutral detergent fiber concentration decreases ruminal hydrogen sulfide concentrations in steers fed high-sulfur diets based on ethanol coproducts. *Journal of Animal Science* 92, 3035–3041.
Morrison, M., Murray, R.M. and Boniface, A.N. (1990) Nutrient metabolism and rumen micro-organisms in sheep fed a poor quality tropical grass hay supplemented with sulfate. *Journal of Agricultural Science* 115, 269–275.
Motta, A.C.V., Araujo, E.M., Broadley, M.R., Young, S.D., Barbosa, J.Z., Prior, S.A. and Schmidt, P. (2020) Minerals and potentially toxic elements in corn silage from tropical and subtropical Brazil. *Revista Brasileira de Zootecnia* 49, e20190214. DOI: 10.37496/rbz492019021.
Neville, B.W., Lardy, G.P., Karges, K.K., Kirschten, L.A. and Schauer, C.S. (2011) Sulfur intake, excretion, and ruminal hydrogen sulfide concentrations in lambs fed increasing concentrations of distillers dried grains with solubles. *Sheep and Goat Research Journal* 26, 13–19.
NRC (2001) *Nutrient Requirements of Dairy Cows*, 5th edn. National Academy of Sciences, Washington, DC.
NRC (2007) *Nutrient Requirements of Horses*, 6th edn. National Academy of Sciences, Washington, DC.
NRC (2016) *Nutrient Requirements of Beef Cattle*, 8th edn. National Academy of Sciences, Washington, DC.
Ojeniyi, S.O. and Kayode, G.O. (1993) Response of maize to copper and sulfur in tropical regions. *Journal of Agricultural Science, Cambridge* 120, 295–299.
Ortolani, E.L. (2001) Sulphur deficiency in dairy calves reared on pasture of *Brachiaria decumbens*. *Ciência Rural* 31, 257–261.
Overton, T.R., LaCount, D.W., Cicela, T.M. and Clark, J.H. (1996) Evaluation of a ruminally protected methionine product for lactating dairy cows. *Journal of Dairy Science* 79, 631–638.
Pacheco-Rios, D., McNabb, W.C., Hill, J.P., Barry, T.N. and McKenzie, D.D.S. (1999) The effects of methionine supplementation upon milk composition and

production of forage-fed cows. *Canadian Journal of Animal Science* 79, 235–241.

Qi, K., Lu, C.D., Owens, F.N. and Lupton, C.J. (1992) Sulfate supplementation of Angora goats: metabolic and mohair responses. *Journal of Animal Science* 70, 2828–2837.

Qi, K., Lu, C.D. and Owens, F.N. (1993) Sulfate supplementation of growing goats: effects on performance, acid–base balance and nutrient digestibilities. *Journal of Animal Science* 71, 1579–1587.

Qi, K., Owens, F.N. and Lu, C.D. (1994) Effects of sulfur deficiency on performance of fiber-producing sheep and goats: a review. *Small Ruminant Research* 14, 115–126.

Qian, K., Xu, J., Zu, H.-C. and Cong, Y-Y. (2020) Research progress of rumen hydrogen sulfide production in ruminants. *Animal Science Journal* 91, e13349.

Raisbeck, M.F. (1982) Is polioencephalomalacia associated with high sulfate diets? *Journal of the American Veterinary Medical Association* 180, 1303–1305.

Revell, D.K., Baker, S.K. and Purser, D.B. (1999) Nitrogen and sulfur mobilised from body tissue can be used for wool growth. *Australian Journal of Agricultural Research* 50, 101–108.

Sarturi, J.O., Erickson, G.E., Klopfenstein, T.J., Rolfe, K.M., Buckner, C.D. and Luebbe, M.K. (2013a) Impact of source of sulfur on ruminal hydrogen sulfide and logic for the ruminal available sulfur for reduction concept. *Journal of Animal Science* 91, 3352–3359.

Sarturi, J.O., Erickson, G.E., Klopfenstein, T.J., Vasconcelos, J.T., Griffin, W.A. *et al.* (2013b) Effect of sulfur content in wet or dry distillers grains fed at several inclusions on cattle growth performance, ruminal parameters, and hydrogen sulphide. *Journal of Animal Science* 91, 4849–4860.

Sauvant, D., Perez, J.M. and Tran, G. (2004) *Tables INRA-AFZ de composition et de valeur nutritive des matières premières destinées aux animaux élevage*, 2nd edn. INRA Editions, Versailles, France.

Schmidt, F. and Monteiro, F.A. (2015) Sulphur supply affects morphology, growth and nutritional status of Tanzania Guinea grass and Mineirão stylo. *Grass and Forage Science* 70, 439–450.

Slyter, L.L., Chalupa, W. and Oltjen, R.R. (1988) Response to elemental sulfur by calves and sheep fed purified diets. *Journal of Animal Science* 66, 1016–1027.

Smart, M.E., Cohen, R., Christensen, D.A. and Williams, C.M. (1986) The effects of sulfate removal from the drinking water on the plasma and liver copper and zinc concentrations of beef cows and their calves. *Canadian Journal of Animal Science* 66, 669–680.

Smith, G.M. and White, C.L. (1997) A molybdenum–sulfur–cadmium interaction in sheep. *Australian Journal of Agricultural Research* 48, 147–154.

Smith, R.H. (1978) S-Methylcysteine sulphoxide: the brassica anaemia factor (a valuable dietary factor for man?). *Veterinary Science Communications* 2, 47–61.

Souri, M., Galbraith, H. and Scaife, J.R. (1998) Comparisons of the effects of genotype and protected methionine supplementation on growth, digestive characteristics and fibre yield in cashmere-yielding and angora goats. *Animal Science* 66, 217–223.

Spears, J.W., Burns, J.C. and Hatch, P.A. (1985) Sulfur fertilisation of cool season grasses and effect on utilisation of minerals, nitrogen and fibre by steers. *Journal of Dairy Science* 68, 347–355.

Spears, J.W., Lloyd, K.E. and Fry, R.S. (2011) Tolerance of cattle to increased dietary sulfur and effect of dietary cation-anion balance. *Journal of Animal Science* 89, 2502–2509.

Stein, H.H., Sève, B., Fuller, M.F., Moughan, P.J. and de Lange, C.F. (2007) Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. *Journal of Animal Science* 85, 172–180.

Sun, X.Z., Waghorn, G.C., Hoskin, S.O., Harrison, S.J., Muetzel, S. and Pacheco, D. (2012) Methane emissions from sheep fed fresh brassicas (Brassica spp.) compared to perennial ryegrass (Lolium perenne). *Animal Feed Science and Technology* 176, 107–116.

Suttle, N.F. (1991) Mineral supplementation of low quality roughages. In: *Proceedings of Symposium on Isotope and Related Techniques in Animal Production and Health*. International Atomic Energy Agency, Vienna, pp. 101–114.

Suttle, N.F., Jones, D.G., Woolliams, C. and Woolliams, J.A. (1987) Heinz body anaemia in lambs with deficiencies of copper and selenium. *British Journal of Nutrition* 58, 539–548.

Thomas, M.R.M., Moir, J.L. and Edwards, G.R. (2016) Grazing and soil fertility effect on naturalized annual clover species in New Zealand high country. *Rangeland Ecology and Management* 69, 444–448.

Thomas, W.E., Loosli, J.K., Williams, H.H. and Maynard, L.A. (1951) The utilisation of urea and inorganic sulfates by lambs. *Journal of Nutrition* 43, 515–523.

Underwood, E.J., and Suttle, N.F. (1999) Sulphur. In: *Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Vanness, S., Klopfenstein, T.J., Erickson, G.E. and Karges, K. (2009) Sulfur in distillers grains. *Nebraska Beef Cattle Reports 538*. University of Nebraska, Lincoln, Nebraska.

Wang, Y., Waghorn, G.C., McNabb, W.C., Barry, T.N., Hedley, M.J. and Shelton, I.D. (1996) Effect of condensed tannins upon the digestion of methionine and cysteine in the small intestine of sheep. *Journal of Agricultural Science, Cambridge* 127, 413–421.

Weston, R.H., Lindsay, J.R., Purser, D.B., Gordon, G.L.R. and Davis, P. (1988) Feed intake and digestion

responses in sheep to the addition of inorganic sulfur to a herbage diet of low sulfur content. *Australian Journal of Agricultural Research* 39, 1107–1119.

Whanger, P.D. (1972) Sulfur in ruminant nutrition. *World Review of Nutrition and Dietetics* 15, 225–237.

White, C.L., Kumagai, H. and Barnes, M.J. (1997) The sulfur and selenium status of pregnant ewes grazing Mediterranean pastures. *Australian Journal of Agricultural Research* 48, 1081–1087.

Whittle, P.J., Smith, R.H. and McIntosh, A. (1976) Estimation of S-methyl sulphoxide (kale anaemia factor) and its distribution among brassica and forage crops. *Journal of Science in Food and Agriculture* 27, 633–642.

Wilkes, J., Cowley, F., Hegarty, R. and Jewell, M. (2019) *Survey of Australian Feedlot Drinking Water Quality*. Project B.FLT.1005. Meat and Livestock Australia Ltd, North Sydney, Australia.

Williams, A.J., Robards, G.E. and Saville, D.G. (1972) Metabolism of cystine by Merino sheep genetically different in wool production. II. The responses in wool growth to abomasal infusion of L-cystine and DL-methionine. *Australian Journal of Biological Science* 25, 1269–1276.

Wu, H., Meng, Q. and Yu, Z. (2016) Evaluation of ferric oxide and ferric citrate for their effects on fermentation, production of sulfide and methane, and abundance of select microbial populations using *in vitro* rumen cultures. *Bioresource Technology* 211, 603–609.

Zain, M., Jamarun, N. and Zulkarnaini (2010) Effect of phosphorus and sulphur supplementation in growing beef cattle diet based on rice straw ammoniated. *Asian Journal of Scientific Research* 3, 184–188.

Zinn, R.A., Aloarez, E., Mendez, M., Montano, M., Ramirez, E. and Shen, Y. (1997) Influence of dietary sulfur level on growth, performance and digestive function in feedlot cattle. *Journal of Animal Science* 75, 1723–1728.

10 Cobalt

Introduction

The essentiality of cobalt in the forages of sheep and cattle emerged from investigations of two naturally occurring disorders: 'coast disease' of sheep in South Australia (Lines, 1935; Marston, 1935) and 'wasting disease' of cattle in Western Australia (Underwood and Filmer, 1935). In both situations, pastures had underlying calcareous soils of Aeolian origin. A condition called 'cobalt pine' was concurrently recognized in sheep on similar soils, known as 'the Machairs', in NW Scotland (Suttle, 1988). Before long, similar severe disorders were recognized in other areas.[i] All described a syndrome with anaemia and failure to thrive, preventable by cobalt supplementation, although geological associations differed. The mode of action of cobalt became clearer when an 'anti-pernicious anaemia factor', subsequently designated vitamin B_{12}, was found to contain Co (Smith, 1948). Cobalt deprivation in ruminants was eventually attributed to a failure of rumen microorganisms to synthesize sufficient vitamin B_{12} to meet the needs of the host and cured by parenteral injections of vitamin B_{12} (Smith *et al*., 1951). Hind-gut fermenters such as the horse were not affected because absorption of B_{12}, synthesized in the caecum, was invariably sufficient to meet needs that were probably lower than those of ruminants (Davies, 1979; Lewis *et al*., 1995). Pigs and poultry obtain their vitamin either pre-formed in food or indirectly by ingesting faeces. There is no evidence that any species requires cobalt other than as vitamin B_{12} and this chapter will again deal almost exclusively with ruminants, whose symbiotic rumen microbial population also needs vitamin B_{12} to produce propionate. Paradoxically, failure of propionate synthesis may create additional problems for the host. There have been no major new findings in ruminants since the last edition but more coherent pathogeneses and diagnoses are presented. Progress has been hindered by failure to appreciate that for decades, B_{12} status has been assessed with an assay (for serum B_{12}) that generally measured different things in sheep as opposed to cattle.

Functions of Vitamin B_{12}

Rumen microbes incorporate Co into vitamin B_{12}, a large molecule with formula $C_{63}H_{88}N_{14}O_{14}PCo$, weight of 1357 daltons and containing 4.4% Co. The corrin (tetrapyrrole) ring structure of the vitamin (cobalamin, Cbl) has physiologically useless analogue molecules (cobinamides), also synthesized in the rumen. The essentiality of cobalt for mammals and microbes is linked to two distinct forms of Cbl, formed by attachment of α ligands that project above the plane of the ring to produce adenosylcobalamin (AdoCbl) and methyl-cobalamin (MeCbl). Dimethylbenzimidazole, a β ligand, projects below the plane and distinguishes Cbl from cobinamides, which have various β ligands (Bannerjee, 1999).

Succinate–propionate exchange

Intermediary energy metabolism requires AdoCbl to keep the tricarboxylic acid cycle turning: AdoCbl assists methylmalonyl-coenzyme A (CoA) mutase to form succinate from propionate, thus facilitating gluconeogenesis, chiefly in the liver (Fig. 10.1). Microbes are equally dependent on AdoCbl, but for the reverse purpose: *Propioni* bacteria form propionate from succinate, a reaction catalysed by the same mutase. Several aminomutases, including leucine 2,3 mutase are also AdoCbl-dependent.

Methylation

Methionine synthase uses MeCbl to transfer methyl groups from 5-methyltetrahydrofolate

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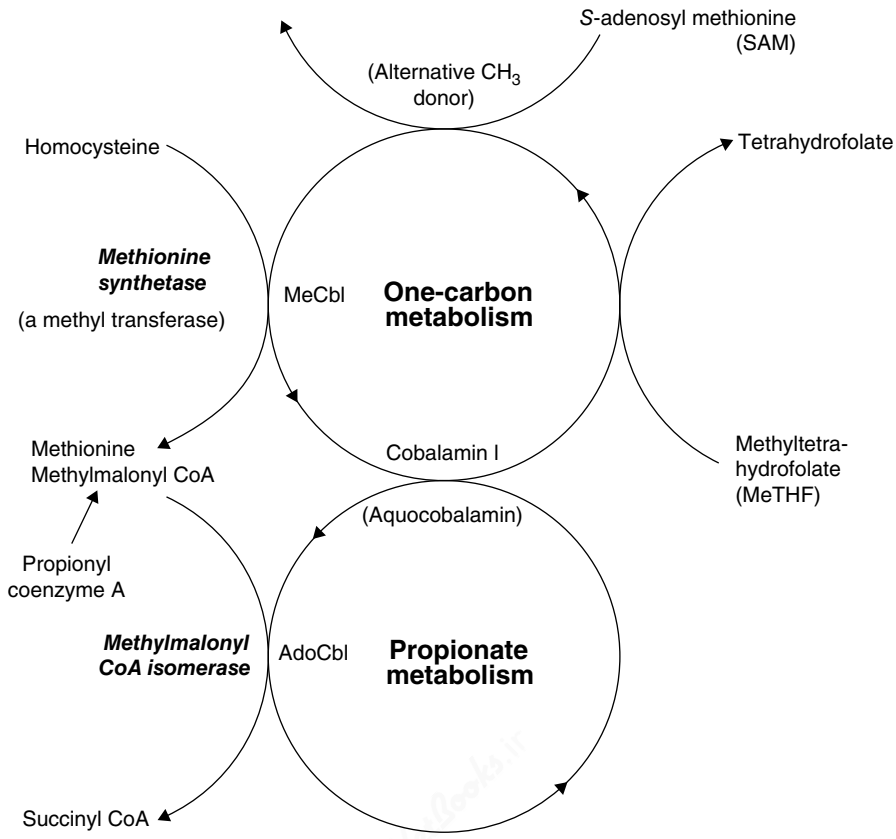

Fig. 10.1. Vitamin B_{12} (cobalamin, Cbl) has two distinct yet linked coenzyme functions: elaborating carbon skeletons via methyl-cobalamin (MeCbl) (the one-carbon pathway) and gluconeogenesis from propionate via adenosyl-cobalamin (AdoCbl) (the propionate pathway): inadequacies are indicated by accumulation of two abnormal metabolites methylmalonyl acid and formiminoglutamic acid, respectively. Rumen microbes use AdoCbl to convert succinate to propionate and failure is indicated by accumulation of succinate in the rumen.

to homocysteine via *S*-adenosyl methionine (SAM), regenerating methionine and tetrahydrofolate in the process (Fig. 10.1). MeCbl thus plays a pivotal role in one-carbon metabolism, building up carbon 'skeletons', facilitating synthesis of nucleotides and many other molecules including formate, noradrenaline, myelin and phosphatidyl ethanolamine (Bassler, 1997). While interest has focused on the hepatic activity of methionine synthase, enzyme activity is three times higher in intestinal mucosa and spleen than in liver (Lambert *et al*., 2002). MeCbl is also required by rumen microbes for methane, acetate and methionine synthesis (Poston and Stadman, 1975).

Clinical Consequences of Cobalt Insufficiency

Ill-thrift and anaemia

The earliest visible abnormality in sheep is excessive lachrymation, which mats the fleece below the eye: the precise cause is unknown. The whole fleece or hair coat usually appears dull, limp and scruffy. Loss of hair colour has been reported in cattle (Judson *et al*., 1982). Progressive anorexia leads to marked weight loss, listlessness, muscular wasting (marasmus) and pica (depraved appetite) but the appearance is indistinguishable from that caused by starvation. Cobalt deprivation was claimed to lower feed digestibility in goats (Kadim *et al*.,

2003) but the protocol was unusual.[ii] Wool growth was more sensitive to lack of dietary Co than liveweight gain (Masters and Peter, 1990) and less efficient after prolonged deprivation in lambs, fed little more than a maintenance ration (Wiese *et al*., 2007). A normocytic and normochromic anaemia eventually develops, visible mucous membranes appear blanched and the skin pale and fragile. Mortality rates can be as high as 30% (Grace and Sinclair, 1999). Internal examination reveals little or no visible fat, heavy haemosiderin deposits in spleen and a hypoplastic bone marrow. Neurological damage may occur (Fell, 1981).

Fatty liver diseases

White-liver disease (WLD) was first reported in grazing lambs at times of prolific pasture growth (Sutherland *et al*., 1979) and was often accompanied by clinical signs of photosensitization and biochemical indications of liver damage. The disease was produced experimentally by feeding lambs a whole barley diet, low in Co (Kennedy *et al*., 1994) and progressed from mild fatty infiltration of hepatocytes to a point where bile ducts proliferated, enzymes leaked into the bloodstream from damaged liver cells and plasma bilirubin rose (Kennedy *et al*., 1997). Affected lambs were also anorexic, anaemic and failed to thrive. The disease is sometimes accompanied by polioencephalomalacia and preventable by either Co or B_{12} supplementation (Sutherland *et al*., 1979; McLoughlin *et al*., 1986). A few cases of WLD were reported in Angora goats in New Zealand (Black *et al*., 1988) and 5.3% of caprine livers were affected in an abattoir survey in Oman (Johnson *et al*., 1999).[iii]

Infertility and perinatal mortality

While infertility may be a secondary consequence of any debilitating condition, apparently heathy ewes deprived of Co during early pregnancy gave birth to fewer lambs than normal (Duncan *et al*., 1981; Quirk and Norton, 1987): those born may be either dead or slow to suck and die prematurely (Fisher and MacPherson, 1991). Anorexia in the mother was not a predisposing factor. Acute impairment of gluconeogenesis at a time of high glucose need around parturition was implicated in the latter study. Depression of milk yield can reduce growth rate of surviving lambs (Quirk and Norton, 1987). Newborn calves are less affected by Co deprivation of their dams, although growth can be depressed (Quirk and Norton, 1988). In a study of beef cows, five out of nine unsupplemented cows failed to conceive compared to only two or three in each of three Co-supplemented groups of 9 to 11 cows (Judson *et al*., 1997).

Disease susceptibility

Increased susceptibility to infection by the abomasal parasite *Ostertagia circumcincta* was reported in lambs (Ferguson *et al*., 1989). Immune responses of cattle to a similar parasite (*O. ostertagi*) were compromised by low dietary Co (MacPherson *et al*., 1987). Faecal egg counts were raised by a natural, mixed nematode infection in grazing Texel lambs with low plasma B_{12} (<100 pmol l^{-1}) (Vellema *et al*., 1996). Resistance to infection by abomasal parasites may be particularly pronounced because these nematodes suppress appetite and destroy the glands that produce intrinsic factor (IF), needed for B_{12} absorption. However, infection with *Trichostrongylus vitrinus* and *O. circumcincta* did not accelerate a decline in plasma B_{12} in grazing lambs (Suttle *et al*., 1986). Susceptibility to *Mycobacterium avium* and bovine herpes infection was not increased by lack of Co (Vellema *et al*., 1996). Although *in vitro* lymphoproliferative responses were increased by Co supplementation, cultured lymphocytes can show a tenfold increase in vitamin B_{12} turnover under such challenges (Quadros *et al*., 1976), calling into question their physiological significance.

Occurrence of Cobalt Insufficiency

Geographical distribution

Severe cobalt deprivation is recognized in many countries (p. 229) but much larger areas exist in which deprivation is mild or marginal and manifested as unsatisfactory growth in 'tail-end' lambs. Cobalt deprivation arises on well-drained soils of diverse geological origin, including coarse volcanic pumice soils of New Zealand (Suttle, 1988), leached podzolized sands of Jutland, sandy loams of Goatland in Sweden (Schwan *et al*., 1987), soils derived from granites and ironstone gravels, as well as on calcareous, wind-blown shell sands that featured in the earliest studies (p. 229). A nationwide survey of minerals in New Zealand pastures reported a median value of 0.1mg Co kg^{-1}DM, the

concentration sheep were thought to require (Knowles and Grace, 2014). High levels of soil Mn depress availability of soil Co to plants and risk of insufficiency may be predicted from equations that relate soil available Co to soil Mn (Suttle *et al*., 2003; Li *et al*., 2004). Cobalt deprivation may be induced by heavy liming, which reduces availability of soil Co to plants, but extremely acid soils (e.g. pH 5.0) can also be low in available Co (Suttle *et al*., 2003). Geochemical mapping of areas of risk has been advocated (Suttle, 2000). Incidents of WLD are less common than 'ill-thrift' but have been reported in lambs in Northern Ireland, Holland and Norway (for review see Suttle, 1988) and a variant may occur in Omani goats (Johnson *et al*., 1999; 2004).

Effects of species, gender and year

Young deer (Clark *et al*., 1986) and goat kids (Clark *et al*., 1987) – like calves and steers – are less likely to show retarded growth than lambs: 68–72% of untreated lambs had to be withdrawn from two New Zealand trials because of substantial weight loss (Grace *et al*., 2003; Gruner *et al*., 2004c). The high vulnerability of sheep and angora goats may be attributable to the high requirements of methionine for fleece growth (see p. 216). Incidence in lambs can vary markedly from year to year on the same property (Lee, 1951), young male lambs within a flock being particularly vulnerable (Shallow *et al*., 1989). Variations in soil Co ingestion, under drought conditions that limit growth, may also be important (Grace, 2006). Disorders occur less often in sheep grazing long than short pasture because less soil is ingested (Andrews *et al*., 1958).

Effects of physiological state and energy substrate

The main sources of energy for ruminants are acetic and propionic acids, produced by rumen fermentation: acetate synthesis is favoured by roughage diets, propionate by cereal-rich diets. Microbial needs for Co, as Adocbl to synthesize propionic acid, increase as readily fermentable carbohydrate content of the diet increases. In lactation, increased energy needs are met partly from endogenous sources (fat), while the increased need for methionine must be met largely from the diet, shifting requirement from the Adocbl to Mecbl pathway. In Norway, incidence of ovine white-liver disease (OWLD) varied between groups on adjacent fields on the same farm and was more closely related to high pasture fructosan than pasture Co, which was relatively high (0.10–0.12mg kg^{-1}DM) (Ulvund, 1995). Pasture fructosan concentrations are increased by sudden decreases in air temperature and may raise rumen propionate, precipitating disorder, or escape rumen degradation, thus limiting rumen synthesis of vitamin B_{12}. Diets rich in readily fermented carbohydrate (e.g. molasses, flaked maize and maize silage) may increase risk of cobalt deprivation, particularly in sheep).

Biochemical Consequences of Cobalt Deprivation

The description and interpretation of abnormalities caused by lack of dietary Co requires an initial understanding of what is normal in the symbiotes.

Normality

Rumen and plasma B_{12} increase exponentially to an early plateau in response to bolus doses of Co in sheep (e.g. Suttle *et al*., 1989) and continuous supplementation can increase serum B_{12} to very high concentrations (Grace and West, 2000) but

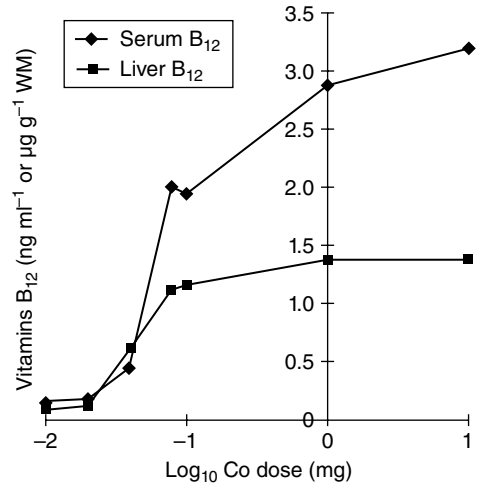

Fig. 10.2. When cobalt-deprived sheep were continuously repleted with diets providing 0.1 to 10 mg Co day^{-1}, vitamin B_{12} concentrations reached an earlier plateau in liver than in serum, suggesting limits to hepatic storage capacity. (Data from Marston, 1970)

liver B_{12} was barely increased during prolonged 100-fold daily supplementation above a 'marginal' intake (0.1mg Co day^{-1}) (Fig. 10.2): similar effects were reported in cattle, when efforts were made to extract all B_{12}, firmly bound to haptocorrins (Strangl *et al*, 2000c). Ruminants are normally well-supplied with Co but this is not reflected by high liver B_{12} reserves: like other water-soluble vitamins, B_{12} is poorly stored in microbial and host tissues. Absorption and metabolism of microbial B_{12} is facilitated by a trio of structurally related, Cbl-transporting proteins: haptocorrins, intrinsic factor (IF) and transcobalamins (TC I and II) (NICE, 2015). Cobalamins leave the rumen either as dead microbes or bound to salivary haptocorrin but are released during subsequent digestion and bound to IF, produced by parietal cells in the abomasum (McKay and McLery, 1981).[iv] Selective endocytosis discriminates against other corrinoids, produced by the microbes, of which only a small fraction of synthesized B_{12} is absorbed. Cbl:IF complexes are bound to TCII in the mucosal villi, exported into the bloodstream (Kozyraki and Cases, 2013) and become bound to specific receptors on cell surfaces. Inside cells, TCII:Cbl complexes are converted to either MeCbl in the cytosol or AdoCbl in mitochondria (NICE, 2015). The two pathways overlap (Riedel *et al*., 1999) during intracellular recycling (Fig. 10.1) and biliary secretion. Transcobalamins differ in distribution and binding properties between mammalian species.[vi] There is usually excess vitamin B_{12}-binding capacity in plasma. Little is known about B_{12} excretion in ruminants but in other species, the kidney plays a primary role in conserving the vitamin: biliary secretion and urinary loss can be small. TCII is strongly expressed in kidney cortex and participates in tubular reabsorption, with the assistance of the lipoprotein megalin (Kozyraki and Cases, 2013).

- The kidney is both the primary regulator of plasma Cbl concentrations and an important lysosomal storage site for vitamin B_{12} in non-ruminants.

In dairy cows, high Co intakes (17.8–31.0 mg day^{-1}) increased urinary vitamin B_{12} to peak values of around 12 μg l^{-1} during lactation, higher than those found in milk and serum (Walker and Elliot, 1972). The predominance of urinary over faecal excretion in disposing of excess B_{12} is a characteristic of all water-soluble vitamins.

Depletion

Cobalt can appear to behave like a Type 1 mineral (p. 40), when measured as plasma vitamin B_{12} in showing an early reduction. However, it can take months to reduce high plasma B_{12} to marginal concentrations in adult sheep (Field *et al*., 1988) and retard growth (Table 10.1). Liver B_{12} does not show an early decrease in sheep (Marston, 1970; Field *et al*., 1988) and poor hepatic storage leaves sheep vulnerable to Co insufficiency. The unresponsiveness of plasma as well as liver B_{12} in cattle (e.g Tiffany *et al*., 2003) is probably illusory and attributable to failure to detect haptocorrin-bound B_{12}, as explained later (p. 239).

- Plasma may serve as a storage pool for B_{12} in both cattle and sheep.

Storage capacity of the kidney has not been studied but may be significant. Placental transfer of B_{12} is inefficient, liver B_{12} in the ovine fetus being less than half of concentrations found in the mother (Grace *et al*., 1986) and not greatly increased by

Table 10.1. Growth rate was not reduced in weaned lambs until a ground oaten hay/lupinseed meal diet, containing <0.026mg Co kg^{-1}DM, had been fed for more than eight weeks compared to those given 0.1 mg Co daily by drench (Plus Co): it took over 11 weeks deprivation to reduce appetite and wool growth (Wiese *et al*. (2007).

	Liveweight gain[a]		Food intake[b]		Wool growth rate	
	Low Co	Plus Co	Low Co	Plus Co	Low Co	Plus Co
Depletion (days)	(kg)		(g day^{-1})		(mg $100cm^{-2}$)	
56–77	1	1.5	780^{AL}	780^{AL}	58 ± 2.5	59 ± 2.3
78–105	–2	–1	550^{AL}	550^{PF}	53 ± 2.3	74 ± 3.0

[a]Approximate figures estimated from graph.
[b]Both groups were fed *ad libitum* (AL) initially, but the food intake of the 'plus Co' group was restricted by pair feeding (PF) to match the AL intake of the 'low Co' group after 78 days.

injecting vitamin B_{12} during pregnancy (Grace *et al*., 2006).

Deficiency

The rumen microflora can show abnormality before the host. A derease in rumen liquor B_{12} can be accompanied by an immediate increase in rumen succinate on energy-rich diets, low in Co: plasma B_{12} declines while plasma succinate increases (Fig. 10.3). At the cellular level in the host, uptake of TCII-Cbl by the liver probably increases delaying a decrease in liver B_{12}.[vii] Secretion of B_{12} in milk decreases in ewes (Hart and Andrews, 1959; Gruner *et al*., 2004a) and cows, declining exponentially as serum B_{12} falls to subnormal levels in the latter (Judson *et al*., 1997). Given the regulatory role of the kidney (p. 233).

- Urinary B_{12} probably falls to negligible levels and may indicate low B_{12} status.

Dysfunctional propionate metabolism

In early depletion studies with sheep, maintained on hay, dysfunction was thought to occur along the AdoCbl pathway. Clearance of propionate from the blood eventually slowed, methylmalonic acid (MMA) accumulated and there was a correlated decrease in appetite (Marston *et al*., 1961, 1972; Gawthorne, 1968). Raised propionate concentrations in portal blood flow induce satiety signals (Farningham *et al*., 1993) that reduce food intake. In a similar, subsequent depletion study with hay, priority was given to the MeCbl pathway, which became dysfunctional later, while appetite remained normal (Fig. 10.4). Onset of dysfunction was much faster in lambs depleted on a whole barley diet that preserves a normal rumen pH in sheep. Growth was immediately slowed and accompanied by large increases in rumen and plasma succinate (Fig. 10.3). Plasma MMA and succinate also increased in grazing lambs (Kennedy *et al*., 1996) and weaner lambs given a ground 3:1 blend of an oaten hay/lupin seed meal diet (Wiese *et al*., 2007); inhibition of propionate-producing microbial species, such as *Selenomonas ruminantium*, was implicated. Plasma MMA showed little change in Co-deprived steers (<6 μmol l^{-1}) on roughage (Stangl *et al*., 2000c) or barley (Kennedy *et al*., 1995) and corn-based diets Tiffany *et al*., 2003; Tiffany and Spears, 2005). Whole grain passes straight through the bovine rumen and must be comminuted to provide fermentable carbohydrate for cattle: more microbial protein leaves the rumen when cattle are given ground barley than ground corn (Yang *et al*., 1997) and rumenal B_{12} synthesis may be poorer on corn-based diets. The metabolic effects of excess succinate have not been studied in ruminants but may be harmful, despite not requiring AdoCbl.

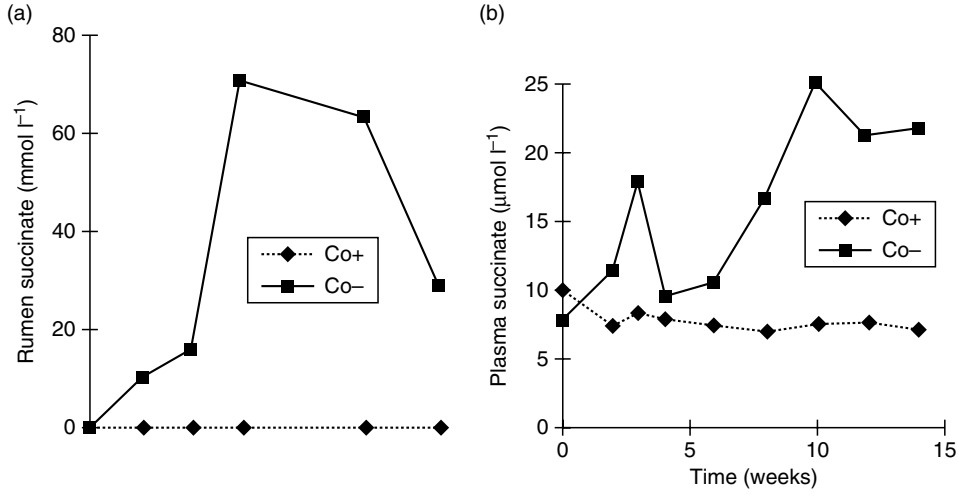

Fig. 10.3. Lambs given a barley-based diet very low in cobalt show marked early rises in rumen succinate followed by increases in plasma succinate, a glucose precursor: this may partially overcome any constraint upon glucogenesis, due to impaired propionate metabolism in the liver. (From Kennedy *et al*., 1991b)

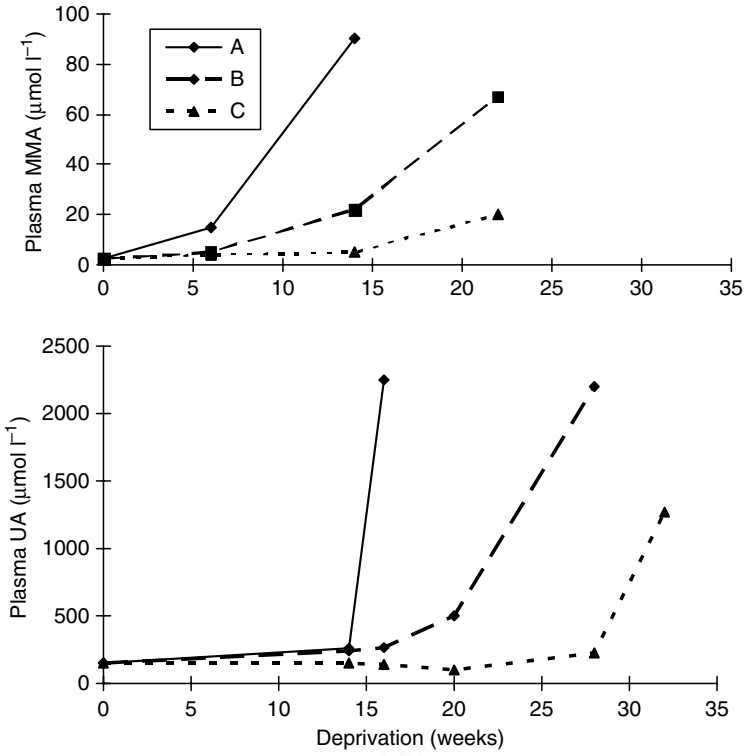

Fig. 10.4. Onset of biochemical dysfunction occurred on the adenosyl cobalamin pathway before the MeCbl pathway in three non-pregnant ewes, given hay low in Co, as indicated by earlier rises in plasma methylmalonyl acid (MMA) than urocanate (UA), an indicator of methyl cobalamin dysfunction (Price, 1991).

- Of particular relevance are inhibition of ketone body utilization by succinate and effects on generation of reactive oxygen species (Tretter *et al.*, 2016), which may cause fat to accumulate and peroxidase in liver.
- Succinate features at the crossroads of several metabolic pathways.

Dysfunctional methionine synthesis

Lambs on the whole-barley diet eventually lost weight when plasma homocysteine (Hcy) increased (Fig. 10.5): final reduction in methionine synthase activity in certain tissues (Table 10.2) confirmed lack of MeCbl. Wool growth was sustained while body weight was lost between 56 and 77 days on a mixed diet but decreased thereafter, again indicating failure along the MeCbl pathway (Table 10.1). Wool growth rates were probably 7–8 g day^{-1} and Fig. 9.2 suggested that >2g SAA day^{-1} was being deposited in the fleece. A similar sequence was described in steers, deprived of Co on a diet based on corn silage (Stangl *et al.*, 2000c) in which concurrent increases in SAM and SAM:*S*-adenosyl Hcy ratio in liver confirmed a reduction in methylation (see Fig. 10.1). MeCbl acts as a methyl donor in folate metabolism (Scott, 1992) and liver folate decreases in sheep (Gawthorne and Smith, 1974) and steers, deprived of Co (Stangl *et al.*, 2000b, c). Increased urinary excretion of formiminoglutamic acid (FIGLU) also indicated reduced methylation of folate (Gawthorne, 1968). Most of the Cbl in milk is present as MeCbl, transferred with the aid of megalin, and mammary secretion of B_{12} may suffer as priority is given to AdoCbl during the synthesis of energy-rich milk. Accumulation of phosphatidyl choline in liver and brain indicates reduced phosphatidyl ethanolamine methyltransferase activity in sheep (Gawthorne and Smith, 1974) (Table 10.2) but not in growing cattle, lacking dietary Co (Kennedy *et al.*, 1995): cattle need less methionine than sheep.[viii]

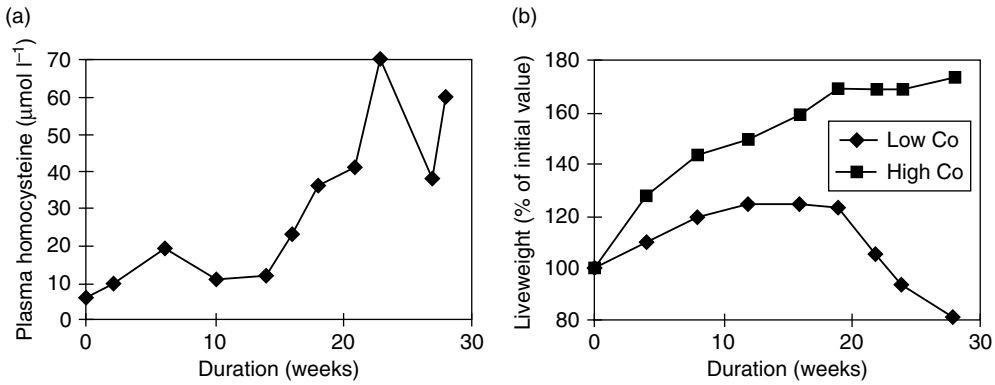

Fig. 10.5. Liveweight gain in lambs deprived of cobalt on a barley diet is a mirror image of concurrent changes in plasma homocysteine. (From Kennedy *et al*., 1992)

Table 10.2. Biochemical markers of cobalt deprivation indicate abnormal lipid and vitamin E metabolism in the livers of lambs with ovine white-liver disease (OWLD) compared with cobalt-sufficient controls (Kennedy *et al*., 1994). Data are mean ± standard error of the mean for three (OWLD) or four (control) lambs.

Marker	Control	OWLD
Vitamin B_{12} (pmol g^{-1})	396 ± 9.7	15 ± 4.1
Vitamin E (nmol g^{-1})	13.1 ± 0.94	5.9 ± 0.56[a]
MMCoA mutase (U g^{-1})	30.8 ± 2.2	4.4 ± 0.6
Methionine synthase (U g^{-1})	869 ± 102	105 ± 26
SAM:SAH	5.4 ± 0.76	2.5 ± 0.68
PC:PE	1.5 ± 0.04	1.1 ± 0.09
Total lipid (g kg^{-1} FW)	50.8 ± 8.0	165 ± 13.9
Triglyceride fatty acids (mg kg^{-1} FW)	5.2 ± 1.00	36.8 ± 14.9
Free fatty acids (mg g^{-1} FW)	2.9 ± 0.80	10.5 ± 1.80

MMCoA, methylmalonyl coenzyme A; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; SAH, *S*-adenosyl homocysteine; SAM, *S*-adenosyl methionine.
[a] Dilution effect of the accumulated fat.

Abnormal lipid metabolism

Major defects in lipid metabolism, involving both Cbl-dependent pathways, may affect the pathogenesis of OWLD in lambs on barley diets (Table 10.2). Accumulating MMA CoA may inhibit β-oxidation of free fatty acids, when fat is mobilized to offset loss of appetite: MMA becomes mis-incorporated into branched-chain fatty acids (BCFA) on diets favouring propionate synthesis (Duncan *et al*., 1981; Kennedy *et al*., 1991a).[ix] Accumulation of free fatty acids normally increases hepatic synthesis of triglycerides and their export as very-low-density lipoprotein (VLDP) but the latter requires MeCbl and methionine synthase, both reduced by lack of Co (Table 10.2). Triglycerides therefore accumulate and may end up as lipofucsin, a characteristic histological anomaly found in sheep that die due to lack of Co.[x]

Rate-limiting function

From the foregoing review, lack of dietary Co can restrict performance in sheep and cattle by one of several pathways:

- AdoCbl deficiency in rumen microbes, causing succinate toxicity in the host;
- AdoCbl deficiency in the host, causing propionate toxicity;
- MeCbl deficiency inhibiting methylation in the host; and
- MeCbl deficiency impairing lipid metabolism by multiple pathways.

The critical rate-limiting pathway is probably affected by:

- the predominant dietary energy source;
- the nature and rate of productivity;
- the duration of deprivation.

In suckled lambs and calves deprived of B_{12}, growth can be retarded before plasma MMA increases (Quirk and Norton, 1987; 1988) and there are two possible explanations: either performance was limited by effects of deprivation on the dam's milk yield or, with lactose as the energy source, priority is given to the AdoCbl

pathway and the MeCbl pathway in lambs has become limiting. In fast-growing, weaned lambs on diets rich in soluble carbohydrate, from cereal (Fig. 10.3) or lush pasture, AdoCbl becomes limiting to microbes but the host may be restricted as succinate accumulates. In slow-growing lambs, lacking Co from a roughage-based diet, microbial dysfunction was ruled out because appetite was rapidly restored by parenteral B_{12} (Wiese *et al*., 2007). Early increases in plasma MMA (to 30 mmol l^{-1}) were tolerated but weight loss was again associated with a rise in plasma Hcy, as in Fig. 10.5, indicating lack of MeCbl pathway, eventually restricting wool growth (Table 10.1). In non-pregnant ewes, given hay, low in Co (0.03 mg kg^{-1}DM), (Fig. 10.4) and in pregnant ewes (Fisher and MacPherson, 1991; Gruner *et al*., 2004c), plasma MMA can increase before appetite and performance are adversely affected: dysfunction along the AdoCbl pathway is tolerable. With onset of lactation, plasma MMA can increase without accompanying reductions in serum or liver vitamin B_{12}, probably induced by higher propionate turnover (Gruner *et al*., 2004c), and may become health-limiting. Possible impacts of metabolic load and animal species on biochemical lesion are illustrated, schematically, in Fig. 10.6. The embodied concept of linked variations in the two Cbl-dependent enzymes was advanced in studies of cultured human glioma cells (Riedel *et al*., 1999).

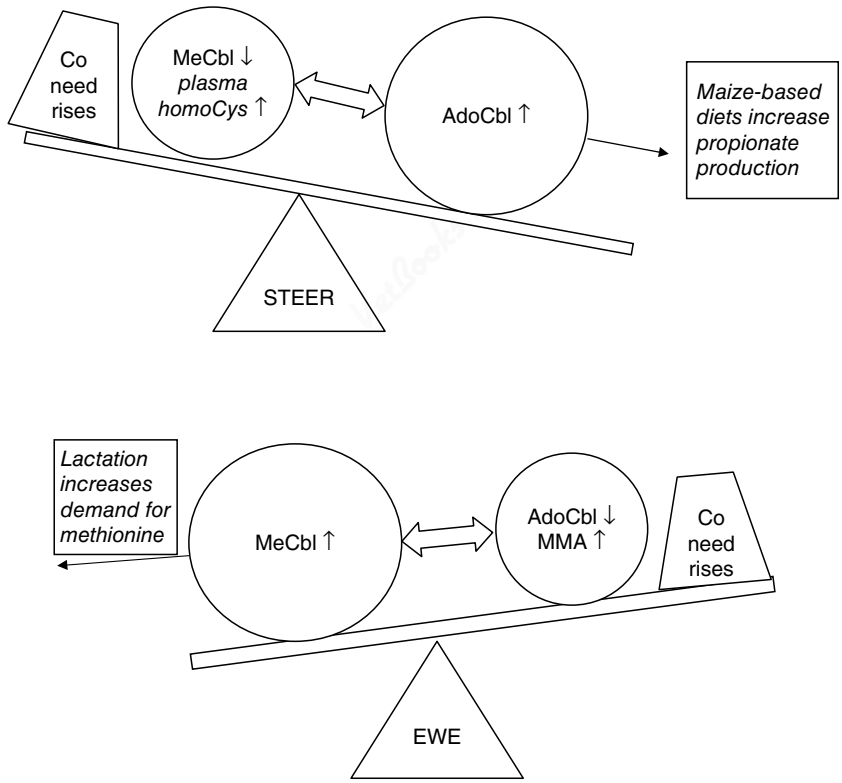

Fig. 10.6. A 'propionate load hypothesis' to explain contrasting metabolic responses of sheep and cattle to cobalt deprivation, based on Fig. 10.1, and an assumption that the balance struck between methyl-cobalamin (MeCbl) and adenosyl-cobalamin (AdoCbl) pathways may be set to favour MeCbl in sheep and AdoCbl in cattle. Dietary or physiological factors that increase activity along the favoured pathway cause dysfunction along the unfavoured pathway: a diet that favours propionate production in cattle will raise plasma homocysteine (Hcy) rather than methylmalonic acid (MMA), while increased demand for methionine associated with onset of lactation in sheep will increase in plasma MMA rather than Hcy.

There is no evidence that lack of AdoCbl constrains either rumen microbes or host in cattle. Rumen succinate was higher and propionate lower than with barley rather than corn supplying a low-Co diet but growth was not retarded (Tiffany and Spears, 2005). Both cereals were fed in ground form and would have resulted in a low rumenal pH and fast digesta outflow, compared to lambs given whole barley. Beef cattle required more Co (0.16 ± 0.01 versus 0.12 ± 0.003 mg kg^{-1} DM) from a corn silage ration, to minimize plasma Hcy than MMA. Plasma MMA was only marginally increased: growth was eventually retarded and liver folate reduced, indicating lack of MeCbl (Schwarz *et al*., 2000; Stangl *et al*., 2000b, c). Anomalies in lipid metabolism were barely evident in Co-deprived cattle given barley (Kennedy *et al*., 1995).

Diagnosis of Cobalt Insufficiency

The basis for interpreting diagnostic criteria is held in Fig. 10.7. Mild deprivation is impossible to diagnose clinically because the unthrifty appearance is indistinguishable from nematodiasis, starvation and lack of digestible nutrients. A number of biochemical criteria are used to aid diagnosis but few are definitive. Although dietary Co has a major influence on B_{12} synthesis and status of the host, other factors determine incidence of disorder, which can vary between years on the same low-Co pasture (Lee, 1951) and between adjacent pastures of similar Co content (Ulvund, 1995). With the added complication of contaminant soil Co, herbage Co is of little value in diagnosis. Liver Co broadly reflects Co intake[xi] but is inferior to criteria related to the functional vitamin.

Liver and kidney vitamin B_{12}

Low liver B_{12} reflects suboptimal Co intake (Fig. 10.2) but considerations of welfare and cost have limited the use of biopsies to obtain samples. Early guidelines were that individual values of 81–140 were 'borderline' and <74 nmol kg^{-1}FW 'low' in sheep

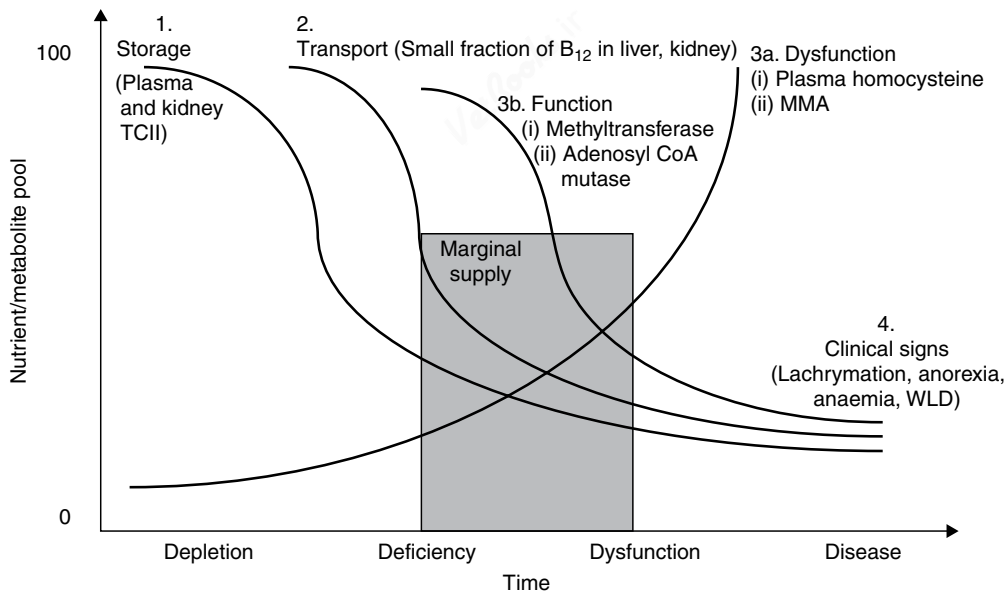

Fig. 10.7. Sequence of biochemical changes preceding clinical symptoms in ruminants deprived of cobalt (and hence vitamin B_{12}). Early decreases in total plasma B_{12} reflect reductions in a storage component (bound to transcobalamin II) and are probably followed by decreases in the transport component (transcobalamin I). Signs of metabolic dysfunction (Fig. 10.3) can accompany reduced performance but precede clinical abnormalities: the rate-limiting pathway, relying on adenosyl- (AdoCbl) or methyl (MeCbl) cobalamin, is partly determined by metabolic load (e.g. propionate load) and demand for methionine (for milk and wool yields; MMA, methylmalonic acid; WLD, white-liver disease).

(Andrews *et al*., 1959; Marston, 1970; Millar and Lorentz, 1979) and broadly supported by subsequent dose–response trials (Grace *et al*., 2003; Gruner *et al*., 2004b). Assessment of mean growth responses to Co supplements in weaned lambs in New Zealand indicated that, on a flock basis, a much higher mean threshold value of 375 nmol kg^{-1} FW was required to avoid growth retardation in summer (Clark *et al*., 1989): a similar relationship was found in housed cattle (Stangl *et al*., 2000c). The apparent contrast had a simple explanation: within all flocks, as in treatment groups (Fig 10.4), there is variation in B_{12} status and Co response: individuals with below-average liver B_{12} will contribute most of the mean growth response.

- Diagnostic limits for individual and mean values should be distinguished for all biochemical criteria (see Table 10.3).

The current 'marginal' band for liver B_{12} for grazing cattle in New Zealand (75–250 nmol kg^{-1} FW) may be too low for cattle on grain-based diets: Co-responsive groups could not be distinguished by reference to liver B_{12} in the range 250–500 nmol kg^{-1} FW on such diets (see Tiffany *et al*., 2003; Tiffany and Spears, 2005). Any fatty infiltration will underestimate effective B_{12} concentrations in the liver.

- Given the importance of the kidney as a regulator of B_{12} status and high expression of TCII there, kidney B_{12} may be diagnostically useful, *post mortem*.

Total serum vitamin B_{12} in sheep

Historically, no attempt has been made to distinguish between haptocorrin-bound and TCII in sheep or cattle. In sheep, diagnostic limits for serum B_{12} depend on stage of development and diet. Transition from milk to grass is accompanied by a tenfold increase in B_{12} intake in lambs, without change in serum B_{12} (Knowles and Grace, 2017) but tolerance of low status changes. Suckling lambs with a mean plasma B_{12} of 140 pmol l^{-1} did not grow faster when supplemented (Grace and Sinclair, 1999; Gruner *et al*., 2004b) but weaned lambs responded when serum B_{12} in mid-trial fell below

Table 10.3. Marginal bands[a] for the most common biochemical indices used to assess the mean cobalt and vitamin B_{12} status of groups of ruminants when grazing or intensively fed on maize-based diets indoors.

	Ruminant species	Grazing	Housed	Interpretive problems
Serum B_{12}[b] (pmol l^{-1})	Bovine: S	**30**–60[c]	Unknown	Prone to gross underestimation and laboratory variation
	Bovine: W	**40**–80[c,d]	**70**–150[d]	
	Ovine and others:[e] S	**100**–200	**100**–200	
	Ovine and others: W	**200**–300	Unknown	Propionate load
Liver B_{12}[b] (nmol kg^{-1} FW)	Bovine: S	**70**–100	**70**–100	
	Bovine: W	**110**–220	**336**–500	Propionate load
	Ovine and others: S	**70**–100	Unknown	
	Ovine and others: W	**110**–220		
Serum homocysteine (μmol l^{-1})	All species:[e] S	8–**12**	8–**12**	Affected by methionine deficiency and possibly rate of transsulfuration in the gut during nematodeinfection
	All species:[e] W	Unreliable[f]		
Serum MMA[b] (μmol l^{-1})	All species: S	Unreliable	Unreliable	Nopropionate load
	Ovine and others: W	5–**10**	Unknown	Propionate load

MMA, methylmalonic acid; S, suckling; W, weaned.

[a]Mean values within a band denote the possibility of sufficient individuals benefiting to justify supplementation for all.

[b]Individual values close to below the **bold** limit are suggestive of production-limiting dysfunction. The higher the proportion in a sampled population, the stronger the case for supplementing with Co or vitamin B_{12}.

[c]Values may also be applied to cervines (Wilson and Grace, 2001).

[d]The apparent contrast between grazing and housed stock may be wholly or partly an artefact, attributable to different analytical methods.

[e]Lack of discrimination between species reflects lack of species-specific data rather than commonality of vitamin B_{12} status.

[f]Furlong *et al*. (2009).

300 pmol l^{-1} (see Fig. 19.5, p. 522; Clark, 2005). In barley-fed lambs, serum MMA was raised in most individuals with serum B_{12} <220 pmol l^{-1} (Rice *et al.*, 1987).

- Tolerance of low total B_{12} status may decrease because dietary energy source changes (p. 232) or changes in the functional B_{12} in serum are masked (p. 240).

Lower thresholds (<220 pmol l^{-1}) for weaned lambs were suggested in some field trials (Grace *et al.*, 2003: Gruner *et al.*, 2004c) but most unsupplemented lambs were withdrawn from the experiments on welfare grounds, leaving selected, relatively tolerant subgroups. Furthermore, plasma B_{12} increased during the study and final liver B_{12} status was normal. Accuracy of prediction from a single sampling will depend on the direction of change: in Scotland, serum B_{12} generally declined between birth and weaning (Suttle *et al.*, 1986). Since a small proportion of lambs benefitting from Co supplementation can cover the expense of treating the whole flock, large numbers of individuals should be sampled at random (at least ten and preferably one-tenth of the flock) and the sampling repeated, if possible (Grace *et al.*, 2003). The guideline values presented in Table 10.3 reflect the above considerations. Residual growth responses in lambs weaned off ewes treated with Co bullets during pregnancy, without response in plasma B_{12} (Gruner *et al.*, 2004b), emphasizes the diagnostic limitations of total serum B_{12}.

Serum vitamin B_{12} in cattle

In all probability, total B_{12} has rarely been measured in bovine samples. Assay results for cattle continue to vary widely between laboratories and diagnostic thresholds remain much lower than those for sheep, using a variety of methods (e.g. Givens and Simpson, 1983; Judson *et al.*, 1997; Underwood and Suttle, 1999).[xii] More recently, calves at pasture tolerated mean serum B_{12} values as low as 80 pmol l^{-1} (Grace *et al.*, 2014). The contrasts are illustrated in Fig. 10.8, which shows responses in grazing lambs and calves, given the same parenteral dose of slow-released B_{12}, albeit in separate trials. Pasture Co was adequate for lambs and generous for calves, yet serum B_{12} was fourfold higher in untreated lambs than in calves and injection of B_{12} widened the difference.

- many techniques are still not recovering all haptocorrin-bound B_{12} in cattle.

Using alkaline extraction and an 'organic enhancer', bovine serum B_{12} was increased from 218 to 905 pmol l^{-1} when dietary Co increased (Stangl *et al.*, 2000c). With the same assay kit but no mention of an 'enhancer', a similar increment in dietary Co had only increased plasma B_{12}, from 48.7 to 132 pmol l^{-1} after 100 days (Tiffany *et al.*, 2003): effects of diet (corn versus barley) on Co-responsiveness were not matched by differences in plasma B_{12} (Tiffany and Spears, 2005). When a chemiluminescence assay was used for the first time in a cattle study (Grace and Knowles, 2012a), serum B_{12} rose to 880–1040 pmol l^{-1} in groups given slow-release injectons of B_{12} (SRB_{12}), higher than any values recorded in cattle by this group.

- Assays that successfully measure total serum B_{12} may need different diagnostic limits from those that fail to do so, although differences between methods will diminish as B_{12} concentrations fall.

'Functional B_{12}' in serum

In human clinical medicine, holotranscobalamin, the 'metabolically active' B_{12}, bound to TCII is now the recommended indicator of B_{12} status (Hunt *et al.*, 2014). Earlier RID methods may have measured only (or largely) the functional (TCII) B_{12} component in bovine samples while avoiding the need for elaborate procedures to extract B_{12} bound to haptocorrin. Assays that detect only TCII in ovine samples may improve specificity.

- Low TCII may be the best indicator for responsiveness to Co and B_{12} supplements in ruminants.

Vitamin B_{12} in milk

Analysis of B_{12} in milk is not complicated by binders and shows large responses to supplementation (e.g. Grace and Knowles, 2012a), even when none are apparent in plasma: values correlated well with liver B_{12}. Milk B_{12} <300 pmol l^{-1} indicate low B_{12} status (Judson *et al.*, 1997) and can be measured by optical biosensor methods (Indyk *et al.*, 2002).

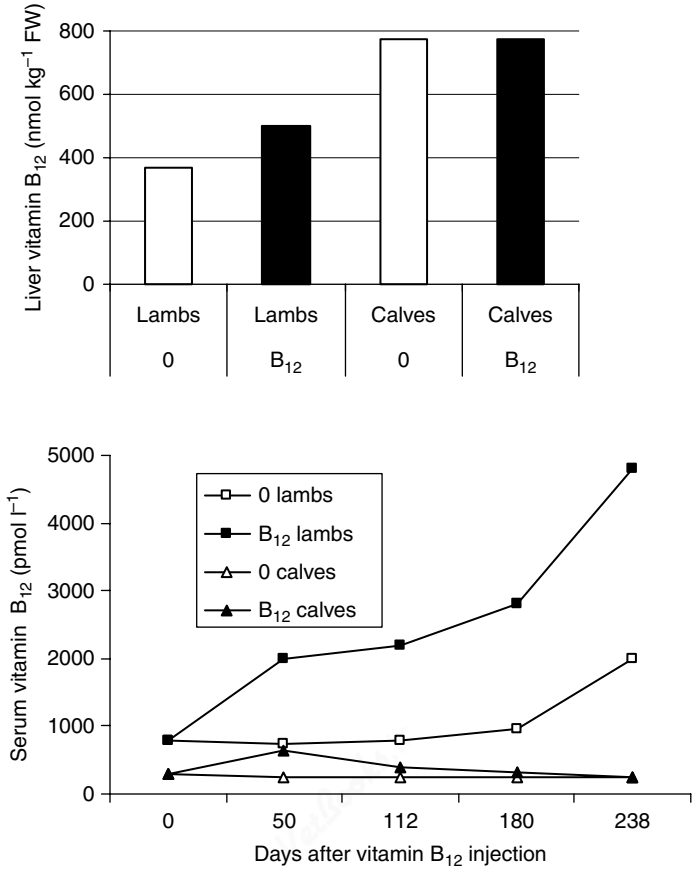

Fig. 10.8. Lambs and calves show contrasting responses in serial serum and final liver vitamin B_{12} concentrations to similar parenteral injections of vitamin B_{12} (0.23 mg kg^{-1} liveweight), given in slow-release (SR) form (lamb data from Grace and Lewis, 1999; calf data from Grace and West, 2000). Pastures were of adequate cobalt content and the same radioisotope dilution method was used in both trials but may greatly underestimate plasma B_{12} in the calves.

Methylmalonic acid in plasma or serum

Plasma or serum MMA is a more sensitive indicator of cobalt responsiveness than serum B_{12} in grazing sheep because the relationship between the two indices is curvilinear (Fig. 10.9; Gruner *et al.*, 2004c), but the point of maximum curvature is influenced by diet. Raised plasma MMA preceded impairment of performance in weaners on mixed diets (Weise *et al.*, 2007) and normal serum MMA in pregnant and lactating ewes does not guarantee that offspring will receive sufficient B_{12} for optimal growth (Quirk and Norton, 1987). Plasma MMA was a relatively insensitive marker in weaned cattle (Stangl *et al.*, 2000c), showing only small increases in Co-responsive animals (Tiffany and Spears, 2005). Plasma succinate and propionate merit consideration as diagnostic aids in sheep (Fig. 10.4).

Indicators of abnormal methylation

Indicators of impaired methylation are the preferred diagnostic criteria in cattle of all ages and in the young suckled lamb. Early work focused on excretion of forminoglutamic acid (FIGLU), which was increased in suckling calves and lambs (Russel *et al.*, 1975), whose growth was retarded by lack of dietary Co. The abnormality arises from a lack of MeTHF (Fig. 10.1) and is neither a primary or specific indicator of lack of dietary Co. Ewes and heifers not excreting FIGLU in

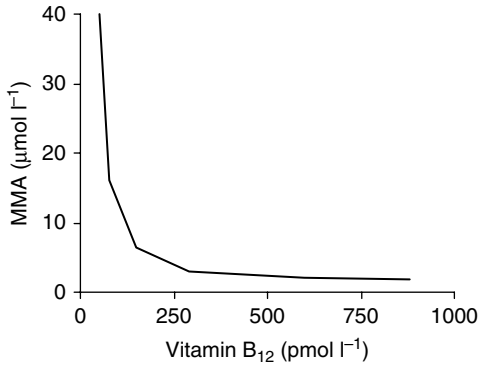

Fig. 10.9. Relationship between vitamin B_{12} and methylmalonic acid (MMA) concentrations in lambs grazing low-cobalt pasture is exponential, with vitamin B_{12} having to reach very low levels before MMA accumulates (Rice *et al*., 1987).

their urine can rear lambs that do so (Quirk and Norton, 1987, 1988), indicating that a different balance is struck between MeCbl- and AdoCbl-dependent pathways in dam and offspring (Fig. 10.6). After weaning, MeCbl dysfunction can become less important (Quirk and Norton, 1987, 1988). Raised plasma Hcy indicates impaired methylation in weaned lambs (Fig. 10.4), (Wiese *et al*., 2007) and calves (Stangl *et al*., 2000a) but the Me Cbl pathway is highly conserved and a late sign (Furlong *et al*., 2010). Plasma Hcy may be influenced by rate of conversion to methionine by trans-sulfuration in the gut mucosa, consequently, by larval nematode infection of that mucosa (Furlong *et al*., 2010). Reductions in liver folate are secondary consequences of impaired methionine synthase activity (e.g. Stangl *et al*., 2000c).

Other indicators of dysfunction

Other biochemical abnormalities have been reported in cobalt-deprived sheep, including low plasma glucose, ascorbic acid and thiamine concentrations and high 'liver enzymes', bile acids and pyruvate values, but these are not consistent or specific features (Ulvund, 1995; Underwood and Suttle, 1999).

- No biochemical marker in a suckling lamb or calf will indicate fully the impact of deprivation that reduces milk yield in the mother.

Sources of Cobalt

Forages

Pasture and forage Co can vary widely between plant species and with soil conditions (Minson, 1990). In early Scottish studies, mixed pastures contained from 0.02–0.22mg Co kg^{-1}DM and legumes, such as red clover, more Co than ryegrass grown in the same conditions (0.35 and 0.18 mg kg^{-1}DM, respectively: Underwood and Suttle, 1999). Lower values were reported for legumes and grasses in Japan but the species difference was proportionately greater (respective means, 0.12 and 0.03mg Co kg^{-1}DM; Hayakawa, 1962). The advantage of legumes can be lost on soils low in Co. Grass species, grown under similar conditions can vary twofold in herbage Co, with *Phleum pratense* and *Dactylis glomerata* poor sources compared to *Lolium perenne*. Pasture Co is reduced as soil pH rises (see Fig. 2.1), increased by poor soil drainage but largely unaffected by advancing plant maturity. Soil contamination can greatly increase herbage Co, particularly in unwashed spring and autumn samples. Washing reduces contamination but may underestimate capacity for B_{12} synthesis, since ingested soil Co is partially available for ruminal synthesis of vitamin B_{12} (Brebner *et al*., 1987), raises serum B_{12} (Grace, 2006) and probably alleviates deficiency at times (Macpherson, 1983; Clark *et al*., 1989). Grass species can differ in proneness to soil contamination (Jumba *et al*., 1995). However, mixed grass/legume pastures in New Zealand varied little in Co, 95% of samples falling within the range 0.095–0.111 mg Co kg^{-1}DM (Knowles and Grace, 2014).

Concentrates

Data on cobalt in grains and other concentrates are meagre, partly because of the difficulty in measuring low concentrations in silica-rich samples. Cereal grains are poor sources, usually containing 0.01–0.06 mg Co kg^{-1}DM, and used to produce diets low in Co (Field *et al*., 1988; Kennedy *et al*., 1991b, 1992). Like most trace elements, Co is highly concentrated in the outer fibrous layers of the grain, so that bran and pollard (middlings) are much richer in Co than the whole grain. Feeds of animal origin, other than liver meal, are generally low in Co.

Milk and milk products

Cow's milk normally contains 0.5–0.9 μg Co l^{-1} but <10% of that is present as vitamin B_{12}, judging from the range of values in fat-free milk (0.3–3.0 nmol B_{12} l^{-1}) (Judson *et al*., 1997), and of nutritional value. Milk is an important source of vitamin B_{12} for the pre-ruminant, although concentrations are low in all species (typically <5 μg l^{-1} in the ewe) (Grace *et al*., 2006). Milk Co and B_{12} can be increased several-fold by large dietary Co and parenteral B_{12} supplements (Grace and Knowles, 2012a, b).

Availability for Synthesis of B_{12}

Dietary Co is only of use to ruminants if incorporated into B_{12} by rumen microbes but they divert most of the supply into physiologically inactive corrinoids (Gawthorne, 1970).

Effects of cobalt intake, source and delivery

Efficiency of capture of ingested Co as Cbl is higher from inherent Co in the feed than from supplementary Co, whether given in pulse doses or continuously. Efficiency of capture decreased from 15% in sheep given hay, low in Co, to 3% from 1mg Co d^{-1}, given by drench (Smith and Marston, 1970). Efficiency of capture diminishes as the amount of supplement increases (Wiese *et al*., 2007) and was proportional to square root of dose, over the range 1–32 mg Co d^{-1} (Suttle *et al*., 1989). Diminishing capture was confirmed in sheep, given a similar range of Co intakes to those used by Smith and Marston (1970) continuously by intra-rumenal infusion with a diet of hay and concentrates (Fig. 10.10). Soluble extrinsic Co gets distributed throughout the rumen fluid phase and is probably less accessible by rumen microbes, particularly those digesting fibre, which colonize the biofilms that surround feed particles.

Effect of species and food substrates

Efficiency of B_{12} synthesis in three cattle studies, involving diverse diets, generously supplemented with Co, ranged from 6.3% to 16.2% (Table 10.4) (Schwab *et al*., 2006), higher than the efficiencies found in sheep under similar conditions and possibly explaining their lower requirement for dietary Co (p. 244). Efficiency of capture was reduced from 13% to 7%, on average, by inclusion of 23% grain (mostly maize) in the ration. In sheep, B_{12} synthesis decreased following addition of cereal to the diet (Sutton and Elliot, 1972; Hedrich *et al*., 1973). In Fig. 10.10, sheep were given a diet of 0.6 parts hay to 0.4 barley and efficiency of B_{12} synthesis was <0.5 of that found previously with a diet of hay alone, equally low in Co (Smith and Marston, 1970).

- Efficiency of B_{12} synthesis may be more efficient from roughage than either concentrate because inherent Co is more accessible to B_{12}-synthesizing microbes that occupy niches on fibre particles.
- Concentrate feeding increases the numbers of rumen protozoa, which acquire B_{12} by ingesting microbes and dwell longer in the rumen.

Roughage and cereal types may also influence rumen synthesis of B_{12}. Efficiency of capture was unaffected by inclusion of maize in one cattle study (trial 3, Zinn *et al*., 1987; Table 10.4). Increasing the forage (mostly maize silage) content of the diet from 35% to 60% reduced efficiency of rumen B_{12} production from 12% to 8% on aver-

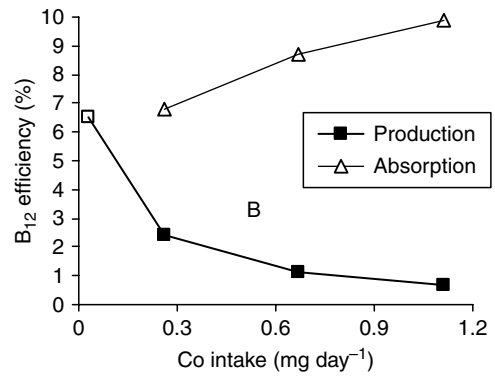

Fig. 10.10. Efficiency of rumen vitamin B_{12} synthesis and subsequent absorption in sheep continuously fed either hay and concentrates, low in cobalt (Co, open square) or given Co by intra-rumenal infusion (solid square). Abomasal cannulation allowed flow of B_{12} from the rumen to be measured and absorption was calculated by calibrating responses in plasma B_{12} against those obtained with continuous intravenous infusions of B_{12} (Ludemann, 2009).

Table 10.4. Efficiency of 'capture' of dietary cobalt as microbial vitamin B_{12} is affected by roughage and carbohydrate composition of cattle diets, and low when intakes of maize silage (MS), ground maize (GM) and ground barley (GB) are high.

Capture of Co as microbial vitamin B_{12}[a] (%)	Roughage (%)	Forage (%)	Carbohydrate (%)	Reference
14.0	45	45 AH	45 FM	Zinn *et al*. (1987)
11.0	20	0	64 DRM	
15.4	59	44 GLS, 15 MS	34 HMM	Sanschi *et al*. (2005)
16.2	86[b]	18 MS, 12 AH, 6 GH	0	Schwab *et al*. (2006)
8.2	61[b]	18 MS, 12 AH, 6 GH	16 GM, 8 GB	
9.5	88[b]	30 MS, 20 AH, 19 GH	0	
6.3	69	30 MS, 20 AH, 19 GH	15 GM, 7 GB	

AH, alfalfa hay; DRM, dried, rolled maize; FM, flaked maize; GH, grass hay; GLS, grass-legume silage; HMM, high-moisture maize
[a]Efficiency calculated from the daily flow of vitamin B_{12} at the duodenum, converted to Co (×0.044) and expressed as a percentage of daily Co intake, which came predominantly from the mineral supplement (0.7 mg added Co kg^{-1}DM in each study). Only Schwab *et al*. (2006) analysed diets for Co and a total Co of 1 mg kg^{-1}DM was assumed for the other studies.
[b]The differences between roughage and total forage percentage were made up by a mixture of soybean hulls and sugarbeet pulp.

age (Schwab *et al*., 2006). Maize and maize silage are characterized by relatively high rates of energy and protein degradability (AFRC, 1993) when compared to barley and grass silage and may sustain relatively low rates of microbial protein and B_{12} synthesis.

In vitro synthesis of vitamin B_{12}

The study of B_{12} (and volatile fatty acid) production in continuous cultures of rumen microbes yielded useful information on bioavailability (Fig. 10.11) (Kawashima *et al*., 1997b; Tiffany *et al*., 2006), after initial disappointments (McDonald and Suttle, 1986), but has only been used to evaluate sources of supplementary Co. Accessibility of Co to rumen microbes probably varies between culture systems. Relatively high B_{12} output from unsupplemented, continuous cultures suggests that Co in oat hulls (Brebner, 1987), hay (McDonald and Suttle, 1986) and corn (Tiffany *et al*., 2006) is of relatively high availability. Availability for B_{12} synthesis probably correlates with solubility, extractability and degradability of the Co source (Kawashima *et al*., 1997a, b). Rumen B_{12} in sheep correlated with the acetate-extractable Co in ingested soil (Brebner *et al*., 1987), confirming *in vitro* findings (Fig. 10.11). Acetate-extractability of Co can vary from 3% to 13% between soil types (Suttle *et al*., 2003). Extractability of Co in feeds could indicate bioavailability to ruminants. Cobalt given as CoEDTA was not incorporated in vitamin B_{12} in continuous cultures (Suttle, unpublished data).

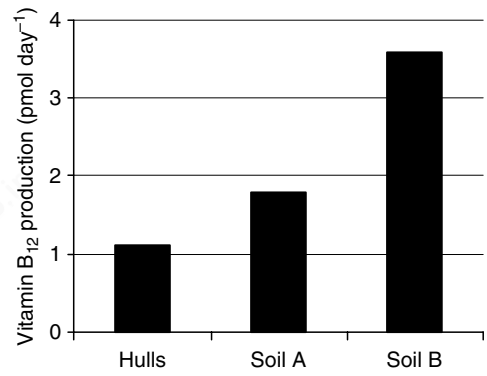

Fig. 10.11. Mean vitamin B_{12} production from three pairs of continuous cultures of rumen microbes 'fed' on oat hulls and liquid substrates. Soils low (A) or high (B) in acetate-extractable cobalt (0.09 and 0.58 mg kg DM^{-1}, respectively) were added to two pairs (Brebner, 1987).

Cobalt Requirements

The differences in vitamin B_{12} synthesis, just described, translate into different cobalt requirements for different species and diets (Table 10.5): stage of reproductive cycle is also important.

Production from grass

Precise estimates of need are difficult to gauge from field studies because of seasonal changes in herbage Co and soil contamination. Cobalt responses were poorly correlated with pasture Co

Table 10.5. Maximum[a] cobalt requirements of ruminants on roughage or mixed diets.

	Roughage diets	Mixed diets		
	Hay	Grass	Grass silage and barley	Maize silage and maize
Cattle and deer[b]	0.04	0.06	0.04	0.15
Sheep and goats	0.08	0.12	0.08	0.30[b]

[a]All classes can perform well with less Co in situations where the quality of the diet and pasture can only sustain moderate levels of performance.
[b]Classifications of deer with cattle and sheep on maize-based diets as having high Co need are speculative.

in sheep (Gruner *et al*., 2004a, b). However, grazing cattle have lower needs than sheep and may not benefit from supplementation on pastures containing 0.06 mg Co kg^{-1}DM that cause ill-thrift in lambs (Clark *et al*., 1986). Variation in animal need is also important. Pasture containing 0.03–0.05 mg Co kg^{-1}DM may be tolerated by mature ewes, but requirements increase with onset of lactation (Quirk and Norton, 1987, 1988; Gruner *et al*., 2004a), when the liver begins to process more propionate and body fat is mobilized to meet energy needs but releases no additional B_{12}.

- High soluble carbohydrate concentrations in fresh pasture may raise requirement for growth and freedom from OWLD to 0.1 mg kg^{-1} DM.
- Fresh or conserved herbages containing 0.05–0.12 mg Co kg^{-1}DM are therefore marginal for grazing sheep.
- Those with 0.03–0.06 mg kg^{-1}DM are marginal for grazing cattle, deer and goats.

Production from cereals

Requirements vary according to the criterion used: those for 'optimal' haemoglobin, plasma Hcy, liver folate, liver B_{12} and plasma B_{12} were estimated to be 0.1, 0.16, 0.19, 0.24 and 0.26 mg kg^{-1}DM, respectively, in steers given corn silage and concentrate (Schwarz *et al*., 2000). Other studies with housed cattle suggested that 0.12–0.15 mg Co kg^{-1}DM was required for optimum growth on maize-based diets (Schwarz *et al*., 2000; Tiffany *et al*., 2003; Tiffany and Spears, 2005). High requirements might reflect inefficient rumen production of B_{12} from corn and corn silage (Table 10.4). With complete diets consisting mostly (85%) of ground corn or barley with monensin, an additive that can favour propionate production, growth requirements were met with only 0.02 mg Co kg^{-1}DM from a barley diet (Tiffany and Spears, 2005). When corn comprised only 49% of the ration of growing steers (Tiffany *et al*., 2003), raising dietary Co from 0.04 to ≥0.09 mg kg^{-1}DM did not improve growth but with 85% corn in a finishing ration, growth was increased. Plasma B_{12} (probably TC II only; see p. 240) in unsupplemented steers in the two phases was equally low (approximately 50 pmol l^{-1}) but rumen propionate was 50% higher (46 versus 31 mmol l^{-1}) on the finishing diet. Cobalt requirements for diets consisting mostly of maize silage or grain may be uniquely high because such diets enhance propionate production (Fig. 10.6). Use of monensin to manipulate rumen metabolism in grazing livestock may increase requirement for Co. Needs for Co in lambs on corn and barley-based diets have not been accurately defined but are likely to exceed those of growing cattle: 0.2–0.3 mg kg^{-1}DM should be allowed until appropriate studies have been conducted (Table 10.5).

Production of milk

Cows in early lactation were postulated to have high systemic requirements for B_{12} to optimize methylation if the diet provided insufficient methionine (Girard, 2002).[xiii] The large amounts of propionate that high-yielding cows have to process may increase the priority given to the AdoCbl pathway and thus expose the MeCbl pathway. However, milk-yield responses to parenteral vitamin B_{12} may be less pronounced on diets providing adequate sulfur and optimum conditions for microbial protein and vitamin B_{12} synthesis. Feeding supra-nutritional dietary Co (up to 1.71 mg kg^{-1}DM) does not increase milk yield (Kincaid and Socha, 2007) but a requirement similar to that of grain-fed cattle (i.e. 0.12 mg kg^{-1}DM) should be allowed for.

Prevention and Control of Deprivation

Vitamin B_{12} deprivation can be cured or prevented by a variety of methods, preference depending on local circumstances and cost–benefit analysis (Grace and Knowles, 2012b).

Soil and pasture fertilizers

Inclusion of cobalt salts or oxides in soil fertilizers increased pasture Co and may stimulate legume growth. Application rates and frequencies varied with the soil type, terrain and husbandry practice.[xiv] However, fertilizer treatment is unreliable on highly alkaline soils, such as calcareous shell sands, heavily limed soil and those high in manganese oxides which all fix Co in an unavailable form (Adams *et al.*, 1969). Cobalt fertilizers are expensive and treating strips of pasture may suffice and cut costs (MacPherson, 1983), avoiding recently limed pastures altogether. Preferably, far smaller rates of application can be used on any field as a foliar spray, shortly before grazing: a single autumn application of 60g $CoSO_4$ ha^{-1} raised herbage Co above 0.1 mg Co kg^{-1}DM for six weeks (Smith, 2004).

Dietary and oral supplements

Cobalt is commonly provided as salts in loose mineral mixtures, licks, blocks, whole rations and drinking water, well in excess of need. A mineral mix containing 40 mg Co kg^{-1}, included at 25 kg t^{-1} in a concentrate comprising one tenth of the total ration is adequate. Provision as salt licks, containing 0.1% Co, is generally effective under range conditions but individual and seasonal uptake can vary (p.22). Oral drenching with Co raises B_{12} status but the frequent dosing required (every 2–4 weeks) is impractical, unless combined with other therapies such as de-worming. Manufacturers of anthelmintic drenches commonly added Co to their products, but the amounts provided ranged widely and increases in plasma B_{12} were short-lived (e.g. Field *et al.*, 1988). Responses increased by 40% when doses for lambs were increased from 8 to 32 mg cobalt (Suttle *et al.*, 1989). Conservative use of anthelmintics to avoid the build-up of 'anthelmintic-resistant' nematodes on pasture restricts the efficacy of combined treatments. Continuous dietary supplementation is recommended for ewes in late pregnancy to avoid loss of appetite and risk of pregnancy toxaemia, while increasing B_{12} status of offspring: in pregnant heifers, milk B_{12} is greatly increased (Quirk and Norton, 1988).

Slow-release oral supplementation

Supplementary Co should maintain rumen liquor Co above 5 µg l^{-1} and this was achieved by Co-containing pellets or bullets were retained in the rumen. Sheep were afforded protection for up to five years by a single pellet (Lee and Marston, 1969) and beef cows for up to 19 months (Judson *et al.*, 1997).[xv] In young lambs and calves, in which the rumen is not fully developed, and in lactating cows, in which feed intake is large and rumination frequent, a few animals regurgitate pellets. A more serious problem was development of an impervious coat of calcium phosphate on the pellet: this was minimized by simultaneously administering an abrasive object or second pellet. Soluble glass boluses are not susceptible to coating and also yield a steady supply of Co for many months (Judson *et al.*, 1988; Zervas, 1988; Masters and Peter, 1990; Kendall *et al.*, 2001).

Parenteral injection of vitamin B_{12}

In early studies, injections of B_{12} rapidly alleviated signs of deprivation, but the commonly used dose in lambs (0.1mg kg^{-1}LW) maintained normal serum B_{12} for <6 weeks (Grace *et al.*, 1998; Underwood and Suttle, 1999) and repeated dosages were prohibitively expensive. Mammary transfer of parenteral B_{12} was inefficient in dairy cows, <6% of weekly 10 mg injections being secreted in milk (Girard and Matte, 2005). With SRB_{12}, B_{12} is incorporated into biodegradable microspheres of lactide/glycolide polymer (Fig. 10.8): single subcutaneous doses, providing 0.14–0.24mg kg^{-1}LW, raised serum B_{12} for ≤187 days in lambs (Grace *et al.*, 2003), 176 days in ewes (Grace *et al.*, 2006) and 90–110 days in calves (Grace and West, 2000; Grace *et al.*, 2014). Efficacy of parenteral SRB_{12} in grazing lambs (Grace *et al.*, 2003) confirms that any changes in rumen microbial metabolism were not growth-limiting. In lactating cows, a single injection of 60 or 90 mg SRB_{12} had caused three- and fivefold increases in milk B_{12} and serum B_{12} 50 days later but the increase in liver B_{12} was only 1.5-fold (Fig. 10.12).

- The distribution of injected B_{12} between TC I and TC II in cattle and sheep plasma merits investigation: large contributions of TC I-bound B_{12} may overestimate functional B_{12} status.

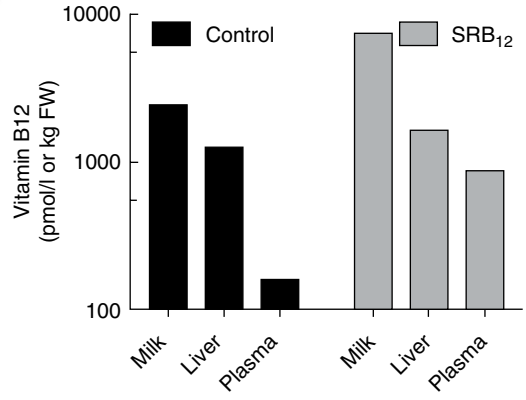

Fig. 10.12. Effect of injecting dairy cows at pasture with 60mg B_{12} of slowly released (SR) B_{12} on vitamin B_{12} concentrations in milk, liver and plasma, 50 days later, compared with values in uninjected control cows. (Data from Grace and Knowles, 2012a). Use of a chemilumescence assay for B_{12} for the first time may have revealed the full increase in serum B_{12}, where radioimmune assays can leave bovine TC I-bound B_{12} undetected.

Cobalt sources

Inorganic Co sources must be readily soluble in the rumen to be efficiently used by ruminants. While successful as a constituent of heavy pellets because of the large doses given, relatively insoluble cobaltous and cobaltic oxides have lower nutritive value than more soluble inorganic salts ($CoCO_3$ and $CoSO_4$) when assessed by comparative increases in liver Co or B_{12} synthesis by cultured rumen microbes (Kawashima *et al*., 1997a, b). In an early study, Co_3O_4 and $CoSO_4$ were equally effective in raising serum and liver B_{12} (Andrews *et al*., 1966), but amounts given (300 mg) far exceeded the capacity for rumenal B_{12} synthesis (Fig. 10.2), putting the more soluble $CoSO_4$ at a disadvantage. Chelates were no more effective in stimulating B_{12} synthesis than simple salts, whether the comparison was made between $CoCl_2$ and CoEDTA (Suttle, unpublished data) or glucoheptonate *in vitro* (Kawashima *et al*., 1997b), between CoEDTA and $CoSO_4$ in grazing lambs (Millar and Albyt, 1984) or between cobalt propionate and $CoCO_3$ in steers (Tiffany *et al*., 2003).

Protection from toxins

Ruminants grazing swards of the perennial grass *Phalaris tuberosa* in restricted areas can develop 'Phalaris-' or 'Ranpha-' staggers. The disorder is preventable but not cured by dosing with Co (Lee and Kuchel, 1953; Lee *et al*., 1957) or by treatment of pastures with a Co-containing fertilizer. *P. tuberosa* contains a neurotoxin that is responsible for the staggers syndrome but the neurotoxin is either inactivated or its absorption is reduced by high rumen Co: administration of vitamin B_{12} is ineffective. The required Co dosage rises with increasing toxic potential of the pastures. In some areas, Co intake from soil and herbage is sufficient to detoxify the neurotoxin. Cobalt supplements can also partially protect sheep from annual ryegrass toxicity. The pathogenic agents are coryne toxins produced by a bacterium, *Clavibacter toxicus*, which is carried into the seed head by an invading nematode, *Anguina agrostis*. Onset of neurological signs was delayed but not prevented by Co (4 or 16 mg d^{-1}), irrespective of amount of toxin (0.15 or 0.30mg kg^{-1}LW day^{-1}) given (Davies *et al*., 1995).

Non-ruminants

Vitamin B_{12} performs the same functions in non-ruminants and ruminants. In pigs, administration of a methionine synthase inhibitor (nitrous oxide) induces symptoms of B_{12} deficiency (Kennedy *et al*., 1991a) and prolonged feeding of a B_{12}-free diet induces mild hyperhomocysteinaemia and macrocytic anaemia (Stangl *et al*., 2000a). Reduced activity of leucine 2,3 aminomutase has been implicated in pernicious anaemia in humans (Poston, 1980) and prolonged B_{12} deprivation of hens impairs synthesis of leucine, but does not induce anaemia or signs of neurological impairment (Ward *et al*., 1986). Non-ruminants have low dietary needs for B_{12} and requirements are said to decline from 20 to 5 μg kg^{-1}DM as pigs grow from 5 to 50 kg LW (NRC, 1998), and from 9 to 3 μg kg^{-1}DM as Leghorn chicks progress between 0–6 and 6–12 weeks of age (NRC, 1984). However, there is no evidence to justify such scaling to body weight or age. Pigs and poultry rations that are derived entirely from plant sources contain little or no vitamin B_{12} but they can be fed for long periods of time without greatly affecting the performance of laying hens (e.g. Ward *et al*., 1986) or growing pigs (Stangl *et al*., 2000a). Vitamin B_{12} is synthesized in the colon, but little or none is absorbed there. Faeces therefore contain B_{12} and useful amounts may be obtained via coprophagy (Stangl *et al*., 2000a). If access to non-dietary B_{12} is denied, growth responses can be obtained from supplementary B_{12} or from the provision of feeds of

animal or microbial origin to pigs and poultry. There are no reports of B_{12} deficiency in horses (Lewis *et al*., 1995). Clinical signs of cobalt (as opposed to B_{12}) deprivation have not been demonstrated in any species.

Cobalt Toxicity

Cobalt is commonly stated to be of low toxicity to all species, but this is only true if toxicity is assessed as a multiple of minimum requirement: then there is a 100-fold margin of safety. In terms of dietary concentrations, cobalt is surpassed only by copper, selenium and iodine among the trace elements as a threat to health. Field cases of suspected cobalt toxicity in ruminants have been reported. There was no distinctive pathology, but liver Co was very high (20–69 mg kg^{-1}DM) (Dickson and Bond, 1974). The ARC (1980) summarized available evidence and – noting that >4mg and >1mg kg^{-1}LW were toxic to sheep and young cattle, respectively – recommended that dietary Co for ruminants should not exceed 30 mg kg^{-1}DM: the NRC (2005) reduced that figure to 25 mg kg^{-1}DM. Tolerance may be higher when cobalt is given continuously in the diet, rather than as a drench and probably reflects sensitivity of the rumen microflora, rather than the host, to excess Co. Pigs tolerated 200 mg kg^{-1}DM, but higher levels (400 and 600 mg kg^{-1}DM) caused anorexia, stiffness, inco-ordination and muscular tremor (Huck and Clawson, 1976). Symptoms were alleviated by supplements of methionine or a combination of Fe, Mn and Zn. Anaemia and decreases in tissue iron indicated a cobalt × iron antagonism. In day-old chicks given 125, 250 or 500 mg Co kg^{-1}DM for 14 days, the lowest level reduced feed intake, weight gain and gain to feed ratios, while the two higher levels caused pancreatic fibrosis, hepatic necrosis and muscle lesions (Diaz *et al*., 1994). In the chick embryo, Co was the third most toxic of eight elements examined, surpassed only by cadmium and arsenic (Gilani and Alibhai, 1990). The mechanism of cobalt toxicity may involve free-radical generation and consequent cytotoxicity (NRC, 2005).

Notes

[i] The disease was known as 'bush sickness' in New Zealand, 'salt sickness' in Florida, 'Nakuruitis' in Kenya, 'lecksucht' in the Netherlands and Germany and 'Grand Traverse disease' in Michigan.

[ii] Individuals were given a fixed intake of concentrate, allowed to express appetite for roughage (given *ad libitum*) and fed only once daily. The finding has yet to be confirmed under 'steady-state' conditions of ration composition and intake.

[iii] The disorder was claimed to have been reproduced experimentally on a diet consisting mostly of Rhodes grass hay, containing <0.1 mg Co kg^{-1}DM, but an uncharacteristic, microcytic and hypochromic anaemia, responsive to B_{12} developed at an early stage (Johnson *et al*., 2004): inadequate microbial protein synthesis may have been a contributory factor.

[iv] In sheep, some 10,000–24,000 IU of IF is secreted in gastric juice each day. Bile salts facilitate binding of Cbl:IF complexes to a multi-purpose receptor, 'cubilin', in the ileal mucosal brush border (Smith, 1997; Andre and Dali-Youcef, 2020).

[v] Only 3–5% of B_{12} leaving the rumen was absorbed by Co-deprived sheep (Kercher and Smith, 1955; Marston, 1970) but 11% was absorbed by cattle on generous cobalt intakes (Zinn *et al*., 1987; Schwab *et al*., 2006). Features of B_{12} absorption in non-ruminant species, such as an increase in absorptive capacity during pregnancy (Nexo and Olesen, 1982) and in colostrum-fed piglets (Ford *et al*., 1975), may also occur in ruminants.

[vi] Plasma contains three Cbl-binding proteins, TC0 (a haptocorrin, now labeled TC III), TCI and TCII. Bovine plasma contains high concentrations of the strong, high-molecular-weight binders TC0 and TC1 (Polak *et al*., 1979; Schulz, 1987a, b; Price, 1991), which can play havoc with some assays (p. 240). TC II carries AdoCbl into mitochondria while TC I conveys MeCbl in the cytosol (NICE, 2015).

[vii] Uptake of B_{12} by hepatocytes from B_{12} rats, deprived of the vitamin, was upregulated and specific activities of NADH/NADPH-linked aquaCbl reductases increased (Watanabe *et al*., 1991) (aquaCbl is an intermediary transfer protein, see Fig. 10.1).

[viii] Methionine supplementation via intravenous (Gawthorne and Smith, 1974) or abomasal infusion (Lambert *et al*., 2002) lowers hepatic methionine synthase activity: a negative balance between methionine supply and demand may increase activity and risk of MeCbl dysfunction. The discovery of another methionine synthase that is zinc-dependent and uses betaine-homocysteine as a substrate (Millian and Garrow, 1998) raises the possibility of compensatory increases that attenuate Co deprivation.

[ix] Intravenous infusion of propionate increases the expression of most lipogenic genes in ovine adipose tissue (Lee and Hossner, 2002). Lipid metabolism may remain unaffected in cattle and deer, deprived of Co, because they cannot activate MMA CoA in adipose tissue (Wahle *et al*., 1979).

[x] High circulating levels of homocysteine could initiate a chain of triglyceride peroxidation, forming lipofuscin, depleting the antioxidant vitamin E and damaging mitochondrial structure in the liver of lambs with OWLD (Kennedy *et al*., 1997). However, homocysteine-induced

oxidant stress was not seen in steers deprived of cobalt on a corn-silage diet (Stangl *et al*., 2000b).

[xi] Early suggestions that liver Co <0.04–0.06 was insufficient and >0.08–0.12 mg kg^{-1}DM adequate were confirmed in cattle (Mitsioulis *et al*., 1995). The relationship between liver Co and liver B_{12} is curvilinear with wide scatter when liver Co is high: the two parameters correlate linearly and well when values are low (Suttle, 1995).

[xii] Incomplete release of vitamin from binders greatly underestimated plasma B_{12} (Price *et al*., 1993). Extraction of B_{12} at 100°C and a consistently high pH (9–12) overcame both non-specific and residual binding problems (Judson *et al*., 2002).

[xiii] Yields of milk and milk protein were increased by weekly intramuscular injections of 10 mg B_{12} in cows given diets with rumen-protected methionine and high in Co (0.66 mg added Co kg^{-1}DM) (Girard and Matte, 2005). The B_{12} injections were believed to have increased the methylation of folate, but milk yield improved in the presence of normal concentrations of plasma MMA and liver B_{12}, apparently confounding conventional assessments of Co status and requirements. When a diet without protected methionine was supplemented with folic acid (2.6 g d^{-1}) and/or vitamin B_{12} (0.5 g d^{-1}), only folic acid increased milk yield (Graulet *et al*., 2007). It would appear that 'supranutritional' supplementation with B_{12} is only necessary when microbial synthesis of methionine is inadequate. The folic acid supplement doubled total liver lipid content, two weeks after calving, unless B_{12} was also given, raising the possibility of involvement of these B vitamins in the aetiology of the fatty liver syndrome.

[xiv] In New Zealand hill country, a single application of 1.5 kg $CoSO_4.7H_2O$ ha^{-1} every 3–4 years was effective, while on more accessible sandy soils, where phosphatic fertilizers were applied every year or two, less Co sufficed (Sherrell, 1984).

[xv] Early types consisted of cobalt oxide (60%): high specific gravity (4.5–5.0) facilitated retention in the rumino-reticulum for months or years, while slow dissolution supplied Co to the rumen bacteria (Underwood and Suttle, 1999). Efficacy was established under a wide range of conditions, including changes of formulation that halved the Co content (Judson *et al*., 1997) and combination with zinc oxide for prevention of facial eczema (Grace *et al*., 1997).

References

Adams, S.N., Honysett, J.L., Tiller, K.G. and Norrish, K. (1969) Factors controlling the increase of cobalt in plants following the addition of a cobalt fertilizer. *Australian Journal of Soil Research* 7, 29–36.

AFRC (1993) *Energy and Protein Requirements of Ruminants*. An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Andres, E, and Dali-Youcef, N. (2020) Cobalamin (vitamin B_{12}) malabsorption. In: Patel, V.B. (ed.) *Molecular Nutrition*. Academic Press, London, pp. 367–386.

Andrews, E.D., Stephenson, B.J., Anderson, J.P. and Faithful, W.C. (1958) The effect of length of pastures on cobalt-deficiency disease in lambs. *New Zealand Journal of Agricultural Research* 31, 125–139.

Andrews, E.D., Hart, L.I. and Stephenson, B.J. (1959) A comparison of the vitamin B_{12} and cobalt contents of livers from normal lambs, cobalt-dosed lambs and others with a recent history of mild cobalt deficiency disease. *New Zealand Journal of Agricultural Research* 2, 274–282.

Andrews, E.D., Stephenson, B.J., Isaccs, C.E. and Register, R.H. (1966) The effects of large doses of insoluble and soluble forms of cobalt given at monthly intervals on cobalt-deficiency disease in lambs. *New Zealand Veterinary Journal* 14, 191–195.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

Bannerjee, R. (1999) *Chemistry and Biochemistry of* B_{12}. John Wiley & Co., New York.

Bassler, K.H. (1997) Enzymatic effects of folic acid and vitamin B_{12}. *International Journal of Vitamin and Nutrition Research* 67, 385–388.

Black, H., Hulton, J.B., Sutherland, R.J. and James, M.P. (1988) White liver disease in goats. *New Zealand Veterinary Journal* 36, 15–17.

Brebner, J. (1987) The role of soil ingestion in the trace element nutrition of grazing livestock. *PhD thesis*, Imperial College, University of London.

Brebner, J., Suttle, N.F. and Thornton, I. (1987) Assessing the availability of ingested soil cobalt for the synthesis of vitamin B_{12} in the ovine rumen. *Proceedings of the Nutrition Society* 46, 766A.

Clark, R.G. (2005) Cobalt deficiency in sheep and diagnostic reference ranges. *New Zealand Veterinary Journal* 53, 265–266.

Clark, R.G., Burbage, J., Marshall, P.M., Valler, T. and Wallace, D. (1986) Absence of vitamin B_{12} weight gain response in two trials with growing red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 34, 199–201.

Clark, R.G., Mantelman, L. and Verkerk, G.A. (1987) Failure to obtain weight gain response to vitamin B_{12} treatment in young goats grazing pasture that was cobalt-deficient for sheep. *New Zealand Veterinary Journal* 35, 38–39.

Clark, R.G., Wright, D.F., Millar, K.R. and Rowland, J.D. (1989) Reference curves to diagnose cobalt deficiency in sheep using liver and serum vitamin B_{12} levels. *New Zealand Veterinary Journal* 37, 1–11.

Davies, M.E. (1979) Studies on the microflora of the large intestine of the horse by continuous culture in an artificial colon. *Veterinary Research Communications* 3, 39–44.

Davies, S.C., White, C.L. and Williams, I.H. (1995) Increased tolerance to annual ryegrass toxicity in sheep given a supplement of cobalt. *Australian Veterinary Journal* 72, 221–224.

Diaz, G.J., Julian, R.J. and Squires, E.J. (1994) Lesions in broiler chickens following experimental intoxication with cobalt. *Avian Diseases* 38, 308–316.

Dickson, J. and Bond, M.P. (1974) Cobalt toxicity in cattle. *Australian Veterinary Journal* 50, 236.

Duncan, W.R.H., Morrison, E.R. and Garton, G.A. (1981) Effects of cobalt deficiency in pregnant and postparturient ewes and their lambs. *British Journal of Nutrition* 46, 337–343.

Farningham, D.A.H., Mercer, J.G. and Lawrence, C.B. (1993) Satiety signals in sheep: involvement of CCK, propionate and vagal CCK binding sites. *Physiology and Behaviour* 54, 437–442.

Fell, B.F. (1981) Pathological consequences of copper deficiency and cobalt deficiency. *Philosophical Transactions of the Royal Society of London, Series B* 294, 153–169.

Ferguson, E.G.W., Mitchell, G.B.B. and MacPherson, A. (1989) Cobalt deficiency and *Ostertagia circumcincta* infection in lambs. *Veterinary Record* 124, 20.

Field, A.C., Suttle, N.F., Brebner, J. and Gunn, G.W. (1988) An assessment of the efficacy and safety of selenium and cobalt included in an anthelmintic for sheep. *Veterinary Record* 123, 97–100.

Fisher, G.E.J. and MacPherson, A. (1991) Effect of cobalt deficiency in the pregnant ewe on reproductive performance and lamb viability. *Research in Veterinary Science* 50, 319–327.

Ford, J.E., Scott, K.J., Sansom, B.F. and Taylor, P.J. (1975) Some observations on the possible nutritional significance of vitamin B_{12}- and folate-binding proteins in milk. Absorption of [^{58}Co] cyano-cobalamin by suckling piglets. *British Journal of Nutrition* 34, 469–492.

Furlong, J.M., Sedcole, J.R. and Sykes, A.R. (2010) An evaluation of plasma homocysteine in the assessment of vitamin B_{12} status of pasture-fed sheep. *New Zealand Veterinary Journal* 58, 11–16**.**

Gawthorne, J.M. (1968) The excretion of methylmalonic and formiminoglutamic acids during the induction and remission of vitamin B_{12} deficiency in sheep. *Australian Journal of Biological Sciences* 21, 789–794.

Gawthorne, J.M. (1970) The effect of cobalt intake on the cobamide and cobinamide composition of the rumen contents and blood plasma of sheep. *Australian Journal of Experimental Biology and Medical Science* 48, 285–292.

Gawthorne, J.M. and Smith, R.M. (1974) Folic acid metabolism in vitamin B_{12}-deficient sheep: effects of injected methionine on methotrexate transport and the activity of enzymes associated with folate metabolism in liver. *Biochemical Journal* 142, 119–126.

Gilani, S.H. and Alibhai, Y. (1990) Teratogenicity of elements to chick embryos. *Journal of Toxicology and Environmental Health* 30, 23–31.

Girard, C.L. (2002) A new look at the requirements of high-producing dairy cows for B-complex vitamins. In: Wiseman, J. and Garnsworthy, P.C. (eds) *Recent Developments in Ruminant Nutrition*. Nottingham University Press, Nottingham, UK, pp. 237–254.

Girard, C.L. and Matte, J.J. (2005) Effects of intramuscular injections of vitamin B_{12} on lactation performance of dairy cows fed dietary supplements of folic acid and rumen protected methionine. *Journal of Dairy Science* 88, 671–676.

Givens, D.I. and Simpson, V.R. (1983) Serum vitamin B_{12} concentrations in growing cattle and their relationship with growth rate and cobalt bullet therapy. *Occasional Publication No. 7, British Journal of Animal Production*, pp. 145–146.

Grace, N.D. (2006) Effect of ingestion of soil on the iodine, copper, cobalt (vitamin B_{12}) and selenium status in grazing sheep. *New Zealand Veterinary Journal* 54, 44–46.

Grace, N.D. and Knowles, S.O. (2012a) Trace element supplementation of livestock in New Zealand: meeting the challenges of free-range grazing systems. *Veterinary Medicine International,* 639472. doi:10.1155/2012/639472

Grace, N.D. and Knowles, S.O. (2012b) Lack of production response in grazing dairy cows supplemented with long-acting injectable vitamin B12. *New Zealand Veterinary Journal* 60, 95-9.

Grace, N.D. and Lewis, D.H. (1999) An evaluation of the efficacy of microencapsulated vitamin B_{12} in increasing and maintaining serum and liver vitamin B_{12} concentrations in lambs. *New Zealand Veterinary Journal* 47, 3–7.

Grace, N.D. and Sinclair, G.R. (1999) Growth responses in lambs injected with a long-acting microencapsulated vitamin B_{12}. *New Zealand Veterinary Journal* 47, 213–214.

Grace, N.D. and West, D. (2000) Effect of an injectable microencapsulated vitamin B_{12} in increasing serum and liver vitamin B_{12} concentrations in calves. *New Zealand Veterinary Journal* 48, 70–73.

Grace, N.D., Clark, R.G. and Mortleman, L. (1986) Hepatic storage of vitamin B_{12} by the pregnant ewe and foetus during the third trimester. *New Zealand Journal of Agricultural Research* 29, 231–232.

Grace, N.D., Munday, R., Thompson, A.M., Towers, N.R., O'Donnell, K., McDonald, R.M., Stirnemann, M. and Ford, A.J. (1997) Evaluation of intraruminal

devices for combined facial eczema control and trace element supplementation in sheep. *New Zealand Veterinary Journal* 45, 236–238.

Grace, N.D., West, D. and Sargison, N.R. (1998) The efficacy of a subcutaneous injection of soluble vitamin B_{12} in lambs. *New Zealand Veterinary Journal* 46, 194–196.

Grace, N.D., Knowles, S.O., Sinclair, G.R. and Lee, J. (2003) Growth responses to increasing doses of microencapsulated vitamin B_{12} and related changes in tissue B_{12} concentrations in cobalt-deficient lambs. *New Zealand Veterinary Journal* 51, 89–92.

Grace, N.D., Knowles, S.O. and West, D. (2006) Dose–response effects of long-acting injectable vitamin B_{12} plus selenium (Se) on the vitamin B_{12} and Se status of ewes and their lambs. *New Zealand Veterinary Journal* 54, 67–72.

Grace, N.D., Knowles, S.O. and Nortjé, R. (2014) Vitamin B12 status and the effects of vitamin B12 supplementation during the first year of life of spring calves from pasture-fed dairy herds. *New Zealand Veterinary Journal* 62, 274–278.

Graulet, B., Matte, J.J., Desrochers, A., Doepel, L., Palin, M.-F. and Girard, C.L. (2007) Effects of dietary supplements of folic acid and vitamin B_{12} on metabolism of dairy cows in early lactation. *Journal of Dairy Science* 90, 3442–3455.

Gruner, T.M., Sedcole, J.R., Furlong, J.M., Grace, N.D., Williams, S.D., Sinclair, G.R., Hicks, J.D. and Sykes, A.R. (2004a) Concurrent changes in serum vitamin B_{12} and methylmalonic acid during cobalt or vitamin B_{12} supplementation of lambs while suckling and after weaning on properties in the South Island of New Zealand considered to be cobalt-deficient. *New Zealand Veterinary Journal* 52, 17–28.

Gruner, T.M., Sedcole, J.R., Furlong, J.M., Grace, N.D., Williams, S.D., Sinclair, G.R. and Sykes, A.R. (2004b) Changes in serum concentrations of MMA and vitamin B_{12} in cobalt-supplemented ewes and their lambs on two cobalt-deficient properties. *New Zealand Veterinary Journal* 52, 117–128.

Gruner, T.M., Sedcole, J.R., Furlong, J.M. and Sykes, A.R. (2004c) A critical evaluation of serum methylmalonic acid and vitamin B_{12} for the assessment of cobalt deficiency of growing lambs in New Zealand. *New Zealand Veterinary Journal* 52, 137–144.

Hart, L.I. and Andrews, E.D. (1959) Effect of cobaltic oxide pellets on the vitamin B_{12} content of ewes milk. *Nature (London)* 184, 1242–1243.

Hayakawa, T. (1962) Amounts of trace elements contained in grass produced in Japan. *National Institute of Animal Health Quarterly* 2, 172–181.

Hedrich, M.F., Elliot, J.M. and Lowe, J.E. (1973) Response in vitamin B_{12} production and absorption to increasing cobalt intake in the sheep. *Journal of Nutrition* 103, 1646–1651.

Huck, D.W. and Clawson, A.J. (1976) Excess dietary cobalt in pigs. *Journal of Animal Science* 43, 1231–1246.

Hunt, A., Harrington, D. and Robinson, S. (2014) Vitamin B_{12} deficiency. *British Medical Journal* 349, g5226.

Indyk, H.E., Persson, B.S., Caselunghe, M.C.B., Moberg, A., Filonzi, E.L. and Woolard, D.C. (2002) Determination of vitamin B_{12} in milk products and selected foods by optical biosensor protein-binding assay: method comparison. *Journal of AOAC International* 85, 72–81.

Johnson, E.H., Muirhead, D.E., Annamalai, K., King, G.J., Al-Busaidy, R. and Hameed, M.S. (1999) Hepatic lipidosis associated with cobalt deficiency in Omani goats. *Veterinary Research Communications* 23, 215–221.

Johnson, E.H., Al-Habsi, K., Kaplan, E., Srikandakumar, A., Kadim, I.T., Annamalai, K., Al-Busaidy, R. and Mahgoub, O. (2004) Caprine hepatic lipidosis induced through the intake of low levels of dietary cobalt. *The Veterinary Journal* 168, 174–179.

Judson, G.J., McFarlane, J.D., Riley, M.J., Milne, M.L. and Horne, A.C. (1982) Vitamin B_{12} and copper supplementation in beef calves. *Australian Veterinary Journal* 58, 249–252.

Judson, G.J., Brown, T.H., Kempe, B.R. and Turnbull, R.K. (1988) Trace element and vitamin B_{12} status of sheep given an oral dose of one, two or four soluble glass pellets containing copper, selenium and cobalt. *Australian Journal of Experimental Agriculture* 28, 299–305.

Judson, G.J., McFarlane, J.D., Mitsioulis, A. and Zviedrans, P. (1997) Vitamin B_{12} responses to cobalt pellets in beef cows. *Australian Veterinary Journal* 75, 660–662.

Judson, G.J., Babidge, P.J., Chen, Z.H. and Sansom, L. (2002) Recent developments in the detection and treatment of vitamin B12 deficiency in sheep and cattle at pasture. *Proceedings of the New Zealand Society of Animal Production* 6, 307–310.

Jumba, I.O., Suttle, N.F., Hunter, E.A. and Wandiga, S.O. (1995) Effects of soil origin and mineral composition and herbage species on the mineral composition of forages in the Mount Elgon region of Kenya. 2. Trace elements. *Tropical Grasslands* 29, 47–52.

Kadim, I.T., Johnson, E.H., Mahgoub, O., Srikandakumar, A., Al-Ajmi, D., Ritchie, A., Annamalai, K. and Al-Halhali, A.S. (2003) Effect of low levels of dietary cobalt on apparent digestibility in Omani goats. *Animal Feed Science and Technology* 109, 209–216.

Kawashima, T., Henry, P.R., Bates, D.G., Ammerman, C.B., Littell, R.C. and Price, J. (1997a) Bioavailability of cobalt sources for ruminants. 2. Estimation of the relative value of reagent grade and feed grade cobalt sources from tissue cobalt accumulation and vitamin B_{12} concentrations. *Nutrition Research* 17, 957–974.

Kawashima, T., Henry, P.R., Bates, D.G., Ammerman, C.B., Littell, R.C. and Price, J. (1997b) Bioavailability

of cobalt sources for ruminants. 3. *In vitro* ruminal production of vitamin B_{12} and total corrinoids in response to different cobalt sources and concentrations. *Nutrition Research* 17, 975–987.

Kendall, N.R., Jackson, D.W., Mackenzie, A.M., Illingworth, D.V., Gill, I.M. and Telfer, S.B. (2001) The effect of a zinc, cobalt and selenium soluble glass bolus on the trace element status of extensively grazed sheep over winter. *Animal Science* 73, 163–169.

Kennedy, D.G., Molloy, A.M., Kennedy, S., Scott, J.M., Blanchflower, W.J. and Weir, D.G. (1991a) Biochemical changes induced by nitrous oxide in the pig. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium of Trace Elements in Man and Animals, Dubrovnik*. IMI, Zagreb, pp. 17–18.

Kennedy, D.G., Young, P.B., McCaughey, W.J., Kennedy, S. and Blanchflower, W.J (1991b) Rumen succinate production may ameliorate the effects of cobalt-vitamin B_{12} deficiency on methylmalonyl CoA mutase in sheep. *Journal of Nutrition* 121, 1236–1242.

Kennedy, D.G., Blanchflower, W.J., Scott, J.M., Weir, D.G., Molloy, A.M., Kennedy, S. and Young, P.B. (1992) Cobalt–vitamin B_{12} deficiency decreases methionine synthase activity and phospholipid methylation in sheep. *Journal of Nutrition* 122, 1384–1390.

Kennedy, D.G., Young, P.B., Blanchflower, W.J., Scott, J.M., Weir, D.G., Molloy, A.M. and Kennedy, S. (1994) Cobalt–vitamin B_{12} deficiency causes lipid accumulation, lipid peroxidation and decreased alpha tocopherol concentrations in the liver of sheep. *International Journal of Vitamin Nutrition Research* 64, 270–276.

Kennedy, D.G., Young, P.B., Kennedy, S., Scott, J.M., Molloy, A.M., Weir, D.G. and Price, J. (1995) Cobalt–vitamin B_{12} deficiency and the activity of methyl malonyl CoA mutase and methionine synthase in cattle. *International Journal for Vitamin and Nutrition Research* 65, 241–247.

Kennedy, D.G., Kennedy, S. and Young, P.B. (1996) Effects of low concentrations of dietary cobalt on rumen succinate concentrations in sheep. *International Journal for Vitamin and Nutrition Research* 66, 86–92.

Kennedy, S., McConnell, S., Anderson, D.G., Kennedy, D.G., Young, P.B. and Blanchflower, W.J. (1997) Histopathologic and ultrastructural alterations of white liver disease in sheep experimentally depleted of cobalt. *Veterinary Pathology* 34, 575–584.

Kercher, C.J. and Smith, S.E. (1955) The response of cobalt-deficient lambs to orally administered vitamin B_{12}. *Journal of Animal Science* 14, 458–464.

Kincaid, R.L. and Socha, M.T. (2007) Effect of cobalt supplementation during late gestation and early lactation on milk and serum measures. *Journal of Dairy Science* 90, 1880–1886.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–310.

Knowles, S.O. and Grace, N.D. (2017) The vitamin B_{12} and Se status of lambs during their transition from milk-fed monogastric to grazing herbivore. *New Zealand Veterinary Journal* 65, 113–118.

Kozyraki, R. and Cases, O. (2013) Vitamin B12 absorption: mammalian physiology and acquired and inherited disorders. *Biochimie* 95, 1002–1007.

Lambert, B.D., Titgemeyer, E.C., Stokka, G.L., DeBey, B.M. and Loest, C.A. (2002) Methionine supply to growing steers affects hepatic activities of methionine synthase and betaine-homocysteine methyl transferase but not cystathione synthase. *Journal of Nutrition* 132, 2004–2009.

Lee, H.J. (1951) Cobalt and copper deficiencies effecting sheep in South Australia. Part 1. Symptoms and distribution. *Journal of Agricultural Science, South Australia* 54, 475–490.

Lee, H.J. and Kuchel, R.E. (1953) The aetiology of Phalaris staggers in sheep. 1. Preliminary observations on the preventive role of cobalt. *Australian Journal of Agricultural Research* 4, 88–99.

Lee, H.J. and Marston, H.R. (1969) The requirement for cobalt of sheep grazed on cobalt-deficient pastures. *Australian Journal of Agricultural Research* 20, 905–918.

Lee, S.H. and Hossner, K.L. (2002) Coordination of ovine adipose tissue gene expression by propionate. *Journal of Animal Science* 80, 2840–2849.

Lee, H.J., Kuchel, R.E., Good, B.F. and Trowbridge, R.F. (1957) The aetiology of Phalaris staggers in sheep. III. The preventive effect of various oral dose rates of cobalt. *Australian Journal of Agricultural Research* 8, 494–501.

Lewis, L.D., Knight, A., Lewis, B. and Lewis, C. (1995) In: Lewis, L.D. (ed.) *Equine Clinical Nutrition: Feeding and Care*. Wiley-Blackwell, New York, pp. 85–86.

Li, Z., McLaren, R.G. and Metherell, R.G. (2004) The availability of native and applied soil cobalt to ryegrass in relation to soil cobalt and manganese status and other soil properties. *New Zealand Journal of Agricultural Research* 47, 33–43.

Lines, E.W. (1935) The effect of the ingestion of minute quantities of cobalt by sheep affected with 'coast disease': a preliminary note. *Journal of the Council for Scientific and Industrial Research, Australia* 8, 117–119.

Ludemann, M. (2009) Development of an experimental approach to measure vitamin B_{12} production and absorption in sheep. PhD thesis, *Lincoln University*, Christchurch, New Zealand.

MacPherson, A. (1983) Oral treatment of trace element deficiencies in ruminant livestock. *Occasional Publication No.* 7. British Society of Animal Production, Edinburgh, UK, pp. 93–103.

MacPherson, A., Gray, D., Mitchell, G.B.B. and Taylor, C.N. (1987) Ostertagia infection and neutrophil function

in cobalt-deficient and cobalt-supplemented cattle. *British Veterinary Journal* 143, 348–355.

Marston, H.R. (1935) Problems associated with 'coast disease' in South Australia. *Journal of the Council for Scientific and Industrial and Industrial Research, Australia* 8, 111–116.

Marston, H.R. (1970) The requirement of sheep for cobalt or vitamin B_{12}. *British Journal of Nutrition* 24, 615–633.

Marston, H.R., Allen, S.H. and Smith, R.M. (1961) Primary metabolic defect supervening on vitamin B_{12} deficiency in the sheep. *Nature, UK* 190, 1085–1091.

Marston, H.R., Allen, S.H. and Smith, R.M. (1972) Production within the rumen and removal from the blood-stream of volatile fatty acids in sheep given a diet deficient in cobalt. *British Journal of Nutrition* 27, 147–157.

Masters, D.G. and Peter, D.W. (1990) Marginal deficiencies of cobalt and selenium in weaner sheep: response to supplementation. *Australian Journal of Experimental Agriculture* 30, 337–341.

McDonald, P. and Suttle, N.F. (1986) Abnormal fermentation in continuous cultures of rumen microorganisms given cobalt deficient hay or barley as the food substrate. *British Journal of Nutrition* 56, 369–378.

McKay, E.J. and McLery, L.M. (1981) Location and secretion of gastric intrinsic factor in the sheep. *Research in Veterinary Science* 30, 261–265.

McLoughlin, M.F., Rice, D.A. and McMurray, C.H. (1986) Hepatic lesions associated with vitamin B_{12} deficiency. In: *Proceedings of Sixth International Conference on Production Disease in Farm Animals, Belfast*. Veterinary Research Laboratory, Stormont, UK, pp. 104–107.

Millar, K.R. and Albyt, A.T. (1984) A comparison of vitamin B_{12} levels in the livers of sheep receiving treatments used to correct cobalt deficiency. *New Zealand Veterinary Journal* 32, 105–108.

Millar, K.R. and Lorentz, P.P. (1979) Urinary methylmalonic acid as an indicator of the vitamin B_{12} status of grazing sheep. *New Zealand Veterinary Journal* 27, 90–92.

Millian, N.S. and Garrow, T.A. (1998) Human betaine-homocysteine transferase is a zinc metalloenzyme. *Archives of Biochemistry and Biophysics* 356, 93–98.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Mitsioulis, A., Bansemer, P.C. and Koh, T.-S. (1995) Relationship between vitamin B_{12} and cobalt concentrations in bovine liver. *Australian Veterinary Journal* 72, 70.

Nexo, E. and Olesen, H. (1982) Intrinsic factor, transcobalamin and haptocorrin. In: Dolphin, D.D. (ed.) B_{12}–*Volume 2. Biochemistry and Medicine*. John Wiley & Sons, New York, pp. 57–86.

NICE (2015) *Active B12 assay for diagnosing vitamin B12 deficiency*. Medtech innovation briefing MIB40, National Institute for Health Care Excellence.

NRC (1984) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (1998) *Nutrient Requirements of Swine*, 10th edn. National Academy of Sciences, Washington, DC.

NRC (2005) *Mineral Tolerance of Animals*, 2nd edn. National Academy of Sciences, Washington, DC.

Polak, D.M., Elliot, J.M. and Haluska, M. (1979) Vitamin B_{12} binding proteins in bovine serum. *Journal of Dairy Science* 62, 697–701.

Poston, J.M. (1980) Cobalamin-dependent formation of leucine and β-leucine by rat and human tissue. *Journal of Biological Chemistry* 255, 10067–10072.

Poston, J.M. and Stadman, T.C. (1975) Cobamides as cofactors: methyl cobamides and the synthesis of methionine, methane and acetate. In: Babior, B.M. (ed.) *Cobalamin: Biochemistry and Pathophysiology*. John Wiley & Sons, New York, pp. 111–140.

Price, J. (1991) The relative sensitivity of vitamin B_{12}-deficient propionate and 1-carbon metabolism to low cobalt intake in sheep. In: Momcilovic, B. (ed.) *Proceedings of Seventh International Symposium on Trace Elements in Man and Animals, Dubrovnik*. IMI, Zagreb.

Price, J., Ueno, S. and Wood, S.G. (1993) Recent developments in the assay of plasma vitamin B_{12} in cattle. In: Anke, M., Meissner, D. and Mills, C.F. (eds) *Proceedings of the Eighth International Symposium on Trace Elements in Man and Animals*. Verlag Media Touristik, Gersdof, Germany, pp. 317–318.

Quadros, E.V., Matthews, D.M., Hoffbrand, A.V. and Linnell, J.C. (1976) Synthesis of cobalamin coenzymes by human lymphocytes *in vitro* and the effects of folates and metabolic inhibitors. *Blood* 48, 609–619.

Quirk, M.F. and Norton, B.W. (1987) The relationship between the cobalt nutrition of ewes and the vitamin B_{12} status of ewes and their lambs. *Australian Journal of Agricultural Research* 38, 1071–1082.

Quirk, M.F. and Norton, B.W. (1988) Detection of cobalt deficiency in lactating heifers and their calves. *Journal of Agricultural Science, Cambridge* 110, 465–470.

Rice, D.A., McLoughlin, M., Blanchflower, W.J., Goodall, E.A. and McMurray, C.H. (1987) Methyl malonic acid as an indicator of vitamin B_{12} deficiency in sheep. *Veterinary Record* 121, 472–473.

Riedel, B., Fiskerstrand, T., Refsum, H. and Ueland, P.M. (1999) Co-ordinate variations in methylmalonyl-CoA mutase and methionine synthase and the cobalamin factors in human glioma cells during nitrous oxide exposure and the subsequent recovery. *Biochemical Journal* 341, 133–138.

Russel, A.J.F., Whitelaw, A., Moberley, P. and Fawcett, A.R. (1975) Investigation into diagnosis and treatment of cobalt deficiency in lambs. *Veterinary Record* 96, 194–198.

Sanschi, D.E., Berthiaume, R., Matte, J.J., Mustafa, A.F. and Girard, C.L (2005) Fate of supplementary B-vitamins in the gastrointestinal tract of dairy cows. *Journal of Dairy Science* 88, 2043–2054.

Schulz, W.J. (1987a) Unsaturated vitamin B_{12} binding capacity in human and ruminant blood serum – a comparison of techniques including a new technique by high performance liquid chromatography. *Veterinary Clinical Pathology* 16, 67–72.

Schulz, W.J. (1987b) A comparison of commercial kit methods for assay of vitamin B_{12} in ruminant blood. *Veterinary Clinical Pathology* 16, 102–106.

Schwab, E.C., Schwab, C.G., Shaver, R.D., Girard, C.L., Putnam, D.E. and Whitehouse, N.L. (2006) Dietary forage and nonfiber carbohydrate contents influence B-vitamin intake, duodenal flow and apparent ruminal synthesis in lactating dairy cows. *Journal of Dairy Science* 89, 174–187.

Schwan, O., Jacobsson, S.-O., Frank, A., Rudby-Martin, L. and Petersson, L.R. (1987) Cobalt and copper deficiency in Swedish Landrace Pelt sheep. *Journal of Veterinary Medicine* 34, 709–718.

Schwarz, F.J., Stangl, G.L. and Kirchgessner, M. (2000) Cobalt requirement of beef cattle feed intake and growth at different levels of cobalt supply. *Journal of Animal Physiology and Nutrition* 83, 121–131.

Scott, J.M. (1992) Folate–vitamin B_{12} interrelationships in the central nervous system. *Proceedings of the Nutrition Society* 51, 219–224.

Shallow, M., Ellis, N.J.S. and Judson, G.J. (1989) Sex-related responses to vitamin B_{12} and trace element supplementation in prime lambs. *Australian Veterinary Journal* 66, 250–251.

Sherrell, C.G. (1984) Cobalt deficiency top-dressing recommendations. *Aglink FPD 814,* Ministry of Agriculture and Fisheries, Wellington.

Smith, E.L. (1948) Presence of cobalt in the anti-pernicious anaemia factor. *Nature, UK* 162, 144–145.

Smith, L.C. (2004) The effectiveness of autumn spray applications of cobalt sulphate, copper chelate and copper sulphate on Southland pastures. *Proceedings of the New Zealand Grassland Association* 66, 171–176.

Smith, R.M. (1997) Cobalt. In: O'Dell, B.L. and Sunde, R.A. (eds) *Handbook of Nutritionally Essential Mineral Elements*. Marcel Dekker Inc., New York, pp. 357–388.

Smith, R.M. and Marston, H.R. (1970) Production, absorption, distribution and excretion of vitamin B_{12} in sheep. *British Journal of Nutrition* 24, 857–877.

Smith, S.E., Koch, B.A. and Turk, K.L. (1951) The response of cobalt-deficient lambs to liver extract and vitamin B_{12}. *Journal of Nutrition* 144, 455–464.

Stangl, G.L., Roth-Maier, D.A. and Kirchgessner, M. (2000a) Vitamin B_{12} deficiency and hyperhomocysteinaemia are partly ameliorated by cobalt and nickel supplementation in pigs. *Journal of Nutrition* 130, 3038–3044.

Stangl, G.L., Schwarz, F.J., Jahn, B. and Kirchgessner, M. (2000b) Cobalt-deficiency-induced hyperhomocysteinaemia and oxidative stress in cattle. *British Journal of Nutrition* 83, 3–6.

Stangl, G.L., Schwarz, F.J., Muller, H. and Kirchgessner, M. (2000c) Evaluation of cobalt requirement of beef cattle based on vitamin B_{12}, folate, homocysteine and methylmalonic acid. *British Journal of Nutrition* 84, 645–653.

Sutherland, R.J., Cordes, D.O. and Carthew, G.C. (1979) Ovine white liver disease: a hepatic dysfunction associated with vitamin B_{12} deficiency. *New Zealand Veterinary Journal* 27, 227–232.

Suttle, N.F. (1988) The role of comparative pathology in the study of copper and cobalt deficiencies in ruminants. *Journal of Comparative Pathology* 99, 241–258.

Suttle, N.F. (1995) Relationship between vitamin B_{12} and cobalt concentrations in bovine liver. *Australian Veterinary Journal* 72, 278.

Suttle, N.F. (2000) Minerals in livestock production. *Asian-Australasian Journal of Animal Science* 13, 1–9.

Suttle, N.F., Jones, D., Clark, M. and Coop, R. (1986) Studies on the trace element/infection interface in lambs. In: *Proceedings of Sixth International Conference on Production Disease in Farm Animals, Belfast*. Veterinary Research Laboratory, Northern Ireland, pp. 195–198.

Suttle, N.F., Brebner, J., Munro, C.S. and Herbert, E. (1989) Towards an optimum dose of cobalt in anthelmintics in lambs. *Proceedings of the Nutrition Society* 48, 87A.

Suttle, N.F., Bell, J., Thornton, I. and Agyriaki, A. (2003) Predicting the risk of cobalt deprivation in grazing livestock from soil composition data. *Environmental Geochemistry and Health* 25, 33–39.

Sutton, A.L. and Elliot, J.M. (1972) Effect of ratio of roughage to concentrate and level of feed intake on ovine ruminal vitamin B_{12} production. *Journal of Nutrition* 102, 1341–1346.

Tiffany, M.E. and Spears, J.W. (2005) Differential responses to dietary cobalt in finishing steers fed corn- versus barley-based diets. *Journal of Animal Science* 83, 2580–2589.

Tiffany, M.E., Spears, J.W., Xi, L. and Horton, J. (2003) Influence of dietary cobalt source and concentration on performance, vitamin B_{12} status and ruminal and plasma metabolites in growing and finishing steers. *Journal of Animal Science* 81, 3151–3159.

Tiffany, M.E., Fellner, V. and Spears, J.W. (2006) Influence of cobalt concentration on vitamin B_{12} production and fermentation of mixed ruminal microorganisms in continuous culture flow-through fermenters. *Journal of Animal Science* 84, 635–640.

Tretter, L., Patocs, A. and Chinopoulos, C. (2016) Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. *Biochimica Biophyica Acta* 1857, 1086–1101.

Ulvund, M.J. (1995) Kobaltmangel hos sau. [Cobalt deficiency in lambs.] *Nordiske Veterinaria Tidsskrift* 107, 489–401.

Underwood, E.J. and Filmer, J.F. (1935) The determination of the biologically potent element (cobalt) in limonite. *Australian Veterinary Journal* 11, 84–92.

Underwood, E.J. and Suttle, N.F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Vellema, P., Rutten, V.P., Hoek, A., Moll, L. and Wentink, G.H. (1996) The effect of cobalt supplementation on the immune response in vitamin B_{12}-deficient Texel lambs. *Veterinary Immunology and Immunopathology* 55, 151–161.

Wahle, K.W.J., Duncan, W.R.H. and Garton, G.A. (1979) Propionate metabolism in different species of ruminant. *Annales de Recherche Veterinaire* 10, 362–364.

Walker, C.K. and Elliot, J.M. (1972) Lactational trends in vitamin B_{12} status on conventional and restricted-roughage rations. *Journal of Dairy Science* 55, 474–478.

Ward, N.E., Jones, J.E. and Maurice, D.V. (1986) Vitamin B12 nutriture of chickens fed raw soybean meal. *Poultry Science* 65,106–113.

Watanabe, F., Nakano, Y., Tachikake, N., Saido, H., Tamura, Y. and Yamanaka, H. (1991) Vitamin B_{12} deficiency increases the specific activities of rat liver NADH- and NADPH-linked aquocobalamin reductase isoenzymes involved in coenzyme synthesis. *Journal of Nutrition* 121, 1948–1954.

Wiese, S.C., White, C.L., Williams, I.H. and Allen, J.G. (2007) Relationships between plasma methylmalonic acid and ruminal succinate in cobalt-deficient and -repleted sheep. *Australian Journal of Agricultural Research* 58, 367–373.

Yang, W.Z., Beauchemin, K.A., Koenig, K.M. and Rode, L.M. (1997) Comparison of hull-less barley, barley, or corn for lactating cows: effects on extent of digestion and milk production. *Journal of Dairy Science* 80, 2475–2486.

Zervas, G. (1988) Use of soluble glass boluses containing Cu, Co and Se in the prevention of trace element deficiencies in goats. *Journal of Agricultural Science, Cambridge* 110, 155–158.

Zinn, R.A., Owens, F.N., Sturat, R.L., Dunbar, J.R. and Norman, B.B. (1987) B-vitamin supplementation of diets for feedlot calves. *Journal of Animal Science* 65, 267–277.

11 Copper

Following the discovery that copper (Cu) was essential for growth and haemoglobin formation in laboratory rats (Hart *et al.*, 1928), a number of naturally occurring wasting diseases in grazing animals were described, in which blood and liver Cu were low and health was restored by supplementary Cu (Suttle, 1991). Severe diarrhoea ('teart'), affecting cattle on molybdenum (Mo)-*rich* pastures on the Somerset Levels in the UK, was also controlled by massive doses of copper, although Cu status was normal (Ferguson *et al.*, 1938, 1943). Concurrent investigations of another 'area' problem, chronic poisoning of sheep in eastern Australia, was characterized by abnormally *low* herbage Mo and controlled by supplementing with Mo (Dick and Bull, 1945). Underlying both problems was a profound effect of Mo on copper metabolism, potentiated by a third dietary factor, thought to be sulphate (SO_4) (Dick, 1956). The primary defect was eventually shown to be impaired Cu absorption, potentiated by organic as well as inorganic S through the formation of unabsorbable, Cu-thiomolybdate complexes in the rumen (Suttle, 1991). This three-way interaction was involved in most outbreaks of copper-responsive disorder (CuRD) in grazing livestock (Suttle, 2012a). Studies with laboratory animals revealed the complex ways in which Cu is safely absorbed, transported and incorporated into functional enzymes and proteins (Prohaska, 2006; Clarkson *et al.*, 2020). Ironically, CuRD rarely occur naturally in non-ruminants, including humans (Suttle, 2012a), but still perplexes agronomists in South Australia by its rarity (Dickson, 2016) and veterinarians in 'teart' areas of Ireland by its persistence (McAloon *et al.*, 2017). Although there have been no major breakthroughs in studies with ruminants, they remain the focus of this chapter because misconceptions still hold sway (Clarkson *et al.*, 2020) and may have lethal consequences (Suttle, 2012a, b).

Functions of Copper

Copper is present in and essential for the activity of numerous enzymes (Table 11.1), cofactors and reactive proteins. Essentiality for processes such as reproduction and bone development is presumed to depend on such functions but only two processes have unequivocal dependency on a specific enzyme: pigmentation on tyrosinase and connective tissue development on lysyl oxidases (Suttle, 1987; Prohaska, 2006). Genes controlling many Cu-dependent enzymes and proteins were identified, including the sheep caeruloplasmin gene, *sCp* (Lockhart and Mercer, 1999) and gene-deletion studies with laboratory animals showed some to be necessary for embryonic survival (e.g. dopamine-β-monooxygenase (DBM) and the transmembrane, Cu-transporting protein, Ctr1). Links between functional molecule and physiological process may be clarified by new techniques that follow the sequence in which the animal tries to avert insufficiency by 'switching on' specific genes.

Cellular respiration

The ubiquitous haem enzyme complex, cytochrome c oxidase (CCO), is responsible for the terminal electron transfer in the respiratory chain and thus for energy generation in all tissues. Low CCO activity may cause several dysfunctions, including impairment of the respiratory burst in neutrophils that is integral to cellular immunity (Jones and Suttle, 1987). In Cu-depleted cattle, a breakdown of basement acinar cell membranes in the pancreas is one of the earliest pathological lesions: reduced CCO activity has been implicated and may have secondary effects on glycosylation or sulfation (Fell *et al.*, 1985). CCO activity in the liver (Mills *et al.*, 1976), gut mucosa (Fell *et al.*, 1975; Suttle and Angus, 1978) and neutrophils is also reduced but there is usually a surplus of CCO in all tissues (Smith

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Table 11.1. Some copper-dependent enzymes found in mammalian tissues,[a] their functions and possible consequences of a marked reduction in activity. (From Bonham *et al*., 2002; Prohaska, 2006)

Enzyme	Functions	Abbreviation	Pathognomic significance
Caeruloplasmin[b] (ferroxidase)	$Fe^{2+} \rightarrow Fe^{3+}$, hence Fe transport; antioxidant	Cp	Anaemia
Cytochrome c oxidase	Terminal electron-transfer respiratory chain	CCO	Anoxia (causing neuronal degeneration, cardiac hypertrophy)
Diamine oxidase	Oxidative deamination of diamines and their derivatives	DAO	Unknown
Dopamine-β-monooxygenase	Catecholamine metabolism	DBM	Behaviour?
Hephaestin	Export of iron from intestine	HEP	Anaemia
Ferroxidase II	Iron oxidation		Anaemia
Lysyl oxidases	Desmosine cross-linkages in connective tissues	LO	Aortic rupture, joint disorders, osteoporosis
Monoamine oxidase	Oxidative deamination of monoamines	MAO	Cell signalling, leucocyte trafficking
Peptidylglycine-α amidating monooxygenase	Elaboration of numerous biogenic molecules (e.g. gastrin)	PAM	Appetite?
Superoxide dismutases	Intracellular and extracellular dismutation of O_2^- to H_2O_2	SOD 1, 2 and 3	Lipid peroxidation, modulation of vascular tone
Thiol oxidase	Disulfide bond formation	TO	Loss of wool and hair strength
Tyrosinase	Tyrosine → melanin	TY	Depigmentation

[a]These enzymes are found in varying concentrations and excesses in most tissues. Some are supplied with copper by specific 'chaperone' proteins (e.g. SOD) and their functions may overlap with each other (e.g. caeruloplasmin (Cp) and HEP) or with those of copper-independent enzymes (e.g. MnSOD). Reductions in activity are not therefore synonymous with dysfunction or copper-responsive disorder.

[b]Multiple forms and/or genes have been identified and may indicate a level of functional importance that is underestimated by survival in specific gene-deletion studies.

et al., 1976) and respiration rates are not necessarily constrained. Gene-deletion studies show that mice cannot survive without COX1, a protein that carries Cu into mitochondria for CCO synthesis (Prohaska, 2006). Furthermore, failure of CCO to sustain critical, rapidly growing cells like neuronal axons may lead to secondary failure in other processes such as myelination in the central nervous system (CNS).

Protection from oxidants

Copper protects tissues against 'oxidant stress' from free radicals, including those generated during respiration, interacting with other antioxidants as it does so. Neutrophils rely upon the 'respiratory burst' to generate free radicals such as superoxide (O_2^-) that kill engulfed pathogens but must have fully functional oxidant defences to avoid 'self-harm'. Blastogenesis (Jones and Suttle, 1987) and phagocytic killing (Boyne and Arthur, 1981; Jones and Suttle, 1981; Xin *et al*., 1991) are compromised in cultured cells from Cu-deprived sheep and cattle. Three superoxide dismutases (SOD1, 2 and 3) convert O_2^- to H_2O_2 and SOD1 activity in neutrophils is reduced when dietary Cu is lacking. Extracellular SOD3 is upregulated by inflammatory stimuli (Jiang *et al*., 2016). The predominant Cu protein in plasma, Cp, is expressed in several tissues (Prohaska, 2006) and scavenges free radicals (Saenko *et al*., 1994). Hepatic activity of the Fe-containing haem enzyme, catalase, was reduced by lack of Cu (Taylor *et al*., 1988). Catalase metabolizes H_2O_2, source of the dangerous OH^- radical and a modulator of cell signalling (Clarkson *et al*., 2020).

Iron metabolism

The historic role of Cp in Fe transport has been overshadowed by recent research into hephaestin, a Cu-protein with 50% homology to Cp and similar properties, including ferroxidase activity. When animals are deprived of Cu, hephaestin concentrations in enterocytes decrease and those of Fe increase, probably because ferroxidase activity was inadequate. Deletion of the *Cp* gene produced only

a mild anaemia, suggesting that hephaestin and ferroxidase II may be more important contributors of ferroxidase activity (Prohaska, 2006). It is now known that Cp facilitates the recycling of Fe by the reticulo-endothelial system and participates in Fe homeostasis in brain, as a complex, conjugated molecule (Collins *et al.*, 2010).

Functions of copper-binding proteins

There are more Cu-binding proteins than there are cuproenzymes, though not all have proven functions (Bonham *et al.*, 2002; Prohaska, 2006). Metallothioneins (MT), especially MTII (Lee *et al.*, 1994), bind Cu and may have protective roles during toxicosis. Two clotting agents, factors 5 and 8, are cuproproteins with Cu-dependent activity (Prohaska, 2006) and lack of dietary Cu may impair blood clotting by weakening platelet adhesion (Lominadze *et al.*, 1997). Factor 8 is structurally similar to Cp (Saenko *et al.*, 1994). A family of Cu-transporter and -chaperone proteins transfer copper across membranes, partition Cu between intracellular enzymes and export excess Cu from enterocytes, hepatocytes and erythroid cells (Clarkson *et al.*, 2020).[i] Prion proteins may facilitate storage of Cu in the CNS, which becomes deranged in bovine spongiform encephalopathy (Hanlon *et al.*, 2002). An hypothesis that the substitution of Mn for Cu on prion proteins was of pathogenic significance found no support (Legleiter *et al.*, 2008).

Clinical Signs that Ruminants Lack Copper

Swayback

Following pioneering field studies in Western Australia (Bennetts and Chapman, 1937), swayback was eventually reproduced experimentally by feeding pregnant ewes diets either low in Cu or high in Cu-antagonists (Suttle, 1988a). Three types of ataxia occur naturally in lambs:

- *neonatal* (paralysis at birth, followed by death; primary anoxic lesions in the brain stem and demyelination of the cerebral cortex);
- *delayed* (uncoordinated hind-limb movements, stiff and staggering gait, swaying hind quarters, often triggered by flock disturbances); and
- *atypical* (older lambs standing transfixed, head quivering and apparently blind; primary lesion, cerebral oedema).

Neonatal ataxia is related to a phase of rapid myelination in the fetal CNS in mid-pregnancy. A second phase of spinal cord myelination occurs a few weeks after birth and copper deprivation from late pregnancy causes delayed ataxia. Demyelination is caused by myelin aplasia, rather than myelin degeneration, associated with degeneration of motor neurons of the brain and spinal cord in sheep (Suttle, 1988) and goats (Wouda *et al.*, 1986). All lesions are irreversible, may commence as early as six weeks before birth, continue to develop afterwards and are the most sensitive clinical consequence of copper deprivation in sheep (Suttle *et al.*, 1970). Swayback may be the only abnormality to develop in a flock deprived of copper. In goat kids, delayed swayback predominates (Hedger *et al.*, 1964) and cerebella hypoplasia is an additional feature (Wouda *et al.*, 1986). Slow regular myelination of the CNS protects calves from swayback (Suttle, 1988a), but deer can be affected (Grace and Wilson, 2002). Adult pregnant yak (*Bos grunniens*) are affected by a CuRD called 'shakeback disease' because of an unsteady gait (Xiao-Yun *et al.*, 2006a, b). However, no neuropathological investigations were conducted and no abnormalities in offspring were reported.

Abnormal structure and colour of wool and hair

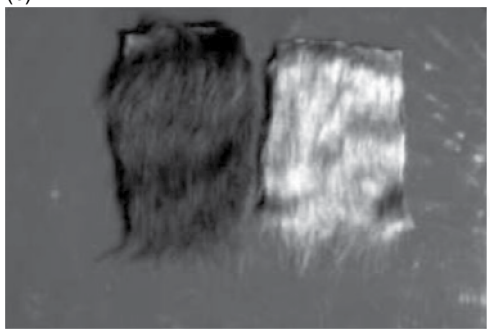
Fleeces display the first sign of abnormality in sheep, deprived of copper after birth. Wool crimp is progressively lost until fibres emerge almost straight, giving rise to 'stringy' and 'steely' wool with diminished tensile strength and elasticity (Lee, 1956). Abnormalities are particularly prominent in breeds with heavily crimped wool such as Merino (Hannam and Reuter, 1977) but supplementation restores crimp to new wool growth (Underwood, 1977). Black fleeces become white and unpigmented bands can be produced by intermittent Cu deprivation (Underwood, 1977). Black-fleeced 'sentinel' sheep in a flock can provide early warning of hypocuprosis. Changes in growth and physical appearance of hair in cattle give the coat a thin, wavy, rough appearance (Suttle and Angus, 1976). Black coats can turn grey or develop a brown tinge (Hansen *et al.*, 2009) while brown coats can become bleached (Fig. 11.1): loss of colour round the eye can give a 'spectacled' appearance, often the earliest and sometimes only clinical abnormality. Loss of coat colour was not a recognizable feature of yak with 'shakeback' (Xiao-Yun *et al.*, 2006a, b).

Diarrhoea

Diarrhoea can be found in hypocuprotic grazing cattle and induced experimentally by depriving calves of Cu (Mills *et al.*, 1976): histochemical and ultrastructural changes were observed in the small intestine (Fell *et al.*, 1975) but no such changes were seen in other clinically affected calves (Suttle and Angus, 1978). Diarrhoea can occur on Mo-rich, 'Teart pastures' *before* liver or blood Cu decrease and may be caused by acute depletion of the caecal mucosa (Suttle, 2012a). Sheep on 'teart pastures' are less affected than cattle but diarrhoea can be induced by MoO_4 supplementation (Suttle and Field, 1968; Hogan *et al.*, 1971). Scouring has been observed in goats on Dutch 'teart' pastures.

(a)

(b)



(c)

Fig. 11.1. Clinical symptoms of copper deficiency in cattle include (a) loss of hair colour, giving a 'spectacled' appearance around the eye; (b) loss of hair; and (c) skeletal abnormalities, including osteoporosis and widening of epiphyses.

Susceptibility to infection

Long-term studies of lamb mortality on a Scottish hill farm revealed a marked increase in mortality after pastures were improved by liming and reseeding (Fig. 11.2a). Losses were higher in breeds of relatively low Cu status and reduced either by Cu supplementation or genetic selection for high Cu status (Fig. 11.2b). Microbial infections were major causes of death.

- Parallel changes in incidence of a proven sign of hypocuprosis – swayback – confirm that susceptibility to infection was increased by lack of dietary Cu.

Subsequent work indicated that perinatal mortality was increased unless Cu supplements were given in late pregnancy, but the precise role of infection was not established (Suttle *et al.*, 1987b).

Bone and connective tissue disorders

Bones develop abnormally in young livestock, deprived of Cu, but pathological features vary widely within and between species. Disturbances of endochondral ossification can cause uneven bone growth (osteochondrosis), morphology being influenced by rate of growth, body-weight distribution, movement and rate of hoof growth at onset of deprivation. Widening of epiphyses in lower-limb bones in growing cattle can give a rachitic appearance (Fig. 11.1c). Osteitis fibrosa was found in vertebrae of calves, slowly deprived of Cu (Mills *et al.*, 1976). 'Beading' of the ribs in sheep and cattle is due to overgrowth of costochondral junctions. A generalized osteoporosis and low incidence of spontaneous bone fractures can occur in grazing cattle and lambs (Cunningham, 1950; Whitelaw *et al.*, 1979).

Connective tissue disorders may not always be recognized. Gross defects in articular cartilages

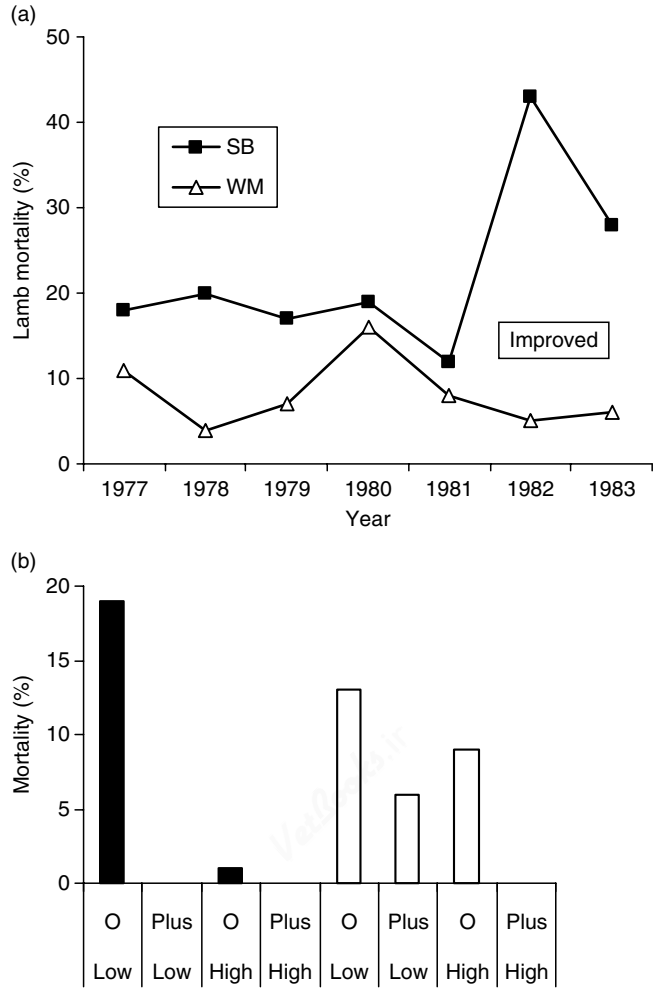


Fig. 11.2. (a) Lamb mortality in Scottish Blackface (SB) and Welsh Mountain (WM) lambs, breeds known to absorb copper with low and high efficiency, respectively, managed as a single flock before and after pasture improvement. (b) Copper supplementation (O versus plus) reduced losses from both swayback (solid columns) and infectious diseases (open columns) in crossbred lambs, selected for low rather than high plasma copper. (Data from Woolliams *et al.,* 1986a)

(Thompson *et al.*, 1994), impaired collagen and elastin development and osteochondrosis in young, farmed deer (Grace and Wilson, 2002) were attributed to lack of dietary Cu. Sub-periosteal haemorrhages and imperfect tendon attachments were found in lambs on Mo-rich pastures (Hogan *et al.*, 1971; Pitt *et al.*, 1980). In cattle, lesions in the *ligamentum nuchae*, supporting neck and scapulae, were found in experimentally deprived calves: in the field, scapulae may dislocate and heads droop, creating a 'humped' posture (B. Ruksan, personal communication, 1989). Abnormal stiff-legged gaits are probably due to a combination of bone and connective tissue disorders.

Anaemia

Anaemia develops after severe or prolonged deprivation in susceptible breeds and is hypochromic and microcytic in lambs, as in iron deficiency. In Jersey cows and Scottish Blackface ewes, anaemia can be hypochromic and macrocytic (Suttle, 1987).

Haemolysis may contribute to the development of anaemia. Heinz bodies, signs of oxidative stress, have been reported in erythrocytes of lambs, low in Cu (Suttle *et al.*, 1987a). Low activity of erythrocyte SOD1 was implicated in haemolytic, Heinz-body anaemia in hypocupraemic cattle, given kale (Barry *et al.*, 1981). Hypochromic, microcytic anaemia was found in pregnant and lactating yak and responded to Cu supplementation (Xiao-Yun *et al.*, 2006a, b).

Cardiovascular disorders

Cardiac lesions were a feature of 'falling disease' of cattle in Western Australia and attributed to lack of dietary Cu (Bennetts and Hall, 1939). Sudden deaths, usually after mild exercise or excitement, were attributed to acute heart failure and associated with myocardial degeneration, replacement fibrosis, extremely low liver and blood Cu and high tissue Fe.[ii] Studies with small rodents and rabbits showed that Cu was needed for angiogenesis (the proliferation of blood vessels) (Harris, 2004). Impaired angiogenesis might impair bone remodelling (Suttle, 2012a). A combination of *in vitro* and *in vivo* methods indicated that inhibition of Cu transport restricted wound healing by slowing smooth muscle cell migration (Ashino *et al.*, 2010).

Infertility

Infertility and abortions were reported in experimentally deprived ewes (Howell and Hall, 1970). Low fertility associated with delayed or depressed oestrus was reported in beef cows, grazing pastures low in Cu, but the relationship with low plasma Cu was inconsistent (Phillippo *et al.*, 1982). In penned heifers, given a diet marginal in Cu (4 mg) with Mo (5 mg as MoO_4) and/or Fe (500–800mg kg^{-1}DM as $FeSO_4$) added as copper antagonists, infertility only occurred in groups given MoO_4, although liver and plasma Cu seemed equally low with only Fe added (Humphries *et al.*, 1983; Phillippo *et al.*, 1987b). Others extrapolated these findings to infer that infertility was widely caused by thiomolybdate toxicity in normocupraemic herds, without valid supporting evidence (Suttle, 2012a). Both improvements and impairments of fertility have occasionally been reported in normocupraemic cows given parenteral Cu (Suttle and Phillippo, 2005).

Subclinical Effects on Diets Lacking Copper

Poor growth was a common feature of hypocuprosis in grazing sheep (Whitelaw *et al.*, 1979, 1983; Woolliams *et al.*, 1986b), cattle (Thornton *et al.*, 1972a, b; Whitelaw *et al.*, 1984) and deer (Grace *et al.*, 2005a) and usually associated with mild exposure to Mo (Phillippo, 1983), as under experimental conditions (Humphries *et al.*, 1983; Phillippo *et al.*, 1987a; Gengelbach *et al.*, 1994). Furthermore, growth can be retarded in the absence of clinical abnormalities and accompanied by poor feed conversion efficiency (FCE), when Mo-exposure begins during pregnancy (Legleiter and Spears, 2007). The possibility that both growth retardation, like infertility, is caused by thiomolybdate toxicity rather than lack of dietary Cu will be discussed later (p. 265). Low milk yield is believed to have caused poor growth in suckled calves, when beef cows grazed pastures high in Mo (Wittenberg and Devlin, 1987).

Occurrence of Copper-responsive Disorders

The wide range of Cu-RD reported (Suttle, 1987) can mostly be described collectively and clinically as 'hypocuproses', susceptibility depending on species and stage of development at the time of deprivation, whether or not Mo is involved (Suttle, 2012a). The hypothesis that most CuRD are 'molybdenoses' is discussed elsewhere (p. 264).

Influence of rumen metabolism

Hypocuprosis is caused by events in the rumen that lower efficiency of absorption, A_{Cu}, rather than lack of dietary Cu. Organic and inorganic S sources are degraded to sulfide (S^{2-}), which potentiates antagonism from Mo and Fe (Suttle 1991). Most of the Cu released during rumen digestion is either precipitated as CuS (Bird, 1970) and FeS or forms unabsorbable complexes with a rumen metabolite, tetrathiomolybdate (TTM) and remains unabsorbed. Copper released during post-ruminal digestion may become partially bound to undigested fibrous constituents. Whereas Cu was absorbed with efficiency (A_{Cu}) of 0.70–0.85 in young milk-fed lambs, A_{Cu} was <0.10 in weaned lambs (Suttle, 1974). Rumen protozoa generate S^{2-} and their removal by rearing in a sterile environment (Ivan, 1988) or defaunation (Barrowman and van Ryssen,

1987) increases A_{Cu}. As herbage S, Mo and/or Fe increase, A_{Cu} can fall as low as 0.01 and risk of hypocuprosis rises. Superimposed on and sometimes amplifying rumen-based effects are those of geochemistry, plant, season and animal genotype.

Geochemical factors

Copper deprivation has been associated with three different soil anomalies: inherently low soil Cu status, as in calcareous sands of Western Australia (Beck, 1951) and heathlands of East Anglia (UK); low plant-available Cu status, as in peat soils rich in organic matter (Haynes, 1997); and those enriched with Mo, which create pastures low in Cu that is available to the grazing animal: most CuRD came into the latter category (Phillippo, 1983). Sheep tolerated herbage with <3mg Cu kg^{-1} DM without becoming hypocupraemic in South Australia but Mo and S were also low (Dickson, 2016). By contrast, hypocuprosis is endemic on pastures underlain by Mo-rich soils, which can raise herbage Mo to >20mg kg^{-1}DM.[iii] Problem areas can be delineated by maps of soil or stream sediment Mo (Thornton and Alloway, 1974; Boila *et al.*, 1984b). Persistence of hypocupraemia on marine shales in Caithness suggested that antagonism from Mo was not readily controlled (Suttle, 2008b). Suggestions that Mo toxicity was responsible for endemic infertility in normocupraemic UK dairy herds, without underlying soil anomalies, were groundless (Suttle, 2012a). Bovine hypocupraemia was associated with Fe-rich soils (rendzinas) in South Australia (McFarlane *et al.*, 1990) and contamination of herbage with soil Fe in England (Jarvis and Austin, 1983). Ingestion of SO_4-rich drinking water from deep aquifers caused hypocupraemia, growth retardation and neonatal mortality in heifers (Smart *et al.*, 1986). In northern India, CuRD in buffalo appeared to be attributable to low Cu status of soil and fodder (Sharma *et al.*, 2008).

Plant factors

Choice of pasture species and pasture management can affect uptake of Cu and Mo from soils (p.22) and hence risk of CuRD. Improvement of Scottish hill pastures by liming and reseeding induced hypocuprosis in lambs (Fig. 11.2) (Whitelaw *et al.*, 1979, 1983) and calves (Whitelaw *et al.*, 1984) at research establishments by raising herbage Mo and S, but did not do so elsewhere in Scotland (Suttle, 2017) or in Australia (Hannam and Reuter, 1977). Reliance on legumes rather than fertilizer as a source of supplementary N on 'organic' farms may increase risk of hypocuprosis on Mo-rich soils because of the propensity of legumes to accumulate Mo. Outbreaks of swayback on coastal pastures in Greece were attributed to ingestion of SO_4-rich halophytic plants by ewes (Spais, 1959) and a similar pathogenesis explained outbreaks in sheep that grazed seaweed in Iceland (Palsson and Grimsson, 1953): some halophytic browse species may be low in available Cu (Ben Salem *et al.*, 2010). Animal production from coastal areas, where pasture S is high, may require intervention with Cu. Heinzbody anaemia and growth retardation, both responsive to Cu supplementation, were reported in cattle consuming a S-rich brassica (kale) crop (Barry *et al.*, 1981) and lambs fattened on rape (Suttle and Peter, 1985).

Seasonal factors

Peaks in herbage Fe in autumn, temperate pasture (Jarvis and Austin, 1983) or seasonally waterlogged pastures (McFarlane *et al.*, 1990) may trigger hypocuprosis and simultaneous increases in Mn may add to the antagonism (p. 273). Irrigation of pasture with Fe-rich bore-water induced diarrhoea in cattle (Campbell *et al.*, 1974). Pasture Mo increased greatly between April and August in Scotland (Suttle and Small, 1993; Suttle, 2017) and Norway (Govasmark *et al.*, 2005). However, herbage S declines as pastures mature (see p. 210) and Cu is probably more absorbable from mature than fresh green herbage. Hypocuprosis is essentially a 'green sward' problem, rarely seen during dry summers or in arid regions, except around irrigated oases (Saleh *et al.*, 2008). Plasma Cu was higher in goats in the dry than in the cool season in Oman (Osman *et al.*, 2003). In parts of southern Australia (Hannam and Reuter, 1977) and UK (Bain *et al.*, 1986), bovine hypocupraemia was associated with months (and years) of heavy rainfall. Swayback of lambs is more prevalent after mild than after severe winters, possibly because less supplementary feeding and more soil ingestion reduce A_{Cu} in mild winters (Suttle *et al.*, 1984). Plasma Cu varied between years in Scottish hill lambs (Suttle, 2017). However, occurrence of hypocuprosis will remain poorly correlated with current herbage composition, being hugely dependent on sufficiency of Cu stores, built up during times of generous supply.

Genetic factors

Breeds of sheep differ in susceptibility to hypocuprosis, Scottish Blackface being more susceptible than Welsh Mountain (Fig. 11.2), Texel, North Ronaldsay and Suffolk sheep: the latter breeds are virtually immune. Differences between breeds of cattle are less pronounced and recognition is complicated by breed and seasonal differences in coat colour. Simmental are more vulnerable to insufficiency than Aberdeen Angus (Gooneratne *et al.*, 1994; Mullis *et al.*, 2003; Stahlhut *et al.*, 2005; Legleiter *et al.*, 2008; Fry *et al.*, 2013). Cattle are more susceptible to hypocuprosis than sheep. In New Zealand, cattle could have low liver Cu and sheep moderate or high liver Cu, while grazing the same property (Korte, 1996; cited by Grace *et al.*, 1998a). Breed differences in plasma Cu have been reported in goats in Oman but only in the dry season and in adult females, not bucks or kids (Osman *et al.*, 2003).

Occurrence in horses

Thoroughbreds grazing the same pastures as hypocuprotic cattle remained healthy and showed little or no response in plasma and liver Cu to supplementation by oral or parenteral routes (Pearce *et al.*, 1999a; Grace *et al.*, 2002). Equine species can be grouped with other non-ruminants in which uninhibited A_{Cu} lessens the need to store Cu (Fig. 11.3). Angular deformities at the pasterns were claimed to respond to Cu supplementation (Barton *et al.*, 1991) but there were other possible causes. Swelling of the distal radial physis in Thoroughbred yearlings did not respond to Cu supplements (Grace *et al.*, 2002). Horses are not adversely affected by pastures high in Mo (Pearce *et al.*, 1999b), presumably because thiomolybdate (TM) cannot be generated until digesta reach the hind gut, by which time most dietary Mo has been absorbed. There are no losses of Cp during clotting in equine samples (Paynter, 1987) to complicate the assessment of serum Cu and a normal range of 16.5–20.0 μmol l^{-1} was suggested for stabled racehorses (Suttle *et al.*, 1996b). However, a seasonal fall took values as low as 9 μmol l^{-1} in grazing fillies in midwinter, possibly due to photoperiodism (Pearce *et al.*, 1999a). Like most newborns, the foal begins life with a large hepatic reserve: as much as 374 ± 130 (s.d.) mg kg^{-1}DM was found regardless of whether mares had been given Cu by intramuscular injection during pregnancy (Gee *et al.*, 2000): most foals showed a rapid decline to normal 'adult' values by 160 days of age (21 ± 6mg kg^{-1}DM), but a small subgroup showed a much slower rate of decline.

Biochemical Evidence of Insufficiency

Normality

The newborn generally begins life with high liver Cu stores (Prohaska, 2006), values rising to 3–6 mmol kg^{-1}DM in newborn calves and lambs from mothers well-supplied with Cu during pregnancy (Underwood, 1977): 15–35% of ovine fetal liver Cu is associated with MT (Bremner *et al.*, 1977). Uptake of dietary Cu is facilitated by both specific (Crt1) and non-specific (divalent metal transporter, DMT) proteins in intestinal mucosa enterocytes (Prohaska, 2006; Hansen *et al.*, 2008). Other proteins shepherd the potentially toxic element to cytosolic and mitochondrial proteins via the golgi apparatus, exporting Cu into the bloodstream at the basal membrane, a process repeated when the liver and other tissues are reached (Clarkson *et al.*, 2020). Adjustment to fluctuations in Cu supply is achieved by changing the expression of genes for transporters and chaperones, which control absorption, hepatic storage and export (as Cp and biliary Cu secretion) (Clarkson *et al.*, 2020). The relative importance of each process and outcome in terms of relationships between dietary and liver Cu vary widely between species. (Fig. 11.3). Non-ruminants have good control over absorption and biliary secretion, allowing entero-hepatic recycling of Cu; liver Cu remains low over a wide range of dietary Cu. By contrast, ruminants have relatively poor control over absorption and store excess dietary Cu avidly in the liver. Losses of biliary Cu in sheep can increase more than fivefold as liver Cu rises (Fig. 11.4) although breeds may differ in this respect (Suttle *et al.*, 2002). Urinary excretion of Cu is normally small, constant and unaffected by Cu intake in all species, although increased in sheep co-exposed to MoO_4 and SO_4 (Smith *et al.*, 1968).

Depletion

Initial liver Cu reflects dietary Cu history and deprivation of the dam can drastically reduce neonatal reserves, hastening subsequent depletion and revealing effects of maternal breed (compare Wiener *et al.*, 1984a versus 1984b). Priority is clearly given to the fetus because cows with low liver Cu (<0.39 mmol kg^{-1}DM) can deliver calves with livers containing 5.2 mmol Cu kg^{-1}DM (Gooneratne and Christensen,

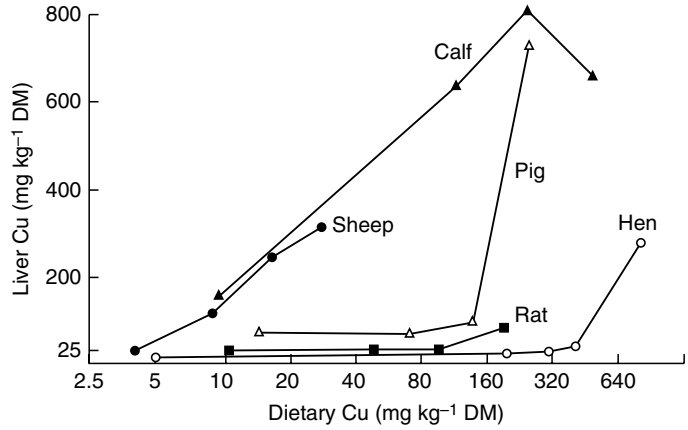


Fig. 11.3. Species differences in hepatic copper storage after exposure to excess dietary copper. Ruminants, for which risk of copper deficiency is ever-present at pasture, store copper avidly while non-ruminant species, which are rarely at such risk, do not: cattle reach an earlier plateau in liver Cu than sheep, although this is not shown in the graph.

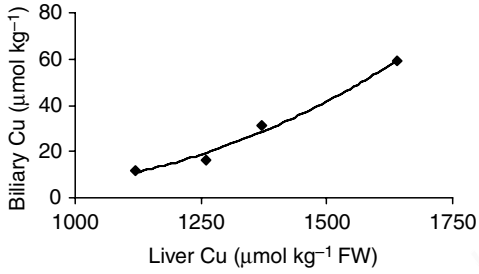


Fig 11.4. Regulation of copper metabolism in grazing Romney ewes, indicated by a linear relationship between biliary Cu and liver Cu above 1250 µmol kg^{-1} DM. Downregulation of absorption would augment biliary regulation by diminishing the reabsorption of secreted Cu. (Data from Grace *et al*., 1998a)

1989). However, with maternal values <0.26 mmol kg^{-1}DM, fetal values declined by up to 50%. The pattern followed by liver Cu during depletion is one of exponential decline at a rate determined by the deficit between supply and demand (Balemi *et al*., 2010; Grace *et al*., 2012; Suttle, 2013).

Deficiency

When liver reserves approach exhaustion or cannot be mobilized fast enough to offset inadequate supply, Cp synthesis decreases and plasma Cu falls below normal (Fig. 11.5). Up to this point, intracellular trafficking of Cu does not change (Fry *et al*., 2013). Blood Cu is slower to decline because erythrocytes are slow to be depleted of SOD1, the major contributor of erythrocyte Cu, and must be released into the bloodstream in sufficient numbers to augment the decline in plasma Cu. Other extra-hepatic changes vary between tissues, with large reductions found in kidney and brain Cu in lambs, adult sheep (Suttle *et al*., 1970) and growing cattle (Suttle and Angus, 1976; Legleiter *et al*., 2008) but not in cardiac muscle (Suttle *et al*., 1970), unless depletion is prolonged (Mills *et al*., 1976). Reduction in cuproenzyme activities proceed at various rates at different sites, indicating progress towards a state of local *dysfunction*, but the rate-limiting pathway is often hard to define, as was the case for CCO in brains of swayback lambs.[iv] While SOD1 also tends to decline in the brain of Cu-deprived calves, MnSOD increased and tissue oxidant capacity did not decrease (Legleiter *et al*., 2008). Plasma diamine oxidase acitivity decreases at a similar rate to Cp oxidase activity and plasma Cu (Leigleiter and Spears, 2007).The catecholamine neurotransmitters dopamine and noradrenaline, as well as monoamine oxidase (MAO) and SOD1 activities, were reduced in the brainstem of ataxic lambs (O'Dell, 1976).[v]

Dysfunction

Experimentally deprived cattle become severely hypocupraemic before growth is retarded (Suttle and Angus, 1976; Legleiter and Spears, 2007; Hansen *et al*., 2008) but liver Fe begins to increase

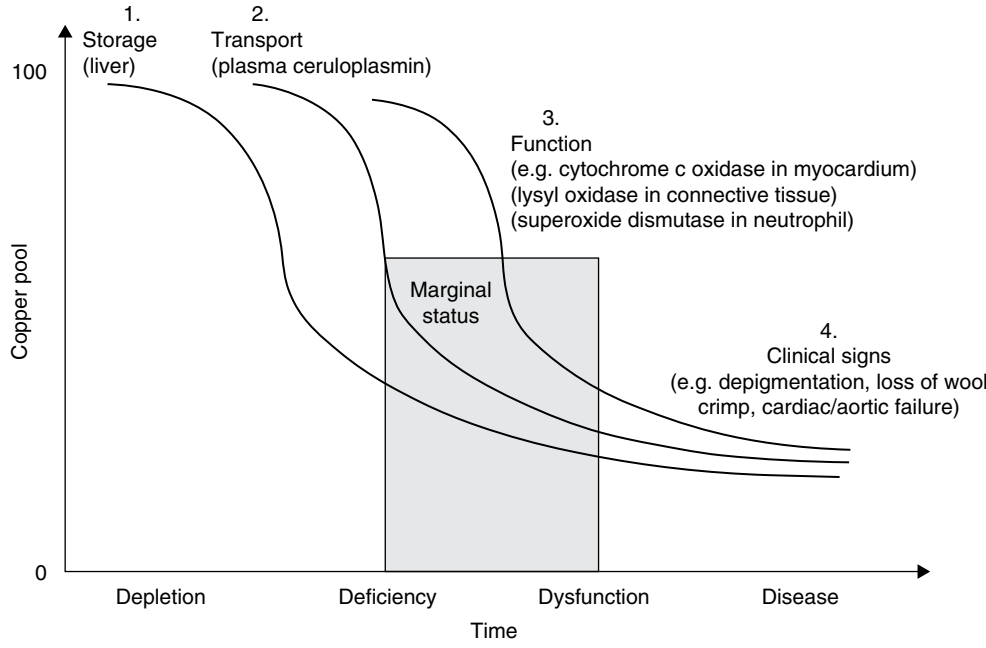


Fig. 11.5. The sequence of biochemical changes preceding clinical signs of copper deprivation.

(Mills *et al.*, 1976), probably as a consequence of low hephaestin activity (Table 11.1): impaired Fe transport may be the first process to dysfunction (Collins *et al.*, 2010). Early depigmentation is caused by low tyrosinase activity (Table 11.1), which inhibits conversion of tyrosine to melanin in the hair or wool follicle (Holstein *et al.*, 1979). Normal crimp, tensile strength and elasticity of wool are dependent on disulfide groups that provide cross-linkages in keratin and align long-chain fibrillae: both processes are adversely affected by lack of dietary Cu (Marston, 1946) and may reflect loss of thiol oxidase activity (see Table 11.1). Haemoglobin synthesis declines at a relatively late stage, but impairment of Fe transport is not necessarily responsible (Suttle, 1987; Prohaska, 2006; Collins *et al.*, 2010). Osteoporosis and reduced osteoblastic activity occurred in young housed lambs, born of Cu-deprived ewes, without morphological bone changes (Suttle *et al.*, 1972). Leucocyte counts increased in Cu-deprived calves (Stabel *et al.*, 1993) and heifers but protection against common pathogens was not compromised (Arthington *et al.*, 1996; Gengelbach *et al.*, 1997). Mo-induced hypocupraemia can lower antibody responses to *Brucella abortus* antigen in cattle (Cerone *et al.*, 1995). However, the precise mechanism(s) whereby resistance to infection is lowered is still unknown and there are many contenders (Bonham *et al.*, 2002).

Disorder

The critical site at which disorder develops probably depends on the metabolic stress placed on the rate-limiting enzyme at critical stages in tissue development, as illustrated by swayback (p. 257): with deprivation occurring *in utero*, low CCO activity in brain stem neurones may cause neonatal disorder; later deprivation affects spinal cord myelination, causing delayed swayback but once myelination has peaked, the cerebral cortex is affected, giving rise to atypical swayback (Suttle, 1988). Low CCO activity in hearts of Cu-deprived calves was associated with abnormal mitochondrial ultrastructure (Leigh, 1975) but was not necessarily the rate-limiting enzyme. Reduction of lysyl oxidase activity could diminish the stability and strength of bone collagen and impair mineralization of the epiphyseal cartilage, with consequent changes in bone morphology and reductions in bone strength, as seen in chicks (Opsahl *et al.*, 1982). A similar

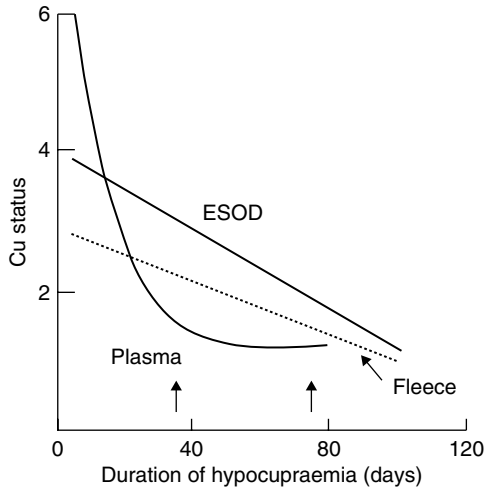


Fig. 11.6. Disparate time courses for changes in three indices of copper status in sheep given a low-copper diet, erythrocyte superoxide dismutase (ESOD), plasma Cu and fleece Cu. Arrows indicate onset of clinical signs and units vary. (From Suttle and McMurray, 1983)

'biochemical lesion' in elastin may weaken connective tissue in calves deprived of Cu.

Physiochemical Changes of Moderate Molybdenum Exposure

The primary biochemical consequence of low exposure to Mo is a reduction in Cu availability, due to formation of unabsorbable Cu–TM complexes in the gut (Suttle, 1991): with dietary Mo ≤5 in sheep and 15 in cattle mg Mo kg^{-1}DM, there is no evidence that moderate, chronic Mo exposure changes the sequence of events. Above dietary Mo thresholds determined partly by dietary Cu:Mo ratio, TM leaves the rumen in absorbable forms and perturbs Cu metabolism (Suttle and Field, 1983; Price *et al.*, 1987). Absorption of TM is indicated by accumulation in plasma of albumin-bound, trichloroacetic acid-insoluble (I) copper complexes (TCAI-Cu; Dick *et al.*, 1975; Smith and Wright, 1975a, b). The rise in TCAI-Cu caused by a given increase in dietary Mo decreases as dietary Cu:Mo ratio increases (Fig. 11.7). Lower dietary Cu:Mo ratios are needed to induce TCAI-Cu in sheep than in deer (Freudenberger *et al.*, 1987) and cattle (Wang *et al.*, 1988). It has been inferred that absorbed TM impairs Cu trafficking in ruminants (Clarkson *et al.*, 2020) but addition of 5 mg Mo and 3 g S to a corn silage diet containing 6mg Cu kg^{-1}DM failed to affect any of the eight transporters and chaperones, measured in cattle after 112 days treatment (Fry *et al.*, 2013). Since TCAI-Cu has yet to be reported in grazing cattle and is not seen in moderately exposed sheep (Smith and Wright, 1975a, b; Suttle and Small, 1993), further discussion will be left to the section dealing with Mo (p. 462). In lambs concurrently exposed to dietary Mo and abomasal parasitic infection, leakage of plasma into the abomasum may have allowed TCAI-Cu to form in the gut but without systemic effect (Ortolani *et al.*, 1993).

Effects on growth and reproduction

Growth retardation and infertility, associated with impaired pulsatile release of luteinizing hormone (LH), was induced in Mo-supplemented heifers, but not in those with an equally severe hypocupraemia induced by Fe (Phillippo *et al.*, 1987b). There are three possible explanations:

- infertility and growth were impaired by excess Mo *per se*;
- extreme hypocupraemia predisposed heifers to thiomolybdate toxicity;
- Mo induced a localized Cu deprivation, not reflected by liver and plasma Cu, that had previously reached respective nadirs.

In the absence of evidence of TM absorption, in the form of low plasma Cp:Cu ratios, the last explanation remains the most plausible (Suttle, 2012a). Close comparison of groups (Fig.11.8) shows that those given Mo, with or without Fe, had lower liver Cu and blood SOD1 activity than the one depleted with Fe alone. When others tried to repeat the findings, heifers remained fertile (Gengelbach *et al.*, 1997).[vi] Diets low in Cu (Mills *et al.*, 1976) and high in Mn (Hansen *et al.*, 2009) can retard cattle growth and produce all other clinical abnormalities associated with moderate exposure to Mo, except infertility. Excess Fe is not always innocuous and can induce hypocupraemia and retard growth in the field. Decreases in food intake and FCE contribute to growth retardation (Mills *et al.*, 1976; Hansen *et al.*, 2009). Neither LH release nor fertility were impaired by Mo supplementation in another study with lower Cu:Mo ratios (0.8 versus 1.9;

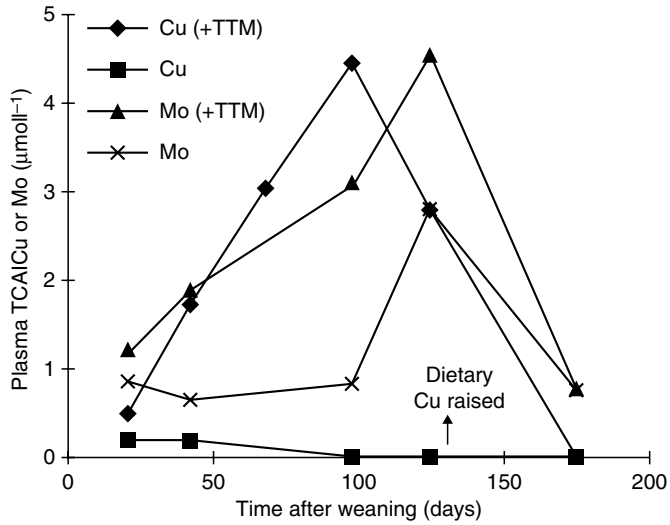


Fig. 11.7. Biochemical consequences of exposing lambs to tetrathiomolybdate (TTM, 5.4mg Mo kg^{-1}DM) via the diet depend on the Cu:Mo created. Initially, when dietary Cu was low (3.5mg kg^{-1}DM, Cu:Mo 0.65), excess TTM was absorbed, causing trichloroacetic acid-insoluble Cu (TCAI-Cu, o) and Mo (▲) to accumulate in plasma. When dietary Cu was raised after 130 days (to 15.5mg kg^{-1}DM, Cu:Mo 2.9), TCAI-Cu disappeared and plasma Mo was markedly reduced, presumably due to insoluble Cu–TTM complex formation in the gut. (From Suttle and Field, 1983)

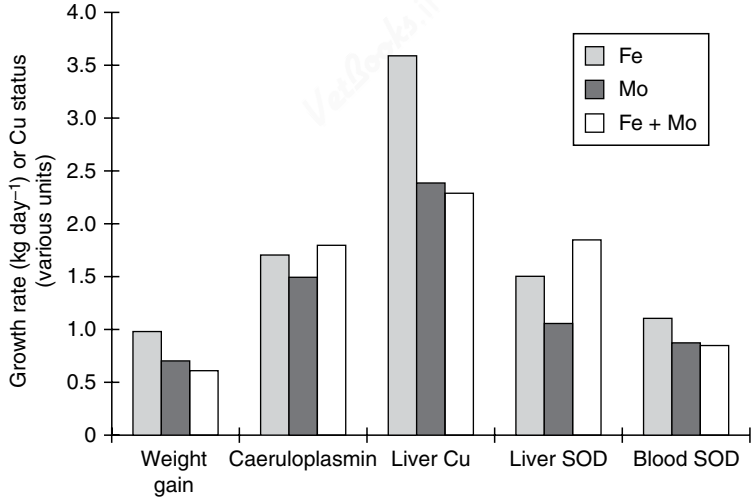


Fig. 11.8. Copper status of three groups of severely copper-deficient heifers, given molybdenum, iron, or both copper antagonists: all had equally low caeruloplasmin concentrations. Only groups given Mo grew poorly but they had lower liver Cu and blood superoxide dismutase (SOD) activity than the group given iron. (Data from Humphries *et al*., 1983). Hypocuprosis rather than molybdenosis was induced.

Xin *et al*., 1993) but neither plasma Cu nor concentrations at sites of suspected influence on LH release (hypothalamus and anterior pituitary) were reduced.

Exposure to dietary MoO_4 may also enhance the inflammatory response whereby sheep expel gut parasites (Suttle *et al*., 1992b; McLure *et al*., 1999) but the role of TM is unclear.

Diagnosis of Hypocuprosis

With the singular exception of swayback, clinical signs of hypocuprosis cannot be relied upon for diagnosis. Nutritional stresses of various kinds can lead to temporary loss of wool strength or crimp in sheep. Loss of coat colour has been noted in many vitamin deficiencies and in cattle deprived of Co (p. 251; Judson *et al.*, 1982). When cattle shed their winter coat, natural depigmentation occurs and the 'foxy-brown' coat discolouration seen in Friesian cattle, for example, is *not* a sign of hypocuprosis (Mee, 1991). Diagnosis rests upon three conditions:

- presence of clinical symptoms or subclinical loss of production;
- biochemical evidence of subnormal liver and/or blood Cu; and
- improvement after treatment with copper, when compared with untreated cohorts.

An incomplete profile will leave diagnosis insecure. Guideline values for biochemical analyses, summarized in Table 11.2, require flexible interpretation of marginal bands to indicate risk of past or future problems.

Dietary copper and its antagonists

Dietary Cu alone has little or no diagnostic value because of vast differences in availability. Dietary Cu:Mo ratios have more merit but thresholds ranged from >2:1 for normal growth in beef cattle (Miltimore and Mason, 1971) to approximately 4:1 for swayback avoidance (Alloway, 1973). In housed steers, depletion was prevented by raising Cu:Mo in a corn-silage-based diet from 1.0 to 1.75 and performance was only marginally improved (Gengelbach *et al.*, 1994). Cu:Mo ratios lack diagnostic precision because:

- current ratios may not reflect previous copper nutrition and hence liver Cu stores;
- the Cu:Mo ratio does not allow for influence of sulfur (see p. 272);
- Cu:Mo ratios vary with season and legume content;
- inhibitory effects wane with >8mg Mo kg^{-1}DM (Suttle, 1983b; Grace *et al.*, 2005b);
- other antagonists, notably iron, may also be influential; and
- the critical ratio may vary with diet and forage diet type (p. 272).

Supplementation with 2mg Mo kg^{-1}DM retarded growth of young cattle given corn silage containing 7mg Cu kg^{-1}DM but not those finished on corn containing 5.4mg Cu kg^{-1}DM (Legleiter and Spears, 2007). Mean Cu:Mo ratio decreased from 3.8 to 2.0 between pasture cuts in Norway but respective ranges were wide (1.1–8.3 and 0.8–5.2; Govasmark *et al.*, 2005). Limits for dietary Fe:Cu ratios are also given in Table 11.2 but allowance for effects of dietary S is complicated by scarcity of data. There is usually sufficient S in green swards to allow expression of antagonisms from Mo and Fe. Sulfur intake from SO_4-rich drinking water must be allowed for in some areas (Smart *et al.*, 1986). Limitations remain because effects of dietary Fe and Mo are not additive (e.g. Table 2.7).

Liver copper

An early diagnostic threshold for liver Cu (<25mg kg^{-1}DM) is still often used in surveys and supplementation studies but a much lower threshold (<10) was justified (Underwood and Suttle, 1999). The critical value may be influenced by breed and/or yield; Holsteins may have a lower threshold for liver Cu than Jerseys for maintenance of normal plasma Cu (Du *et al.*, 1996). In growing heifers, CuRD was associated with liver Cu <3–4mg kg^{-1}DM (Humphries *et al.*, 1983; Phillippo *et al.*, 1987a, b; Legleiter and Spears, 2007). Such extreme tolerance justifies the use of marginal bands but higher bands are needed in newborn than in older animals (Table 11.2) and may also be needed for deer (Grace and Wilson, 2002).

- Liver Cu lacks precision as an index of hypocuprosis.

Plasma copper

Marginal bands are needed because individuals and groups with plasma Cu between 3–9 µmol l^{-1} can remain healthy (Underwood and Suttle 1999; Nicol *et al.*, 2003) but values of 3–4.5 µmol Cu l^{-1} are found in clinically affected sheep (Whitelaw *et al.*, 1979, 1983; Woolliams *et al.*, 1986b), cattle (McFarlane *et al.*, 1991) and growth-retarded deer (Grace *et al.*, 2005b). Infectious diseases and vaccinations increase plasma Cu substantially by inducing Cp synthesis, even in initially hypocupraemic animals (Suttle, 1994), thus overestimating status. Simultaneous assay of acute-phase reactants

Table 11.2. Marginal bands[a] for copper and antagonist concentrations in diets and copper in bloodstream and soft tissues for diagnosis and prognosis of copper-responsive disorders in ruminants on fresh herbage- (H) or forage- (F) based diets.

Criterion[b]	Diet	Sheep and cattle	Deer[c] and goats	Interpretive limits
Diet Cu:Mo	H	**1.0**–3.0	**0.5**–2.0	Diet S >2g kg^{-1}DM
	F	**0.5**–2.0	**0.3**–1.2	Diet Mo <8mg kg^{-1}DM for sheep, deer and goats, <15mg kg^{-1}DM for cattle
Diet Fe:Cu	H	50–**100**	**50**–100	
Diet Cu (mg kg^{-1}DM)	H	**6**–8	**6**–8	Diet Mo <1.5mg kg^{-1}DM
	F	**4**–6	**4**–6	
Liver Cu (µmol kg^{-1}DM)[d,e]	H or F	**100**–300	**180**–300	
Plasma Cu (µmol l^{-1})[e]	H or F	**3**–9	**5**–8	Diet Mo <8mg kg^{-1}DM for sheep, deer and goats, <15mg kg^{-1}DM for cattle: no acute phase response; age >1 week
Blood Cu (µmol l–1)	H or F	**6**–10	**6**–10	
Hair or wool Cu (mg kg^{-1}DM)	H or F	**2**–4 (sheep)	**6**–9 (deer)	Clean sample, recent growth
		4–8 (cattle)	**3**–5 (goats)	

[a]Means within marginal band indicate probability of hypocuprosis increases with proximity to the **bold** limit, while values beyond the band do not. As the number of individual values falling within a marginal band increases, so does the likelihood of herd or flock benefit from Cu supplementation.
[b]Alternative criteria such as caeruloplasmin and erythrocyte CuZnSOD activities are not included because they introduce additional problems of interpretation or standardization without adding significantly to the precision of diagnosis.
[c]Some data for cervines from Wilson and Grace (2001).
[d]Divide by 3.0 to obtain values on fresh weight basis.
[e]Multiply by 0.064 to obtain values in mg.

(low plasma Zn, fibrinogen >1 g l^{-1} or haptoglobin) can identify such anomalies. Assays of Cp offer little advantage over plasma Cu because they should be highly correlated (e.g. Legleiter and Spears, 2007; Suttle, 2008a; Laven and Lawrence, 2010) and may be affected by starch intake (McCaughearn *et al*., 2020). Analysis of TCA-soluble Cu is a reliable surrogate for Cp in most circumstances (Suttle, 2008a) and avoids the need for viscosity correction when assaying plasma samples by atomic absorption methods (Suttle, 1994). Measurement of TCAI-Cu is of little diagnostic use (Paynter, 1987; Suttle, 2012a) for several reasons: values usually fall within background error in sheep (Suttle, 2008b), are not raised by moderate Mo exposure in cattle (Suttle, 2008a); sometimes anomalously high (e.g. in goats) (Osman *et al*., 2003). Direct measurement of non-Cp Cu (Beattie *et al*., 2001) and plasma SOD2 and 3 activities merit study in livestock. Note that:

- normal values for plasma Cu and Cp are around 50% lower at birth (Table 11.2) (Suttle, 1994) than in later life but increase rapidly during the first week in calves (McMurray, 1980), kids (Osman *et al*., 2003) and lambs (Grace *et al*., 2004).
- Serum samples should *not* be used for herbivore species other than equines and cervines (Laven and Lawrence, 2010) because Cp may be 'lost' during clotting and values are underestimated[vii] (cattle, Laven *et al*., 2007; sheep, Laven and Smith, 2008; Laven and Lawrence, 2012).
- The relationship between insufficiency plasma Cu is closer than with liver Cu.

Copper in blood and blood cells

Whole-blood Cu is rarely measured although it has certain advantages. Approximately one third of blood Cu is contributed by erythrocytes, which have a long half-life. Anomalies caused by induction of Cp or sudden changes in Cu supply have less impact on blood than plasma Cu and none on erythrocyte Cu (Arthington *et al*., 1996). Low activity of erythrocyte (E)SOD indicates prolonged deficiency (Ward and Spears, 1997) and ESOD was more closely correlated with growth rate than Cp (Fig. 11.9) and with mortality, than plasma Cu

(Fig. 11.2) (see Jones and Suttle, 1987). However, the conventional units of ESOD activity are relative and 'enzyme equivalence' may vary between laboratories. Erythrocytes must be washed to remove plasma inhibitors and heavily diluted to minimize interference from haemoglobin (Herbert *et al.*, 1991). Tentative diagnostic ranges for one particular kit assay method have been proposed (Herbert *et al.*, 1991) but may be subject to a S × Cu interaction at very high S intakes (Grace *et al.*, 1998b). Neutrophil Cu SOD1 reduced (from 13.0 to 5.1 pg/10^7cells and from 0.65 to 0.31 IU/10^6 cells, respectively) in steers given a diet with 15mg Mo kg^{-1}DM and Cu:Mo ratios of 1.0–1.8 for eight months, by which time mean liver Cu had become marginal but plasma Cu was normal (Xin *et al.*, 1991). *In vitro* killing capacity of neutrophils fell from 27.7% to 17.3%, although animals were clinically normal. Neutrophil SOD1 activity was also lowered in calves with Mo-induced hypocuprosis, but not in equally hypocupraemic, clinically normal calves given supplementary Fe (Gengelbach *et al.*, 1997).

- Decreases in neutrophil Cu or SOD1 warn of impending dysfunction.

Hair and fleece copper

Hair and fleece Cu have been widely measured without becoming established as reliable diagnostic aids, although they can confirm or deny subjective impressions formed from changes in appearance (Fig 11.1). Values can reflect suboptimal Cu uptake in both sheep (Fig. 11.6) and cattle (Suttle and Angus, 1978) but a slow rate of decline means that low values indicate prolonged deprivation.

- Hair and fleece are samples of choice from grossly deteriorated carcases and Cu profiles along the fibre may record historic fluctuations in Cu intake.

Other possible criteria

Plasma MAO, liver CCO and liver SOD1 activities decreased in cattle deprived of Cu (Mills *et al.*, 1976) but may precede dysfunction (Fig. 11.5). Plasma diamine oxidase showed similar changes to Cp and ESOD1 in sheep (Kendall *et al.*, 2001) and to plasma Cu and Cp in Cu-deprived cattle (Legleiter and Spears, 2007). Terminal responses in gene expression after prolonged deprivation in cattle showed upregulation of the chaperone protein supplying Cu to SOD1 (CCS) in duodenal mucosa and downregulation of CCO in liver, where SOD1 expression was unchanged (Fig. 11.9). Early changes in circulating levels of mRNA for CCS merit attention.

- Rates of decline in tyrosinase and thiol oxidase activity in hair or wool follicles should be studied in CuRD, since sampling would be minimally invasive.
- A genealogical approach, similar to that used in the pathogenesis of ovine copper poisoning (p. 281) might reveal breed differences in response

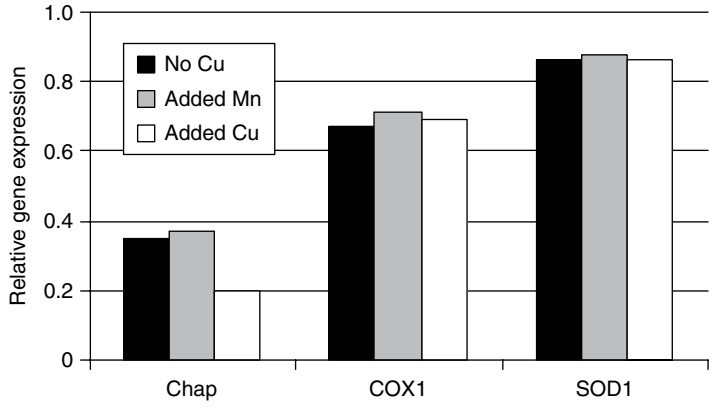


Fig. 11.9. Effects of long-term copper deprivation, with or without excess dietary manganese, on expression of three 'Cu genes' in cattle showing signs of hypocuprosis. The chaperone gene that facilitates the incorporation of Cu into superoxide dismutase 1 (SOD1) in duodenal mucosa (*Chap*) is upregulated by Cu deprivation, but expression of SOD1 and cytochrome oxidase (COX1) in liver are not. (From Hansen *et al.*, 2008)

to copper proteomes of host and/or symbiotic rumen microbes to lack of Cu.

A variety of immunological markers are affected by subclinical Cu deprivation in laboratory animals (Bonham *et al.*, 2002). Exposure of single-cell cultures to Cu chelators in the absence of albumin will shock proteomes into action and generate publications without advancing prognosis of CuRD.

Sources of Copper and Its Antagonists

Copper in pasture

Pasture and forage Cu varies with species, strain and maturity of the plant, with certain soil conditions (not pH) and with fertilizer usage (Table 11.3) (McFarlane *et al.*, 1990). Temperate grasses tend to contain less Cu than temperate legumes (4.7 versus 7.8mg kg^{-1}DM, respectively) but the position is reversed under tropical conditions (7.8 versus 3.9mg kg^{-1}DM, respectively) (Minson, 1990). On the Cu-deficient soils of South Australia, herbage Cu is often <3mg kg^{-1}DM (Dickson, 2016). Different grass species grown on the same soil can contain from 4.5 to 21.1mg Cu kg^{-1}DM (Underwood and Suttle, 1999) but differences were smaller in the dry season in west Kenya (Jumba *et al.*, 1995). Copper is unevenly distributed in temperate grasses, leaves being 35% richer in Cu than stems; values in the whole plant therefore decline during the growing season (Minson, 1990), sometimes markedly (Jarvis and Austin, 1983; Dickson 2016). Variation was greater within than between hays and silages in Alberta (4.3 ± 2.45 and 5.0 ± 1.98mg (s.d.) Cu kg^{-1}DM, respectively; Suleiman *et al.*, 1997) but lower values in hay than in silage were also evident in UK data (MAFF, 1990) and may partly reflect respective maturities.

Copper antagonists in and on pasture

Herbage Mo, S and Fe also vary between species (Jumba *et al.*, 1995), pasture management systems (Whitelaw *et al.*, 1983) and seasons. Pasture Mo increased from 1.5 to 3.3mg kg^{-1}DM between cuts in Norway (Govasmark *et al.*, 2005) and had shown similar increases between May and August in grazed, improved pastures in Scotland (Suttle and Small, 1993). Pasture Mo increases as soil pH increases (Fig. 2.1) and both Mo and S can be high on soils high in organic matter (Table 11.3). Temperate legumes tend to be richer in Mo than grasses in the same sward and a tenfold difference was reported on a Mo-rich soil (Kincaid *et al.*, 1986). Fodders and grains in British Columbia had Cu:Mo ratios ranging from 0.1 to 52.7 (Miltimore and Mason, 1971). A maize silage, grown in the Mo-rich 'teart' area of Somerset, was relatively low in Mo (2.3mg kg^{-1}DM; Suttle, 1986). Herbage S and protein are correlated and both decrease as swards mature (Dickson, 2016). Spring pastures are often rich in Fe from contaminating soil and a waning iron × copper antagonism has been invoked to explain recovery from hypocuprosis in cattle grazing in summer (Jarvis and Austin, 1983). Herbage Fe is high in overgrazed pastures and in those on soils prone to waterlogging (e.g. red gums and rendzinas, Table 11.3). Values can vary greatly between farms (150–1345mg Fe kg^{-1}DM) (Nicol *et al.*, 2003) and increase exponentially, as does herbage Mn, in autumn (Suttle, 1998). Contamination of pastures and silages with soil Fe is commonplace and should be taken into account when assessing their value as copper sources. Further details of the range of forage S and Fe are given in Chapters 9 and 13, respectively.

Table 11.3. Mean (range) of copper, molybdenum, sulfur and iron concentrations in subterranean and/or strawberry clover-tops sampled on two to four occasions from sites of major soil types in South Australia. (From McFarlane *et al.*, 1990)

Soil type	No. of sites	Cu (mg kg^{-1})	Mo (mg kg^{-1})	S (g kg^{-1})	Fe (mg kg^{-1})
Sand/clay	24	6.6 (3.0–14.6)	1.4 (0.1–4.1)	2.5 (1.5–4.0)	160 (45–346)
Red gum	22	9.5[a] (4.1–15.9)	1.8 (0.1–5.4)	2.6 (1.5–4.3)	520[a] (81–2300)
Groundwater rendzina	20	8.5 (4.2–12.8)	1.6 (0.2–5.4)	2.5 (1.9–5.1)	510[a] (200–1000)
Deep sand	16	7.3 (4.0–14.0)	1.1 (0.1–3.8)	2.8 (2.1–4.6)	140 (119–154)
Peat	4	7.2 (4.4–11.4)	8.3[a] (4.7–16.2)	3.6[a] (2.8–4.5)	110 (48–320)
Calcareous sand	11	5.3 (1.9–9.5)	10.1[a] (1.6–21.8)	2.5 (1.7–3.4)	100 (70–130)

Mean values followed by [a] differ significantly ($P < 0.05$) from others. Each is comprised of 5–10 samples.

Composition of concentrate feeds

Species differences in cereal Cu, Mo and S are relatively small and values low compared with most other feeds. In Northern Ireland, oats and barley contained, on average, 3.9– 4.9 mg Cu, 0.25–0.30 mg Mo and 0.8–1.5g S kg^{-1}DM, respectively (Todd, 1972). While Mo is invariably low, more recent data from the USA indicates that Cu and S can vary, maize being particularly low in Cu (Table 11.4). Large amounts of by-products from the biofuel industry are being fed to feedlot cattle in the USA: use of H_2SO_4 in the process raises S in DDGS well above that in the grain but Cu is unaffected. Whisky distillery by-products were notoriously rich sources of Cu, with 86, 138, 44 and 129mg kg^{-1}DM reported for pot ale syrup, wheat and barley distillery dried grains and ensiled grain residues, respectively (Suttle *et al.*, 1996a): Cu is dissolved from copper stills during distillation. Leguminous and oilseed meals were and remain much richer in copper, 25–40 mg kg^{-1}DM being found in palm-kernel cake (Chooi *et al.*, 1988), but are also richer in Mo than cereals, containing 1–4mg Mo kg^{-1}DM. Dairy milk and milk products are inherently low in Cu (approximately 1mg kg^{-1}DM) but other feeds of animal origin are richer, with meat meal and fish meal typically containing 5–15mg Cu kg^{-1}DM. Feeds of animal origin are relatively low in Mo. Milk Mo is highly dependent on dietary intake and, in contrast to copper, can be raised several-fold above the normal (0.06 mg Mo l^{-1} in ewes and cows). However, milk Mo will bypass the rumen and probably have little effect on copper status of the suckling.

Availability of Copper in Feeds for Ruminants

There are two keys to understanding copper nutrition in ruminants: the first is to appreciate the importance of availability rather than post-absorptive events like Cu-trafficking, which occupy so much research effort.

- Efficiencies of absorption (A_{Cu}) vary more widely than for any other mineral.
- Feeds of unexceptional Cu and Mo contents vary tenfold (Table 11.2).
- Risk of insufficiency and excess are largely determined by A_{Cu}.

Variation has little to do with the forms of Cu in feeds, about which little is known,[viii] but much to do with rumen fermentation. Key events are the rapid degradation of S sources to sulfide (p. 208) and fermentation of carbohydrates to yield organic acids. Addition of starch to the diet of dairy cows raised liver Cu and was thought to have done so by lowering pH and disulfide (HS) in rumen liquor (McCaughern *et al.*, 2020): at lowered pH, disulfide is converted to H_2S and accumulates in the rumen gas cap (Fig. 9.2). Reductions in liver Cu in lambs allowed access to fresh straw bedding, while receiving a concentrate (Crosby *et al.*, 2004; Day *et al.*, 2006) and increases in A_{Cu} in sheep fed once daily rather than every 4 hours (Luo *et al.*, 1996) are both probably explained by changes in pH (raised by straw feeding, lowered by single feeding).

- Variation in availability between feeds, shown in Table 11.5, is probably due, in the main, to differences in pH and rumen disulfide, which forms insoluble and unavailable CuS (Suttle, 1991).
- Low A_{Cu} in fresh grass and high A_{Cu} in cereals and cereal by-products probably reflect extremes of pH produced in the rumen after feeding. The A_{Cu} in blends of forage with concentrates will probably be determined by pH attained in the rumen. Low pH and high A_{Cu} in total mixed rations may explain the high liver Cu status being found in cull cows (Hunter *et al.*, 2013; Suttle, 2016).

Copper–molybdenum–sulfur interactions

The second key is to appreciate the wide variation in A_{Cu}, mediated by S^{2-} and MoO_4. Thiomolybdates

Table 11.4. Mean copper and sulfur concentrations (± s.d.) in cereals and distillers dried grain solubles (DDGS) from the USA (Kerr *et al.*, 2008); bracketed whole numbers indicate number of samples analysed.

Element	DDGS (19)	Maize (23)	Oats (5)	Rice (9)	Wheat (10)
Cu (mg kg^{-1}DM)	4.1 ±1.31	0.9 ± 0.65	5.1 ± 0.46	3.3 ±1.04	5.9 ± 0.52
S (g kg^{-1}DM)	6.9 ±2.36	1.1 ± 0.11	2.3 ± 0.11	1.3 ± 0.16	1.8 ± 0.22

Table 11.5. Mean true absorption of copper (A_{Cu}, %) from natural foodstuffs of normal copper (<10) and molybdenum concentration (< 2 mg kg^{-1}DM), determined by the same repletion technique, in Scottish Blackface ewes.

	A_{Cu} (mean ± s.d)	Number of estimates
Grazed herbage (July)	2.5 ± 1.09	7
Grazed herbage (September/October)	1.4 ± 0.86	6
Silage	4.9 ± 3.2	7
Hay	7.3 ± 1.8	5
Root brassicas	6.7 ± 0.9	2
Whole cereals	9.1 ± 0.97	3
Distillers' grains[a]	5.0 ± 0.70	3
Leafy brassicas[b]	12.8 ± 3.20	5

[a]The relatively low value for grain-based by-products is attributable to use of a basal diet supplemented with S (Suttle *et al*., 1996a).
[b]The high value for a feed high in S may be attributable to the presence of S in non-degradable forms (see p. 223).

(TM) can be synthesized in the rumen by progressive substitution of S for O in MoO_4^{2-}. Maximal substitution produces tetrathiomolybdate (MoS_4^{2-}), a powerful Cu-chelator that can complex Cu and become irreversibly bound to particulate matter in the rumen (Allen and Gawthorne, 1987), leaving little that is absorbable (Price and Chesters, 1985). Effects of forage Mo and S on A_{Cu} were large and predictable, early increments within commonly encountered ranges having the largest inhibitory effects, but relationships differed between forage types (Fig. 11.10). Antagonisms were strongest in fresh herbage (Fig 11.10c) and the weighting for Mo was confirmed in grazing sheep, using stable isotope methodology (Knowles *et al*., 2000): they explain occurrence of hypocuprosis in lambs grazing improved pastures with raised herbage Mo and S (Fig. 11.2) (Whitelaw *et al*., 1979; Woolliams *et al*., 1986b). The interaction was equally strong in cattle (Suttle, 1986), which also develop hypocuprosis on improved hill pastures (Whitelaw *et al*., 1984). Quantitative support was given by the negative exponential decline in liver Cu as herbage Mo increased in grazing in New Zealand (Grace and Knowles, 2010). Similar exponential effects of forage S (Fig. 11.10a) may largely explain the lack of effect on liver Cu when supplements raised dietary S from 2.7 to 6g kg^{-1}DM in grazing lambs (Grace *et al*., 1998b) (see p. 222 for more on Cu-S interactions). Conservation as hay attenuated antagonism from Mo and S (Fig. 11.10b). Relevant attributes of hay are that as hay intakes rise, rumen liquor pH increases but H_2S in the gas space decreases (Morine *et al*., 2014). More H_2S may be lost during eructation on fibrous diets and more HS in rumen liquor may become incorporated into microbial protein rather than forming TM. Antagonism from Mo appeared to be relatively weak in the only Mo-rich, grass silage studied (Suttle, 1986). Recent studies of changes in liver Cu in cattle suggested that the antagonisms were even weaker on corn silage (Sinclair *et al*., 2013, 2017) and attenuated by adding starch to corn silage, on which rumen pH was lower (McCaughearn *et al*., 2020). However, specific effects of forage type can only be ascertained in forages closely matched for Mo. Antagonism from Mo + SO_4 in corn silage has been consistently stronger with 2 (e.g. Hansen and Spears, 2008) than with 5–7mg kg^{-1}DM added Mo. Fitting a parabolic relationship to pooled data (Fig. 11.11) accounted for 82% of variation in liver Cu and shows the effect of silage type to be small relative to that of dietary Mo. There have been other indications that responses to Mo are parabolic (Smith and Wright, 1975b; McClure *et al*., 1999).

- Differences in antagonism from Mo+S between forages are, like those between feeds low in Mo, partly attributable to differences in rate of carbohydrate fermentation, rumen pH, disulfide concentration and TM synthesis.
- Reversal of antagonism when forage diets contain >5mg Mo kg^{-1}DM may reflect transition from inhibition of A_{Cu} to a systemic increase in half-life of absorbed Cu.

The possibility is discussed in more detail in the section dealing with excess Mo (p. 464), since such high values are rarely found on farms.

Copper–iron–manganese interactions

Increases in dietary Fe as soil or Fe salts slowed repletion of plasma Cu in sheep (Suttle *et al*., 1984), accelerated depletion (Humphries *et al*., 1983) and slowed repletion (Rabiansky *et al*., 1999) of liver Cu in cattle. The antagonism can be mediated by dietary S in sheep (Suttle *et al*., 1984; Suttle and Peter, 1985) and cattle (Bremner *et al*., 1987) and is probably caused by reductions in A_{Cu}. Bovine copper status has been lowered by as little as 250 mg Fe kg^{-1}DM as saccharated iron carbonate

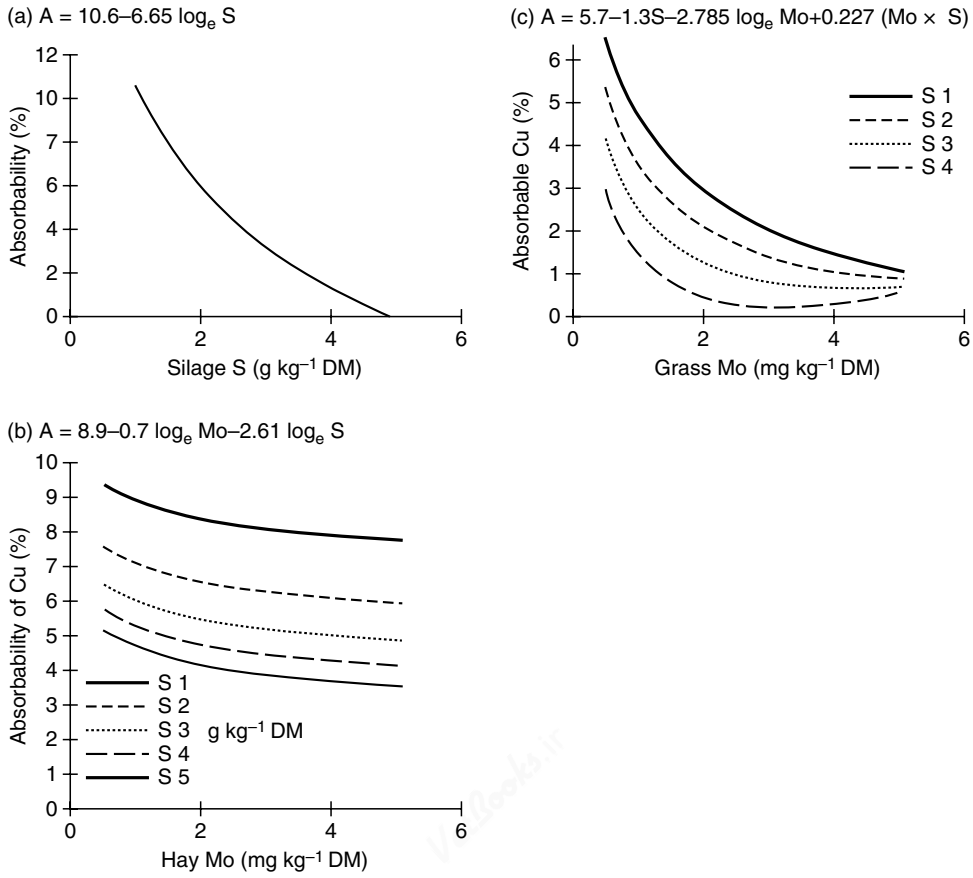


Fig. 11.10. Value of fresh and conserved grass as sources of copper (Cu) for ruminants is determined largely by interactions with sulfur (S) and molybdenum (Mo) that reduce copper absorption (A_{Cu}) but vary with forage type: (a) in silages, S markedly reduces A_{Cu} in a logarithmic manner; (b) in hays, the effect of S is less pronounced but inhibitory effects of Mo are detectable; (c) in fresh grass, A_{Cu} starts low and is further greatly reduced by small increments in Mo and S. (Data from Suttle, 1983a, b obtained using the technique outlined by Suttle and Price, 1976).

($FeCO_3$) (Bremner *et al.*, 1987). Raising dietary Fe downregulated the non-specific carrier (DMT1) in intestinal mucosa (Hansen *et al.*, 2008). Supplements of Fe_2O_3, providing 800mg Fe kg^{-1}DM and simulating effects of ingesting soil Fe, significantly lowered A_{Cu} from 0.06 to 0.04 in sheep (Suttle and Peter, 1985); a similar effect was obtained with $FeSO_4$ (Fig. 11.12). Effects of iron on A_{Cu} cannot be predicted but with 800 mg added Fe kg^{-1}DM, A_{Cu} is roughly halved. The Cu–Fe interaction is not manifested in pre-ruminant calves (Bremner *et al.*, 1987) or in sheep on concentrate diets, low in S (Rosa *et al.*, 1986).

Addition of 500 mg Mn (as $MnSO_4$), as well as 8 g S and 2mg Mo kg^{-1}DM, to a corn-based diet for finishing steers exacerbated hypocupraemia on a diet marginal in Cu (5.4 mg) (Legleiter and Spears, 2007). Increases in dietary Mn also downregulate DMT1 and the Cu-Mn antagonism was stronger in growing steers given corn silage (Hansen *et al.*, 2009). While housed cattle are not normally exposed to high dietary Mn in available forms, grazing cattle are exposed to less available Mn in ingested soils. Soil Mn is relatively labile (Brebner *et al.*, 1985) and may be partially solubilized, like Fe, by ensilage. The Cu-Mn and Cu-Fe antagonisms may be mediated by shared mechanisms that inhibit A_{Cu} beyond the rumen by one of three independent mechanisms:

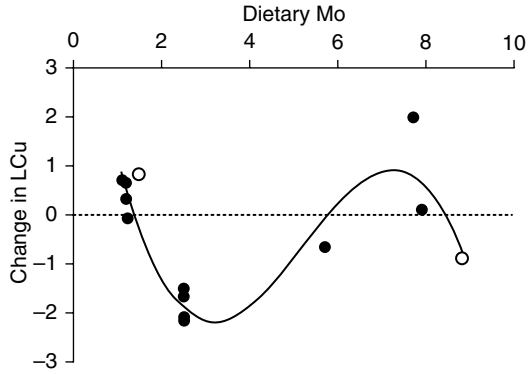


Fig. 11.11. Third-order polynomial relationship between rate of change in liver copper (LCu; mg $kg^{-1}d^{-1}$) and molybdenum (Mo) concentration in corn silage diets for steers (Hansen *et al*., 2008) and dairy cows (Sinclair *et al*., 2013, 2017; McCaughearn *et al*., 2020) (solid symbols) and two grass silages (Sinclair *et al*., 2017; open symbols): dietary Cu varied from 13 to 26mg kg^{-1}DM).

- soluble Fe and Mn may trap S^{2-} as insoluble sulfides in rumen liquor but release S^{2-} in the acid abomasum, which then converts soluble Cu to insoluble CuS;
- Cu may be adsorbed by oxides of Mn, as with those of Fe (Suttle and Peter, 1985);
- downregulation of DMT1.

The Cu–Fe and Cu–Mo–S interactions are not additive in cattle (Humphries *et al*., 1983; Phillippo *et al*., 1987a, b; Gengelbach *et al*., 1994) or sheep (Suttle, unpublished data) and the Cu-Mn antagonism may also behave non-additively.

- Competition for S_2^- may make antagonisms from Mn, Fe and Mo non-additive.
- Each antagonism probably becomes stronger as rumen pH increases (by reducing loss of volatile H_2S (p. 271).

Other influences upon copper availability

Large Zn supplements (200–400mg kg^{-1}DM) reduced liver Cu in housed sheep (Bremner *et al*., 1976): the antagonism is unlikely to occur across the normal range of forage Zn but may be elicited during treatment of facial eczema with large doses of zinc salts (Morris *et al*., 2006). Small supplements of Cd can reduce the placental transfer of Cu in ewes and cows (p. 489). Antagonisms between

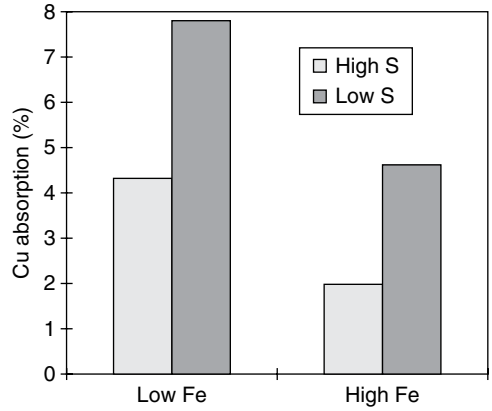


Fig. 11.12. Absorbability of copper (A_{Cu}) in diets for weaned ruminants is affected by interactions between copper and iron, which are partly dependent on sulfur.

Cd and Cu may be important where herbage Cd has been increased by the use of superphosphates, high in Cd (p. 487). Malabsorptive conditions of humans are associated with poor A_{Cu} (Prohaska, 2006) and a similar association in sheep could explain why intestinal parasitism lowered plasma Cu in lactating ewes with suppressed immunity (Fig. 11.13). Similarly, A_{Cu} values for lambs grazing in autumn grass may be reduced by an autumn rise in larval nematode infection. Early reports of inhibitory effects of high dietary Ca on A_{Cu} were not repeatable (Underwood and Suttle, 1999).

Copper Requirements of Ruminants

Net requirements in sheep and cattle

Components of net requirement for factorial models of minimum needs (see p. 9) are not easily determined, particularly for Cu (ARC, 1980). The maintenance component consists mainly of faecal endogenous losses and, since faeces is the major route for excreting excess absorbed Cu, only obligatory losses ($^{o}FEL_{Cu}$) need to be replaced (Suttle, 1983c). The growth component is relatively small because muscle, fat and bone contain little Cu and hepatic Cu, substantial in animals fed excess dietary Cu for several months, should be excluded. Muscle is the major contributor of extra-hepatic Cu in sheep carcases (Fig. 11.14) and contained only 0.8 and 1.2 mg Cu kg^{-1}FW in cattle (Simpson *et al*., 1981) and sheep (Grace, 1983), respectively: extra-hepatic Cu increases when excess Cu is fed

(Simpson *et al.*, 1981; Suttle, 1983c). Several papers have appeared recently that purport to define net Cu requirements for maintenance and growth in exotic breeds of lambs and kids by unvalidated regression methods (e.g. Zhang *et al.*, 2018). Body weight was varied by feeding restricted amounts of concentrate diets, rich in Cu. Comparative slaughter methods with complete diets rich in Cu will overestimate net need.

Gross requirements for sheep: influence of diet

Requirements of weaned animals vary tenfold under the powerful influence of interactions with

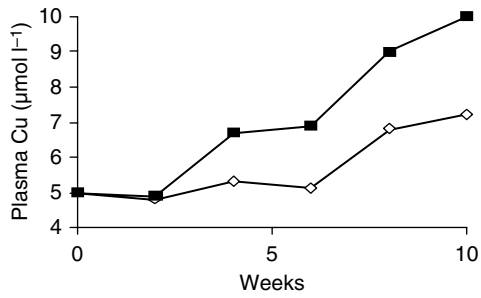


Fig. 11.13. Administration of a bolus (solid symbol) that slowly released anthelmintic hastened the spontaneous recovery in plasma copper in lactating ewes at pasture. Ewes showed suppressed immunity to gut parasites, which may have indirectly increased copper requirements (Suttle, 1996).

Fe, Mo and S that cause A_{Cu} to vary from 0.01 to 0.10 (p. 371).

- Gross requirements should not be presented as single figures.

Assessments of A_{Cu} in feeds given to inefficient absorbers of Cu, such as the Scottish Blackface (Table 11.5 and Fig. 11.10), allow adequate needs on diets with contrasting availabilities (A_{Cu}) to be budgeted for (Table 11.6). Appropriate A_{Cu} choices are:

- A_{Cu} 0.06 for dry mixed rations, corn silage or dry-season grazing;
- A_{Cu} 0.03 for normal green pastures or grass silage;
- A_{Cu} 0.015 for green pasture containing ≥5mg Mo kg^{-1}DM.

For sheep consuming forages and swards contaminated with soil, containing >800mg Fe kg^{-1}DM, interpolated requirements for an A_{Cu} of 0.02 are suggested.

Gross requirements for sheep: influence of genotype

Genetic variation in requirements was first indicated by variation in incidence of swayback between breeds under a common management regimen (Fig. 11.2) and later by substantial differences in liver Cu between breeds given the same diet (Woolliams *et al.*, 1982; van der Berg *et al.*, 1983). Evidence was strengthened when crosses between

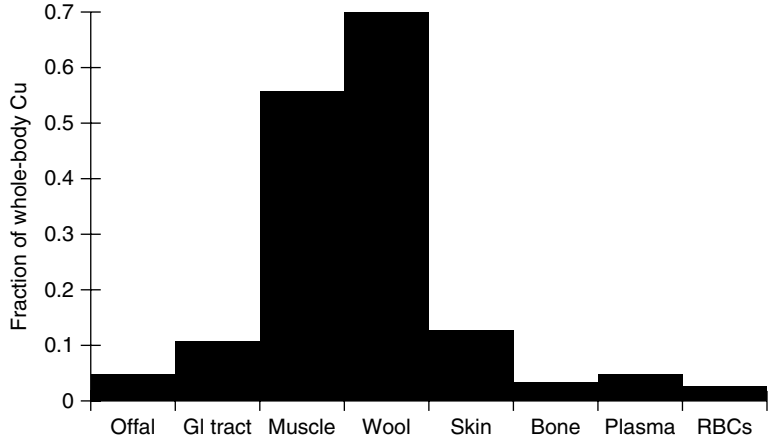


Fig. 11.14. Distribution of copper in the shorn, empty body of mature sheep. The relatively large amounts in fleece are also shown (data from Grace, 1983). GI, gastrointestinal; RBC, red blood cell

Table 11.6. Factorial estimates of the dietary copper requirements of sheep and cattle given three types of diet with contrasting copper absorbabilities[a] (A_{Cu}).

	Live- weight (LW) (kg)	Growth rate or milk yield (kg day^{-1})	Net requirement[b] (mg day^{-1})	Food intake[c] (kg DM day^{-1})	Gross requirement		
					A_{Cu}0.06	0.03	0.015
					(mg kg^{-1} DM)		
Lamb	20	0.1	0.13	0.5	4.3	8.6	17.2
		0.2	0.18	0.7	4.3	8.6	17.2
Ewe (16-week twin fetuses)	75	0	0.63	1.5	7.0	14.0	21.0[d]
Lactating ewe	75	1	0.52	1.5	5.8	11.6	23.2
		3	1.24	2.9	7.1	14.2	28.4
Calf	100	0.5	0.65	3.0	3.6	7.2	14.4
		1.0	0.90	3.6	4.2	8.4	16.8
Cow (40-week fetus)	500	0	4.1	9.1	7.5	15.0	30.0[d]
Lactating	500	20	4.0	15.2	4.4	8.8	17.6
		40	6.0	19.3	5.2	10.4	20.8

[a]Approximate A_{Cu} values for particular feed classes are as follows: 0.06 for roughage and concentrates; 0.03 for normal green swards; 0.015 for molybdenum-rich swards (>2mg Mo kg^{-1}DM); intermediate values apply for brown, dry season swards (0.04) and iron-rich (<800 mg Fe kg^{-1}DM) green swards (0.02).

[b]Components of factorial model: M, 4 µg kg^{-1} LW; G, 0.5 mg kg^{-1} LW gain; L, 0.1 and 0.22 mg l^{-1} for cow and sheep milk, respectively; and F, 4 mg Cu kg^{-1} clean wool growth.

[c]Real food intakes will vary according to diet quality (see Table 16.6).

[d]Requirements for late pregnancy allow for build up of a large fetal reserve of copper.

breeds had a copper status intermediate between those of parent breeds (Wiener, 1987). Proof came when sire selection for high and low plasma Cu within a cross between breeds of relatively high (Welsh Mountain) and low (Scottish Blackface) status produced two lines that matched parental breed rank (Woolliams *et al.*, 1986b). Comparisons of liver Cu between sire families in Merino (Judson *et al.*, 1994), Suffolk and Texel (Suttle *et al.*, 2002) breeds showed that liver Cu can be highly heritable (h^2 > 0.6). Genetic differences probably reflect differences in A_{Cu} (Wiener *et al.*, 1978; Woolliams *et al.*, 1983) but differences in hepatic storage capacity (Haywood *et al.*, 2005) and FEL_{Cu} may contribute (Woolliams *et al.*, 1982, 1983; Suttle *et al.*, 2002). Differences in net requirement, attributed by default to genotype (Zhang *et al.*, 2018), are probably artefacts of experimental protocols (p. 8). Requirements in Table 11.6 are appropriate for Scottish Blackface but should be reduced by >50% for more efficient utilizers of dietary Cu, such as the Texel. Weighting of A_{Cu} for effects of herbage Mo in Fig. 11.10c is similar to that found in studies with another breed under grazing conditions in New Zealand (Knowles *et al.*, 2000) but the baseline A_{Cu} was higher and requirements would therefore be marginally lower.

Gross requirements for cattle

Cattle are generally thought to absorb Cu less efficiently than sheep but are similarly affected by increases in forage Mo and S to Scottish Blackface sheep (p. 272). A_{Cu} values obtained with such sheep are therefore suitable for estimating minimum requirements for cattle (Table 11.7): outcomes for mixtures of roughage and concentrate are speculative (Suttle, 1994). The weighting for Mo is supported by the report that increasing dietary Cu by 58% countered the depleting effects of 5mg Mo kg^{-1}DM in steers (Ward and Spears, 1997). A similar Mo supplement induced only a marginal decline in liver Cu in cows given a total mixed ration (TMR) based on corn silage (Sinclair *et al.*, 2017) but initial liver Cu was very high and not fully responsive to changes in A_{Cu}. High liver Cu in dairy cows after removal of supplements from a whole-crop silage-based TMR indicated that requirements were met by 6mg Cu kg^{-1}DM (Hunter *et al.*, 2013). Severe hypocupraemia developed in steers on a

corn-silage-based diet, low in Mo but containing 1000 mg added Fe (as $FeSO_4$) kg^{-1}DM, indicating a requirement >8 mg Cu kg^{-1}DM (Mullis *et al.*, 2003) and confirming that needs are increased when dietary Fe is high. Evidence for genetic variations in cattle is growing. Friesian calves had higher liver Cu than Galician Blond calves in Spain, while crossbred calves were of intermediate status (Miranda *et al.*, 2006). Requirements may be marginally higher in Jersey than in Friesian cows (Du *et al.*, 1996) and in Simmental than Aberdeen Angus (Mullis *et al.*, 2003; Stahlhut *et al.*, 2005). A difference in liver Cu between the latter breeds was influenced by dietary Mo and S, being absent unless Mo and S were added to a corn silage diet, whereupon Simmentahl had the lower values (Fry *et al.*, 2013). Lactation in cows does not raise required dietary concentrations because milk is low in Cu (approximately 3 µmol Cu l^{-1}) and food intakes increase in lactation.

Requirements of deer, goats and other ruminants

Using the ARC (1980) factorial model with an A_{Cu} coefficient of 0.04 and a net growth component of 1.6 mg Cu kg^{-1} LWG, growing hinds were estimated to need 8mg kg^{-1}DM (Grace *et al.*, 2008). Earlier field trials on low-Mo farms suggested that less Cu was needed to avoid growth retardation but not hypocupraemia (Fig. 11.15). Although deer seem less susceptible to the Cu–Mo antagonism than cattle (Mason *et al.*, 1984), exposure to pasture high in Mo increased severity of hypocupraemia and requirements were not met by 7–8mg Cu kg^{-1}DM (Nicol *et al.*, 2003).

In the absence of valid data, caprine requirements are assumed to be similar to those of sheep. However, breed and seasonal differences in plasma Cu raise the possibility of genetic differences (Osman *et al.*, 2003). Growth rate, digestibility of dietary fibre and growth of cashmere were increased by increasing dietary Cu from 4.7mg kg^{-1}DM in a diet of wild rye and alfalfa hay, supplementation increasing the number of secondary follicles (Zhang *et al.*, 2011); with dietary S at 2.1 g and Mo increased from 1.7 to 6.7, 24mg Cu kg^{-1}DM was no longer sufficient for normal body growth but did not affect cashmere growth. Copper supplementation also improved, serum Cp, SOD and antioxidant status, while GPX activity was increased by supplementary Cu and Mo (Zhang *et al.*, 2012). An earlier experiment indicated requirement for optimum performance was >7.4 mg Cu kg^{-1}DM (Zhang *et al.*, 2008), a higher need than that of sheep.

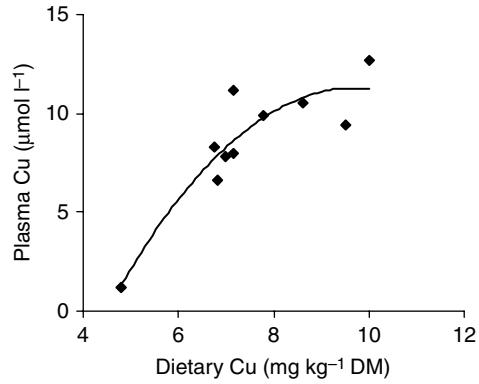


Fig. 11.15. Curvilinear relationship between mean plasma and dietary Cu in deer on ten farms in Canterbury, New Zealand, indicate a requirement of 7–8mg kg^{-1}DM to avoid hypocupraemia by the beginning of winter on pasture low in Mo (0.7mg kg^{-1}DM). (Data from Nicol *et al*., 2003)

Avoiding Copper Insufficiency in Ruminants

Pasture fertilizers

Applications of Cu-containing fertilizers can increase plant yields and raise herbage and crop Cu but animal needs from pasture (see Tables 11.6 and 11.7) are higher than plant needs (≤4mg Cu kg^{-1}DM) (McFarlane, 1989; Haynes, 1997). Required application rates vary with soil type and climatic conditions.[ix] Fertilizer treatment is unsuitable on soils high in organic matter because Cu becomes 'fixed' in unavailable humic acid complexes, is not advised when induced hypocuprosis occurs on pastures high in Mo (McFarlane *et al.*, 1990) and uneconomic under range conditions. Foliar application is more costly than top-dressing but more effective in the short term: the target should be to maintain a herbage Cu:Mo ration >3 but the method should be used warily where mixed stocking with sheep and cattle is intended.

Dietary supplementation: inorganic sources

Salt licks containing 0.5–1.9% Cu were effective on extensive farms. With paddock grazing and a

piped water supply, $CuSO_4$ can be added by metering devices: small additions elicited rapid clinical responses in cattle on Mo-rich pasture (Farmer *et al.*, 1982), possibly because some Cu bypasses the 'hostile' rumen environment. Salts such as the acetate, carbonate (Ledoux *et al.*, 1995), citrate and chloride are no better as dietary supplements than $CuSO_4$ in raising liver Cu (Engle and Spears, 2000). The relatively insoluble source, tribasic copper chloride ($Cu_2[OH_3]Cl$) was almost twice as effective in raising liver Cu in calves as $CuSO_4$ when added to a corn-silage diet with added Mo and SO_4 but had no advantage in the absence of antagonists (Spears *et al.*, 2004), indicating scope for other sources that protect copper from TM in the rumen.

Dietary supplementation: 'organic' sources

Extravagant claims have been made that Cu–protein hydrolysates ('metalosates') and specific amino acid complexes such as Cu_2–lysine afforded protection from rumen-based antagonisms: neither source had any consistent advantage over $CuSO_4$ in five studies with cattle and one with sheep (for review, see Underwood and Suttle, 1999). Subsequent studies with sheep (Luo *et al.*, 1996), steers (Rabiansky *et al.*, 1999; Engle and Spears, 2000; Yost *et al.*, 2002; Dorton *et al.*, 2003; Mullis *et al.*, 2003), cows (Sinclair *et al.*, 2013) and kids (Mondal *et al.*, 2007) failed to demonstrate consistent or sustained advantage for complexation. Against the tide of opinion in peer-reviewed papers, copper proteinate stimulated growth of goat kids more than equivalent supplements as $CuSO_4$ (Datta *et al.*, 2006) but growth stimulation produced by $CuSO_4$ may have waned as subclinical toxicity developed. Calves given 5 and 10mg Cu kg^{-1}DM as either glycinate complex or $CuSO_4$ in corn-silage-based diets only showed responses in plasma and liver Cu when dietary Mo was raised from 2 (Fig. 11.10) to 6mg kg^{-1}DM (Hansen *et al.*, 2008) and source did not affect performance. Production advantages that do accrue may begin in the feed bag during storage, where fat-soluble vitamins are protected from oxidation by $CuSO_4$.

- Since dry rations for sheep and cattle rarely need additional Cu, the solution to any autooxidation problem is to avoid adding Cu, not chelation.

Oral drenching and parenteral dosage

Intermittent treatment can be effective because ruminants store copper in liver when supplied with a sudden excess and draw on those stores when supplies become inadequate. Drenching sheep with $CuSO_4$ at monthly or longer intervals can be effective (e.g. Palsson and Grimsson, 1953). Although delayed swayback is untreatable, it may be preventable (Suttle, 1988) and oral copper doses of 1mg kg^{-1}LW are recommended for all suckling lambs after a first clinical case in a flock. In Fig. 11.2b, swayback incidence in 1982 was reduced by parenteral Cu (10 mg) given at 30 ± 10 days of age (Woolliams *et al.*, 1986a). In adult cattle, thrice-weekly drenches, providing 150 mg Cu d^{-1} as $CuSO_4$, glycinate or metalosates for 100 days, each stemmed liver copper depletion as effectively as two parenteral injections of 100 mg CuCaEDTA (Balemi *et al.*, 2010). However, wide variation in initial liver Cu had a dominant effect on depletion rate and the test was insensitive.

- Variation in initial liver Cu commonly undermines or delays efficacy tests of copper supplements but can be tackled pharmacologically and/or statistically.

Parenteral tetrathiomolybdate can be used to rapidly deplete liver copper stores and subsequent fractional decline in liver Cu used to evaluate responses (Suttle, 2012c). Thus, a new aqueous formulation of CuCaEDTA was shown to be less effective than its emulsified predecessor in steers (Suttle, 2013). Copper in the aqueous product was thought to have been the more rapidly translocated and less efficiently retained in the liver. The parenteral route is not recommended for acute molybdenum-induced diarrhoeas, where the critical locus is the gut mucosa. The common practice of injecting copper prior to insemination to improve conception rate in dairy cows can have the opposite effect (Hawkins, 2014).

Slow-release oral supplementation

Boluses of CuO wire particles (CuOWP) of high specific gravity are retained in the abomasum, where Cu is released for several weeks (Dewey, 1977; Deland *et al.*, 1979). Although CuO provides Cu of low availability (25% relative to $CuSO_4$) (Baker, 1999) large doses can be given safely. Optimum dosage for sheep and cattle is 0.1g

kg^{-1}LW (Suttle, 1981), sufficient to maintain liver Cu stores for many months (McFarlane *et al.*, 1991) and alleviate hypocuprosis in lambs (Whitelaw *et al.*, 1983; Woolliams *et al.*, 1986b) and calves (Whitelaw *et al.*, 1984) on improved hill pastures. In farmed deer, reared on Mo-fertilized pasture, dosing with CuOWP slowed depletion of liver Cu and development of hypocupraemia, but was far less effective on pasture containing 9.0 rather than 4.4mg Mo kg^{-1}DM (Wilson and Grace, 2005) and can present handling difficulties (Nicol *et al.*, 2003). Efficacy may be reduced where diarrhoea is a problem. Protection from hypocupraemia was afforded for 56 and 150 in two groups of lambs, given the same dose at the same time but grazing different fields: protection was the lesser in a badly scouring group and abomasal parasitism was implicated (Suttle and Brebner, 1995). Nematode infections of the abomasum raise abomasal pH and decrease the release of Cu from CuOWP (Bang *et al.*, 1990.[x] Copper oxide is also effective when given to sheep in coarse powder form (Cavanagh and Judson, 1994) and to cattle in a multi-mineral and vitamin bolus (Parkins *et al.*, 1994). A soluble glass bolus has been devised that releases Cu, along with Co and Se for long periods in cattle (Koh and Judson, 1987; Givens *et al.*, 1988; McFarlane *et al.*, 1991).

- Slow-release Cu should only be administered to animals of proven low-copper status as the sole source to reduce risk of toxicity.
- Dosing with CuOWP prior to turnout may increase particle retention.

Genetic selection

The existence of genetic variation in copper metabolism could be exploited to prevent deprivation and lessen the dependence on supplementation where CuRD is endemic. Selection of ram lambs for four generations on the basis of plasma Cu has eliminated hypocuprosis in an inbred, vulnerable 'breed' (Woolliams *et al.*, 1986a, b). Alternatively, the use of rams of a 'tolerant' breed, such as the Texel for crossing with the Scottish Blackface, should reduce hypocuprosis problems on hill pastures. Comparisons of liver Cu accretion between offspring of Suffolk and Texel sires has revealed a high heritability for liver Cu, suggesting scope for selection for high liver Cu based on samples retrieved from the abattoir (Suttle *et al.*, 2002).

- The heritability of serum Cu in an Aberdeen Angus herd (0.28) was sufficiently high to suggest that Cu status could be manipulated by breeding (Morris *et al.*, 2006), and the heritability of plasma Cu might be even higher.

Minimizing antagonisms

Given the strong influence of antagonisms from Fe and Mo on occurrence of Cu-RD, prevention can be achieved in part by minimizing exposure to such antagonists: by avoiding excessive use of lime and improving soil drainage, antagonism from Mo and Fe can be reduced. Advantage might also be taken of a lower uptake of Mo by corn than grass on 'teart' pastures and the use of corn silage as a winter feed in place of grass silage. By avoiding overgrazing and 'poaching', particularly on 'temporary' pasture, antagonisms from soil Fe (and possibly Mn) are minimized. Iron should *never* be used in mineral supplements for grazing or housed, weaned ruminants, even as Fe_2O_3 to give colour.

Copper Poisoning in Sheep

Occurrence of haemolytic copper poisoning

The detailed pathology of chronic copper poisoning (CCP), as reviewed by Howell and Gooneratne (1987), develops in two phases, pre-haemolytic and haemolytic: the latter is marked by an acute haemolytic crisis, indicated by jaundice, and haemoglobinuria and accompanied by a marked rise in serum acid phosphatase and creatine kinase, as well as plasma Cu. Sheep are more susceptible than any other species to CCP but incidents were rare and had exotic explanations.[xi] Incidents of CCP in the UK became as common as those of hypocuprosis, having risen steadily to about 100 per annum between 1970 and 1990. The rise in CCP was partly due to the growing popularity of susceptible breeds such as the Texel. The equally susceptible North Ronaldsay was unique in succumbing to CCP while grazing pasture (Wiener *et al.*, 1978). Nowadays, CCP often occurs in housed lambs, milk sheep and pedigree rams in unexceptional circumstances, other than receiving large amounts of concentrates.[xii] Complete sheep diets should contain <15mg Cu kg^{-1} air-dry feed (Hartmans, 1975) because Cu is well absorbed from such diets (Table 11.5). It is difficult to keep dietary Cu consistently below this limit, particularly when Cu-rich by-products such as distillers grains (Suttle *et al.*,

1996a) and palm-kernel cake, are fed (Chooi *et al.*, 1988). Abbatoir surveys of liver Cu confirm that in many countries, sheep are receiving far more dietary Cu than they need (Kendall *et al.*, 2015). Having access to straw bedding lowered liver Cu (Crosby *et al.*, 2004; Day *et al.*, 2006).

Pre-haemolytic copper poisoning

Pre-haemolytic CCP should be recognized as a separate disorder (PCP) (Suttle, 2012d): though clinically silent, it is neither biochemically nor histologically silent, yet typical of a Type 1 mineral (Fig 3.2): Initially, liver Cu can increase to >1000mg kg^{-1}DM over weeks or months but biochemical abnormalities become detectable, such as increases in plasma glutamate dehydrogenase (GDH), released from damaged hepatocytes into the bloodstream: serum Cu does not increase. Eventually, plasma bile acids and ESOD activity are raised and serum Cu may marginally increase (Fig. 11.16): γ-glutamyltransferase (GGT) activity (not shown) also increases, indicating chronic damage to bile ducts. Histological (King and Bremner, 1979) and biochemical (Woolliams *et al.*, 1982) evidence of liver damage can be seen with liver Cu as low as 350 mg kg^{-1}DM and it is conceivable that liver function might be compromised at times of heavy metabolic load, such as late pregnancy in the twin-bearing ewe: the precautionary principle should be applied and a marginally toxic range of 350–1500mg kg^{-1}DM replace the commonly used single threshold of 750mg kg^{-1}DM for liver Cu (Table 11.7); marginal bands should also be used to interpret liver enzyme activity and plasma Cu. Transition from PCP to CCP marks a breakdown in trafficking Cu safely to storage, bound to MT in lysosomes, and biliary secretion (Clarkson *et al.*, 2020). The factors that trigger breakdown are unknown but appear to be less influential in pastured than in housed sheep. No sheep developed CCP in a grazing study in which liver Cu reached 5000mg kg^{-1}DM and biliary Cu kept increasing (Fig. 11.4). Neonatal infection with a gut pathogen, *Mycobaterium avium*, decreased incidence of PCP in weaned lambs (Fig. 11.16): impaired absorption, increased turnover or impaired hepatic retention of Cu may have been responsible (Suttle, 2012b).

Diagnosis of haemolytic copper poisoning

Diagnosis of haemolytic copper poisoning (HCP or CCP) rests upon clinical evidence of haemolysis

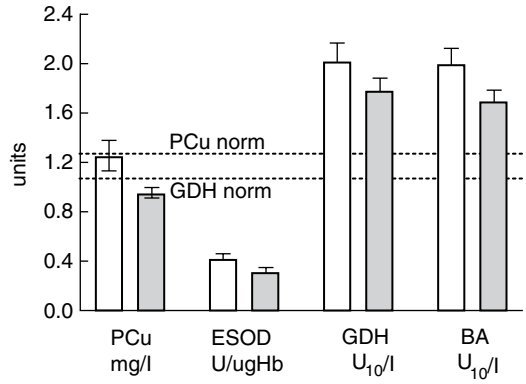


Fig. 11.16. Blood biochemistry of pre-haemolytic copper poisoning (PCP) in sheep (data from Suttle, 2012b). Results are shown for plasma Cu (PCu), glutamate dehydrogenase (GDH), bile acids (BA) and erythrocyte superoxide dismutase (ESOD) in five cohorts (clear columns), following a case of haemolytic copper poisoning, and 12 sheep, treated identically but infected with *M. avium* at birth (shaded columns), which was protective from PCP; note that log transformation is needed to accommodate wide variation in GDH and BA, which was reduced in infected sheep.

(jaundice and haemoglobinuria) and is confirmed by high liver *and* kidney Cu *and* high kidney Fe (Table 11.7). If liver Cu alone is high, the possibility of PCP should be considered. Raised 'liver enzyme' activities in surviving, clinically normal cohorts confirm pre-haemolytic liver injury. Colostral intake of γ-glutamyltransferase invalidates its diagnostic use in the first weeks of life. Acute CP, usually caused by maladministration of parenteral or oral Cu supplements, is distinguished from CCP by raised kidney Cu and Fe but relatively low liver Cu.

Genotype and tolerance

The ranking of sheep breeds in terms of susceptibility to CCP is the inverse of their susceptibility to hypocuprosis, something forgotten in the rush to link susceptibility to CCP to a genetic defect in hepatic handling excess intracellular Cu (Haywood *et al.*, 2005; Simpson *et al.*, 2006; Clarkson *et al.*, 2020). Differences in hepatic gene expression have been reported between susceptible and tolerant breeds but breeds differ in the rate at which they absorb Cu from the diet and differences in Cu trafficking in the liver may originate in the gut, i.e.

Table 11.7. Marginal bands[a] for copper to antagonist concentrations in the diet, copper concentrations in the bloodstream and tissues and serum enzyme activities (indicative of liver or muscle damage) in the diagnosis and prognosis of chronic copper poisoning (CCP) in livestock.

	Sheep	Cattle	Goats	Pigs	Poultry	Interpretive limits
Diet Cu:Mo	10–**20**	30–60	30–60	NA	NA	
Diet Cu (mg kg^{-1}DM)	12–**36**	50–**300**	30–**100**	250–**750**	200–**400**	
Liver Cu (mmol kg^{-1}DM)[b]	6.4–**16.0**	6.4–**16.0**	6.4–**16.0**	32.0–**48.0**	1.0–**2.0**	
Kidney Cu[c] (mmol kg^{-1} DM)	0.6–**0.8**	0.6–**0.8**	0.6–**0.8**	10.0–**16.0**		
Kidney Fe (mmol kg^{-1} DM)	16–**18**	**16–18**	16–**18**			
Plasma[d] Cu (µmol/l)	20–**25**	20–**25**	20–**25**	40–**60**		No acute-phase response
Serum bilirubin (mmol l^{-1})	4–**20**					
Serum AAT[e]	60–**100**	20–**60**				Also released during muscle damage
Serum GLDH[e]	12–**51**	12–**51**				Consider other possible causes (e.g. liver fluke)
Serum GGT[e]	45–**100**	45–**100**				

AAT, aspartate aminotransferase; Fe, iron; GGT, γ-glutamyltransferase; GLDH, glutamate dehydrogenase.
[a]Values below the lower limit rule out CCP; values above the upper limit are strongly suggestive of CCP; marginal values for several criteria indicate probable CCP.
[b]Multiply by 63.4 to obtain units in mg kg^{-1}DM and by 21.1 for mg kg^{-1} fresh weight.
[c]High kidney but normal liver Cu indicates acute toxicity; high kidney Cu with normal kidney Fe indicates hepatic crisis without haemolytic crisis.
[d]Serum samples should not be used for sheep or cattle because values can be underestimated by up to 6 µmol Cu l^{-1}: the corresponding marginal range for ovine serum Cu is 15.8–19.2 µmol Cu l^{-1}, judging by data of Laven and Smith (2008).
[e]All units of enzyme activity are given as IU measured at 30°C.

effect rather than cause. CCP is not the ovine equivalent of Wilson's disease.

Treatment

Sheep affected by CCP (Gooneratne *et al.*, 1981) or PCP (Suttle, 2012b, c) can be treated with TTM, mediator of the Cu–Mo–S antagonism. Sheep with HCP were conveniently and effectively treated with a course of three subcutaneous injections of 3.7 mg ammonium salt (ATTM) kg^{-1} LW, given on alternate days (Humphries *et al.*, 1988). Increases in total plasma Cu occur after ATTM treatment due to accumulation of TCAI-Cu, and analysis of TCA soluble-Cu is recommended (Suttle, 2008b). The success of treatment can only be assessed by liver enzyme assays in plasma (Suttle *et al.*, 1992a; Suttle, 2008b). There is a temporary, marked fall in blood ESOD after ATTM treatment (Suttle *et al.*, 1992a) but this probably has no important physiological consequences (p. 465).

Prevention

Dietary antagonists of Cu can be used to prevent PCP and CCP. Provision of Mo-containing salt licks eliminated mortality from toxaemic jaundice in Australia (Dick and Bull, 1945) and led to the preventive use of dietary MoO_4 plus SO_4 to reduce Cu availability (Suttle, 1977). In outbreaks of CCP, unaffected sheep with raised liver enzyme activities should be treated subcutaneously with ATTM (Suttle, 2012b) and all sheep transferred to a diet low in available Cu (Suttle, 2012c). This can be achieved by replacing the proprietary concentrate with urea-treated whole grain and/or adding gypsum (15 g) and Na_2MoO_4 (19.2mg kg^{-1}DM) to the forage: other antagonists such as Fe (Rosa *et al.*, 1986) and Zn (Bremner *et al.*, 1976; Akpan *et al.*, 2005) can be used. It is common for mixtures of antagonists to be added by feed compounders, although there is no evidence that additive effects will be obtained. A 'cocktail' of Mo, Zn and S reduced but did not prevent PCP in Texel sheep, given a concentrate high in Fe (Fig. 11.17): use of S is limited by risk of S toxicity (p. 222). With vulnerable breeds or dairy sheep at recurrent risk while housed, the grazing of strips of pasture fertilized with Na_2MoO_4 (0.27kg ha^{-1}) could reduce liver Cu between periods of housing. Genetic selection could lower the susceptibility of vulnerable breeds. Selection of Texel sires for low plasma Cu reduced

liver Cu in their offspring (Woolliams, personal communication, 1990): selection for low liver Cu should achieve similar results (Suttle *et al.*, 2002) and merits consideration in dairy sheep, which are generously fed with concentrates.

Copper Poisoning in Cattle and Goats

Occurrence in cattle

Bovine CCP was rarely encountered, other than in calves given Cu-rich milk substitutes (Shand and Lewis, 1957), from which Cu is well absorbed. CCP was induced after six weeks supplementation of a milk substitute with 200mg Cu kg^{-1}DM: 50 mg kg^{-1}DM was tolerated for nine weeks (Jenkins and Hidiroglou, 1989). Tolerance will depend on the copper nutrition of the mother and liver Cu in the newborn calf. Administration of CuOWP to newborn calves, against proprietary instructions, contributed to an outbreak of CCP in which the dams also received parenteral Cu in late pregnancy (Steffen *et al.*, 1997). After weaning, cattle are less vulnerable to CCP and up to 900mg Cu kg^{-1}DM was tolerated for several months by beef cattle (Felsman *et al.*, 1973). However, incidence greatly increased in the UK (Livesey *et al.*, 2002): between 2000 and 2007 there were 31 ± 9.2 (s.d) incidents annually compared to 50 ± 9.3 outbreaks of lead poisoning, the commonest metal poisoning (VIDA, 2007). Incidence remains high (Clarkson *et al.*, 2020), although this is partly due to growing recognition that cattle can be poisoned by Cu without going through a haemolytic crisis (Bidewell *et al.*, 2012).

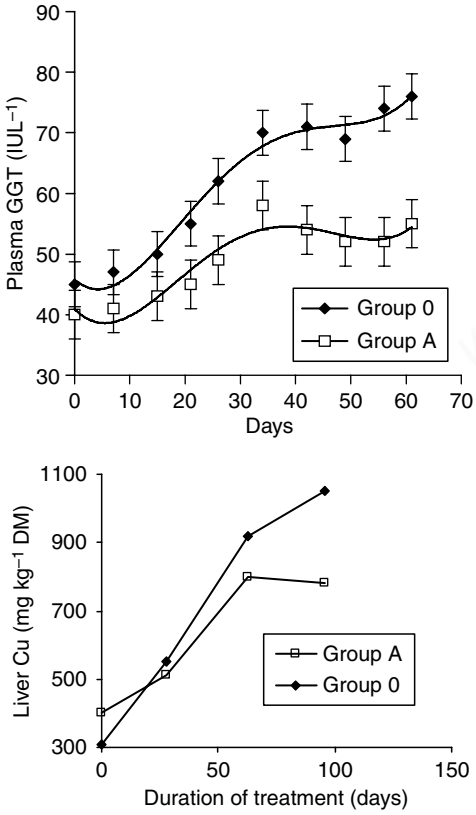


Fig. 11.17. Effects of dietary supplementation with a cocktail of copper antagonists on liver Cu and a biochemical indicator of liver damage (γ-glutamyl transferase, GGT) in Texel rams given a diet high in Cu and iron (26 and 345mg kg^{-1}DM, respectively). Group 0 were unsupplemented and Group A received added Mo, 2, S, 3000 Zn, 300mg kg^{-1}DM) (Suttle, 2012c).

- So-called 'atypical copper poisoning' describes a hepatopathy and calls for a similar distinction to that proposed for sheep, i.e. between pre-haemolytic and haemolytic disorders (PCP and HCP).

As with sheep, abattoir surveys of liver Cu reveal widespread overfeeding of Cu; 40% of culled dairy cows and 17% of beef cows had values >8 mmol kg^{-1}DM and were at risk of PCP (Kendall *et al.*, 2015): a similar situation was described in New Zealand (Grace and Knowles, 2015) and attributed in part to increased use of PKC, rich in Cu. However, the problem is broader and had two components: failure to appreciate how little Cu is needed in a TMR (*c.*6mg Cu kg^{-1}DM); a widely promoted misconception that cows with normal plasma Cu often needed 'sacrificial' supplementary Cu to counteract the flow of toxic TM from the rumen (Suttle, 2016). The 'thiomolybdate myth' is dealt with elsewhere (p. 464). Liver fluke infections, which are also on the increase, may also be partly responsible, lowering the capacity of the liver to safely store copper (Suttle, unpublished data).

Cows with high liver Cu give birth to calves with even higher, possibly lethal, values (Hunter *et al.*, 2013). Residual effects may be seen in the form of raised plasma GDH around weaning. Some instances of CCP have involved massive pollution from mining activities (Gummow *et al.*, 1991). Like sheep, cattle can succumb to acute CP when given excessive amounts by injection (NRC, 2005) and doses should always be carefully scaled according to LW.

Occurrence in goats

Goat kids have tolerated doses of 200 mg Cu day^{-1} for 98 days (Cummins *et al.*, 2008) and added dietary Cu of 60mg kg^{-1}DM for 137 days, whereas lambs succumbed to CCP (Zervas *et al.*, 1990). Goats are a copper-tolerant species. Addition of 20 or 40mg Cu kg^{-1}DM to a diet adequate in Cu (9.5kg^{-1}DM) for 84 days *improved* LWG in kids and was more effective when added as proteinate rather than as $CuSO_4$ (Datta *et al.*, 2006). This is either a unique example of hyperavailability or lower toxicity for a 'copper chelate'. Since the diet was low in Mo (0.28mg kg^{-1}DM) and contained 51% bran but no long roughage, it is arguable that the growth stimulation, produced by the more available source, $CuSO_4$, waned as PCP developed.

Subclinical hepatotoxicity

The possibility of harmful subclinical consequences of excessive Cu intakes in cattle, prior to development of PCP, is gaining support. Blood biochemistry in cohorts of cows that died of HCP presented a similar picture to that shown for sheep in Fig. 11.16 (Johnston *et al.*, 2014). Plasma GDH was raised until weaning in calves that had been born with high liver Cu (Hunter *et al.*, 2013). Performance of finishing steers was lowered by supplementation with 20–40mg Cu kg^{-1}DM and impaired rumen function was implicated (Engle and Spears, 2000): no adverse effects were evident in the growing phase but liver Cu could have exceeded 600mg kg^{-1}DM. Steers dosed with CuOWP digested feed less well in the second than in the first phase of an experiment, when liver contained 556 and 640 mg Cu kg^{-1}DM, respectively (Arthington, 2005). A supplement of 120mg Cu kg^{-1}DM in a concentrate, offered to heifers with *ad libitum* access to hay, decreased LWG. Plasma Cu entered the marginally toxic range (20–25μmol l^{-1}) in cattle finished on corn-based diets containing monensin, an enhancer of A_{Cu}, and 17mg Cu kg^{-1}DM (Hansen *et al.*, 2008). Copper supplements have also retarded growth in goat kids (Mondal *et al.*, 2007) and cashmere goats (Zhang *et al.*, 2008).

Diagnosis

The criteria for a diagnosis of HCP are the same for cattle and goats as for sheep (p. 279). The assessment of high liver Cu in the newborn calf requires higher diagnostic limits than those used for older cattle: 10 mmol Cu kg^{-1}DM was reported in histologically normal livers in the newborn (Hunter *et al.*, 2013). In the absence of corresponding data for caprines, their newborn should be assessed using the limits given for calves. Serum samples should never be used in surveillance work (see p. 268) because underestimation is proportionate to concentration and can be as high as 7 μmol Cu l^{-1} (Laven *et al.*, 2007).

Prevention

The prevention of HCP and PCP in cattle requires an end to routine supplementation in housed herds, especially when there is no evidence of low Cu status (Steffen *et al.*, 1997; Livesey *et al.*, 2002; Suttle and Phillippo, 2005; Suttle, 2016). Although monensin is now a prohibited additive in Europe, its use elsewhere with supplementary Cu is contraindicated.

- The permitted concentration of copper in milk substitutes in Europe, 15mg kg^{-1}DM, is far too generous and should be cut by two thirds (Hunter *et al.*, 2013).

Dietary supplementation with antagonists should follow the guidelines given for sheep and would be more convenient and efficacious than drenching with Mo + SO_4 (e.g. Johnston *et al.*, 2014).

Copper Nutrition of Non-ruminants

Detailed description of feeds and calculations of needs are superfluous because coefficients for AA_{Cu} are consistently high (*c*.0.5) and dietary provision from natural sources generous. There are wide differences between species in both colostral and main milk Cu. Colostrums contain two to three times more Cu than main milk and sow's milk five times more than cow's or goat's milk (10 μmol versus 2 μmol Cu l^{-1}). High Cu in sow's milk reflects the need to sustain large, rapidly growing litters during a brief lactation. Milk Cu is reduced in dams deprived of Cu but cannot be increased by supplementing adequate diets (Underwood and Suttle, 1999). Newborn of all species can absorb copper-containing molecules by endocytosis, contributing to a high efficiency of absorption. Variations in A_{Cu} between dry feeds have been extensively studied, particularly in poultry, although low values are unlikely to cause insufficiency.[xiii]

Gross requirements of pigs and poultry

A purified diet supplying 6mg Cu kg^{-1}DM fully met requirements of baby pigs (Okonkwo *et al.*, 1979) and 4mg kg^{-1}DM was considered sufficient for growing pigs (ARC, 1981). Diets composed of cereals and normal protein supplements will meet needs, despite pelleting, which can greatly reduce AA_{Cu} (Larsen *et al.*, 1999). The additional requirement of 4–6 mg Cu d^{-1} for lactation (5–7 kg milk containing 0.75 mg Cu kg^{-1}) would be met by additional food intake (3 kg d^{-1}) on a diet containing 4mg Cu kg^{-1}DM, with absorptive efficiency at 50%. Definitive requirements for chick growth and egg production have not been reported. NRC (1994) recommendations (all mg kg^{-1}DM) were: starting chicks (0–8 weeks), 5; growing leghorn-type chickens (8–18 weeks), 4; broilers (all ages), 8; starting turkey poults, 8; growing turkeys, 6; and breeding turkeys, 6. No requirements were given for laying or breeding hens, presumably because previous low recommendations (3–4mg kg^{-1}DM) had not been questioned.

Occurrence of Insufficiency in Non-ruminants

Normal pig and poultry rations usually contain >6mg kg^{-1}DM and rarely require supplementation. Where dietary Cu is low, enhancement of A_{Cu} probably allows biliary Cu to be recycled more efficiently and guard against deprivation. Normal ranges for plasma Cu differ greatly between species (Table 11.7).

Gross copper requirements of horses

Using a factorial model with A_{Cu} at 0.30 and a net growth component at 1.0 mg placed need for a 200-kg horse gaining 1 kg day^{-1} at only 4.5mg Cu kg^{-1}DM (Grace *et al.*, 1999). This low requirement was supported by the fact that horses thrive on pastures with 6–9 mg Cu kg^{-1}DM and neither growth nor bone development was enhanced by copper supplementation (Pearce *et al.*, 1999a). Exposure to molybdenum does not impair copper metabolism or raise copper requirements in horses (Strickland *et al.*, 1987; Pearce *et al.*, 1999b), probably because molybdenum is extensively absorbed before the site conducive to thiomolybdate formation – the caecum – is reached. Equine species can be grouped with other non-ruminants in which uninhibited A_{Cu} lessens the need to store copper (Fig. 11.3). Angular deformities at the pasterns have been claimed to respond to Cu supplementation (Barton *et al.*, 1991), but there are other possible causes. Swelling of the distal radial physis in Thoroughbred yearlings did not respond to 130 mg Cu d^{-1} (Grace *et al.*, 2002). There are no losses of Cp during clotting in equine samples (Paynter, 1987) to complicate the assessment of serum Cu, and a normal range of 16.5–20.0 µmol l^{-1} has been suggested (Suttle *et al.*, 1996b). However, a seasonal fall has been seen to take values as low as 9 µmol l^{-1} in grazing fillies in midwinter and may have been attributable to photoperiodism (Pearce *et al.*, 1999a). Like most newborns, the foal begins life with a large hepatic reserve (374 ± 130 (s.d.) mg kg^{-1}), whether or not the mare has been injected with Cu during pregnancy (Gee *et al.*, 2000). Most foals show a rapid decline in liver Cu to normal 'adult' values by 160 days of age (21 ± 6mg kg^{-1}DM) but a small subgroup showed a much slower rate of decline.

Pigs

Copper deprivation can be induced by weaning pigs early on to bovine milk or its products. Plasma and liver Cu fall simultaneously from the outset, a microcytic, hypochromic anaemia soon develops and survival time of erythrocytes is shortened (Bush *et al.*, 1956). Iron accumulates in the gut mucosa at a pre-clinical stage. The piglet is particularly susceptible to the anaemia of iron deprivation, but the rate-limiting factor may be haem biosynthesis rather than iron transport. Addition of 100mg Zn kg^{-1}DM to a corn/SBM ration reduced AA_{Cu} from 0.22 to 0.13 in young pigs (Adeola *et al.*, 1995) and a Cu-responsive anaemia has been reported in pigs fattened on swill that had been stored in galvanized bins (Pritchard *et al.*, 1985).[xiv] In severely deficient young pigs, osteoblastic activity became depressed, epiphyseal plates widened

Table 11.8. Normal ranges for plasma and liver copper concentrations in some monogastric species.

	Plasma Cu (µmol l^{-1})	Liver Cu (µmol kg^{-1}DM)
Horse	8–12	100–200
Pig	14–16	100–1000
Chicken	3–6	100–500

and gross bone disorders developed, with fractures, and severe deformities, and marked bowing of the forelegs (Baxter *et al.*, 1953). Abnormal elastin development can cause aortic rupture and high mortality (Carnes *et al.*, 1961) for reasons explained below.

Poultry

Experimental deprivation in chicks produces a normocytic and normochromic anaemia and low MAO or CCO activity in bones may compromise osteoblastic activity, leading to abnormal bone morphology (Rucker *et al.*, 1969, 1975). Abnormal elastin development can also cause high mortality due to aortic rupture in chicks under experimental conditions (O'Dell *et al.*, 1961) and turkey poults under commercial conditions (Guenthner *et al.*, 1978). Lysyl oxidase activities (Table 11.1) in plasma, liver and aorta are subnormal in Cu-deprived chicks (and piglets), diminishing the capacity to form desmosine from lysine and the cross-links that give the aorta strength and elasticity (O'Dell, 1976). Chicks and piglets are particularly susceptibile to aortic rupture because growth of the aorta is rapid in the first few weeks of life. Similar biochemical abnormalities may impair shell formation in the laying hen (Baumgartner *et al.*, 1978) and the air–blood capillary network in the avian lung (Buckingham *et al.*, 1981). Hens deprived of Cu lay eggs of abnormal size and shape, either lacking shells or with wrinkled, rough shells, due to malformation of the shell membranes (Baumgartner *et al.*, 1978). Egg production and hatchability are markedly reduced and, after incubation, the embryos from these hens exhibit anaemia, retarded development and a high incidence of haemorrhage (Savage, 1968). Such problems do not occur on natural diets.

Laboratory animals

The lethal effects of aortic rupture drew attention away from the role of copper in other parts of the cardiovascular system, including the heart. In mice deprived of Cu, DBM becomes rate-limiting in the heart (Prohaska, 2006). Other enzymes are also likely to be depleted, including SOD1, but there are compensatory increases in MnSOD activity (Lai *et al.*, 1994). Gene-deletion studies have shown that while absence of Cp causes iron to accumulate in the liver and elsewhere, the associated anaemia is relatively mild and animals survive (Prohaska, 2006). Increased expression of Cp homologues such as hephaestin (Table 11.1) may provide cover for iron transfer. Immunological markers such as interleukin-2 production and lymphocyte subset phenotyping may be particularly sensitive measures of Cu status (Bonham *et al.*, 2002) and merit studies in ruminants.

Copper as a Growth Promoter

Copper was widely used at supra-nutritional levels, usually as $CuSO_4$, to enhance growth and FCE in pigs (Braude, 1967)[xv] and poultry (Jenkins *et al.*, 1970) but responses were more variable in birds.[xvi] The environmental pollution caused by the copper-enriched effluents from pig and poultry units has eliminated the practice in Europe (see Chapter 20). Differences in 'copper value' amongst inorganic Cu compounds exist for pigs (Baker and Ammerman, 1995; Ewing *et al.*, 1998) and poultry (Koh *et al.*, 1996; Baker, 1999) but have been reduced to academic significance. Organic sources have not shown consistently superior availability over $CuSO_4$ (Baker and Ammerman,1995; Waldroup *et al.*, 2003; Pekel and Alp, 2011). Faecal Cu excretion in pigs given Cu-proteinates or $CuSO_4$ indicates that they were equally available (Veum *et al.*, 2004) (p. 531).

Chronic Copper Poisoning in Pigs and Poultry

In non-ruminants, CCP follows a similar two-phase pattern to that seen in sheep, except that the haemolytic crisis is less pronounced and less life-threatening while growth retardation is more prominent. The disorder occurred in a minority of pigs given copper to stimulate growth in rations not suitably balanced with other minerals. Protection was afforded by adding zinc and iron in broadly equivalent concentrations (Suttle and Mills, 1966a, b). Sows were given similar supplements throughout six gestations without causing toxicity, although liver Cu increased to 1899mg kg^{-1}DM (Cromwell *et al.*, 1993). Tolerance of dietary Cu in chicks was influenced by type of diet, increasing from 200 to 800mg kg^{-1}DM when dehulled SBM replaced casein in diets high in Ca (Funk and Baker, 1991). Copper accretion increased when chicks were given diets low in Ca (Leach

et al., 1990). Antagonism from phytate may therefore protect poultry from toxicity, while addition of phytase may lower tolerance. Growth of turkeys was inhibited when 120 or 240mg Cu kg^{-1}DM was added to a corn/SBM diet, fed in mash form: again, enhanced phytase activity may have reduced tolerance (Kashani *et al*., 1986). Erosion of the gizzard is an additional feature of CCP in poultry and occurs with dietary Cu around 500mg kg^{-1}DM (Christmas and Harms, 1979; Jensen and Maurice, 1979). Methionine supplementation lessened the growth retardation but did not prevent gizzard erosion (Kashani *et al*., 1986). Insoluble salts such as $CuCO_3$ may cause less gizzard corrosion. Chicks were more vulnerable when a diet with 800mg Cu kg^{-1}DM was marginal in Se: mortality was associated with exudative diathesis and prevented by raising dietary Se from 0.2 to 0.7mg kg^{-1}DM (Jensen, 1975).

Notes

[i] CrT1 and 2 carry Cu across membranes; CCS chaperones Cu for synthesis of SOD, COX1 carries Cu for cytochrome oxidase synthesis and ATP7A exports Cu from cells: the latter is induced by lack of dietary Fe.

[ii] Liver Cu in affected animals was 32 µmol kg^{-1}DM, plasma Cu 1.6 µmol l^{-1} and pasture Cu (1–3 mg kg^{-1}DM).

[iii] In 'teart' pastures, underlain by Mo-rich Lower Lias clay (Thornton and Alloway, 1974). Some peat soils in New Zealand (Cunningham, 1950), southern Australia (McFarlane *et al.*, 1990), Washington state (Kincaid *et al.*, 1986) and Ireland (Thornton and Alloway, 1974; McAloon *et al*., 2017) are high in Mo. Alluvial plains of Argentina, marine shales of the English Midlands (Thornton and Alloway, 1974), Scotland (Caithness; Suttle, 2008b) and north-east Manitoba (Boila *et al*., 1984a, b), grey soils of NE Saskatchewan (Gooneratne and Christensen, 1989) and Taupo ash soils of New Zealand (Grace *et al.*, 2005b), all provide sufficient Mo to induce hypocuprosis.

[iv] For example, a study of field cases of swayback indicated a threshold relationship between brain Cu, CCO and pathological change, with brain Cu <3mg kg^{-1}DM critical (Mills and Williams, 1962). However, the relationship was less clear in experimentally induced swayback (Suttle *et al.*, 1970) and early depletion of CCO activity may not be sufficient to constitute a respiratory constraint (Smith *et al.*, 1976).

[v] Peptidylglycine α-amidating monooxygenase (PAM) decreases in the brain of newborn rats from Cu-depleted dams but there have been no studies of PAM in the CNS of ruminants.

[vi] Late reductions in SOD1 activity in neutrophils were found in offspring of Mo-supplemented groups, but not an equally hypocupraemic high Fe group (Gengelbach *et al.*, 1997).

[vii] Glycosylphosphatidylinositol-linked CP or GI-CP.

[viii] Pot ale syrup, a distillery by-product, contains Cu that is six times less available than $CuSO_4$ and distillers' grains (A_{Cu} 0.01 and 0.06, respectively) and may be present as an unavailable complex (Suttle *et al*., 1996a). Most of the copper in poultry waste (64%) was extractable with EDTA and equalled $CuSO_4$ in availability to sheep (A_{Cu} 0.08 versus 0.07) (Suttle and Price, 1976).

[ix] Early Australian experience indicated that a single dressing of 5–7 kg $CuSO_4$ ha^{-1} was effective for at least 3–4 years, except on calcareous soils (Underwood *et al.*, 1943) and for up to 23 years on sandy soils. However, on yellow-brown/yellow-grey earths in New Zealand hill country, the response in herbage Cu only lasted for a year and 3 kg $CuSO_4$ ha^{-1} raised pasture Cu for barely 100 days (Grace *et al.*, 2005a).

[x] Although CuOWP can have anthelmintic activity (Burke *et al.*, 2005), the dose required is far higher than that used to supplement with Cu.

[xi] The disease rarely occurred in outdoor flocks unless pastures had been contaminated with exogenous Cu fungicides, used in orchards, or copper-rich pig slurry (Suttle and Price, 1976; Kerr and McGavin, 1991). In parts of Australia and elsewhere, uncontaminated pastures, very low in Mo (0.1–0.2mg kg^{-1}DM), or infested with hepatotoxic weeds such as *Heliotropium europaeum*, caused 'toxaemic jaundice' or 'yellows', both forms of CCP (Howell *et al.*, 1991). Exposure to a fungal hepatotoxin, phomopsin, in fields of lupins also triggered CCP (White *et al.*, 1994).

[xii] Cases of ovine CCP have been caused by contamination of pastures and feed with copper-supplemented pig or cattle rations, and by the excessive consumption of copper-containing salt licks and the unwise use of copper supplements (oral or parenteral). The addition of monensin (Barrowman and van Ryssen, 1987) and possibly selenium (Hartmans and van Ryssen, 1997) to sheep feeds predisposes to CCP.

[xiii] Corn gluten meal, cottonseed meal, peanut hulls and soybean meal (SBM) all had values approximately 40% lower than $CuSO_4$ for poultry (Baker and Ammerman, 1995). Feeds with low availability transfer that property to copper supplements. Hepatic retention of copper from $CuSO_4$ in chicks decreased substantially when soy protein was substituted for casein (Funk and Baker, 1991). Since low relative absorption in vegetable proteins is partly attributed to antagonism from phytate, cereals are likely to contain Cu of equally low value. Addition of phytase to corn/SBM diets raised AA_{Cu} from 0.22 to 0.37 in pigs (Adeola *et al.*, 1995) but excess dietary Ca has the opposite effect, enhancing the phytate antagonism (Leach *et al*., 1990). Such antagonisms only had practical significance in the context of growth promotion. Certain animal by-products have low relative A_{Cu} (feather meal <1%, meat and bone meal 4–28%),

possibly due to heat denaturation during processing, but others (poultry waste, 67%) are good sources (Baker and Ammerman, 1995).

[xiv] The swill was high in Zn (2.6–5.1g kg^{-1}DM) and low in copper (3.2-4.7mg kg^{-1}DM). Other swills being fed in the locality but uncontaminated with zinc contained slightly more copper (3.5-9.0mg kg^{-1}DM) and caused no problems. It was concluded that swills should be supplemented with copper and not stored in galvanized bins.

[xv] Comparisons of $Cu(OH)_3Cl$ (Cromwell *et al.*, 1998) and citrate (Armstrong *et al.*, 2004) with $CuSO_4$ showed no effect of copper source on growth promotion. Optimal dietary Cu lay between 100 and 250mg Cu kg^{-1}DM, but this figure has varied between studies and may depend on the strength of antagonism from calcium and phytate. Growth promotion may result from improved digestibility or appetite (Pekel and Alp, 2011), antibiotic activity, detoxification of goitrogens (see p. 305) or a systemic endocrine stimulus. Intravenously administered copper histidinate can stimulate growth and release of the mRNA for growth hormone is stimulated by both the oral and intravenous routes of copper supplementation, which significantly increase brain Cu (Pekel and Alp, 2011). 'Supra-nutritional' copper supplementation is not always a safe procedure. The practice has reduced iron stores in the liver (Bradley *et al.*, 1983) and induced anaemia, copper toxicosis or zinc deficiency. Concurrent supplementation with iron and zinc, each at 150mg kg^{-1}DM, protected against the adverse effects of copper at 425mg Cu kg^{-1} DM in one early study (Suttle and Mills, 1966a). Birth and weaning weights can be increased by 9% by similarly supplementing the sow's diet with copper (Cromwell *et al.*, 1993).

[xvi] Chick growth was increased in a wheat/fish meal diet, but depressed when a maize/SBM diet was similarly supplemented and stimulated with a maize/SBM diet, but not on a casein/dextrose diet, on which higher availability may have resulted in hepatotoxicity (Funk and Baker, 1991). With only 63 mg Cu kg^{-1} added as citrate, chick growth to 45 days of age was increased (Ewing *et al.*, 1998) but in turkey poults, growth has been retarded by 204mg Cu kg^{-1}DM in a short-term (10-day) study (Ward *et al.*, 1994) and by 120mg kg^{-1}DM in a longer-term (24-week) study (Kashani *et al.*, 1986). Brief addition of copper to the drinking water as well as diet is often practised, but is not recommended (Ward *et al.*, 1994).

References

Adeola, O., Lawrence, B.V., Sutton, A.L. and Cline, T.R. (1995) Phytase induced changes in mineral utilization in zinc-supplemented diets for pigs. *Journal of Animal Science* 73, 3384–3391.

Akpan, H.D., Udosen, E.O., Udofia, A.A., Akpan, E.J. and Joshua, A.A. (2005) The effect of phytase and zinc supplementation on palm kernel cake toxicity in sheep. *Pakistan Journal of Nutrition* 4, 148–153.

Allen, J.D. and Gawthorne, J.M. (1987) Involvement of the solid phase rumen digesta in the interaction between copper, molybdenum and sulphur in sheep. *British Journal of Nutrition* 58, 265–276.

Alloway, B.J. (1973) Copper and molybdenum in sway-back pastures. *Journal of Agricultural Science, Cambridge* 80, 521–524.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

ARC (1981) *The Nutrient Requirements of Pigs*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 215–248.

Armstrong, T.A., Cook, D.R., Ward, M.M., Williams, C.M. and Spears, J.W. (2004) Effect of copper source (cupric citrate or cupric sulfate) and concentration on growth performance and fecal copper excretion in weanling pigs. *Journal of Animal Science* 82, 1234–1240.

Arthington, J.D. (2005) Effects of copper oxide bolus administration or high-level copper supplementation on forage utilization and copper status in beef cattle. *Journal of Animal Science* 83, 2894–2900.

Arthington, J.D., Corah, L.R. and Blecha, F. (1996) The effect of molybdenum-induced copper deficiency on acute phase protein concentrations, superoxide dismutase activity, leukocyte numbers and lymphocyte proliferation in beef heifers inoculated with bovine herpes virus-I. *Journal of Animal Science* 74, 211–217.

Ashino, T., Sudhahar, V., Urao, N., Oshikawa, J., Chen, G.-F. *et al.* (2010) Unexpected role of the copper transporter atp7a in pdgf-induced vascular smooth muscle cell migration. *Circulation Research* 107, 787–799.

Bain, M.S., Spence, J.B. and Jones, P.C. (1986) An investigation of bovine serum copper levels in Lincolnshire and South Humberside. *Veterinary Record* 119, 593–595.

Baker, D.H. (1999) Cupric oxide should not be used as a copper supplement for animals or humans. *Journal of Nutrition* 129, 2278–2279.

Baker, D.H. and Ammerman, C.B. (1995) Copper bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 127–156.

Balemi, S.C., Grace, N.D., West, D.M., Smith, S.L. and Knowles, S.O. (2010) Accumulation and depletion of liver copper stores in dairy cows challenged with a Cu-deficient diet and oral and injectable forms of Cu supplementation. *New Zealand Veterinary Journal* 58, 137–141.

Bang, K.S., Familton, A.S. and Sykes, A.R. (1990) Effect of ostertagiasis on copper status in sheep: a study

involving use of copper oxide wire particles. *Research in Veterinary Science* 49, 306–314.

Barrowman, P.R. and van Ryssen, J.B.J. (1987) Effect of ionophores on the accumulation of copper in the livers of sheep. *Animal Production* 44, 255–261.

Barry, T.N., Reid, T.C., Millar, K.R. and Sadler, W.A. (1981) Nutritional evaluation of kale (*Brassica oleracea*) diets. 2. Copper deficiency, thyroid function and selenium status in young cattle and sheep fed kale for prolonged periods. *Journal of Agricultural Science, Cambridge* 96, 269–282.

Barton, J., Hurtig, M. and Green, S. (1991) The role of copper in developmental orthopaedic disease in foals. In: *Highlights of Agricultural and Food Research in Ontario*, September. University of Guelph, Canada, pp. 14–18.

Baumgartner, S., Brown, D.J., Salevsky, E. Jr and Leach, R.M. Jr (1978) Copper deficiency in the laying hen. *Journal of Nutrition* 108, 804–811.

Baxter, J.H., Van Wyk, J.J. and Follis, R.H. Jr (1953) A bone disorder associated with copper deficiency. *Bulletin of the Johns Hopkins Hospital* 93, 1–39.

Beattie, J.H., Reid, M.D., Fairweather-Tait, S., Dainty, J.R., Majsak-Newman, G. and Harvey, L. (2001) Selective extraction of blood plasma exchangeable copper for isotope studies of dietary copper absorption. *Analyst* 126, 2225–2229.

Beck, A.B. (1951) *A survey of the copper content of Western Australian pastures*. Leaflet No. 678, Department of Agriculture, Western Australia.

Ben Salem, H., Norman, H.C., Nefzaouic, A., Mayberry, D.E., Pearce, K.L. and Revell, D.K. (2010) Potential use of oldman saltbush (Atriplex nummularia Lindl.) in sheep and goat feeding. *Small Ruminant Research* 91, 13–28.

Bennetts, H.W. and Chapman, F.E. (1937) Copper deficiency in sheep in Western Australia: a preliminary account of the aetiology of enzootic ataxia of lambs and on anaemia of ewes. *Australian Veterinary Journal* 13, 138–149.

Bennetts, H.W. and Hall, H.T.B. (1939) 'Falling disease' of cattle in the south-west of Western Australia. *Australian Veterinary Journal* 15, 152–159.

Bidewell, C.A., Drew, J.R., Payne, J.H., Sayers, A.R., Higgins, R. and Livesey, C.T. (2012) Case study of copper poisoning in a British dairy herd. *Veterinary Record* 170. DOI:10.1136/vr.100267

Bird, P.R. (1970) Sulfur metabolism and excretion studies in ruminants. III. The effect of sulfur intake on the availability of copper in sheep. *Proceedings of the Australian Society of Animal Production* 8, 212–218.

Boila, R.J., Devlin, T.J., Drydale. R.A. and Lillie, L.E. (1984a) Geographical variation in the copper and molybdenum contents of forages grown in northwestern Manitoba. *Canadian Journal of Animal Science* 64, 899–918.

Boila, R.J., Devlin, T.J., Drydale, R.A. and Lillie, L.E. (1984b) The severity of hypocupraemia in selected herds of beef cattle in northwestern Manitoba. *Canadian Journal of Animal Science* 64, 919–936.

Bonham, M., O'Connor, J.M., Hannigan, B.M. and Strain, J.J. (2002) The immune system as a physiological indicator of marginal copper status. *British Journal of Nutrition* 87, 393–403.

Boyne, R. and Arthur, J.R. (1981) Effect of selenium and copper deficiency on neutrophil function in cattle. *Journal of Comparative Pathology* 91, 271–276.

Bradley, B.D., Graber, G., Condon, R.J. and Frobish, L.T. (1983) Effects of graded levels of dietary copper on copper and iron concentrations in swine tissues. *Journal of Animal Science* 56, 625–630.

Braude, R. (1967) Copper as a stimulant in pig feeding. *World Review of Animal Production* 3, 69–82.

Brebner, J., Thornton, I., McDonald, P. and Suttle, N.F. (1985) The release of trace elements from soils under conditions of simulated rumenal and abomasal digestion. In: Mills, C.F., Bremner, I. and Chesters, J.K. (eds) *Proceedings of the Fifth International Symposium on Trace Elements in Man and Animals, Aberdeen*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 850–852.

Bremner, I., Young, B.W. and Mills, C.F. (1976) Protective effect of zinc supplementation against copper toxicosis in sheep. *British Journal of Nutrition* 36, 551–561.

Bremner, I., Young, B.W. and Mills, C.F. (1977) Copper sheep foetus. *British Journal of Nutrition* 37.

Bremner, I., Humphries, W.R., Phillippo, M., Walker, M.J. and Morrice, P.C. (1987) Iron-induced copper-deficiency in calves: dose–response relationships and interactions with molybdenum and sulfur. *Animal Production* 45, 403–414.

Buckingham, K., Heng-khou, C.S., Dubick, M., Lefevre, M., Cross, C., Julian, L. and Rucker, R. (1981) Copper deficiency and elastin metabolism in avian lung. *Proceedings of the Society for Experimental Biology and Medicine* 166, 310–319.

Burke, J.M., Miller, J.E. and Brauer, D.K. (2005) The effectiveness of copper oxide wire particles as an anthelmintic in pregnant ewes and safety of dosing. *Veterinary Parasitology* 131, 291–297.

Bush, J.A., Jensen, W.N., Athens, J.W., Ashenbrucker, H., Cartwright, G.E. and Wintrobe, M.M. (1956) Studies on copper metabolism. 19. The kinetics of iron metabolism and erythrocyte life-span in copper-deficient swine. *Journal of Experimental Medicine* 103, 701–712.

Campbell, A.G., Coup, M.R., Bishop, W.H. and Wright, D.E. (1974) Effect of elevated iron intake on the copper status of grazing cattle. *New Zealand Journal of Agricultural Research* 17, 393–399.

Carnes, W.H., Shields, G.S., Cartwright, G.E. and Wintrobe, M.M. (1961) Vascular lesions in copper deficient swine. *Federation Proceedings* 20, 118.

Cavanagh, N.A. and Judson, G.J. (1994) Copper oxide powder as a copper supplement for sheep. *Journal of Trace Element and Electrolytes in Health and Disease* 8, 183–188.

Cerone, S., Sansinea, A. and Nestor, A. (1995) Copper deficiency alters the immune response of bovine. *Nutrition Research* 15, 1333–1341.

Chooi, K.F., Hutagalung, R.I. and Wan Mohammed, W.E. (1988) Copper toxicity in sheep fed oil palm products. *Australian Veterinary Journal* 66, 156–157.

Christmas, R.B. and Harms, R.H. (1979) The effect of supplemental copper and methionine on the performance of turkey poults. *Poultry Science* 58, 382–384.

Clarkson, A., Paine, S., Martín-Tereso, J. and Kendall, N. (2020) Copper physiology in ruminants: trafficking of systemic copper, adaptations to variation in nutritional supply and thiomolybdate challenge. *Nutrition Research Review* 33, 43–49.

Collins, J.F., Prohaska, J.R. and Knutson, M.D. (2010) Metabolic crossroads of iron and copper. *Nutrition Reviews* 68, 133–147.

Cromwell, G.L., Monegue, H.J. and Stahly, T.S. (1993) Long-term effects of feeding a high copper diet to sows during gestation and lactation. *Journal of Animal Science* 71, 2996–3002.

Cromwell, G.L., Lindemann, M.D., Monegue, H.J., Hall, D.D. and Orr, D.E. (1998) Tribasic copper chloride and copper sulfate as copper sources for weanling pigs. *Journal of Animal Science* 76, 118–123.

Crosby, T.F., Quinn, P.J., Callan, J.J. and O'Hara, M. (2004) Effect of floor type and dietary molybdenum content on the liver copper concentration at slaughter and performance of intensively finished lambs. *Livestock Production Science* 90, 181–190.

Cummins, K.A., Solaimin, S.G. and Bergen, W.G. (2008) The effect of dietary copper supplementation on fatty acid profile and oxidative stability of adipose tissue depots in Boer × Spanish goats. *Journal of Animal Science* 86, 390–396.

Cunningham, I.J. (1950) Copper and molybdenum in relation to diseases of cattle and sheep in New Zealand. In: McElroy, W.D. and Glass, B. (eds) *Copper Metabolism: A Symposium on Animal, Plant and Soil Relationships.* Johns Hopkins Press, Baltimore, Maryland, pp. 246–273.

Datta, C., Mondal, M.K. and Biswas, P. (2006) Influence of dietary inorganic and organic form of copper salt on performance, plasma lipids and nutrient utilisation of Black Bengal (*Capra indicus*) goat kids. *Animal Feed Science and Technology* 135, 191–209.

Day, J.P., Boland, T.M. and Crosby, T.F. (2006) The effects of plastic slatted floor or straw bedding on performance, liver weight and liver copper concentrations in intensively reared lambs. *Livestock Science* 100, 270–275.

Deland, M.P.B., Cunningham, P., Milne, M.L. and Dewey, D.W. (1979) Copper administration to young calves: oral dosing with copper oxide compared with subcutaneous copper glycinate injection. *Australian Veterinary Journal* 55, 493–494.

Dewey, D.W. (1977) An effective method for the administration of trace amounts of copper to ruminants. *Search, Australia* 8, 326–327.

Dick, A.T. (1956) Molybdenum in animal nutrition. *Soil Science* 81, 229–258.

Dick, A.T. and Bull, L.B. (1945) Some preliminary observations on the effect of molybdenum on copper metabolism in herbivorous animals. *Australian Veterinary Journal* 21, 70–72.

Dick, A.T., Dewey, D.W. and Gawthorne, J.M. (1975) Thiomolybdates and the copper–molybdenum–sulfur interaction in ruminant nutrition. *Journal of Agricultural Science, Cambridge* 85, 567–568.

Dickson, H. (2016) Copper deficiency: a review of the economic cost and current constraints to effective management of copper deficiency in southern Australian sheep flocks. B.AHE.0209 Final Report. Meat and Livestock Australia Limited Locked Bag 1961, Sydney, Australia.

Dorton, K.L., Engle, T.E., Hamar, D.W., Siciliano, P.D. and Yemm, R.S. (2003) Effects of copper source and concentration on copper status and immune function in growing and finishing steers. *Animal Feed Science and Technology* 110, 31–44.

Du, Z., Hemken, R.W. and Harmon, R.J. (1996) Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. *Journal of Dairy Science* 79, 1873–1880.

Engle, T.E. and Spears, J.W. (2000) Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. *Journal of Animal Science* 78, 2446–2451.

Ewing, H.P., Pesti, G.M., Bakalli, R.I. and Menten, J.F.M. (1998) Studies of the feeding of cupric sulfate pentahydrate, cupric citrate and copper oxychloride to broiler chickens. *Poultry Science* 77, 445–448.

Farmer, P.E., Adams, T.E. and Humphries, W.R. (1982) Copper supplementation of drinking water for cattle grazing molybdenum-rich pastures. *Veterinary Record* 111, 193–195.

Fell, B.F., Dinsdale, D. and Mills, C.F. (1975) Changes in enterocyte mitochondria associated with deficiency of copper in cattle. *Research in Veterinary Science* 18, 274–281.

Fell, B.F., Farmer, L.J., Farquharson, C., Bremner, I. and Graca, D.S. (1985) Observations on the pancreas of cattle deficient in copper. *Journal of Comparative Pathology* 95, 573–590.

Felsman, R.J., Wise, M.B., Harvey, R.W. and Barrick, E.R. (1973) Effect of graded levels of copper sulfate and antibiotic on performance and certain blood constituents of calves. *Journal of Animal Science* 36, 157–160.

Ferguson, W.S., Lewis, A.H. and Watson, S.J. (1938) Action of molybdenum in milking cattle. *Nature, UK* 141, 553.

Ferguson, W.S., Lewis, A.H. and Watson, S.J. (1943) The teart pasture of Somerset: the cause and cure of teartness. *Journal of Agricultural Science, Cambridge* 33, 44–51.

Freudenberger, D.O., Familton, A.S. and Sykes, A.R. (1987) Comparative aspects of copper metabolism in silage-fed sheep and deer (*Cervus elaphus*). *Journal of Agricultural Science, Cambridge* 108, 1–7.

Fry, R.S., Spears, J.W., Lloyd, K.E., Nan, A.T. and Ashwell, M.S. (2013) Effect of dietary copper and breed on gene products involved in copper acquisition, distribution, and use in Angus and Simmental cows and fetuses. *Journal of Animal Science* 91, 861–871.

Funk, M.A. and Baker, D.H. (1991) Toxicity and tissue accumulation of copper in chicks fed casein and soy-based diets. *Journal of Animal Science* 69, 4505–4511.

Gee, E.K., Grace, N.D., Firth, E.C. and Fennessy, P.F. (2000) Changes in liver copper concentration of Thoroughbred foals from birth to 160 days of age and the effect of prenatal copper supplementation. *Australian Veterinary Journal* 78, 347–353.

Gengelbach, G.P., Ward, J.D. and Spears, J.W. (1994) Effects of dietary copper, iron and molybdenum on growth and copper status of beef cows and calves. *Journal of Animal Science* 72, 2722–2727.

Gengelbach, G.P., Ward, J.D. and Spears, J.W. (1997) Effect of copper deficiency and copper deficiency coupled with high dietary iron or molybdenum on phagocytic function and response of calves to a respiratory disease challenge. *Journal of Animal Science* 75, 1112–1118.

Givens, D.I., Zervas, G., Simpson, V.R. and Telfer, S.B. (1988) Use of soluble rumen boluses to provide a supplement of copper for suckled calves. *Journal of Agricultural Science, Cambridge* 110, 199–204.

Gooneratne, S.R. and Christensen, D.A. (1989) A survey of maternal copper status and foetal tissue copper concentrations in Saskatchewan bovine. *Canadian Journal of Animal Science* 69, 141–150.

Gooneratne, S.R., Howell, J. and Gawthorne, J.M. (1981) Intravenous administration of thiomolybdate for the prevention of chronic copper poisoning in sheep. *British Journal of Nutrition* 46, 457–467.

Gooneratne, S.R., Symonds, H.W., Bailey, J.V. and Christensen, D.A. (1994) Effects of dietary copper, molybdenum and sulfur on biliary copper and zinc secretion in Simmental and Angus cattle. *Canadian Journal of Animal Science* 74, 315–325.

Govasmark, E., Steen, A., Bakken, A.K., Strom, T., Hansen, S. and Bernhoft, A. (2005) Copper, molybdenum and cobalt in herbage and ruminants from organic farms in Norway. *Acta Agriculturae Scandinavica* 55, 21–30.

Grace, N.D. (1983) Amounts and distribution of mineral elements associated with the fleece-free empty body weight gains of the grazing sheep. *New Zealand Journal of Agricultural Research* 26, 59–70.

Grace, N.D. and Knowles, S. (2010) Copper. In: Grace, N.D. and Knowles, S. and Sykes, A. (eds) *Managing Mineral Deficiencies in Grazing Livestock*. Occasional Publication No. 15, New Zealand Society of Animal Production, Hamilton, New Zealand.

Grace, N.D. and Knowles, S. (2015) Taking action to reduce the risk of copper toxicity in cattle. *Veterinary Record* 177, 490–491.

Grace, N.D. and Wilson, P.R. (2002) Trace element metabolism, dietary requirements, diagnosis and prevention of deficiencies in deer. *New Zealand Veterinary Journal* 50, 252–259.

Grace, N.D., Knowles, S.O., Rounce, J.R., West, D.M. and Lee, J. (1998a) Effect of increasing pasture copper concentrations on the copper status of grazing Romney sheep. *New Zealand Journal of Agricultural Research* 41, 377–386.

Grace, N.D., Rounce, J.R., Knowles, S.O. and Lee, J. (1998b) Changing dietary sulfur intakes and the copper status of grazing lambs. *New Zealand Journal of Agricultural Research* 40, 329–334.

Grace, N.D., Pearce, S.G., Firth, E.C. and Fennessy, P.F. (1999) Content and distribution of macro- and trace-elements in the body of young pasture-fed horses. *Australian Veterinary Journal* 77, 172–176.

Grace, N.D., Gee, E.K., Firth, E.C. and Shaw S.L. (2002) Digestible energy intake, dry matter digestibility and mineral status of grazing New Zealand thoroughbred yearlings. *New Zealand Veterinary Journal* 50, 63–69.

Grace, N.D., Knowles, S.O., West, D.M. and Lee, J. (2004) Copper oxide needles administered during early pregnancy improve the copper status of ewes and their lambs. *New Zealand Veterinary Journal* 52, 189–192.

Grace, N.D., Wilson, P.R. and Quinn, A.K. (2005a) The effects of copper-amended fertiliser and copper oxide wire particles on the copper status of farmed red deer (*Cervus elaphus*) and their progeny. *New Zealand Veterinary Journal* 53, 31–38.

Grace, N.D., Wilson, P.R. and Quinn, A.K. (2005b) Impact of molybdenum on the copper status of red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 53, 31–38.

Grace, N.D., Castillo-Alcala, F. and Wilson, P.R. (2008) Amounts and distribution of mineral elements associated with live-weight gains of grazing red deer (*Cervus elaphus*). *New Zealand Journal of Agricultural Research* 51, 439–449.

Grace, N.D., Knowles, S.O., West, D.M. and Smith, S.L. (2012) The role of liver Cu kinetics in the depletion of

reserves of Cu in dairy cows fed a Cu-deficient diet. *New Zealand Veterinary Journal* 60, 142–145.

Guenthner, E., Carlson, C.W. and Emerick, R.J. (1978) Copper salts for growth stimulation and alleviation of aortic rupture losses in turkeys. *Poultry Science* 57, 1313–1324.

Gummow, B., Botha, C.J., Basson, C.J. and Bastianello, S.S. (1991) Copper toxicity in ruminants: air pollution as a possible cause. *Onderstepoort Journal of Veterinary Research* 58, 33–39.

Hanlon, J., Minks, E., Hughes, C., Weavers, E. and Rogers, M. (2002) Metallothionein in bovine spongiform encephalopathy. *Journal of Comparative Pathology* 127, 280–289.

Hannam, R.J. and Reuter, D.J. (1977) The occurrence of steely wool in South Australia, 1972–75. *Agricultural Record* 4, 26–29.

Hansen, S.L. and Spears, J.W. (2008) Impact of copper deficiency in cattle on proteins involved in iron metabolism. *FASEB Journal* 22, 443–445.

Hansen, S.L., Schlegel, P., Legleiter, L.R., Lloyd, K.E. and Spears, J.W. (2008) Bioavailability of copper from copper glycinate in steers fed high dietary sulfur and molybdenum. *Journal of Animal Science* 86, 173–179.

Hansen, S.L., Ashwell, M.S., Legleiter, L.R., Fry, R.S., Lloyd, K.E. and Spears, J.W. (2009) The addition of high manganese to a copper-deficient diet further depresses copper status and growth of cattle. *British Journal of Nutrition* 101, 1068–1078.

Harris, E.D. (2004) A requirement for copper in angiogenesis. *Nutrition Reviews* 62, 60–64.

Hart, E.B., Steenbock, H., Waddell, J. and Elvehjem, C.A. (1928) Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. *Journal of Biological Chemistry* 77. Accessible as a 'Nutrition Classic' in *Nutrition Reviews* (1987) 45,181–183.

Hartman, F. and van Ryssen, J.B.J. (1997) Metabolism of selenium and copper in sheep with and without sodium bicarbonate supplementation. *Journal of Agricultural Science, Cambridge* 128, 357–364.

Hartmans, J. (1975) The incidence of copper poisoning and the role of concentrates asa causative factor: report of an inquiry. *Tijdschrift voor Diergeneeskunde* 100, 379–382.

Hawkins, D. (2014) Effects of subcutaneously injected Ca Cu EDTA on concentrations of Cu in liver, milk production and reproductive performance in New Zealand dairy cows. *New Zealand Veterinary Journal* 62, 244–249.

Haynes, R.J. (1997) Micronutrient status of a group of soils in Canterbury, New Zealand, as measured by extraction with EDTA, DTPA and HCl, and its relationship with plant response to applied Cu and Zn. *Journal of Agricultural Science, Cambridge* 129, 325–333.

Haywood, S, Simpson, D.M., Ross, G. and Beynon. R.J. (2005) The greater susceptibility of North Ronaldsay sheep compared with Cambridge sheep to copper-induced oxidative stress, mitochondrial damage and hepatic stellate cell activation. *Journal of Comparative Pathology* 133, 114–127.

Hedger, R.S., Howard, D.A. and Burdin, M.L. (1964) The occurrence in goats and sheep in Kenya of a disease closely similar to swayback. *Veterinary Record* 76, 493–497.

Herbert, E., Small, J.N.W., Jones, D.G. and Suttle, N.F. (1991) Evaluation of superoxide dismutase assays for the routine diagnostic assessment of copper status in blood samples. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals, Dubrovnik*. IMI, Zagreb, pp. 5–16.

Hogan, K.G., Money, D.F.L., White, D.A. and Walker, R. (1971) Weight responses of young lambs to copper and connective tissue lesions associated with grazing pasture of high molybdenum content. *New Zealand Journal of Agricultural Research* 14, 687–701.

Holstein, T.J., Fung, R.Q., Quevedo, W.C. and Bienieki, T.C. (1979) Effect of altered copper metabolism induced by mottled alleles and diet on mouse tyrosinase. *Proceedings of the Society for Experimental Biology and Medicine* 162, 264–268.

Howell, J.M. and Gooneratne, S.R. (1987) The pathology of copper toxicity. In: Howell, J.M. and Gawthorne, J.M. (eds) *Copper in Animals and Man*. Volume II. CRC Press Ltd, Boca Raton, Florida, pp. 53–78.

Howell, J.M. and Hall, G.A. (1970) Infertility associated with experimental copper deficiency in sheep, guinea-pigs and rats. In: Mills, C.F. (ed.) *Trace Element Metabolism in Animals*. E. & S. Livingstone, Edinburgh, pp. 106–109.

Howell, J.M., Deol, H.S., Dorling, P.R. and Thomas, J.B (1991) Experimental heliotrope and copper intoxication in sheep: morphological changes. *Journal of Comparative Pathology* 105, 49–74.

Humphries, W.R., Phillippo, M., Young, B.W. and Bremner, I. (1983) The influence of dietary iron and molybdenum on copper metabolism in calves. *British Journal of Nutrition* 49, 77–86.

Humphries, W.R., Morrice, P.C. and Bremner, I. (1988) A convenient method for the treatment of chronic copper poisoning in sheep using subcutaneous ammonium tetrathiomolybdate. *Veterinary Record* 123, 51–53.

Hunter, A.G., Suttle, N., Martineau, H.M., Spence, M.A., Thomson, J.R., Macrae, A.I. and Brown, S. (2013) Mortality, hepatopathy and liver copper concentrations in artificially reared Jersey calves before and after reductions in copper supplementation. *Veterinary Record* 172, 46.

Ivan, M.M. (1988) The effect of faunation on rumen solubility and liver content of copper in sheep fed low or high copper diets. *Journal of Animal Science* 66, 1498–1501.

Jarvis, S.C. and Austin, A.R. (1983) Soil and plant factors limiting the availability of copper to a beef suckler herd. *Journal of Agricultural Science, Cambridge* 101, 39–46.

Jenkins, K.J. and Hidiriglou, M. (1989) Tolerance of the calf for excess copper in milk replacer. *Journal of Dairy Science* 72, 150–156.

Jenkins, N.K., Morris, T.R. and Valamotis, D. (1970) The effect of diet and copper supplementation on chick growth. *British Poultry Science* 11, 241–248.

Jensen, L.S. (1975) Precipitation of a selenium deficiency by high dietary levels of copper and zinc (in fowls). *Proceedings of the Society for Experimental Biology and Medicine* 149, 113–116.

Jensen, L.S. and Maurice, V. (1979) Influence of sulfur amino acids on copper toxicity in chicks. *Journal of Nutrition* 109, 91–97.

Jiang, D., Muschhammer, J., Qi, Y., Kügler, A., de Vries, J.C., Saffarzadeh, M. *et al.* (2016) Suppression of neutrophil-mediated tissue damage: a novel skill of mesenchymal stem cells. *Stem Cells* 34, 2393–2406.

Johnston, H., Beasley, L. and MacPherson, N. (2014) Copper toxicity in a New Zealand dairy herd. *Irish Veterinary Journal* 67, 20. DOI: 10.1186/2046-0481-67-20

Jones, D.G. and Suttle, N.F. (1981) Some effects of copper deficiency on leukocyte function in sheep and cattle. *Research in Veterinary Science* 31, 151–156.

Jones, D.G. and Suttle, N.F. (1987) Copper and disease resistance. In: *Trace Substances in Environmental Health – XXI*. University of Missouri, Columbia, Missouri, pp. 514–525.

Judson, J.G., Walkley, J.R., James, P.J., Kleeman, D.O. and Ponzoni, R.W. (1994) Genetic variation in trace element status of Merino sheep. *Proceedings of the Australian Society of Animal Production* 20, 438.

Jumba, I.O., Suttle, N.F., Hunter, E.A. and Wandiga, S.O. (1995) Effects of soil origin and mineral composition and herbage species on the mineral composition of forages in the Mount Elgon region of Kenya. 2. Trace elements. *Tropical Grasslands* 29, 47–52.

Kashani, A.B., Samie, H., Emerick, R.J. and Carlson, C.W. (1986) Effect of copper with three levels of sulfur-containing amino acids in diets for turkeys. *Poultry Science* 65, 1754–1759.

Kendall, N.R., Jackson, D.W., Mackenzie, A.M., Illingworth, D.V., Gill, I.M. and Telfer, S.B. (2001) The effect of a zinc, cobalt and selenium soluble glass bolus on the trace element status of extensively grazed sheep over winter. *Animal Science* 73, 163–169.

Kendall, N.R., Holmes-Pavord, H.R., Bone, P.A., Ander, E.L. and Young, S.D. (2015) Liver copper concentrations in cull cattle in the UK: are cattle being copper loaded? *Veterinary Record* 177, 493.

Kerr, L.A. and McGavin, H.D. (1991) Chronic copper poisoning in sheep grazing pastures fertilized with swine manure. *Journal of the American Veterinary Medical Association* 198, 99–101.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S.L., Bearson, B.L *et al*. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Kincaid, R.L., Gay, C.C. and Krieger, R.I. (1986) Relationship of serum and plasma copper and caeruloplasmin concentrations of cattle and the effects of whole sample storage. *American Journal of Veterinary Research* 47, 1157–1159.

King, T.P. and Bremner, I. (1979) Autophagy and apoptosis in liver during the pre-haemolytic phase of chronic copper poisoning in sheep. *Journal of Comparative Pathology* 89, 515–530.

Knowles, S.O., Grace, N.D., Rounce, J.R., Litherland, D.M. and Lee, J. (2000) Dietary Mo as an antagonist to Cu absorption: stable isotope (^{65}Cu) measurements in grazing sheep. In: Roussel, A.M., Anderson, R.A. and Favrier, A.E. (eds) *Proceedings of the 10th International Symposium on Trace Elements in Man and Animals, Etian, France*. Springer, pp. 717–722.

Koh, T.-S. and Judson, G.J. (1987) Copper and selenium deficiency in cattle: an evaluation of methods of oral therapy and an observation of a copper–selenium interaction. *Veterinary Record Communications* 11, 133–148.

Koh, T.-S., Peng, R.K. and Klasing, K.C. (1996) Dietary copper level affects copper metabolism during lipopolysaccharide-induced immunological stress in chicks. *Poultry Science* 75, 867–872.

Lai, C.-C., Huang, W.-H., Askari, A., Wang, Y., Sarvazyan, N., Klevay, L.M. and Chin, T.H. (1994) Differential regulation of superoxide dismutase in copper deficient rat organs. *Free Radical Biology and Medicine* 16, 613–620.

Larsen, T., Skoglund, E., Sandberg, A.S. and Engberg, R.M. (1999) Soaking and pelleting of pig diets alters the apparent absorption and retention of minerals. *Canadian Journal of Animal Science* 79.

Laven, R.A. and Lawrence, K.E. (2010) Analysis of the value of measurement of the activity of caeruloplasmin as an alternative to measurement of the concentration of elemental copper in plasma and serum of farmed red deer, *New Zealand Veterinary Journal* 58, 207–212.

Laven, R.A. and Lawrence, K.E. (2012) An evaluation of the effect of clotting on the recovery of copper from caprine blood. *Veterinary Journal* 192, 232–235.

Laven, R.A. and Smith, S.L. (2008) Copper deficiency in sheep: an assessment of relationship between concentration of copper in serum and plasma. *New Zealand Veterinary Journal* 56, 334–338.

Laven, R.A., Lawrence, K.E. and Livesey, C.T. (2007) The assessment of blood copper status in cattle: a

comparison of measurements of caeruloplasmin and elemental copper in serum and plasma. *New Zealand Veterinary Journal* 55, 171–176.

Leach, R.M. Jr, Rosenblum, C.I., Amman, M.J. and Burdette, J. (1990) Broiler chicks fed low-calcium diets. 2. Increased sensitivity to copper toxicity. *Poultry Science* 69, 1905–1910.

Ledoux, D.R., Pott, E.B., Henry, P.R., Ammerman, C.B., Merritt, A.M. and Madison, J.B. (1995) Estimation of the relative bioavailability of inorganic copper sources for sheep. *Animal Feed Science and Technology* 15, 1803–1813.

Lee, H.J. (1956) The influence of copper deficiency on the fleece of British breeds of sheep. *Journal of Agricultural Science, Cambridge* 47, 218–244.

Lee, J., Treloar, B.P. and Harris, P.M. (1994) Metallothionein and trace element metabolism in sheep tissues in response to high and sustained zinc dosages. 1. Characterisation and turnover of metallothionein isoforms. *Australian Journal of Agricultural Research* 45, 303–320.

Legleiter, L.R. and Spears, J.W. (2007) Plasma diamine oxidase: a biomarker of copper deficiency in the bovine. *Journal of Animal Science* 85, 2198–2204.

Legleiter, L.R., Spears, J.W. and Liu, H.C. (2008) Copper deficiency in the young bovine results in dramatic decreases in brain copper concentration but does not alter brain prion protein biology. *Journal of Animal Science* 86, 3069–3078.

Leigh, L.C. (1975) Changes in the ultrastructure of cardiac muscle in steers deprived of copper. *Research in Veterinary Science* 18, 282–287.

Livesey, C.T., Bidewell, C.A., Crawshaw, T.R. and David, G.P. (2002) Investigation of copper poisoning in adult cows by the Veterinary Laboratories Agency. *Cattle Practice* 10, 289–294.

Lockhart, P.J. and Mercer, J.F.B. (1999) Cloning and expression analysis of the sheep ceruloplasmin gene. *Gene* 236, 251–257.

Lominadze, D., Saari, J.T., Miller, F.N., Catalfamo, J.L. and Schuschke, D.A. (1997) Von Willebrand factor restores platelet thrombogenesis in copper-deficient rats. *Journal of Nutrition* 127, 1320–1327.

Luo, X.G., Henry, P.R., Ammerman, C.B. and Madison, J.B. (1996) Relative bioavailability of copper in a copper–lysine complex or copper-sulfate for ruminants as affected by feeding regimen. *Animal Feed Science and Technology* 57, 281–289.

MAFF (1990) UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Marston, H.R. (1946) Nutrition and wool production. *Proceedings of a Symposium on Fibrous Proteins*. Society of Dyers and Colourists, Leeds, UK.

Mason, J. (1986) Thiomolybdates: mediators of molybdenum toxicity and enzyme inhibitors. *Toxicology* 42, 99–109.

Mason, J., Williams, S., Harrington, R. and Sheahan, B. (1984) Some preliminary studies on the metabolism of ^{99}Mo-labelled compounds in deer. *Irish Veterinary Journal* 38, 171–175.

McAloon, C.G., Carty, C. and Mulligan, F. (2017) Molybdemosis in calves. *Veterinary Ireland Journal* 7, 678–680.

McCaughern, J.H., Mackenzie, A.M. and Sinclair, L.A. (2020) Dietary starch concentration alters reticular pH, hepatic copper concentration, and performance in lactating Holstein-Friesian dairy cows receiving added dietary sulfur and molybdenum. *Journal of Dairy Science* 103, 9024–9036.

McClure, S.J., McClure, T.J. and Emery, D.L. (1999) Effects of molybdenum intake on primary infection and subsequent challenge by the nematode parasite *Trichostrongylus colubriformis* in weaned Merino lambs. *Research in Veterinary Science* 67, 17–22.

McFarlane, J.D. (1989) The effect of copper supply on vegetative and seed yield of pasture legumes and the field calibration of a test for detecting copper deficiency I. *Subterranean clover. Australian Journal of Agricultural Research* 40, 817–132.

McFarlane, J.D., Judson, J.D. and Gouzos, J. (1990) Copper deficiency in ruminants in the south east of Australia. *Australian Journal of Experimental Agriculture* 30, 187–193.

McFarlane, J.D., Judson, J.D., Turnbull, R.K. and Kempe, B.R. (1991) An evaluation of copper-containing soluble glass pellets, copper oxide particles and injectable copper as supplements for cattle and sheep. *Australian Journal of Experimental Agriculture* 31, 165–174.

McMurray, C.H. (1980) Copper deficiency in ruminants. In: *Biological Roles of Copper*. Ciba Foundation 79. Elsevier, New York, pp. 183–207.

Mee, J.F. (1991) Coat colour and copper deficiency in cattle. *Veterinary Record* 129, 536.

Mills, C.F. and Williams, R.B. (1962) Copper concentration and cytochrome-oxidase and ribonuclease activities in the brains of copper-deficient lambs. *Biochemical Journal* 85, 629–632.

Mills, C.F., Dalgarno, A.C. and Wenham, G. (1976) Biochemical and pathological changes in tissues of Friesian cattle during the experimental induction of copper deficiency. *British Journal of Nutrition* 35, 309–311.

Miltimore, J.E. and Mason, J.L. (1971) Copper to molybdenum ratio and molybdenum and copper concentrations in ruminant feeds. *Canadian Journal of Animal Science* 51, 193–200.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Miranda, M., Cruz, J.M., Lopez-Alonso, M. and Benedito, J.L. (2006) Variations in liver and blood copper concentrations in young beef cattle raised in north-west Spain: associations with breed, sex, age and season. *Animal Science* 82, 253–258.

Mondal, M.K., Biswas, P., Roy, B. and Mazumdar, D. (2007) Effect of copper sources and levels on serum lipid profiles in Black Bengal (*Capra hircus*) kids. *Small Ruminant Research* 67, 28–35.

Morine, S.J., Drewnoski, M.E. and Hansen, S.L. (2014) Increasing dietary neutral detergent fiber concentration decreases ruminal hydrogen sulfide concentrations in steers fed high-sulfur diets based on ethanol co-products. *Journal of Animal Science* 92, 3035–3041.

Morris, C.A., Amyes, N.C. and Hickey, S.M. (2006) Genetic variation in serum copper concentration in Angus cattle. *Animal Science* 82, 799–803.

Mullis, L.A., Spears, J.W. and McGraw, R.L. (2003) Effects of breed (Angus v Simmental) and copper and zinc source on mineral status of steers fed high dietary iron. *Journal of Animal Science* 81, 318–322.

Nicol, A.M., Keeley, M.J., Giuld, C.D.H., Isherwood, P. and Sykes, A.R. (2003) Liveweight gain and copper status of young deer treated or untreated with copper oxide wire particles on ten deer farms in Canterbury. *New Zealand Veterinary Journal* 51, 14–20.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2005) *Mineral Tolerances of Animals*, 2nd edn. National Academy of Sciences, Washington, DC.

O'Dell, B.L. (1976) Biochemistry and physiology of copper in vertebrates. In: Prasad, A.S. (ed.) *Trace Elements in Human Health and Disease*. Academic Press, New York, pp. 391–413.

O'Dell, B.L., Hardwick, B.C., Reynolds, G. and Savage, J.E. (1961) Connective tissue defect in the chick resulting from copper deficiency. *Proceedings of the Society for Experimental Biology and Medicine* 108, 402–405.

O'Dell, B.L., Smith, R.M. and King, R.A. (1976) Effect of copper status on brain neurotransmitter metabolism in the lamb. *Journal of Neurochemistry* 26, 451–455.

Okonkwo, A.C., Ku, P.K., Miller, E.R., Keahey, K.K. and Ullrey, D.E. (1979) Copper requirement of baby pigs fed purified diets. *Journal of Nutrition* 109, 939–948.

Opsahl, W., Zeronian, H., Ellison, M., Lewis, D., Rucker, R.B. and Riggins, R.S. (1982) Role of copper in collagen cross-linking and its influence on selected mechanical properties of chick bone and tendon. *Journal of Nutrition* 112, 708–716.

Ortolani, E., Knox, D.P., Jackson, F., Coop, R.L. and Suttle, N.F. (1993) Abomasal parasitism lowers liver copper status and influences the Cu × Mo × S antagonism in lambs. In: Anke, M., Meissner, D. and Mills, C.F. (eds) *Proceedings of the Eighth International Symposium on Trace Elements in Man and Animals*. Verlag Media Touristik, Gersdorf, Germany, pp. 331–332.

Osman, N.I.E.D., Johnson, E.H., Al-Busaidi, R.M. and Suttle, N.F. (2003) The effects of breed, neonatal age and pregnancy on the plasma copper status of goats in Oman. *Veterinary Research Communications* 27, 219–229.

Palsson, P.A. and Grimsson, H. (1953) Demyelination in lambs from ewes which feed on seaweeds. *Proceedings of the Society for Biology in Medicine* 83, 518–522.

Parkins, J.J., Hemingway, R.G., Lawson, D.C. and Ritchie, N.S. (1994) The effectiveness of copper oxide powder as a component of a sustained-release multitrace element and vitamin rumen bolus system for cattle. *British Veterinary Journal* 150, 547–553.

Paynter, D.I. (1987) The diagnosis of copper insufficiency. In: Howell, J.M. and Gawthorne, J.M. (eds) *Copper in Animals and Man. Volume* II. CRC Press Ltd, Boca Raton, Florida, pp. 101–119.

Pearce, S.G., Firth, E.C., Grace, N.D. and Fennessy, P.F. (1999a) The effect of copper supplementation on the copper status of pasture-fed young thoroughbreds. *Equine Veterinary Journal* 30, 204–210.

Pearce, S.G., Firth, E.C., Grace, N.D. and Fennessy, P.F. (1999b) The effect of high pasture molybdenum concentrations on the copper status of grazing horses in New Zealand. *New Zealand Journal of Agricultural Research* 42, 93–99.

Pekel, M. and Alp, A.Y. (2011) Effects of different dietary copper sources on laying hen performance and egg yolk cholesterol. *Journal of Applied Poultry Research* 20, 506–513.

Phillippo, M. (1983) The role of dose response trials in predicting trace element deficiency disorders. *Occasional Publication of the British Society of Animal Production* 7, pp. 51–59.

Phillippo, M., Humphries, W.R., Lawrence, C.B. and Price, J. (1982) Investigation of the effect of copper therapy on fertility in beef suckler herds. *Journal of Agricultural Science, Cambridge* 99, 359–364.

Phillippo, M., Humphries, W.R. and Garthwaite, P.H. (1987a) The effect of dietary molybdenum and iron on copper status and growth in cattle. *Journal of Agricultural Science, Cambridge* 109, 315–320.

Phillippo, M., Humphries, W.R., Atkinson, T., Henderson, G.D. and Garthwaite, P.H. (1987b) The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrus cycles in cattle. *Journal of Agricultural Science, Cambridge* 109, 321–336.

Pitt, M., Fraser, J. and Thurley, D.C. (1980) Molybdenum toxicity in the sheep: epiphysiolysis, exostoses and biochemical changes. *Journal of Comparative Pathology* 90, 567–576.

Price, J. and Chesters, J.K. (1985) A new bioassay for assessment of copper bioavailability and its application in a study of the effect of molybdenum on the distribution of available copper in ruminant digesta. *British Journal of Nutrition* 53, 323–336.

Price, J., Will, M.A., Paschaleris, G. and Chesters, J.K. (1987) Identification of thiomolybdates in digesta and plasma from sheep after administration of

^{99}Mo-labelled compounds into the rumen. *British Journal of Nutrition* 58, 127–138.

Pritchard, G.C., Lewis, G., Wells, G.A.H. and Stopforth, A. (1985) Zinc toxicity, copper deficiency and anaemia in swill-fed pigs. *Veterinary Record* 117, 545–548.

Prohaska, J.R. (2006) Copper. In: Filer, L.J. and Ziegler, E.E. (eds) *Present Knowledge in Nutrition*, 7th edn. International Life Science Institute–Nutrition Foundation, Washington, DC.

Rabiansky, P.A., McDowell, L.R., Velasquez-Pereira, J., Wilkinson, N.S., Percival, S.S. *et al.* (1999) Evaluating copper lysine and copper sulfate sources for heifers. *Journal of Dairy Science* 82, 2642–2650.

Rosa, I.V., Ammerman, C.B. and Henry, P.R. (1986) Interrelationships of dietary copper, zinc and iron on performance and tissue mineral concentration in sheep. *Nutrition Reports International* 34, 893–902.

Rucker, R.B., Parker, H.E. and Rogler, J.C. (1969) Effect of copper deficiency on chick bone collagen and selected bone enzymes. *Journal of Nutrition* 98, 57–63.

Rucker, R.B., Riggins, R.S., Laughlin, R., Chan, M.M., Chen, M. and Tom, K. (1975) Effect of nutritional copper deficiency on the biomechanical properties of bone and arterial elastin metabolism in the chick. *Journal of Nutrition* 105, 1062–1070.

Saenko, E.L., Yaroplov, A.I. and Harris, E.D. (1994) Biological functions of caeruloplasmin expressed through copper-binding sites. *Journal of Trace Elements in Experimental Medicine* 7, 69–88.

Saleh, M.A., Al-Salahy, B. and Sanousi, S.A. (2008) Corpuscular oxidative stress in desert sheep naturally deficient in copper. *Small Ruminant Research* 80, 33–38.

Savage, J.E. (1968) Trace minerals and avian reproduction. *Federation Proceedings* 27, 927–931.

Shand, A. and Lewis, G. (1957) Chronic copper poisoning in young calves. *Veterinary Record* 69, 618–620.

Sharma, M.C., Joshi, C. and Das, G. (2008) Therapeutic management of copper deficiency in buffalo heifers: impact on immune function. *Veterinary Research Commuications* 32, 49–63.

Simpson, A.M., Mills, C.F. and McDonald, I. (1981) Tissue copper retention or loss in young growing cattle. In: Howell, J.McC., Gawthorne, J.M. and White, C.L. (eds) *Proceedings of the Fourth International Symposium on Trace Element Metabolism in Man and Animals*. Australian Academy of Sciences, Canberra, pp. 133–136.

Simpson, D.M., Mobasheri, A., Haywood, S. and Beynon, R.J. (2006) A proteomics study of the response of North Ronaldsay sheep to copper challenge. *BMC Veterinary Research* 2, 36. DOI: 10.1186/1746-6148-2-36

Sinclair, L.A., Hart, K.J., Johnson, D. and Mackenzie, A.M. (2013) Effect of inorganic or organic copper fed without or with added sulfur and molybdenum on the performance, indicators of copper status, and hepatic mRNA in dairy cows. *Journal of Dairy Science* 96, 4355–4367.

Sinclair, L.A., Johnson, D., Wilson, S. and Mackenzie, A.M. (2017) Added dietary sulfur and molybdenum has a greater influence on hepatic copper concentration, intake, and performance in Holstein-Friesian dairy cows offered a grass silage- rather than corn silage-based diet. *Journal of Dairy Science* 100, 4365–4376.

Smart, M.E., Cohen, R., Christensen, D.A. and Williams, C.M. (1986) The effects of sulfate removal from the drinking water on the plasma and liver copper and zinc concentrations of beef cows and their calves. *Canadian Journal of Animal Science* 66, 669–680.

Smith, B.S.W., Field, A.C. and Suttle, N.F. (1968) Effect of intake of copper, molybdenum and sulfate on copper metabolism in the sheep. III. Studies with radioactive copper in male castrated sheep. *Journal of Comparative Pathology* 78, 449–461.

Smith, B.S.W. and Wright, H. (1975a) Effect of dietary molybdenum on copper metabolism: evidence for the involvement of molybdenum in abnormal binding of copper to plasma proteins. *Clinica Chimica Acta* 62, 55–62.

Smith, B.S.W. and Wright, H. (1975b) Copper:molybdenum interactions: effects of dietary molybdenum on the binding of copper to plasma proteins in sheep. *Journal of Comparative Pathology* 85, 299–305.

Smith, R.M., Osborne-White, W.S. and O'Dell, B.L. (1976) Cytochromes in brain mitochondria from lambs with enzootic ataxia. *Journal of Neurochemistry* 26, 1145–1148.

Spais, A.G. (1959) *Askeri veteriner dergisi* 135, 161.

Spears, J.W., Kegley, E.B. and Mullis, J.A. (2004) Bioavailability of copper from tribasic copper chloride and copper sulfate in growing cattle. *Animal Feed Science and Technology* 116, 1–13.

Stabel, J.R., Spears, J.W. and Brown, T.T. (1993) Effect of copper deficiency on tissue, blood characteristics and immune function of calves challenged with infectious bovine rhinotracheitis virus and pasteurella haemolytica. *Journal of Animal Science* 71, 1247–1255.

Stahlhut, H.S., Whishant, C.S. and Spears, J.W. (2005) Effect of chromium supplementation and copper status on performance and reproduction of beef cows. *Animal Feed Science and Technology* 128, 266–275.

Steffen, D.J., Carlson, M.P. and Casper, H.H. (1997) Copper toxicosis in suckling beef cattle with improper administration of copper oxide boluses. *Journal of Veterinary Diagnostic Investigation* 9, 443–446.

Strickland, K., Smith, F., Woods, M. and Mason, J. (1987) Dietary molybdenum as a putative copper antagonist in the horse. *Equine Veterinary Journal* 19, 50–54.

Suleiman, A., Okine, E. and Goonewardne, L.A. (1997) Relevance of National Research Council feed

composition tables in Alberta. *Canadian Journal of Animal Science* 77, 197–203.
Suttle, N.F. (1974) Effects of age and weaning on the apparent availability of dietary copper to young lambs. *Journal of Agricultural Science, Cambridge* 84, 255–261.
Suttle, N.F. (1977) Reducing the potential toxicity of concentrates to sheep by the use of molybdenum and sulfur supplements. *Animal Feed Science and Technology* 2, 235–246.
Suttle, N.F. (1981) Effectiveness of orally administered cupric oxide needles in alleviating hypocupraemia in sheep and cattle. *Veterinary Record* 108, 417–420.
Suttle, N.F. (1983a) Assessing the mineral and trace element status of feeds. In: Robards, G.E. and Packham, R.G. (eds) *Proceedings of the Second Symposium of the International Network of Feed Information Centres*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 211–237.
Suttle, N.F. (1983b) Effects of molybdenum concentration in fresh herbage, hay and semi-purified diets on the copper metabolism of sheep. *Journal of Agricultural Science, Cambridge* 100, 651–656.
Suttle, N.F. (1983c) The nutritional basis for trace element deficiencies in ruminant livestock. In: Suttle, N.F., Gunn, R.G., Allen, W.M., Linklater, K.A. and Wiener, G. (eds) *Trace Elements in Animal Production and Veterinary Practice*. British Society of Animal Production Special Publication No. 7, Edinburgh, pp. 51–60.
Suttle, N. (1986) Copper deficiency in ruminants; recent developments. *Veterinary Record* 119, 519–522.
Suttle, N.F. (1987) The nutritional requirement for copper in animals and man. In: Howell, J. M. and Gawthorne, J.M. (eds) *Copper in Animals and Man. Volume* I. CRC Press, Boca Raton, Florida, pp. 21–44.
Suttle, N.F. (1988a) The role of comparative pathology in the study of copper and cobalt deficiencies in ruminants. *Journal of Comparative Pathology* 99, 241–258.
Suttle, N.F. (1988b) Relationships between the trace element status of soils, pasture and animals in relation to the growth rate of lambs. In: Thornton, I. (ed.) *Geochemistry and Health*. Science Reviews Limited, Northwood, UK, pp. 69–79.
Suttle, N.F. (1991) The interactions between copper, molybdenum and sulfur in ruminant nutrition. *Annual Review of Nutrition* 11, 121–140.
Suttle, N.F. (1994) Meeting the copper requirements of ruminants. In: Garnsworthy, P.C. and Cole, D.J.A. (eds) *Recent Advances in Animal Nutrition*. Nottingham University Press, Nottingham, UK, pp. 173–188.
Suttle, N.F. (1996) Non-dietary influences on mineral requirements of sheep. In: Masters, D.G. and White, C.L. (eds) *Detection and Treatment of Mineral Nutrition Problems in Sheep*. ACIAR Monograph, Canberra, pp. 31–44.
Suttle, N.F. (1998) Relationships between the trace element status of soils, pasture and animals in relation to growth rate in lambs. In: Thornton, I. (ed.) *Proceedings of the Second International Symposium on Geochemistry and Health*. Science Reviews Ltd, Chippenham, UK.
Suttle, N.F. (2008a) Relationships between the concentrations of tri-chloroactic acid-soluble copper and caeruloplasmin in the serum of cattle from areas with different soil concentrations of molybdenum. *Veterinary Record* 162, 237–240.
Suttle, N.F. (2008b) Lack of effect of parenteral thiomolybdate on ovine caeruloplasmin activity: diagnostic implications. *Veterinary Record* 162, 593–594.
Suttle, N.F. (2012a) Copper imbalances in ruminants and humans: unexpected common ground. *Advances in Nutrition* 3, 666–674.
Suttle, N.F. (2012b) Residual effects of *Mycobacterium avium* infection on susceptibility of sheep to copper toxicity and efficacy of treatment with tetrathiomolybdate. *Veterinary Record* 17, 18. doi:10.1136/vr.100716
Suttle, N.F. (2012c) Control of hepatic copper retention in Texel ram lambs by dietary supplementation with copper antagonists followed by a copper depletion regimen. *Animal Feed Science and Technology* 173, 194–200.
Suttle, N.F. (2012d) Responsiveness of prehaemolytic copper poisoning in sheep from a specific pathogen-free environment to a relatively high dose of tetrathiomolybdate. *Veterinary Record* 171, 246-246. DOI: 10.1136/vr.100722
Suttle, N.F. (2013) Rates of change in liver copper concentration in cattle given a copper-deficient diet, with or without pre-treatment with tetrathiomolybdate, for evaluation of two parenteral copper supplements. *New Zealand Veterinary Journal* 61, 154–158.
Suttle, N.F. (2016) Reducing risk of copper toxicity in dairy cattle. *Veterinary Record.* doi:10.1136/vr.i793
Suttle, N.F. (2017) Challenges in predicting needs for mineral supplementation in grazing animals. In: Singh, B.R., McLoughlin, M.J. and Brevik, E.C. (eds) *The Nexus of Soils, Plants and Animals and Human Health*. Catena Schwezerbart, Stuttgart, pp. 105–110.
Suttle, N.F. and Angus, K.W. (1976) Experimental copper deficiency in the calf. *Journal of Comparative Pathology* 86, 595–608.
Suttle, N.F. and Angus, K.W. (1978) Effects of experimental copper deficiency on the skeleton of the calf. *Journal of Comparative Pathology* 82, 137–145.
Suttle, N.F. and Brebner, J. (1995) A putative role for larval nematode infection in diarrhoeas which did not respond to anthelmintic drenches. *Veterinary Record* 137, 311–316.
Suttle, N.F. and Field, A.C. (1968) The effect of intake of copper, molybdenum and sulfate on copper metabolism in sheep 1. Clinical condition and blood copper

distribution in the pregnant ewe. *Journal of Comparative Pathology* 78, 351–363.

Suttle, N.F. and Field, A.C. (1983) Effects of dietary supplements of thiomolybdates on copper and molybdenum metabolism in sheep. *Journal of Comparative Pathology* 93, 379–389.

Suttle, N.F. and McMurray, C.H. (1983) Use of erythrocyte copper:zinc superoxide dismutase activity and hair or fleece copper concentrations in the diagnosis of hypocuprosis in ruminants. *Research in Veterinary Science* 35, 47–52.

Suttle, N.F. and Mills, C.F. (1966a) Studies of the toxicity of copper to pigs. 1. Effects of oral supplements of zinc and iron salts on the development of copper toxicosis. *British Journal of Nutrition* 20, 135–148.

Suttle, N.F. and Mills, C.F. (1966b) Studies of the toxicity of copper to pigs. 2. Effect of protein source and other dietary components on the response to high and moderate intakes of copper. *British Journal of Nutrition* 20, 149–161.

Suttle, N.F. and Peter, D.W. (1985) Rumen sulfide metabolism as a major determinant of the availability of copper to ruminants. In: Mills, C.F., Bremner, I. and Chesters, J.K. (eds) *Proceedings of the Fifth International Symposium on Trace Elements in Man and Animals, Aberdeen*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 367–370.

Suttle, N.F. and Phillippo, M. (2005) Effect of trace element supplementation on the fertility of dairy herds. *Veterinary Record* 156, 155–156.

Suttle, N.F. and Price, J. (1976) The potential toxicity of copper-rich animal excreta to sheep. *Animal Production* 23, 233–241.

Suttle, N.F. and Small, J.N.W. (1993) Evidence of delayed availability of copper in supplementation trials with lambs on molybdenum-rich pasture. In: Anke, M., Meissner, D. and Mills, C.F. (eds) *Proceedings of the Eighth International Symposium on Trace Elements in Man and Animals*. Verlag Media Touristik, Gersdorf, Germany, pp. 651–655.

Suttle, N.F., Field, A.C. and Barlow, R.M. (1970) Experimental copper deficiency in sheep. *Journal of Comparative Pathology* 80, 151–162.

Suttle, N.F., Angus, K.W., Nisbet, D.I. and Field, A.C. (1972) Osteoporosis in copper-depleted lambs. *Journal of Comparative Pathology* 82, 93–97.

Suttle, N.F., Abrahams, P. and Thornton, I. (1984) The role of a soil × dietary sulfur interaction in the impairment of copper absorption by soil ingestion in sheep. *Journal of Agricultural Science, Cambridge* 103, 81–86.

Suttle, N.F., Jones, D.G., Woolliams, C. and Woolliams, J.A. (1987a) Heinz body anaemia in lambs with deficiencies of copper or selenium. *British Journal of Nutrition* 58, 539–548.

Suttle, N.F., Jones, D.G., Woolliams, J.A. and Woolliams, C. (1987b) Copper supplementation during pregnancy can reduce perinatal mortality and improve early growth in lambs. *Proceedings of the Nutrition Society* 46, 68A.

Suttle, N.F., Brebner, J., Small, J.N. and McLean, K. (1992a) Inhibition of ovine erythrocyte superoxide dismutase activity (ESOD; EC1.14.1.1) *in vivo* by parenteral ammonium tetrathiomolybdate. *Proceedings of the Nutrition Society* 51, 145A.

Suttle, N.F., Knox, D.P., Angus, K.W., Jackson, F. and Coop, R.L. (1992b) Effects of dietary molybdenum on nematode and host during *Haemonchus contortus* infection in lambs. *Research in Veterinary Science* 52, 230–235.

Suttle, N.F., Brebner, J. and Pass, R. (1996a) A comparison of the availability of copper in four whisky distillery by-products with that in copper sulfate for lambs. *Animal Science* 62, 689–690.

Suttle, N.F., Small, J.N.W., Collins, E.A., Mason, D.K. and Watkins, K.L. (1996b) Serum and hepatic copper concentrations used to define normal, marginal and deficient copper status in horses. *Equine Veterinary Journal* 28, 497–499.

Suttle, N.F., Lewis, R.M. and Small, J.N. (2002) Effects of breed and family on rate of copper accretion in the liver of purebred Charollais, Suffolk and Texel lambs. *Animal Science* 75, 295–302.

Taylor, C.G., Bettger, W.J. and Bray, T.M. (1988) Effect of dietary zinc or copper deficiency on the primary free radical defence system in rats. *Journal of Nutrition* 118, 613–621.

Thompson, K.G., Audige, L., Arthur, D.G., Juhan, A.F., Orr, M.B., McSporran, K.D. and Wilson, P.R. (1994) Osteochondrosis associated with copper deficiency in young farmed red deer and wapiti × red deer hybrids. *New Zealand Veterinary Journal* 42, 137–143.

Thornton, I. and Alloway, B.J. (1974) Geochemical aspects of the soil–plant–animal relationship in the development of trace element deficiency and excess. *Proceedings of the Nutrition Society* 33, 257–266.

Thornton, I., Kershaw, G.F. and Davies. M.K. (1972a) An investigation into copper deficiency in cattle in the southern Pennines. 1. Identification of suspect areas using geochemical reconnaissance followed by blood copper surveys. *Journal of Agricultural Science, Cambridge* 78, 157–163.

Thornton, I., Kershaw, G.F. and Davies, M.K. (1972b) An investigation into copper deficiency in cattle in the southern Pennines. 2. Response to copper supplementation. *Journal of Agricultural Science, Cambridge* 78, 165–171.

Todd, J.R. (1972) Copper, molybdenum and sulfur contents of oats and barley in relation to chronic copper poisoning in housed sheep. *Journal of Agricultural Science, Cambridge* 79, 191–195.

Underwood, E.J. (1977) *Trace Elements in Human and Animal Nutrition*, 4th edn. Academic Press, London.

Underwood, E.J. and Suttle, F. (1999) Copper. In: *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK, pp. 283–342.

Underwood, E.J., Robinson, T.J. and Curnow, D.H. (1943) The influence of topdressing with copper sulfate on the copper content and the yield of mixed pasture at Gingin. *Journal of the Department of Agriculture of Western Australia* 20, 80–87.

Van der Berg, R., Levels, F.H.R. and van der Schee, W. (1983) Breed differences in sheep with respect to the accumulation of copper in the liver. *Veterinary Quarterly* 5, 26–31.

Veum, T.L., Carlson, M.S., Bollinger, D.W. and Ellersieck, M.R. (2004) Copper proteinate in weanling pig diets for enhancing growth performance and reducing faecal copper excretion compared with copper sulfate. *Journal of Animal Science* 82, 1062–1070.

VIDA (2007) *Veterinary Investigation Surveillance Report 2007*. Veterinary Laboratories Agency, Weybridge, UK.

Waldroup, P.W., Fritts, C.A. and Yan, F. (2003) Utilization of Bio-Mos© mannan oligosaccharide and Bioplex© copper in broiler chicks. *International Journal of Poultry Science* 2, 44–52.

Wang, Z.Y., Poole, D.B.R. and Mason, J. (1988) The effects of supplementation of the diet of young steers with Mo and S on the intracellular distribution of copper in liver and on copper fractions in blood. *British Veterinary Journal* 114, 543–551.

Ward, J.D. and Spears, J.W. (1997) Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance and carcass characteristics of cattle. *Journal of Animal Science* 75, 3057–3065.

Ward, T.L., Watkins, K.L. and Southern, L.L. (1994) Interactive effects of dietary copper and water copper level on growth, water uptake and plasma and liver copper concentrations of poults. *Poultry Science* 73, 1306–1311.

White, C.L., Masters, D.G., Paynter, D.I., Howell, J.M., Roe, S.P., Barnes, M.J. and Allen, J.G. (1994) The effects of supplementary copper and a mineral mix on the development of lupinosis in sheep. *Australian Journal of Agricultural Research* 45, 279–291.

Whitelaw, A., Armstrong, R.H., Evans, C.C. and Fawcett, A.R. (1979) A study of the effects of copper deficiency in Scottish Blackface lambs on improved hill pasture. *Veterinary Record* 104, 455–460.

Whitelaw, A., Russel, A.J.F., Armstrong, R.H., Evans, C.C. and Fawcett, A.R. (1983) Studies in the prophylaxis of induced copper deficiency in sheep grazing reseeded hill pastures. *Animal Production* 37, 441–448.

Whitelaw, A., Fawcett, A.R. and McDonald, A.J. (1984) Cupric oxide needles for the prevention of bovine hypocuprosis. *Veterinary Record* 115, 357.

Wiener, G. (1987) The genetics of copper metabolism in man and animals. In: Howell, J. M. and Gawthorne, J.M. (eds) *Copper in Man and Animals. Volume* I. CRC Press, Boca Raton, Florida, pp. 45–61.

Wiener, G., Suttle, N.F., Field, A.C., Herbert, J.G. and Woolliams, J.A. (1978) Breed differences in copper metabolism in sheep. *Journal of Agricultural Science, Cambridge* 91, 433–441.

Wiener, G., Wilmut, I., Woolliams, C. and Woolliams, J.A. (1984a) The role of the breed of dam and the breed of lamb in determining the copper status of the lamb. 1. Under a dietary regime low in copper. *Animal Production* 39, 207–217.

Wiener, G., Wilmut, I., Woolliams, C. and Woolliams, J.A. (1984b) The role of the breed of dam and the breed of lamb in determining the copper status of the lamb. 2. Under a dietary regime moderately high in copper. *Animal Production* 39, 219–227.

Wilson, P.R. and Grace, N.D. (2001) A review of tissue reference values used to assess the trace element status of farmed red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 49, 126–132.

Wilson, P.R. and Grace, N.D. (2005) Effects of molybdenum on tissue copper concentrations and response to copper wire particle supplementation in deer. *Proceedings of the Deer Branch of the New Zealand Veterinary Association*, pp. 34–36.

Wittenberg, K.M. and Devlin, T.J. (1987) Effects of dietary molybdenum on productivity and metabolic parameters of lactating beef cows and their offspring. *Canadian Journal of Animal Science* 67, 1055–1066.

Woolliams, J.A., Suttle, N.F., Wiener, G., Field, A.C. and Woolliams, C. (1982) The effect of breed of sire on the accumulation of copper in lambs, with particular reference to copper toxicity. *Animal Production* 35, 299–307.

Woolliams, J.A., Suttle, N.F., Wiener, G., Field, A.C. and Woolliams, C. (1983) The long-term accumulation and depletion of copper in the liver of different breeds of sheep fed diets of different copper content. *Journal of Agricultural Science, Cambridge* 100, 441–449.

Woolliams, C., Suttle, N.F., Woolliams, J.A., Jones, D.G. and Wiener, G. (1986a) Studies on lambs from lines genetically selected for low or high plasma copper status. 1. Differences in mortality. *Animal Production* 43, 293–301.

Woolliams, J.A., Woolliams, C., Suttle, N.F., Jones, D.G. and Wiener, G. (1986b) Studies on lambs from lines genetically selected for low or high plasma copper status. 2. Incidence of hypocuprosis on improved hill pasture. *Animal Production* 43, 303–317.

Wouda, W., Borst, G.H.A. and Gruys, E. (1986) Delayed swayback in goat kids: a study of 23 cases. *The Veterinary Quarterly* 8, 45–56.

Xiao-Yun, S., Guo-Zhen, D. and Hong, L. (2006a) Studies of a naturally occurring molybdenum-induced copper deficiency in the yak. *The Veterinary Journal* 171, 352–357.

Xiao-Yun, S., Guo-Zhen, D., Ya-Ming, C. and Bao-Li, F. (2006b) Copper deficiency in yaks on pasture in western China. *Canadian Veterinary Journal* 47, 902–905.

Xin, Z., Waterman, D.F., Hemken, R.W. and Harmon, R.J. (1991) Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in steers. *Journal of Dairy Science* 74, 3078–3085.

Xin, Z., Silvia, W.J., Waterman, D.F., Hemken, R.W. and Tucker, W.B. (1993) Effect of copper status on luteinizing hormone secretion in dairy steers. *Journal of Dairy Science* 76, 437–444.

Yost, G.P., Arthington, J.D., McDowell, L.R., Martin, F.G., Wilkinson, N.S. and Swenson, C.K. (2002) Effect of copper source and level on the rate and extent of copper repletion in Holstein heifers. *Journal of Dairy Science* 85, 3297–3303.

Zervas, G., Nikolau, E. and Mantzios, A. (1990) Comparative study of chronic copper poisoning in lambs and young goats. *Animal Production* 50, 497–506.

Zhang, W., Wang, R., Kleemann, D.O., Lu, D., Zhu, X.P. and Jia, Z. (2008) Effects of dietary copper on nutrient digestibility, growth performance and plasma copper status in cashmere goats. *Small Ruminant Research* 74, 188–193.

Zhang, W., Zhang, Y.S., Zhu, X.P., Wang, R.L. and Jia, Z.H. (2011) Effect of different levels of copper and molybdenum supplements on performance, nutrient digestibility, and follicle characteristics in cashmere goats. *Biological Trace Element Research* 143, 1470–1479.

Zhang, W., Zhang, Y.S., Song, X.Z., Jia, Z.H. and Wang, R.L. (2012) Effect of different levels of copper and molybdenum supplements on serum lipid profiles and antioxidant status in cashmere goats. *Biological Trace Element Research* 148, 309–315.

Zhang, H., Nie, H., Wang, Z. and Wang, F. (2018) The net iron, manganese, copper, and zinc requirements for maintenance and growth of Dorper × Hu ewe lambs. *Italian Journal of Animal Science* 17, 941–949.

12 Iodine

Iodine is unique among mineral elements in that deprivation causes an easily recognized clinical abnormality, 'goitre', caused by enlargement of the thyroid gland. Attempts to treat goitre can be traced back to 3000 BC when a Chinese emperor successfully used seaweed as a remedy. The relationship between iodine nutrition and goitre emerged in the l9th century, with the isolation of iodine from seaweed and the discovery that iodine was highly concentrated in normal thyroid glands but dilute in goitrous thyroids. In 1927, a putative hormone, containing 65% iodine by weight, was isolated from thyroid tissue and called 'thyroxine' (see Harington, 1953). A link between thyroid activity and basal metabolic rate was suggested by the similarity of requirements for species as diverse as the rat and cow (Mitchell and McClure 1937), when expressed in terms of calorie intake or heat production. Early field observations associated goitre in poultry, pigs and sheep with diets containing 0.1–0.2mg kg^{-1}DM (Orr and Leitch, 1929), but were substantially lower for cows. Treatment and prevention of goitre with thyroid extracts or iodized salt became routine. Extensive 'goitrous' areas were eventually discovered on every continent (Fig. 12.1), often associated with an environmental deficiency of iodine, and still cause problems in livestock (Singh *et al*., 2005) and humans (Zimmerman *et al*., 2008).

However, the link between geochemical I status and incidence of goitre can be weak (Stewart and Pharaoh, 1996): many incidents are caused by organic goitrogens in feeds (Stoewsand, 1995) and some cannot be cured or prevented by iodine supplementation (p. 313). Subsequent discoveries that lack of dietary I harmed brain development (Hetzel, 1991) and embryonic viability (Sargison *et al*., 1998) and were exacerbated by lack of dietary Se (see p. 378) (Beckett and Arthur, 1994) meant that 'iodine deficiency' was not synonymous with 'goitre': a new classification, 'iodine deficiency disorders' (IDD), replaced 'goitre' (Hetzel and Welby, 1997). Environmental stressors that affect basal metabolic rate, such as extreme cold, place stress on the thyroid gland. Thyroid activity controls productivity in all classes of livestock, particularly small ruminants (Todini, 2007), and impairment by goitrogens presents an obstacle to the exploitation of certain shrubs in conditions unfavourable for pasture growth. Iodine presents an excellent example of the interdependency of mineral nutrition in humans and animals. Cow's milk is a major contributor of iodine to the human diet but overfeeding dietary I to the cow poses a risk of excess to the consumer. Permitted additions to livestock feeds have been reduced tenfold in the new millennium (EFSA, 2013) but, ironically, the restriction may benefit the newborn calf.

Function of Iodine

Iodine has only one but vital, function, as a constituent of thyroid hormones. The thyroxine molecule has four atoms of iodine attached in two similar positions on linked 'outer' and 'inner' tyrosine rings (OR and IR) and became known as 3,3',5,5'-tetraiodothyronine (T4), an inactive prohormone. Removal of one atom by two OR-deiodinase enzymes (5'ORD types I and II) generates the active hormone, 3,3',5-triiodothyronine (T3). Both T4 and T3 can be inactivated by an IR deiodinase (type III) and physiological activity is regulated peripherally by these three deiodinases (Fig. 12.2) (Kulper *et al*., 2005; Todini, 2007). The rate of gene transcription is controlled by T3 (Bassett *et al*., 2003), which thus influences protein synthesis in all cells and has major effects on fetal development (Erenberg *et al*., 1974; Hopkins, 1975). T3 also has a thermoregulatory role, controlling cellular respiration and energy production, with widespread effects on intermediary metabolism, growth, muscle function, immune defence and circulation.

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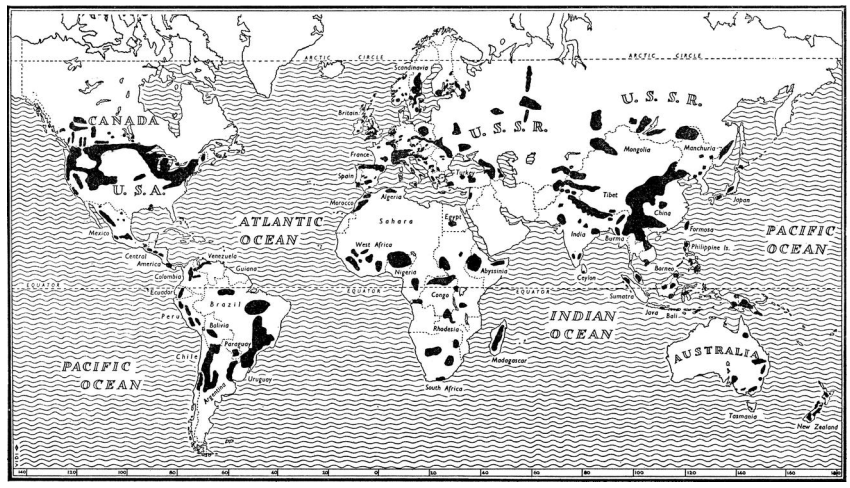

Fig. 12.1. Areas of the world where goitre was endemic. (Map courtesy of the Iodine Education Bureau, London)

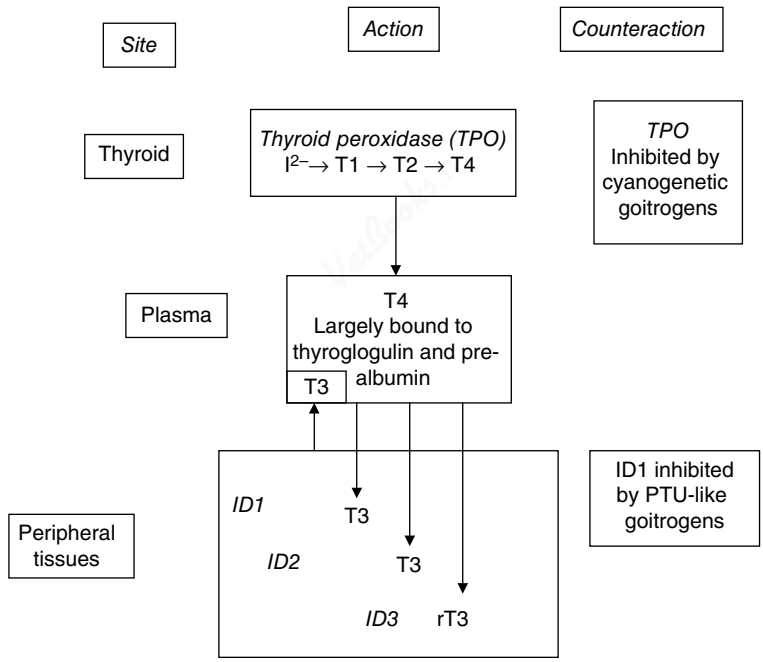

Fig. 12.2. Principal steps in the regulation of thyroid hormone metabolism: incorporation of iodide (I^{2-}) into mono-, di- and tetraiodothyronines (T1, T2 and T4) in the thyroid, catalysed by thyroperoxidase (TPO); transportation of bound T4 to peripheral tissues; activation to T3 by two outer-ring deiodinases (ID1 and 2); release of T3 into the bloodstream. T3 is also formed by similar processes in the thyroid gland, but extra-thyroidal synthesis is more important. All enzymes are selenium-dependent and upregulated during primary iodine deprivation. Secondary T3 deprivation can arise through inhibition of TPO by thiocyanates and inhibition of ID1 by goitrins, formed from ingested precursors, causing upregulation of inhibited enzymes. The relative importance of ID1 and 2 varies between organs and species. Low-circulating concentrations of free T3 stimulate release of thyrotrophic hormones from the brain. Excess T3 induces a third selenium deiodinase that removes iodine from the inner tyrosine ring of T4 (ID3) to form an inactive analogue, reverse T3 (rT3).

These functions are particularly important in facilitating the change from a fetal to free-living stage (Symonds and Clarke, 1996). The seasonality of reproduction in ewes is related to seasonal changes in thyroid activity (Follett and Potts, 1990) and required co-operation from the male is also probably facilitated by thyroid responses to change in day length (Todini, 2007). As a determinant of metabolic rate, T3 interacts with hormones, such as insulin, growth hormone and corticosterone (Ingar, 1985), and regulatory proteins of exocrine origin. For example, leptin production by adipose tissue is induced by thyrotrophic hormones and controls appetite (Menendez *et al*., 2003); thyroidectomy raises plasma leptin in the ovine fetus (O'Connor *et al*., 2007) and alters the leptin response to underfeeding in the horse (Buff *et al*., 2007). In the long term, life is unsustainable without a thyroid gland.

Clinical Abnormalities Due to Lack of Dietary I

Goitre

The size of the thyroid gland increases with the intensity and duration of I deprivation in an attempt to compensate for insufficient production of thyroid hormones. This and other manifestations occur whether hormonogenesis is impaired by lack of dietary I or is induced by goitrogens that block uptake of I by the thyroid gland. Goitre is manifested predominantly in the newborn animal, which is usually delivered by a clinically normal dam. In adults, the thyroid can be enlarged in otherwise healthy animals (Villar *et al*., 1998).

Impaired fertility

Infertility and poor conception rates, due to irregular or suppressed oestrus, were allegedly features of thyroid dysfunction in cows, attributable to enhanced losses of I at peak lactation, when conception usually occurs (Hemken, 1960). Male fertility is also affected, libido and semen quality declining in I-deprived bucks (Pattanaik *et al*., 2001), rams, bulls and stallions. In Brown Leghorn cockerels deprived of iodine, the testes are small and lack spermatozoa. Thyroidectomy reduces egg production in hens and the seasonal cycle of egg production in poultry is related to seasonal variations in thyroid activity.

Impaired fetal development and birth

In pregnant sheep given a diet very low in iodine (5–10 μg kg^{-1}DM), fetal brain maturation was impaired and lambs were stillborn, without wool (Potter *et al*., 1981, 1982). Brain abnormalities were reversed by giving I during the third trimester. Fetal development may be arrested at any stage by thyroid dysfunction, leading to early embryonic death and resorption (e.g. in ewes, Sargison *et al*., 1998), abortion or subnormal birth weights, but such abnormalities can have other causes (e.g. in calves, Smyth *et al*., 1992). Experimental iodine deprivation *in utero* lowers basal metabolic rate and resistance to hypothermia: sucking behaviour is impaired in the newborn lamb (Potter *et al*., 1982). Neonatal mortality can increase in lambs from ewes fed on goitrogenic kale or swedes during pregnancy (Andrews and Sinclair, 1962; Knowles and Grace, 2007) but exposure to kale for 62 days in mid-to-late pregnancy tripled thyroid weight in necropsied lambs without increasing mortality (Knowles and Grace, 2015). Residual effects of exposure to goitrogens in white clover in early pregnancy may have increased lamb mortality in New Zealand (Sargison *et al*., 1998). The need for thyroid hormones to produce lung surfactants may be an important determinant of neonatal survival (Erenberg *et al*., 1974; Symonds and Clarke, 1996). Poultry can withstand considerable iodine deprivation. In one study, it took two years of feeding an I-deficient diet to retard embryo development, prolong hatching time and decrease hatchability in hens (Rogler, 1958).

Post-natal mortality and growth retardation

In mildly iodine-deficient areas where the occurrence of goitre was low and variable, productivity was seriously impaired. In a flock of Polwarth ewes in Tasmania showing minimal thyroid enlargement, lamb mortality was 36% (King, 1976): the findings were confirmed elsewhere (Knights *et al*., 1979; Andrewartha *et al*., 1980; McGowan, 1983). Given the importance of thermogenesis from brown adipose tissue (BAT) to survival in animals born into a cold environment, iodine deprivation may increase susceptibility to cold stress (Arthur *et al*., 1992). There is, however, little convincing evidence that marginal iodine deprivation impairs calf survival (McCoy *et al*., 1995, 1997; Mee *et al*., 1995). Addition of goitrogens to a pig diet, low in iodine, severely restricts growth and stunted skeletal development (Sihombing *et al*., 1974), while the inclusion of RSM

in a natural diet for weanling pigs reduced growth rate by 50% (Lüdke and Schöne, 1988). Iodine supplementation increased growth rate and food intake in castrated and intact male goats given a diet high in goitrogens (Pattanaik *et al*., 2001): similar responses were found on a diet containing goitrogen-rich *Leucaena* leaves (Pattanaik *et al*., 2011)

Disorders of the integument

Abnormalities of the skin and its outgrowths are common features of thyroid dysfunction. Pigs (and calves) born to I-deprived mothers had thick pulpy skins due to subcutaneous oedema (Sihombing *et al*., 1974), an early sign recognized in various species in goitrous regions. Myxoedema also develops when weanling pigs are given goitrogen-rich diets (Lüdke and Schöne, 1988). Milder deficiencies are characterized by a rough, dry skin and a harsh coat in pigs, by scanty wool and hairiness of the fleece in sheep. Thyroid insufficiency in young lambs permanently impairs quality of the adult fleece, since normal development of wool-producing, secondary follicles is highly dependent on thyroid activity (Ferguson *et al*., 1956). Administration of methylthiouracil to cashmere goats reduced the proportion of active secondary hair follicles and delayed onset of moult (Rhind and McMillen, 1996). Thyroidectomy of the ram reduced wool growth but replacement therapy only needed to raise plasma T4 to 30% of normal to restore yield (Maddocks *et al*., 1985). Supplementation of the ewe with iodine increased wool production by 6% (Statham and Koen, 1981). Thyroid insufficiency can be very conspicuous in some types of bird, particularly the male in which the comb decreases in size, moulting is inhibited and characteristic plumage is lost. Broilers exposed to cyanogenetic goitrogens (CG) show incomplete feathering (Schone *et al*., 1992).

Low milk yield

Reductions in milk yield, conspicuous in dairy cows on diets lacking I, probably occur in all mammals. The hypothyroid state induced by prolonged feeding of goitrogen-rich foods is accompanied by loss of appetite, impaired growth and/or depressed milk yield (Hill, 1991). The earlier in post-natal life that maternal exposure to goitrogens begins, the greater the impact on milk yield and hence lamb growth. However, overall relationships between serum (or plasma) T4 or T3 and milk yield in cows are *negative* (for supporting references, see Pezzi *et al*., 2003), possibly due to the confounding effect of stage of lactation, redistribution of thyronines in tissues and their secretion into milk.

Occurrence of Iodine Deficiency Disorders

Geochemical and geographical factors

Occurrence of IDD was traditionally indicated by maps showing the incidence of goitre, one of the most widespread of all mineral deficiency diseases (Fig. 12.1) but local variations, even within small countries (Statham and Bray, 1975; Wilson, 1975) were easily masked. Maps of plant-available soil I would appear similar to Fig. 12.1, 'blackspots' indicating comparatively recent glaciation, where I-rich surface soils have been removed and had yet to be replenished with airborne I (Hetzel and Welby, 1997). Low soil I can generally be attributed to leaching loss, scant marine deposition and low inputs in rainfall (Fuge, 2005). It was estimated that 54–122 mg I ha^{-1} fell annually in rain on the Atlantic coastal plain, compared with only 1.7 mg ha^{-1} on the inland Great Lakes region of North America, where goitre was endemic (Hercus *et al*., 1925). Soils low in organic matter (e.g. sandy soils) tend to be low in I because they trap little if any deposited iodine. Goitre is also a problem in the highland interior of Papua New Guinea (Walton and Humphrey 1979). The influence of proximity to the coast is illustrated in Fig. 12.3 but less beneficial if it does not face the prevailing wind.

Soil–plant interactions

Where soil I is marginal, incidence and severity of IDD are influenced by climate and season: these affect grazing animals directly and indirectly through changes in pasture productivity and degree of contamination with marine or soil I. Goitre incidence in lambs is higher in 'good' than in 'poor' seasons in Tasmania: luxuriant herbage growth in 'good', high-rainfall seasons may reduce soil I ingestion (Statham and Bray, 1975). Responses to iodine supplements in outwintered ewes can be greater in a 'wet' than in a 'dry' year and increases in serum I between mating and lambing mask an early threat to embryonic survival (Sargison *et al*., 1998). The four- to fivefold reduction in pasture I that can accompany advancing maturity of a sward (Table 12.1) means that the risk of IDD tends to

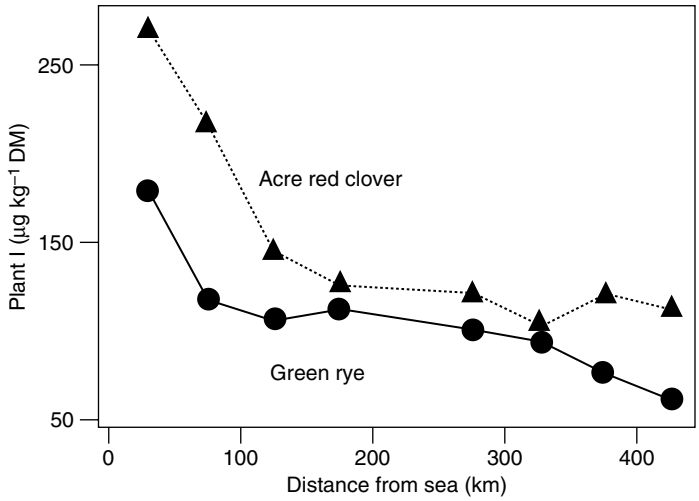

Fig. 12.3. Iodine concentrations in crops and grasses are greatly influenced by marine deposition and therefore decrease with distance from the sea. (Data from Groppel and Anke, 1986)

Table 12.1. Effects of sampling time on plant iodine concentrations (µg kg^{-1}DM) in different plant species (Groppel and Anke, 1986).

	24 April	16 June
Meadow fescue	184 ± 24	20 ± 4
Green rye	305 ± 108	43 ± 20
Green wheat	215 ± 53	18 ± 4
Acre red clover	294 ± 34	103 ± 37
Lucerne	358 ± 81	149 ± 37

increase as the grazing season progresses in herds that calve throughout the year.

Occurrence in herbivores

Goitre has been described in both mares and foals but horses normally receive sufficient I from their diet. In pregnant camels, addition of 1mg KI kg^{-1} DM to a diet of concentrate, berseem hay and rice straw of unspecified Co concentration improved reproductive performance, milk yield and calf performance (Abd El-Salaam et al., 2014): goitre was not reported.

Secondary iodine disorders

Occurrence of goitre is increased by the consumption of plants containing goitrogens, which can either impair thyroid uptake of iodine into T4 or the deiodination of T4 to T3. Figure 12.4 shows the dramatic effect of feeding kale or swedes to ewes in late pregnancy on incidence of neonatal goitre. When goitrogen exposure occurs in early pregnancy, conception rate may be decreased without evidence of neonatal goitre (Sargison *et al.*, 1998). Exposure to kale in mid-pregnancy can cause residual thyroid enlargement in newborn lambs, without compromising flock performance (Knowles and Grace, 2015). Use of kale to supplement autumn-calving cows may adversely affect conception rate. However, growth in lambs fattened on rape was rarely improved by iodine supplementation, despite thyroid enlargement (Fitzgerald, 1983). The feeding of mustard cake has induced IDD in goats (Pattanaik *et al.*, 2001). Barbari goats (18.8 kg initial LW) reared on a concentrate containing mustard-cake goitrogens gained twice as much weight (5.4 kg versus 2.6 kg), when supplemented with just 75 µg I day^{-1} for 180 days as those given no supplement. It is possible that interception of depositing iodine by broad-leaved species raises leaf I in autumn.

Pigs and poultry are also affected by goitrogens. Inclusion of as little as 80g kg^{-1}DM of a high-glucosinolate rapeseed meal (RSM) in pig rations will induce hypothyroidism and suppress growth, effects that supplementary iodine can only partially alleviate (Fig. 12.5). The feeding of cassava can induce IDD, but diets containing 500mg HCN kg^{-1}DM have been fed to sows throughout gestation and lactation

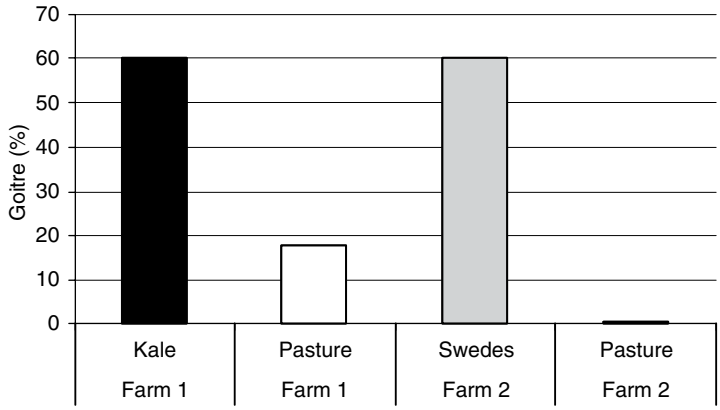

Fig. 12.4. The incidence of goitre in newborn lambs can greatly increase when ewes are fed on kale or swedes, rather than pasture, for the latter half of pregnancy. Mean iodine (mg kg^{-1}DM) was <0.05 in kale leaf and 0.15 in pasture on farm 1, and <0.05 in swede root and 0.11 in pasture on farm 2. Goitre incidence on pasture did not relate to pasture iodine. (Data from Grace *et al*., 2001)

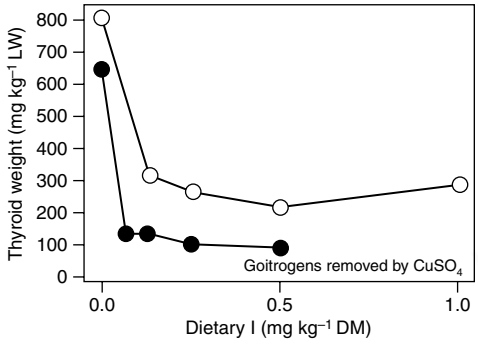

Fig. 12.5. Thyroid enlargement in pigs given diets containing some thiouracil-type goitrogens (as rapeseed meal) cannot be fully controlled by iodine supplementation, but their removal by treatment with copper sulfate allows full recovery. (Data from Schöne *et al*., 1988)

without adverse effects on sow or litter; bitter varieties pose the greatest risk of inducing IDD.

Physiochemical Features of Iodine Insufficiency

Normality

Like all the anionic elements, iodine is absorbed very efficiently, enabling iodine secreted prior to absorptive sites to be extensively recycled: in ruminants, iodine is recycled via the abomasum (Miller *et al*., 1974). Absorbed iodine is largely transported in the bloodstream loosely bound to plasma proteins but a small proportion circulates in free ionic form and accumulates in soft tissues when excess I is consumed (Downer *et al*., 1981). Recycling of thyroidal I occurs via the iodide pool. Excess dietary I is excreted predominantly via urine as iodide but significant amounts can be secreted in milk (Miller *et al*., 1965; Hillman and Curtis, 1980). The slope of the linear relationship between dietary and milk I (Fig. 12.6) indicates that about 30% of ingested I is secreted in milk by cows but transfer can be halved by exposure to glucosinolates (Franke *et al*., 2009): that proportion may be even higher in sheep, since milk I can be much higher than in cows, grazing the same pasture (50 versus 20 µg l^{-1}) (Grace *et al*., 2001).

Approximately 80% of body I is found in the thyroid gland because 90% of I entering that organ goes no further (Hetzel and Welby, 1997). Captured iodine is used to iodinate tyrosine and form T4 (Fig. 12.2) (Howie *et al*., 1995). Efficiency of capture is regulated by two hormones with thyrotropin-releasing and T4-stimulating properties (TRH and TSH). Plasma T4 is under feedback control by free (f) T4 and T3, tiny fractions (<1%) the total plasma T4 and T3 pools (Beckett and Arthur, 1994; Todini, 2007). Intracellular receptors on inner mitochondrial membranes and in the cell nucleus, take up T4, which is activated by deiodinases, ID1. There are major differences between ruminants and non-ruminants, ID1 being particularly important in BAT in

the newborn ruminant as a source of T3 for other tissues (Nicol *et al*., 1994): 80% or more of T3 is normally formed extra-thyroidally, principally in the liver and kidneys (Ingar, 1985) but also in the skin (Hetzel and Welby, 1997; Villar *et al*., 2000), testes (Brzezinska-Slebodzinska *et al*., 2002) and mammary gland. In early lactation in cows, ID2 activity decreases in liver and mammary tissue, while plasma T4 and T3 decrease (Pezzi *et al*., 2003). In newborn mammals, serum T4 and T3 rise immediately after birth (Rowntree *et al*., 2004), following ingestion of colostrum, rich in T4 and T3 (Pezzi *et al*., 2003), and reflecting the importance of T3 to survival.[i] Ingested T3 may promote development of the gut mucosa. In the chick embryo, there are large increases in T3 and ID activity after 19 days development, when lungs begin to function and glucocorticoid regulation changes (Darras *et al*., 1996). Departure from normality when dietary I is lacking is marked by the sequence of biochemical events illustrated in Fig. 12.7.

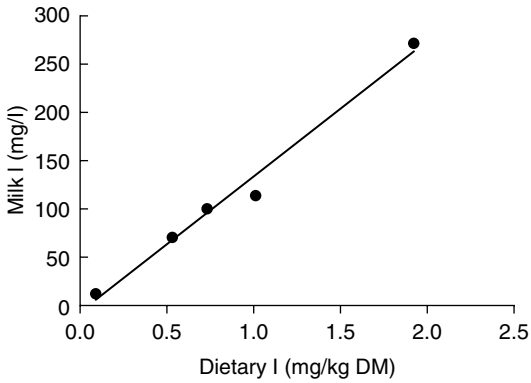

Fig 12.6. Iodine concentrations in cow's milk are directly proportional to iodine intake (van der Reijden *et al*., 2019).

Depletion

Although the thyroid is small (weighing approximately 4 g DM in adult cows), the amounts of stored I can be substantial (8–16 g), relative to minimum daily need (0.3 mg day^{-1} in heifers) (McCoy *et al*., 1997) and the depletion phase can therefore be prolonged. Initially, colloid is depleted and thyroid I declines below the normal level for mammals (2–5g kg^{-1}DM). Concentrations of total and protein-bound iodine (PBI) in the bloodstream decrease, as do those in milk and urine. The average

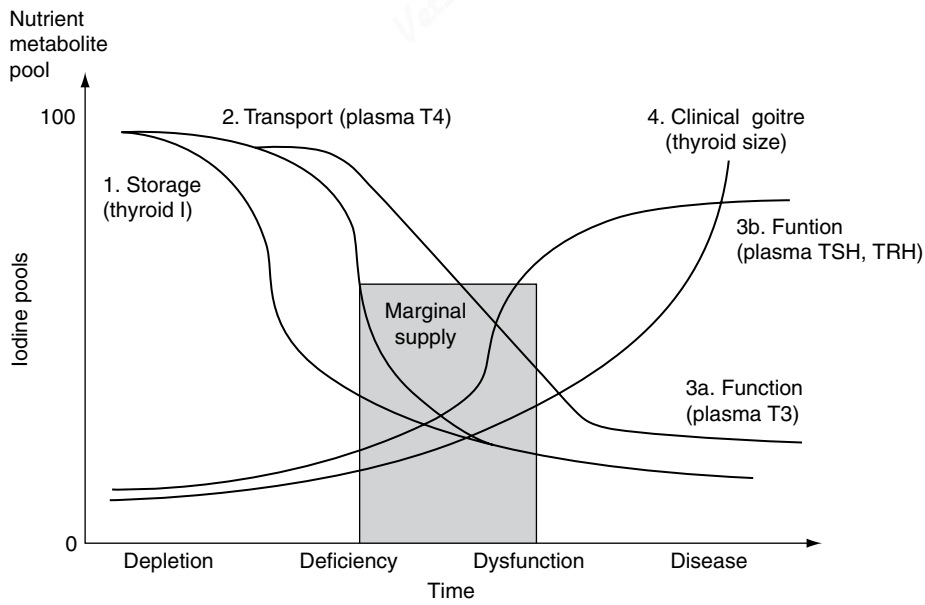

Fig. 12.7. A general scheme for the sequence of biochemical changes in livestock deprived of iodine; where thiouracil-type goitrogens are involved, impaired conversion of T4 to T3 in and beyond the thyroid changes the picture.

hen's egg contains some 4–10 μg of iodine, mostly in the yolk. This amount can be increased as much as 100-fold by feeding excess iodine but is reduced during iodine depletion. Ingestion of goitrogens that impair iodine uptake, such as glucosinolates, accelerates depletion.

Deficiency

The deficiency phase is marked by hyperplasia of the cuboidal epithelium, lining the thyroid follicles, further reducing iodine concentration in the enlarging thyroid. Deficiency is also accelerated by glucosinolates. Ratios of T3 to T4 and T1 to T2 in serum and tissues both increase in an attempt to use the diminishing supply of iodine more efficiently (e.g. Karmarkar *et al*., 1974). Enhanced activity in the thyroid gland causes oxidative stress and both cattle and sheep respond by increasing cytosolic GPX activity in the gland (Fig. 12.8) (Zagrodski *et al*., 1998). Selenium deprivation can induce IDD and disrupt the activity of ID1 in BAT but the brain and pituitary are protected, allowing ID2 activity to increase during I deprivation, regardless of Se status (Mitchell *et al*., 1997). When weanling rats were exposed to low temperatures (4°C for 18 h), ID2 activity in BAT increased. However, the increase was slowed and eventually greatly diminished by Se deprivation (Arthur *et al*., 1992). In pigs, serum T4 decreases, causing the serum T4:T3 ratio to decrease and induction of a hypothyroid state by the administration of methimazole increased ID2 in the thyroid and in skeletal muscle (Wassen *et al*., 2004).

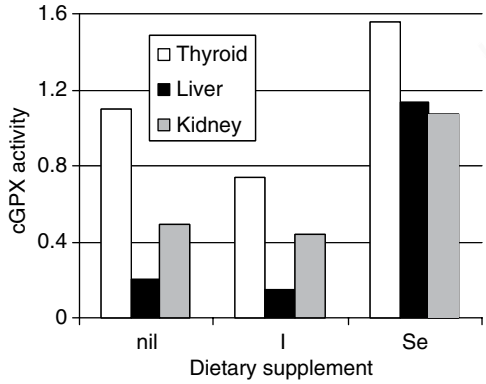

Fig. 12.8. Effects of prolonged iodine (I) and selenium (Se) deficiencies, singly or combined, on cytosolic glutathione peroxidase (cGPX) in three tissues of lambs, expressed as a fraction of activities in a fully supplemented group. In the thyroid, large increases in activity in the group depleted of iodine alone ('Se') and minimal decreases with Se depletion ('I') indicate adaptations to protect the thyroid from peroxidation: sensitivity and efficacy of adaptation is indicated because I depletion was not sufficient to cause either thyroid enlargement or alter plasma T4 and T3. (From Voudouri *et al*., 2003)

Dysfunction

The approach of dysfunction is indicated by hypertrophy of the cuboidal epithelium and increases in the thyrotrophic hormones TRH and TSH in plasma (e.g. in lambs, Clark, 1998; in horses, Johnson *et al*., 2003). Cellular and humoral immune responses in goitrous goats were improved by treatment with either colloidal I or T4, both given by injection (Singh *et al*., 2005) but may have been secondary responses to improved appetite.

Physiochemical Effects of Goitrogens

Biochemical responses to thiouracil goitrogens are far more pronounced than physiological responses and interpretation is complicated by adaptations to challenge (Arthur *et al*., 1988). In Fig. 12.9, the smallest dose markedly inhibited ID1 activity in the thyroid but >4.4 mg was required to raise T4:T3 and induce thyroid hyperplasia. A compensatory two- to threefold increase in ID2 activity in skin indicated adaptation to such inhibitors, peripheral production of T3 even increasing. Deiodinase activity should be measured at several sites (Villar *et al*., 2000). The cashmere goat is less susceptible to thiouracil goitrogens than cattle (Villar *et al*., 1998).[ii]

When both CG and propylthiouracil (PTU) goitrogens are ingested, biochemical changes may be different and the relative changes in ID1 and ID2 may indicate the predominant goitrogen in the diet. In pigs given RSM, which contains both goitrogen types, extra-thyroidal responses also accompanied those occurring within the thyroid (Spiegel *et al*., 1993). Presence of CG in RSM was confirmed by a rise in serum SCN and a sixfold rise in thyroid weight, but liver size also doubled. Serum T4 decreased but T3 was maintained and T4:T3 ratios therefore decreased. Hepatomegaly was believed to have increased T3 synthesis from T4, allowing growth to proceed unimpaired. In broilers, however, feeding more than 190g RSM kg^{-1}DM, high in glucosinolates,

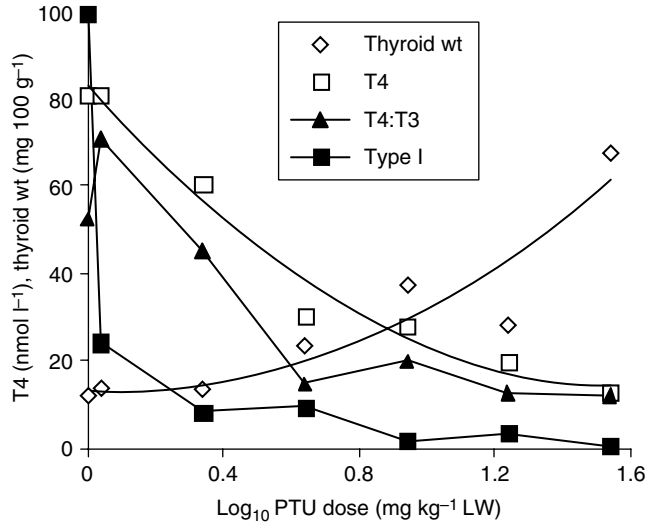

Fig. 12.9. Responses of hepatic type I deiodinase activity (% of untreated group), plasma tetraiodothyronine (T4), T4:T3 (triiodothyronine) ratio and thyroid weight in cashmere goats given daily oral doses of a goitrin, propylthiouracil (PTU) for 2 months. (from Villar *et al.*, 1998).

caused hepatomegaly and lowered serum T3 while leaving T4 unaffected (Taraz *et al.*, 2006). Such results begin to explain the diagnostic limitations of measurements of thyroid size and serum T4.

Diagnosis of Iodine Deficiency Disorders

Thyroid morphology, histology and composition

Diagnosis of IDD is based on indices of questionable precision, whether they are morphological, histological or biochemical (Clark *et al.*, 1998). Measurements involving the thyroid can only confirm one particular IDD, albeit the most conspicuous one, goitre, but absence of neonatal goitre does not rule out IDD (Sargison *et al.*, 1998). Severe goitre can be diagnosed from a visibly or palpably enlarged thyroid, but milder forms require thyroids to be weighed. Early studies indicated that the thyroid status of newborn lambs was normal if the fresh gland weighed <1.3 g, doubtful between 1.3 and 2.8 g and 'hyperplastic' if >2.8 g (Sinclair and Andrews, 1961); corresponding values for calves were <10, 10–13 and >13 g (Wilson, 1975). However, histologically normal calf thyroids can vary widely in size (McCoy *et al.*, 1997). Hyperplasia may be a more reliable index (Smyth *et al.*, 1992) but can also be found in healthy calves (Mee *et al.*, 1995; McCoy *et al.*, 1997); the terms 'colloid goitre' and 'hyperplastic goitre' are therefore diagnostically misleading. Thyroid weight can vary greatly in newborn lambs in an outbreak of goitre. A single thyroid weight:LW ratio >0.8g kg^{-1} indicated a 90% probability that the lamb came from an iodine-responsive flock, with a marginal band of 0.4–0.8 (Knowles and Grace, 2007) and confirming a mean value >0.4 (g kg^{-1}) as diagnostic (Clark *et al.*, 1998). Thyroid I also requires flexible interpretation. Hyperplasia was associated with thyroid I <1–1.2g kg^{-1}DM (Marine and Williams, 1908; Andrews *et al.*, 1948; Sinclair and Andrews, 1958) but lower values are found in histologically normal calf thyroids (Wilson, 1975; McCoy *et al.*, 1995, 1997).

Iodine in the bloodstream

Total serum I, PBI and butanol-extractable consist largely of T4 in bound forms and reflect stage of depletion rather than dysfunction. PBI values of 30–40 µg l^{-1} were taken as 'normal' for adult sheep and cattle but were found in an outbreak of neonatal goitre (Statham and Koen, 1981); lower values are found in healthy fowl and horses (Irvine, 1967). Misleadingly high PBI values are found after administering diiodosalicylic acid, which becomes strongly bound to serum proteins (Miller and Ammerman, 1995). Total serum I became a popu-

lar measure of current intake but does not indicate thyroid hormone function (Mee *et al.*, 1995), particularly when CG are being consumed. Since exposure to such goitrogens reduces uptake of I by the thyroid and secretion in milk.

- Serum I probably overestimates I status on diets rich in CG goitrogens.

Serum I tended to be higher in ewes at lambing, ten days after kale feeding, than in pastured ewes (50 ± 12 versus 39 ± 5 μg l^{-1}) despite a far higher incidence of goitre (Fig. 12.4). In a similar comparison, consumption of pasture containing more than three times more I than kale (0.15 ± 0.07 versus <0.05mg I kg^{-1}DM) did not affect serum I (45 ± 1 μg l^{-1}) (Knowles and Grace, 2015).

- A high ratio of serum I to T4 may indicate exposure to CG goitrogens.

T4 assays

Serum T4 indicates past I nutrition and stage of depletion but is a poor indicator of current thyroid dysfunction or disorder.[iii] Interpretation is influenced by a number of factors that affect serum T4: season (higher values in winter than summer due to photoperiodism; Todini, 2007); pregnancy (values fall in late pregnancy); early lactation (at a time of negative energy balance, values fall to 20–40 nmol l^{-1} in healthy dairy cows, and similar changes occur in goat (Pezzi *et al.*, 2003; Todini, 2007); birth (values are high in normal newborn animals; Clark *et al.*, 1998); infection (values decreased by intestinal parasitism; Prichard *et al.*, 1974); food intake, which is often positively correlated with plasma T4 (Todini, 2007). Diagnostic uncertainty surrounding interpretation of serum T4 was indicated by similar confidence limits for values in samples from dairy cows where lack of iodine was suspected and samples submitted for other reasons (50–90 nmol l^{-1}; Livesey *et al.*, 2014). In lambs, serumT4 declines to adult levels after about eight weeks and a ratio of <1 in lamb:ewe T4 in serum at birth may indicate deficiency in offspring (Andrewartha *et al.*, 1980). Similarly, T4 values in newborn calves from heifers given adequate dietary I are three times higher than those of dams, but the critical ratio may be higher than in sheep (<2.5) (McCoy *et al.*, 1997). Serum T4 in the newborn calf and piglet can fall by 50% within a week (Mostyn *et al.*, 2006). Where the cause of hypothyroidism is impaired conversion of T4 to T3, as in selenium deprivation or ID1 inhibition by goitrogens, serum T4 may be particularly misleading. However, low plasma T4 accompanied clinically significant goitre in pigs, induced by RSM (e.g. Schöne *et al.*, 1988, 1992). Assays of free T4 are rarely utilized.

T3 assays

Assays of T3 are preferred by some as a measure of dysfunction, particularly in Se-induced IDD. Serum T3 was lower in a dairy herd that responded to Se than in an unresponsive herd (1.81 versus 2.06 nmol l^{-1}; Wichtel *et al.*,1996). Serum T4 was high in untreated heifers (66 and 99 nmol l^{-1}) but reduced by giving Se. Mean serum T3, measured at scanning and late pregnancy, was poorly correlated with I-responsive lamb mortality in New Zealand (Clark *et al.*, 1998). Serum T3 is also affected by many factors other than iodine status, including: feed and water restriction (↓) (Blum and Kunz, 1981); cold (↑) (sheep, Nazifi *et al.*, 2003) and heat stress (↓) (beef cattle, Christopherson *et al.*, 1979; sheep, Nazifi *et al.*, 2003; poultry, (Sahin and Kocuk, 2007); parasitic infection (↓) (Hennessy and Pritchard, 1981); protein (↓) (Ash *et al.*, 1985) and iron deficiencies (↓) (Beard *et al.*, 1989); pregnancy (↓) (Todini *et al.*, 2007); birth (an early reduction in calves is proportionately greater than that in T4; see Fig. 12.10); and ageing (serum T3 falls from 2.8 ± 0.2 to 1.7 ± 0.1 μg l^{-1}, mean ± standard error)

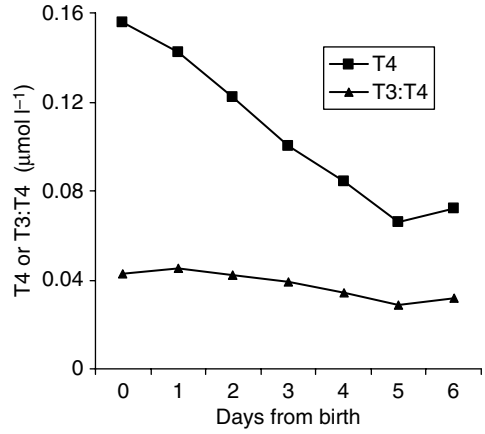

Fig. 12.10. Serum tetra- and triiodothyronine (T4 and T3) decline in many species in the first week after birth: the effect, shown here for calves, is greater for T3 than T4, and the T3:T4 ratio also decreases. (Data from Rowntree *et al.*, 2004)

between 1 and 5 years of age in goats (Todini, 2007). Serum T3 is not dependent on genotype in piglets (Mostyn *et al*., 2006).

Iodine in urine and milk

Iodine excretion in milk and urine reflects I intakes and milk I surveys can indicate the 'goitre status' of an area. Dietary I was considered to be suboptimal for dairy cows if milk contained <25 μg l^{-1} (Alderman and Stranks, 1967) but milk yield was not increased by supplementing a herd with milk I <20 μg l^{-1} (Grace and Waghorn, 2005). Higher thresholds (possibly >50 μg l^{-1}) are needed for optimal colostrum I (McCoy *et al*., 1997). Average milk I in ewes predicted field incidence of goitre in lambs, determined by palpable thyroid enlargement, values <100 μg l^{-1} pointing to a possible dysfunctional state (Mason, 1976). However, relationships are perturbed by ingestion of CG, which lower milk I (Van der Reijden *et al*., 2019) but raises urinary I (Fig 12.11).

- Where thyroid dysfunction is induced by CG, urine I will probably be deceptively high and milk I deceptively low.

Conclusions on diagnostic guidelines

Confirmation of IDD in livestock needs a new approach. Serum T4, T3 and T4:T3 ratios did not predict IDD in newborn lambs in New Zealand dose–response trials (Clark *et al*., 1998). Simple microplate ELISA assays for TSH have long been used to predict goitre prevalence in infants (Sullivan *et al*., 1997), are not without critics (Wainwright and Cook, 2019), should be investigated in livestock. Adaptive increases in ORD activity clearly indicate imminent thyroid dysfunction and have potential diagnostic value in livestock. Interpretation of conventional indices of I status can be improved by the adoption of marginal ranges, assessments of variability in a sampled population and use of more than one index of status. Response to iodine supplementation will depend on the proportion of individuals that lie within or beyond the limits of marginality shown in Table 12.2.

Sources of Iodine

Forages

Plant I is highly variable, due to species and strain differences, climatic and seasonal conditions, soil I

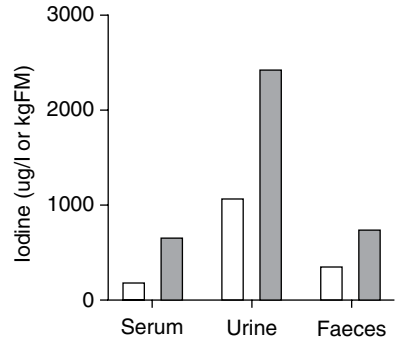

Fig. 12.11. Excretion of supplementary iodine (5mg I kg^{-1}DM) in urine and faeces by dairy cows was increased by exposure to glucosinolates (shaded columns × mmol g^{-1}DM) and serum I was increased (data from Franke *et al*., 2009, pooling data for iodide and iodate supplements for urine and faeces but not for serum, where only iodate groups were compared).

and interactions between these factors. Soils high in I, such as boulder clays and alluvial soils, generally produce plants richer in I than iodine-low soils, such as those derived from granites (Groppel and Anke, 1986). However, correlations between soil and plant I are often poor because plant species and strains differ widely in ability to absorb and retain soil iodine (Table 12.3) and there are interactions between species and seasonal effects. Plant I can decline rapidly as the season progresses (Fig. 12.3) but the effect was proportionally lower in leguminous than in gramineous species. Elsewhere, pasture I ranged from 0.16 to 0.18mg kg^{-1}DM and from 0.06 to 0.14mg kg^{-1}DM in white clover and grasses, respectively, in the Netherlands (Hartmans, 1974); means of 0.2–0.3mg I kg^{-1}DM were reported for mixed swards in Wales (Alderman and Jones, 1967) and Northern Ireland (McCoy *et al*. 1997).

Proximity to the sea has a major influence on plant I (Fig. 12.3). Marine deposits over winter may be diluted by new growth in spring and contribute to the marked seasonal decline in pasture I between spring and summer (Table 12.1). Plant I can be much higher in the leaf than stem of swedes (0.28 versus <0.05mg kg^{-1}DM, respectively) (Knowles and Grace, 2007) and white clover (Crush and Caradus, 1995) but not kale (Knowles and Grace, 2007). The only artificial fertilizer naturally high in I is Chilean nitrate of soda and applications can double or treble plant I. Heavy applications of seaweed can increase pasture I 10- to 100-fold (Gurevich, 1964) but nitrogenous

Table 12.2. Indices of marginal iodine status[a] in livestock.

	Condition	Pigs	Poultry	Sheep	Cattle
Diet (mg/kg DM)	Summer	**0.04**–0.08	**0.06**–0.08	**0.10**–0.15	**0.075**–0.10
	Winter	NA (+)	NA (+)	**0.20**–0.30	**0.15**–0.20
	Goitrogens[b]	**0.14**–0.28	**0.18**–0.24	**0.75**–1.00	**0.75**–1.00
Thyroid – wet weight (g kg^{-1} LW)	Newborn	NA	NA	0.4–**0.9**	0.5–**1.0**
	Weanling	**0.2**–0.4	**0.06**–0.10	0.4–**0.8**	
Iodine (g kg^{-1}DM)	Adult				0.1–**0.2**
Serum PBI (μg l^{-1})	Adult	20–33	**30**–40	**30**–45	**30**–40
Serum T4 (nmol l^{-1})[c]	D:NBAdult	**20**–30	NA	**40**–60	**30**–60
		40–60	**5**–9	**20**–45	**18**–40
Serum T3 (μmol l^{-1})	D:NBAdult	0.6–0.8	NA	2–**3.4**	<**4**–2
		NA	1.5–2.0	1.0–**1.7**	
Milk (μg l^{-1})		NA	NA	**70**–100	**15**–30

D, dam; NB, newborn; NA, not available (but requirements may be raised (+) in the free-ranging, cold-stressed animal); PBI, protein-bound iodine; T3, triiodothyronine; T4, tetraiodothyronine

[a] Mean values for sample falling within the ranges given indicate a possibility of response to iodine supplementation (or goitrogen removal) if sustained. If values for ≥10% of individuals sampled lie beyond the limit in **bold**, supplementation with iodine will probably be beneficial.

[b] Three- to fivefold increases in iodine requirement can be caused by the ingestion of diets rich in cyanogenetic goitrogens.

[c] Exposure to cyanogetic goirogens may give false high values.

Table 12.3. Iodine content of New Zealand pasture species grown on soils of different type and iodine concentration. (From Johnson and Butler, 1957)

	Herbage I (μg kg^{-1}DM)		
	Silt loam	Sandy loam	Hill soil
Total soil I (μg kg^{-1}DM)	8530	3000	1760
Perennial ryegrass	1500	1600	1350
Short-rotation ryegrass	150	168	230
Cocksfoot	175	258	225
Dallis grass*	1280	1280	1700
White clover	500	725	800

**Paspalum dilatatum*

fertilizers generally reduce herbage I by increasing plant growth (Alderman and Jones, 1967).

Soil iodine

Addition of soil to a pelleted lucerne diet for 63 days significantly increased serum I in sheep, clearly demonstrating that soil I can be absorbed (Grace, 2006). Extremely high pasture I in spring (15–60mg kg^{-1}DM) was attributed to soil contamination and linked to year-to-year variations in incidence of I-responsive mortality in lambs (Clark *et al.*, 1998). Ingested soil can be an important source of I for grazing and foraging livestock.

Other foodstuffs

Animal feedstuffs vary widely as sources of I and differences first reported in 1952 were mostly confirmed 30 years later (Table 12.4). Cereals and oilseed meals are poor sources, containing on average 0.05mg I kg^{-1}DM but are influenced by soil I. Fish meals are exceedingly rich in I and animal protein sources are generally intermediate, though increased by iodine supplementation. Seaweed may contain as much 4–6g I kg^{-1}DM and its inclusion in rations of cows and hens greatly increases the milk and egg I. Sheep that graze seaweed along the coastlines of northern Europe have a surfeit of dietary I.

Water

Iodine in drinking water largely reflects I content of surrounding rocks and soils and correlates with local plant I (Fuge, 2005). In some areas, drinking water I was inversely correlated with incidence of endemic goitre in man but in others there was no such correlation (Stewart and Pharaoh, 1996): similar inconsistencies no doubt exist for farm animals. Seawater is rich in I, containing >50 μg l^{-1}, and deposition of marine I influences drinking water I: in East Germany, values fell from 7.6 ± 4.4 to 1.1 ± 0.9 (s.d.) μg l^{-1} as distance from the coast increased from <50 to >400 km (Groppel and Anke, 1986). Exceptionally, drinking water can contain more I than seawater and may be sufficient

Table 12.4. Confirmation from the German Democratic Republic (GDR) (Groppel and Anke, 1986) of wide differences in iodine concentrations (μg kg^{-1}) between animal foodstuffs, first reported by the Chilean Iodine Education Bureau (CIEB) in 1952.

		GDR			
Feedstuff	CIEB range	Feedstuff	Mean	*n*	Standard deviation
Hays and straws	100–200	Hay	136	13	61
		Cereal straw	368	5	283
Cereal grains	40–90	Maize	44	9	18
		Barley	95	69	69
Oilseed meals	100–200	Rapeseed (ext.)	67	7	30
		Soybean (ext.)	97	8	33
Meat meals	100–200	Meat meals	–	–	–
Milk products	200–400	Dried skimmed milk	376	8	196
Fish meals	800–8000	Fish meal	6688	16	2879

to suppress thyroid activity. If other supplementary sources of iodine are consumed (Pedersen *et al.*, 2007). However, drinking water does not normally contribute significantly to I intakes.

Milk and milk products

Milks from all species vary widely in I due to variation in intake and the ease with which iodine crosses the mammary barrier (Fig. 12.6). Winter milk in the UK contained over twice as much I as summer milk (200 versus 90 μg l^{-1}) (MAFF, 2000), largely due to oversupplementation of concentrates given to housed dairy cows (Phillips *et al.*, 1988). Milk I is also influenced by stage of lactation: colostrum is two to three times richer in I than main milk and concentrations decline towards the end of lactation. Milk I was increased by the use of iodophors to maintain dairy hygiene and decreased use probably contributed to a large reduction in milk I in New Zealand from 435 to 120 μg l^{-1} between 1977–1978 and 1987–1988 (Sutcliffe, 1990). Iodine differs from most minerals in milk in that it is associated with milk fat. Milk products such as dried skimmed milk, buttermilk and whey usually contain less I than the milk from which they are derived.

Sources of Goitrogens

Goitrogens occur more widely than was once thought and are normally divided into two categories according to mode of action and counteraction, although there may be some overlap (Freyberger and Ahr, 2005).

Thyroperoxidase inhibitors

Plants can contain one or more of about 100 different glucosinolates, along with β-glucosidases such as myrosinase, that release HCN after structural damage to plant tissue, during harvesting or processing (Mawson *et al.*, 1993). The glucosinolate content of kale was higher at a site of high than low soil S but unaffected by applications of N and S that did not increase plant yield (Fletcher *et al.*, 2010) but may dilute goitrogen content where yields are increased (Alderman and Stranks, 1967; McDonald *et al.*, 1981; Wheeler and Vickery, 1989, in which P was applied). Thiocyanates inhibit thyroperoxidase (Fig. 12.2). White clover is a common cyanogenic pasture component, yielding HCN concentrations that vary widely between strains (0.1–1.1mg kg^{-1}DM) and seasons, decreasing by 75% between spring and early summer (Crush and Caradus, 1995). Leafy and root *Brassica* species are renowned for their CG content. Kale (*B. oleracea* var. *acephala*) contained, 10.4 mg sinigrin kg^{-1}DM, as its predominant glucosinolate (Kushadet *et al.*, 1999) and swedes (*B. napus*) are also rich in glucosinolates. Both kale and swedes are important 'winter gap' feeds for outwintered ruminants. Use of cassava as a livestock feed can be limited by its content of the glucosinolates linamarin and lotaustralin (Khajarern *et al.*, 1977) but HCN concentrations in both leaf and root can be reduced by appropriate processing and drying. Mustard cake is

a goitrogen-rich by-product after extracting oil from mustard seeds (*B. juncea*) (Pattanaik *et al*., 2001). The effects of CG goitrogens are attenuated by iodine supplementation.

Deiodinase inhibitors

The synthetic goitrogen PTU can induce IDD by inhibiting ORD1 in mammals and inhibition can therefore take place in peripheral tissues, outside the thyroid (Fig. 12.2). Some plant species contain similar substances, such as the glucosinolate goitrin (vinyl thio oxazolidinethione, VTO) and precursors (e.g. progoitrin in oilseed rape (*Brassica campestris* and *B. napus*)). There are 44 mmol progoitrin kg^{-1}DM in RSM its major goitrogenic component (Taraz *et al*., 2006) but meals prepared from 'low' glucosinolate varieties may contain only 2–5 mmol total glucosinolates kg^{-1}DM (Hill, 1991). Iodine supplementation does not counter the effects of ORD1 inhibitors (Emanuelson, 1994) and short-term exposure may cause goitre that is not an IDD. However, thiocyanates can be formed by hydrolysis of compounds such as progoitrin: the deiodinase inhibitor thus yields TPO inhibitors, whose effects can be countered by supplementary iodine.

Goitrogenicity

Unheated soybeans contain heat-labile goitrogens (Hemken, 1960). CG and PTU-like goitrogens have additive effects and overall goitrogenicity of a feed or ration depends on the respective concentrations, their fate during digestion and the ratio of CG to iodine. CG and non-CG strains of white clover, grown in the same area in New Zealand, contained 0.2 and 0.04mg I kg^{-1}DM, respectively, indicating that goitrogenicity may be exaggerated by the concentration of CG alone (Johns, 1956). In pot trials with strains that varied widely in HCN and I, there was no correlation between the two parameters (Crush and Caradus, 1995).

Genetic selection

Early selection for disease and pest resistance also increased goitrogenicity because goitrogens protected the plant from pathogens. The most popular strains of white clover in New Zealand were rich in HCN. Ladino types of *T. repens* were low in HCN (0.17mg kg^{-1}DM) but popular in the USA (Crush and Caradus, 1995). The pendulum swung and strains were selected for 'low' goitrogen content, particularly oilseed rape, widely cultivated for edible oil and generating large quantities of RSM, a valuable protein supplement for livestock. Canadian plant breeders led the way in producing low-glucosinolate cultivars and Canola meal, a by-product containing <30 mmol glucosinolate kg^{-1}DM; such products have relatively small effects on iodine status (Emanuelson, 1994). Winter varieties of rape grown in middle and western Europe are a different proposition and their by-products required treatment to remove glucosinolates. Selection for high plant iodine status held little promise (Crush and Caradus, 1995).

Metabolism of Goitrogens

Glucosinolates are less goitrogenic in ruminants than non-ruminants because they are extensively degraded in the rumen (Hill, 1991; Emanuelson, 1994). Nevertheless, goitrin and SCN excretion in cow's milk rises when RSM is fed, a clear indication of incomplete destruction. Excretion of SCN is accompanied by a reduction in milk I and relatively small increases in milk I when I supplements are fed. The apparent paradox may arise from inhibition by SCN of I uptake by the mammary gland, which conserves iodine (Iwarsson, 1973), but only at the expense of any suckled offspring. Increases in TSH secretion in cows on high-glucosinolate diets (Laarveld *et al*., 1981) indicate net impairment of T3 function: however, the effect may be less in lactating than non-lactating animals, especially ewes which secrete far more iodine in milk than cows. The browse legume *Leucaena leucocephala* contains mimosine, which is degraded to a goitrogen in the rumen and thus causes thyroid enlargement in steers in some locations. Cattle on Hawaii possessed a rumen microorganism that detoxified the goitrogen and could consume such browse with impunity. Transfer of a rumen inoculum from Hawaii to other locations prevented the induction of IDD elsewhere in stock consuming *L. leucocephala* (Jones and Megarrity, 1983). Inclusion of *L. leucocephala* leaves in the diet of goats caused weight loss and thyroid enlargement unless dietary I was increased (Pattanaik *et al*., 2011).

- Cross-infection with rumen inocula may be needed more widely to obtain full benefit from

the capacity of such goitrogenic shrubs to yield digestible OM.

Conversion of HCN to SCN or isothiocyanate can continue during digestion in non-ruminants through the activity of myrosinases produced by gut microbes. Inclusion of 40% cold-pressed RSM in the diet of nursery pigs reduced growth rate, increased relative liver and thyroid sizes by 27% and 64%, respectively and reduced serum T4 from 30 to 18 μg l^{-1} (Lee *et al*., 2020). Thiocyanate production in the caecum and thyrotoxicity were reduced by a dietary supplement that reduced colonic pH.

Iodine Requirements on Goitrogen-free Diets

Early estimates of daily need for I (Mitchell and McClure, 1937), converted into dietary concentrations assuming a normal daily DM consumption, lay between 0.05 and 0.10 mg kg^{-1}DM for poultry, pigs and sheep and 0.03–0.06mg kg^{-1}DM for milking cows. As with all minerals, requirement depends on criterion of adequacy: needs for growth are not necessarily those for reproduction, lactation or for maintenance of thyroid structure and serum T4. Furthermore, critical levels for a given species are likely to vary with environmental temperature, rate and stage of production and efficiency of energy utilization.

- A functional assessment of hypothyroidism (i.e. TRH or TSH induction) should be used to define iodine requirements.

Ruminants

Environmental temperature affects I requirement. Uptake of I by the thyroid in lactating goats was about tenfold higher when animals were kept at 5°C rather than 33°C (Lengemann, 1979). At the lower temperature, less ^{125}I was excreted in milk (2.6% versus 16.8% of dose) but more was lost in urine (71.2% versus 52.5%). A physiological estimation of requirement to sustain TSR assumed a capture efficiency of 33% for iodine reaching the thyroid, predicted mean requirements of 0.11 in summer and 0.54 mg I kg^{-1}DM, respectively, in winter for sheep and cattle (ARC, 1980). However, needs were overestimated because no allowance was made for I recycling. Experiments[iv] and field trials in Northern Ireland with winter-calving herds (Mee *et al*., 1995) indicated that herbage with 0.2–0.3mg I kg^{-1}DM was marginal (Mee *et al*., 1995) but needs for the pregnant and lactating ewe were placed higher at 0.7mg kg^{-1}DM (Boland *et al*., 2005). Herbage with 0.18–0.27mg I kg^{-1}DM sustained normal growth in cattle (Wichtel *et al*., 1996), milk yield in cows (Grace and Waghorn, 2005) and wool growth in sheep (Barry *et al*., 1983) in summer in New Zealand. There is no evidence of breed differences in requirements. Lambs from hill breeds of sheep have better thermoregulatory powers than lowland breeds but the difference is probably attributable to differences in birth coat characteristics (Todini, 2007). Associations between plasma T4 and wool growth rate, body size or reaction to food restriction are not matched by reports of breed differences in susceptibility to IDD (Todini 2007).

Herbivores

NRC (2005) suggested that horses require 0.35mg I kg^{-1}DM but there is little evidence to affirm or refute the figure or that it may not be safely used by default for other herbivores.

Pigs and poultry

Growing pigs on cereal and soybean meal rations require between 0.09 and 0.15mg I kg^{-1}DM to avoid thyroid hyperplasia (Cromwell *et al*., 1975; Schöne *et al*., 1988). Growth of 6–7-week-old pigs was only slightly and inconsistently compromised when the diet contained 0.03–0.04mg I kg^{-1}DM, after prior depletion of iodine reserves but thyroid weight was invariably and markedly reduced by adding 0.1mg I kg^{-1}DM (Sihombing *et al*., 1974). NRC (2012) recommended 0.14mg I kg^{-1}DM for all pig rations. For pigs and poultry, requirements based on minimum thyroid weight exaggerate iodine requirements. Highly variable needs were reported for growth in early feeding trials with chicks and laying birds (0.03–1.0mg kg^{-1}DM) but requirements for normal thyroid structure were more consistent (approximately 0.3mg kg^{-1}DM) (Underwood and Suttle, 1999) and similar for both growth and normal thyroid development in pheasants and quail (Scott *et al*., 1960). NRC (1994) recommended 0.3–0.4mg I kg^{-1}DM for growing and laying birds, which should cover all eventualities in farm practice.

Influence of Goitrogens on Iodine Requirements

Ruminants

The impact of organic goitrogens on iodine requirements is poorly defined in ruminants. ARC (1980) suggested an increase in iodine provision to 2mg kg^{-1}DM in the presence of substantial quantities of goitrogens. Thyroid weight increased linearly from 67.4 to 120.8 mg kg^{-1}LW as glucosinolate in the lambs' diet (from RSM) increased up to 17.5mmol kg^{-1}DM but neither food intake nor growth rate was affected (Hill *et al*., 1990). The principal concentrate component of the diet contained 0.8 mg added I kg^{-1}DM but goitre may not have been wholly iodine-responsive. Administration of 6 mmol KSCN day^{-1} by vaginal pessary for the last two thirds of pregnancy in ewes lowered plasma T3 and T4 concentrations by 11% without affecting values in either the newborn or subsequent lamb growth (Donald *et al*., 1993). Feeding kale to pregnant ewes for eight weeks in mid-pregnancy increased thyriod:LW ratio in lambs born at pasture from 0.51 to 1.27 g/kg (Knowles and Grace, 2015) but intramuscular injections of iodine, which raised total plasma iodine, did not improve survival or weaning weight. The ARC (1980) recommendation for goitrogen-rich diets is probably excessive and a doubling of what are generous recommendations.

Pigs and poultry

Feeding RSM (80g kg^{-1}DM) of a high glucosinolate type to weaned pigs increased requirements from around 0.1 to 0.5 mg I kg^{-1} DM (Schöne *et al*., 1988). The addition of 50 mmol KCNS kg^{-1}DM to the diet of young pigs induced goitre and a precipitous fall in serum protein-bound iodine (PBI) (from 45 to 5 μg l^{-1} in 21 days) but the challenge was severe: values recovered almost as quickly when the goitrogen was removed from the low-iodine diet (0.04 mg kg^{-1}DM) (Sihombing *et al*., 1974). Exposure of broilers to RSM high in glucosinolates doubled thyroid size without affecting growth and I supplements did not reduce thyroid size (Schöne *et al*., 1992). Soaking diets in myrosinase, a process that would generate CG, increased thyroid size fivefold but effects were then countered by I supplementation. Heating myrosinase-treated RSM diets also increased goitrogenicity. In broilers, early growth was inhibited by >250 g RSM (providing >4.2 mmol glucosinolates kg^{-1}DM) but by the finishing stage the tolerable level of RSM had doubled (dietary I unspecified but no supplement was used) (Taraz *et al*., 2006). Iodine requirements may be decreased by soaking and pelleting, which degrade goitrogens.

Inorganic goitrogens

Human goitre has been associated with high arsenic and fluoride intakes and consumption of drinking waters high in calcium, magnesium and nitrates. Evidence for an antagonism between F and I in livestock is unconvincing. Antithyroid activity has been demonstrated for several cations and anions when ingested in excess, including sodium, chloride and especially perchlorate. Inorganic goitrogens may increase urinary I and/or impair I uptake by the thyroid. Like organic goitrogens they may only achieve clinical significance when dietary I is marginal.

Prevention and Control of Iodine Insufficiency

Disorders caused by simple or CG-induced iodine deprivation can be prevented and cured by iodine supplementation. Those caused by thiouracil-type goitrogens or selenium deprivation require other measures.

Oral supplementation

Housed livestock can be given iodized salt licks, kelp (seaweed) or iodine salts in mineral mixtures (0.1g I kg^{-1}DM) or concentrates. With the exception of diiodosalicylic acid, commonly used iodides and iodates (e.g. KI and $Ca(IO_3)_2$) are well-absorbed by ruminants and non-ruminants (Miller and Ammerman, 1995). There is little evidence that iodide and iodate are metabolized differently (Franke *et al*., 2009), although the former is reductive and the latter oxidative, making admixture unwise (EFSA, 2013). Considerable losses of I can occur from iodides by volatilization and leaching, particularly in hot, humid climates (Kelly, 1953). Cuprous iodide and orthoperiodates (e.g. $Ca_5(IO_6)_2$) are stable and readily absorbed despite a low solubility. Consumption of all free-access sources can be extremely variable (p. 47). Herbage I can be increased by applying I in fertilizers but uptake is poor, especially if iodide is used and lime applied (Whitehead, 1975). Dosing or drenching with iodine salts is effective but is time-consuming

and costly, unless animals are being handled for other reasons. Two oral doses of 280 mg KI or 360 mg KIO_3, after months 3 or 4 of pregnancy, prevented neonatal mortality and associated goitre in lambs from flocks outwintered on goitrogenic kale (Clark, 1998).

Slow-release methods

A single 1ml intramuscular injection of iodized poppy-seed oil, containing 40% I (w/v), at mating or 7–9 weeks before lambing, reduced goitre incidence and improved survival to weaning (Sinclair and Andrews, 1958, 1961; Statham and Koen, 1981; McGowan, 1983; Clark *et al*., 1998). Residual effects can improve wool yield in the year after treatment and control goitre at a further lambing (Statham and Koen, 1981). The recommended dose of iodized poppy oil for cows is only 4 ml (i.e. not proportional to body weight *vis-à-vis* sheep). Improved oil-based products became available for sheep and cattle: they raised iodine status for up to a year (Grace and Waghorn, 2005). Serum T4 was raised for 150 days in ewes after administration in mid-pregnancy (Knowles and Grace, 2015). Milk I was substantially increased and serum T4 in suckling lambs was raised for nine weeks. An intraruminal device, relying on simple diffusion from a polyethylene sack, released I for several years (Mason and Laby, 1978). Rigid capsules achieved a similar result (Siebert and Hunter, 1982) and efficacy was demonstrated (Ellis and Coverdale, 1982; Rogers *et al*., 1998).

Countering goitrogenicity

Soaking RSM in copper sulfate ($CuSO_4$) solution (25g l^{-1}) and drying (Ludke and Schöne, 1988) or supplementation of diets with 250mg Cu kg^{-1}DM can eliminate both VTO and the related indolyl compound (ITO), restoring normal growth and thyroid size in weaned pigs (Schöne *et al*., 1988) and broilers (Schone *et al*., 1992), whereas supplementary iodine alone can be ineffective. The RSM goitrogens were predominantly of the thiouracil type (11.9g VTO and 3.8g ITO kg^{-1}DM). Fresh cassava leaf and tuber contains potentially harmful concentrations of CG-type goitrogens but simple processes such as wilting, drying and ensiling can largely eliminate them (Lukuyu *et al*., 2014).

Iodine Toxicity

Iodine is a cumulative, chronic poison but tolerable doses for all sources vary. Exposure to excess dietary I paradoxically results in hypothyroidism because of feedback inhibition of T3 synthesis. However, there is a wide margin between required (<0.5 mg) and tolerated concentrations (<50mg kg^{-1}DM) for species other than the horse. Beef cattle given diets containing 100–200mg I kg^{-1}DM for three to four months grew poorly and developed respiratory problems: minimum safe concentration ranges were placed at 25–50mg kg^{-1}DM (Newton *et al*., 1974) but 8.3mg I kg^{-1}DM was sufficient to cause thyroid enlargement in fattening bulls (Meyer *et al*., 2008). The only natural diets that present a risk of toxicity are where large proportions of seaweed, cultured marine algae or their derivatives are fed since these can contain 1–11g I kg^{-1}DM (Indergaard and Minsaas, 1991; Rey-Crespo *et al*., 2014). Smaller livestock species appear to be more tolerant than large animals but this is partly because large-animal experiments usually last longer than those with pigs and poultry. Nevertheless, tolerances have been placed at 300–400 mg for pigs and poultry and 50mg I kg^{-1}DM for sheep and cattle (NRC, 2005). The horse appears to be exceptionally vulnerable, goitre being reported in mares and their foals given large amounts of seaweed (McDowell, 1992): tolerance of equines has been placed as low as 5mg kg^{-1}DM. Periods of high soil I ingestion may suppress serum T4 in ewes (Clark *et al*., 1998). High iodine intakes from cows' milk increase risk of thyrotoxicosis in humans (Phillips *et al*., 1988). A limit of 2mg kg^{-1}DM for dairy cow rations has been proposed in Europe (EFSA, 2013). Toxicoses were caused by misuse of ethylenediamine dihydriodide (EDDI), used at 74–402 mg d^{-1} in orally treated foot rot and soft tissue lumpy jaw in the USA[v]: EDDI is no more toxic than other sources of iodine.[vi]

Subclinical effects of moderate iodine exposure on neonates

Components of a mineral block, consumed by ewes during pregnancy, were believed to have greatly reduced serum immunoglobulin (IgG) in newborn lambs (Boland *et al*., 2004): the suspects were iodine, which inhibited IgG uptake by the lamb (Boland *et al*., 2005). The inhibitory effect occurred

with 20 mg I d^{-1}, 40% of the amount then regarded as tolerable, given for only the last three weeks of pregnancy: free and total serum T3 were also reduced in the lamb (Boland *et al*., 2008). Exposure of the late-pregnant ewe to 26.6 mg I d^{-1} as iodide or iodate changed the expression of genes related to the passive transfer of antibodies, before colostrum was ingested (Fig. 12.12). Excess I also reduced plasma tocopherol in the neonate, suggesting impairment of vitamin E absorption (Boland *et al*., 2008). With iodate incorporated into a concentrate, fed at up to 0.87 kg d^{-1} in late pregnancy, effects on serum IgG in lambs, 24 hours after birth, suggested that 5mg kg^{-1}DM in the concentrate (equivalent to about 2.5 mg of total DMI and 5 g total I) began to suppress immunoglobulin absorption (Fig. 12.13).

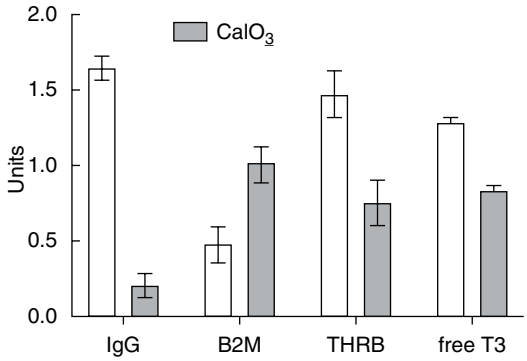

Fig. 12.12. Effect of adding iodine (26.6 mg d^{-1} as $CaIO_3$) to the silage diet of ewes from the 119th day of gestation on serum and free tri-iodothyroxine (T3, pmol $100ml^{-1}$prior to intake of colostrum, serum immunoglobulin (IgG, g) after 24 h and relative expression of two genes that control synthesis of β2 microglobulin (B2M) and thyroid hormone receptor β (THRB): the supplement was chosen as an amount that could be ingested in free-access minerals. (Data from McGovern *et al*., 2016)

- An urgent review of the effects of iodine supplements during pregnancy, including slow-release injections (p. 316) and seaweed meal supplements, is called for.

Notes

[i] In mare's milk, T3 rises to a peak of 0.71 nmol l^{-1}, 4 days after parturition, and there are similar increases in ID1 and 2 activities (Slebodzinski *et al*., 1998).

[ii] Daily doses of 15 mg PTU kg^{-1}LW for a month in pre-pubertal Rambouillet ewes, given alfalfa hay, had reduced plasma T4 from 79 to 9 ± 2.5μg l^{-1} within 7 days and reductions in T3 were similar (Wells *et al*., 2003). Recovery was almost complete, one month after PTU treatment ceased, when respective T4 concentrations were 54 and 43 ± 2.9 μg l^{-1} and there were no residual effects on onset of puberty or pregnancy rate.

[iii] In lactating ewes, a mean plasma T4 of <50 nmol l^{-1} was said to indicate thyroid insufficiency from field studies in Australia (Wallace *et al*., 1978); but a slightly lower threshold was adopted in New Zealand. Flocks with mean serum T4 values of 47 nmol l^{-1} at mating were iodine responsive (Clark *et al*., 1998).

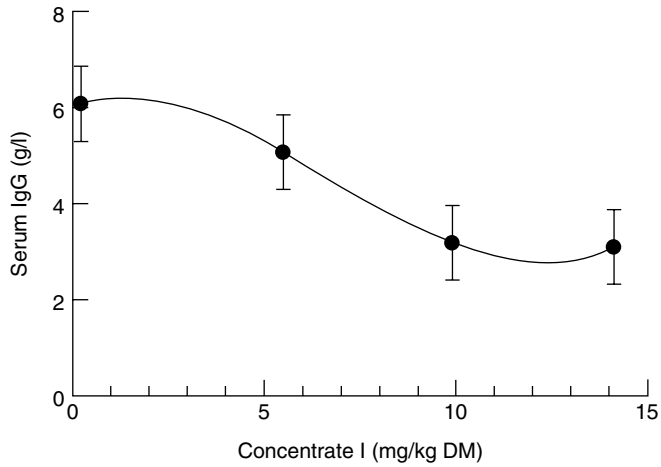

Fig. 12.13. Effects of dietary cobalt concentration during late pregnancy on serum IgG, 24 hours after birth, in lambs removed at birth and hand-fed a standard colostrum. (Rose *et al*., 2007)

[iv] McCoy *et al.* (1997) reported a 120% increase in thyroid weight in calves born to housed cows given experimental diets when an experimental diet contained 0.06 rather than 0.27mg I kg^{-1}DM during pregnancy.

[v] Bolus doses of EDDI equivalent to 100mg I kg^{-1}DM feed were tolerated for 13 months. Onset of lactation reduces serum I and risk of toxicity declines because excess I can be secreted in milk (Fish and Swanson, 1983). When EDDI was incorporated into a free-access salt mixture (1.25 I kg^{-1}) for a herd of beef cows at pasture, consumption of the mixture ranged from 20 to 70 g animal-$unit^{-1}$ day^{-1} and provided sufficient iodine (24–88mg day^{-1}) to reduce the incidence of foot rot without harming calf performance. Mean serum I did not exceed 0.46 mg l^{-1} (Maas *et al*., 1984). Total serum I increased logarithmically as dose increased, reaching about 4 mg l^{-1} when 0.77mg EDDI kg^{-1} LW is given daily for 28 days (Maas *et al*., 1989) and providing a rough index of exposure. Milk I can exceed 2 mg l^{-1} and also confirms iodosis, but serum T4 can remain normal (Hillman and Curtis, 1980). Neonatal losses do not present with typical morphological or histological evidence of goitre, although serum T4 is decreased (Fish and Swanson, 1983).

[vi] Symptoms include nasal and lachrymal discharge, conjunctivitis, coughing, broncho-pneumonia, hair loss and dermatitis (Hillman and Curtis, 1980). Metabolic rate is elevated, as indicated by a raised body temperature and heart rate. Blood glucose and urea concentrations are raised and immunosuppression has been reported (Haggard *et al*., 1980).

References

Abd El-Salaam, A.M., El-Tahan, A.A.H. and Bakr, A.A. (2014) Impact of dietary iodine supplementation on productive and reproductive performance of Maghrebian She-camels. *IOSR Journal of Agriculture and Veterinary Science* 11, 59–69.

Alderman, G. and Jones, D.I.H. (1967) The iodine content of pastures. *Journal of the Science of Food and Agriculture* 18, 197–199.

Alderman, G. and Stranks, M.H. (1967) The iodine content of bulk herd milk in summer in relation to estimated dietary iodine intake of cows. *Journal of the Science of Food and Agriculture* 18, 151–153.

Andrewartha, K.A., Caple, I.W., Davies, W.D. and McDonald, J.W. (1980) Observations on serum thyroxine concentrations in lambs and ewes to assess iodine nutrition. *Australian Veterinary Journal* 56, 18–21.

Andrews, E.D. and Sinclair, D.P. (1962) Goitre and neonatal mortality in lambs. *Proceedings of the New Zealand Society of Animal Production* 22, 123–132.

Andrews, F.N., Shrewsbury, C.L., Harper, C., Vestal, C.M. and Doyle, L.P. (1948) Iodine deficiency in newborn sheep and swine. *Journal of Animal Science* 7, 298–310.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

Arthur, J.R., Morrice, P.C. and Beckett, G.J. (1988) Thyroid hormone concentrations in selenium-deficient and selenium-sufficient cattle. *Research in Veterinary Science* 45, 122–123.

Arthur, J., Nicol, F., Guo, Y. and Trayhurn, P. (1992) Progressive effects of selenium deficiency on the acute, cold-induced stimulation of type II deiodinase activity in rat brown adipose tissue. *Proceedings of the Nutrition Society* 51, 63A.

Ash, C.P.J., Crompton, D.W.T. and Lunn, P. G. (1985) Endocrine responses of protein-malnourished rats infected with *Nippostrongylus brasiliensis* (Nematoda). *Parasitology* 91, 359–368.

Barry, T.N., Duncan, S.J., Sadler, W.A., Millar, K.R. and Sheppard, A.D. (1983) Iodine metabolism and thyroid hormone relationships in growing sheep fed kale (*Brassica oleracea*) and ryegrass (*Lolium perenne*) – white clover (*Trifolium repens*) fresh forage diets. *British Journal of Nutrition* 49, 241–254.

Bassett, J.H.D., Harvey, C.B. and Williams, G.R. (2003) Mechanisms of thyroid hormone receptor-specific nuclear and extra-nuclear actions. *Molecular and Cellular Endocrinology* 213, 1–11.

Beard, J., Tobin, B. and Green, W. (1989) Evidence for thyroid hormone deficiency in iron-deficient anemic rats. *Journal of Nutrition* 119, 772–778.

Beckett, G.J. and Arthur, J. (1994) The iodothyronine deiodinases and 5′deiodination. *Baillieres Clinical Endocrinology and Metabolism* 8, 285–304.

Blum, J.W. and Kunz, P. (1981) The effects of fasting on thyroid hormone levels and kinetics of reverse triiodothyronine in cattle. *Acta Endocrinologica* 98, 234–239.

Boland, T., Brophy, P., Callan, J., Quinn, P., Nowakowski, P. and Crosby, T. (2004) The effects of mineral-block components when offered to ewes in late pregnancy on colostrum yield and immunoglobulin G absorption in their lambs. *Animal Science* 79, 293–302.

Boland, T., Guinan, M., Brophy, P., Callan, J., Quinn, P., Nowakowski, P. and Crosby, T. (2005) The effect of varying levels of mineral and iodine supplementation to ewes during late pregnancy on serum immunoglobulin G concentrations in their progeny. *Animal Science* 80, 209–218.

Boland, T., Hayes, L., Sweeney, T., Callan, J., Baird, A., Keely, S. and Crosby, T. (2008) The effects of cobalt and iodine supplementation of the pregnant ewe diet on immunoglobulin G, vitamin E, T3 and T4 levels in the progeny. *Animal* 2, 197–206.

Brzezinska-Slebodzinska, E., Slebodzinski, A.B. and Kowalska, K. (2002) Evidence for the presence of 5′-deiodinase in mammalian seminal plasma and for

the increase in enzyme activity in the prepubertal testis. *International Journal of Andrology* 23, 218–224.

Buff, P.R., Messer, N.T., Cogswell, A.M., Wilson, D.A., Johnson, P.J., Keisler, D.H. and Ganjam, V.K. (2007) Induction of pulsatile secretion of leptin in horses following thyroidectomy. *Journal of Endocrinology* 193, 353–359.

Chilean Iodine Educational Bureau (1952) *Iodine Content of Foods*. CIEB, London.

Christopherson, R.J., Gonyon, H.W. and Thompson, J.R. (1979) Effects of temperature and feed intake on plasma concentrations of thyroid hormones in beef cattle. *Canadian Journal of Animal Science* 59, 655–661.

Clark, R.G. (1998) Thyroid histological changes in lambs from iodine supplemented and untreated ewes. *New Zealand Veterinary Journal* 46, 216–222.

Clark, R.G., Sargison, N.D., West, D.M. and Littlejohn, R.P. (1998) Recent information on iodine deficiency in New Zealand sheep flocks. *New Zealand Veterinary Journal* 46, 216–222.

Cromwell, G.L., Sihombing, D.T.H. and Hays, V.W. (1975) Effects of iodine level on performance and thyroid traits of growing pigs. *Journal of Animal Science* 41, 813–818.

Crush, J.R. and Caradus, J.R. (1995) Cyanogenesis potential and iodine concentration in white clover (*Trifolium repens* L.) cultivars. *New Zealand Journal of Agricultural Research* 38, 309–316.

Darras, V.M., Kotanen, S.P., Geris, K.L., Berghman, L.R. and Kuhn, E.R. (1996) Plasma thyroid hormone levels and iodothyronine deiodinase activity following acute glucocorticoid challenge in embryonic development compared with post-hatch chickens. *General and Comparative Endocrinology* 104, 203–212.

Donald, G.E., Langlands, J.P., Bowles, J.E. and Smith, A.J. (1993) Subclinical selenium insufficiency. 4. Effects of selenium, iodine and thiocyanate supplementation of grazing ewes on their selenium and iodine status and on the status and growth of their lambs. *Australian Journal of Experimental Agriculture* 33, 411–416.

Downer, J.V., Hemken, R.W., Fox, J.D. and Bull, L.J. (1981) Effect of dietary iodine on tissue iodine content of the bovine. *Journal of Animal Science* 52, 413–417.

EFSA (2013) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP): Scientific opinion on the safety and efficacy of iodine compounds (E2) as feed additives for all species: calcium iodate anhydrous and potassium iodide, based on a dossier submitted by HELM AG. *EFSA Journal* 11, 3101.

Ellis, K.J. and Coverdale, O.R. (1982) The effects on new-born lambs of administering iodine to pregnant ewes. *Proceedings of the Australian Society of Animal Production* 14, 660.

Emanuelson, M. (1994) Problems associated with feeding rapeseed meal to dairy cows. In: Garnsworthy, P.C. and Cole, D.J.A. (eds) *Recent Advances in Animal Nutrition – 1994*. Nottingham University Press, Nottingham, UK, pp. 189–214.

Erenberg, A., Omori, K., Menkes, J.N., Oh, W. and Fisher, D.A. (1974) Growth and development of the thyroidectomised ovine foetus. *Pediatrics Research* 8, 783–789.

Ferguson, K.A., Schinckel, P.G., Carter, H.B. and Clarke, W.H. (1956) The influence of the thyroid on wool follicle development in the lamb. *Australian Journal of Biological Sciences* 9, 575–585.

Fish, R.E. and Swanson, E.W. (1983) Effects of excessive iodide administered in the dry period on thyroid function and health of dairy cows and their calves in the periparturient period. *Journal of Animal Science* 56, 162–172.

Fitzgerald, S. (1983) The use of forage crops for store lamb fattening. In: Haresign, W. (ed.) *Sheep Production*. Butterworths, London, pp. 239–286.

Fletcher, A.L. Wilson, D.R., Maley, S., McCallum, J. and Shaw, M. (2010) The effect of sulphur and nitrogen fertiliser on levels of antinutritional compounds in kale. *Proceedings of the New Zealand Grassland Association 72, 79–84.*

Follett, B.K. and Potts, C. (1990) Hypothyroidism affects reproductive refractoriness and the seasonal oestrus period in Welsh Mountain ewes. *Journal of Endocrinology* 127, 103–109.

Franke, K, Meyer, U., Wagner, H. and Flachowsky, G. (2009 Influence of various iodine supplementation levels and two different iodine species on the iodine content of the milk of cows fed rapeseed meal or distillers dried grains with solubles as the protein source. *Journal of Dairy Science* 92, 4514–4523.

Freyberger, A. and Ahr, H.-J. (2005) Studies on the goitrogenic mechanism of action of N,N,N',N' tetramethylthiourea. *Toxicology* 217, 169–175.

Fuge, R. (2005) Soils and iodine deficiency. In: Selinus, O. and Alloway, B.J. (eds) *Essentials of Medical Geology*. British Geological Survey, Edinburgh, pp. 417–434.

Grace, N.D. (2006) Effect of ingestion of soil on the iodine, copper, cobalt (vitamin B_{12}) and selenium status of grazing sheep. *New Zealand Veterinary Journal* 54, 44–46.

Grace, N.D. and Waghorn, G.C. (2005) Impact of iodine supplementation of dairy cows on milk production and iodine concentrations in milk. *New Zealand Veterinary Journal* 53, 10–13.

Grace, N.D., Knowles, S.O. and Sinclair, G.R. (2001) Effect of pre-mating iodine supplementation of ewes fed pasture or a brassica crop pre-lambing on the incidence of goitre in newborn lambs. *Proceedings of the New Zealand Society of Animal Production* 61, 164–167.

Groppel, B. and Anke, M. (1986) Iodine content of feedstuffs, plants and drinking water in the GDR. In: Anke, M., Boumann, W., Braunich, H., Bruckner, B. and Groppel, B. (eds) *Spurenelement Symposium Proceedings*, Vol. 5. Iodine. Friedrich Schiller University, Jena, Germany, pp. 19–28.

Gurevich, G.P. (1964) Soil fertilization with coastal iodine sources as a prophylactic measure against endemic goiter. *Federation Proceedings* 23, T511–T514.

Haggard, D.L., Stowe, H.D., Conner, G.H. and Johnson, D.W. (1980) Immunological effects of experimental iodine toxicosis in young cattle. *American Journal of Veterinary Research* 41, 539–543.

Harington, C.R. (1953) *The Thyroid Gland, Its Chemistry and Physiology*. Oxford University Press, London.

Hartmans, J. (1974) Factors affecting the herbage iodine content. *Netherlands Journal of Agricultural Science* 22, 195–206.

Hemken, R.W. (1960) Iodine. *Journal of Dairy Science* 53, 1138–1143.

Hennessey, D.R. and Pritchard, R.K. (1981) Functioning of the thyroid gland in sheep infected with *Tricostrongylus colubroformis. Research in Veterinary Science* 30, 87–92.

Hercus, C.E., Benson, W.N. and Carter, C.L. (1925) Endemic goitre in New Zealand, and its relation to the soil-iodine. *Journal of Hygiene* 24, 321–402.

Hetzel, B.S. (1991) The international public health significance of iodine deficiency. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals, Dubrovnik*. IMI, Zagreb, pp. 7-1–7-3.

Hetzel, B.S. and Welby, M.C. (1997) Iodine. In: O'Dell, B.L. and Sunde, R.A. (eds) *Handbook of Nutritionally Essential Mineral Elements*. Marcel Dekker, New York, pp. 557–582.

Hill, R. (1991) Rapeseed meal in the diet of ruminants. *Nutrition Abstracts and Reviews, Series B* 61, 139–155.

Hill, R., Vincent, I.C. and Thompson, J. (1990) The voluntary food intake and weight gain of lambs given concentrate foods containing rapeseed meals with a range of glucosinolate contents. *Animal Production* 50, 587.

Hillman, D. and Curtis, A.R. (1980) Chronic iodine toxicity in dairy cattle: blood chemistry, leucocytes and milk iodine. *Journal of Dairy Science* 63, 55–63.

Hopkins, P.S. (1975) The development of the foetal ruminant. In: McDonald, I.W. and Warner, A.C.I. (eds) *Metabolism and Digestion in the Ruminant. Proceedings of the IVth International Symposium on Ruminant Physiology*, 7th edn. University of New England Publishing Unit, Armidale, Australia, pp. 1–14.

Howie, F., Walker, S.W., Akesson, B., Arthur, J.R. and Beckett, G.J. (1995) Thyroid extracellular glutathione peroxidase: a potential regulator of thyroid hormone synthesis. *Biochemical Journal* 308, 713–717.

Indergaard, M. and Minsaas, J. (1991) Animal and human nutrition. In: Gury, M.D. and Blunden, G. (eds) *Seaweed Resources in Europe: Uses and Potential*. John Wiley & Sons, New York.

Ingar, S.H. (1985) The thyroid gland. In: Wilson, J.D. and Foster, D.W. (eds) *Williams Textbook of Endocrinology*. Saunders, Philadelphia, Pennsylvania, pp. 682–815.

Irvine, C.H.G. (1967) Protein-bound iodine in the horse. *American Journal of Veterinary Research* 28, 1687–1692.

Iwarsson, K. (1973) Rapeseed meal as a protein supplement for dairy cows. 1. The influence of certain blood and milk parameters. *Acta Veterinaria Scandinavica* 14, 570–594.

Johns, A.T. (1956) The influence of high-production pasture on animal health. In: *Proceedings of the 7th International Grassland Congress,* Palmerston North, New Zealand, pp. 251–261.

Johnson, J.M. and Butler, G.W. (1957) Iodine content of pasture plants. 1. Method of determination and preliminary investigation of species. *Physiologia Plantarum* 10, 100–111.

Johnson, P.J., Messer, N.T., Ganjam, V.K., Thompson, D.L., Erefsal, K.R., Loch, W.E. and Ellersieck, M.R. (2003) Effects of propylthiouracil and bromocryptine on serum concentrations of thyrotropin and thyroid hormones in normal female horses. *Equine Veterinary Journal* 35, 296–301.

Jones, R.J. and Meggarity, R.G. (1983) Comparative responses of goats fed on *Leucena leucephola* in Australia and Hawaii. *Australian Journal of Agricultural Research* 34, 781–790.

Karmarkar, M.G., Deo, M.G., Kochupillai, N. and Ramalingaswami, V. (1974) Pathophysiology of Himalayan endemic goiter. *American Journal of Clinical Nutrition* 27, 96–103.

Kelly, F.C. (1953) Studies on the stability of iodine compounds in iodized salt. *Bulletin of the World Health Organization* 9, 217–230.

Khajarern, S., Khajarern, J.M., Kitpanit, N. and Muller, Z.O. (1977) Cassava in the nutrition of swine. In: Nestel, B. and Graham, M. (eds) *Cassava as Animal Feed. Proceedings of a Workshop Held at the University of Guelph*. International Development Research Centre, Ottawa, pp. 56–64.

King, C.F. (1976) Ovine congenital goitre associated with minimal thyroid enlargement. *Australian Journal of Experimental Agriculture and Animal Husbandry* 16, 651–655.

Knights, G.I., O'Rourke, P.K. and Hopkins, P.S. (1979) Effects of iodine supplementation of pregnant and lactating ewes on the growth and maturation of their offspring. *Australian Journal of Experimental Agriculture and Animal Husbandry* 19, 19–22.

Knowles, S.O. and Grace, N.D. (2007) A practical approach to managing the risks of iodine deficiency in flocks using thyroid-weight:birthweight ratios of lambs. *New Zealand Veterinary Journal* 55, 314–318.

Knowles, S.O. and Grace, N.D. (2015) Serum total iodine concentrations in pasture-fed pregnant ewes and newborn lambs challenged by iodine supplementation and goitrogenic kale. *Journal of Animal Science* 93, 425–432.

Kulper, G.G., Kester, M.H., Peeters, R.P. and Visser, T.J. (2005) Biochemical mechanisms of thyroid hormone deiodination. *Thyroid* 15, 787–798.

Kushadet, M.M., Brown, A., Kurilich, A.C., Juvik, J.A., Klein, B.P., Wallig, M.A. and Jeffery, E.H. (1999) Variation in glucosinolates in vegetable crops of *Brassica oleracea. Journal of Agricultural and Food Chemistry* 47, 1541–1548.

Laarveld, B., Brockman, R.P. and Christensen, D.A. (1981) The effects of Tower and Midas rapeseed meals on milk production and concentrations of goitrogens and iodine in milk. *Canadian Journal of Animal Science* 61, 131–139.

Lee, J.W., Wang, S., Huang, Y., Seefeldt, T., Donkor, A., Logue, B.A., and Woyengo, T.A. (2020) Toxicity of canola-derived glucosinolates in pigs fed resistant starch-based diets. *Journal of Animal Science* 98, skaa111. DOI: 10.1093/jas/skaa111

Lengemann, F.W. (1979) Effect of low and high ambient temperatures on metabolism of radioiodine by the lactating goat. *Journal of Dairy Science* 62, 412–415.

Livesey, C.T., Payne, J.H., Sayers, A.R., Whitaker, D.A., Burrough, E., MacRae, A.I. (2014) Thyroxine concentrations in ruminant health monitoring and diagnostic blood samples. *Veterinary Record* 174, 47.

Lüdke, H. and Schöne, F. (1988) Copper and iodine in pigs diets with high glucosinolate rapeseed meal. I. Performance and thyroid hormone status. *Animal Feed Science and Technology* 22, 33–43.

Lukuyu, B., Okike, I., Duncan, A., Beveridge, M. and Blümmel, M. (2014) *Use of cassava in livestock and aquaculture feeding programs*. ILRI Discussion Paper 25. International Livestock Research Institute, Nairobi.

Maas, J., Davis, L.E., Hempsteed, C., Berg, J.N. and Hoffman, K.A. (1984) Efficacy of ethylenediamine dihydriodide in the prevention of naturally occurring foot rot in cattle. *American Journal of Veterinary Research* 45, 2347–2350.

Maas, J., Berg, J.N. and Petersen, R.G. (1989) Serum distribution of iodine after oral administration of ethylenediamine dihydriodide in cattle. *American Journal of Veterinary Research* 50, 1758–1759.

Maddocks, S., Chandrasekhar, Y. and Setchell, B.P. (1985) Effect on wool growth of thyroxine replacement in thyroidectomised lambs. *Australian Journal of Biological Science* 38, 405–410.

MAFF (2000) Iodine in milk. *Food surveillance information sheet No. 198,* Joint Food Safety and Standards Group.

Marine, D. and Williams, W.W. (1908) The relation of iodine to the structure of the thyroid gland. *Archives of Internal Medicine* 1, 349–384.

Mason, R.W. (1976) Milk iodine content as an estimate of the dietary iodine status of sheep. *British Veterinary Journal* 132, 374–379.

Mason, R.W. and Laby, R. (1978) Prevention of ovine congenital goitre using iodine-releasing intramuscular devices: preliminary results. *Australian Journal of Experimental Agriculture and Animal Husbandry* 18, 653–657.

Mawson, R., Heaney, R.K., Piskula, M. and Kozlowska, H. (1993) Rapeseed meal-glucosinolates and their nutritional effects. Part I: Rapeseed production and chemistry of glucosinolates. *Die Nahrung* 37, 131–140.

McCoy, M.A., Smyth, J.A., Ellis, W.A. and Kennedy, D.G. (1995) Parenteral iodine and selenium supplementation in stillbirth perinatal weak calf syndrome. *Veterinary Record* 136, 124–126.

McCoy, M.A., Smyth, J.A., Ellis, W.A., Arthur, J.R. and Kennedy, D.G. (1997) Experimental reproduction of iodine deficiency in cattle. *Veterinary Record* 141, 544–547.

McDonald, R.C., Manley, T.R., Barry, T.N., Forss, D.A. and Sinclair, A.G. (1981) Nutritional evaluation of kale (*Brassica oleracea*) diets. 3. Changes in plant composition induced by fertility practices, with special reference to SMCO and glucosinolate concentrations. *Journal of Agricultural Science, Cambridge* 19, 27–33.

McDowell, L.R. (1992) *Iodine in: Minerals in Animal and Human Nutrition.* Academic Press, New York.

McGovern, F., Magee, D., Browne, J., MacHugh, D. and Boland, T. (2016) Iodine supplementation of the pregnant dam alters intestinal gene expression and immunoglobulin uptake in the newborn lamb. *Animal* 10, 598–606.

McGowan, A.C. (1983) The use of 'Lipiodol' for sub-clinical iodine deficiency in livestock. *Proceedings of the New Zealand Society of Animal Production* 43, 135–136.

Mee, J.F., Rogers, P.A.M. and O'Farrell, K.J. (1995) Effect of feeding a mineral-vitamin supplement before calving on the calving performance of a trace element deficient dairy herd. *Veterinary Record* 137, 508–512.

Menendez, C., Baldelli, R., Camina, J.P., Escudero, B., Peino, R., Dieguez, C. and Casanueva, F.F. (2003) TSH stimulates leptin secretion by a direct effect on adipocytes. *Journal of Endocrinology* 176, 7–12.

Meyer, U., Weigel, K., Schöne, F. and Flachowsky, G. (2008) Effect of dietary iodine on growth and iodine status of growing fattening bulls. *Livestock Science* 115, 219–225.

Miller, E.R. and Ammerman, C.B. (1995) Iodine bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 157–168.

Miller, J.K., Swanson, E.W. and Hansen, S.M. (1965) Effects of feeding potassium iodide, 3,5 diiodosalicylic acid or L-thyroxine on iodine metabolism of lactating dairy cows. *Journal of Dairy Science* 48, 888–894.

Miller, J.K., Swanson, E.W., Spalding, G.E., Lyke, W.A. and Hall, R.F. (1974) The role of the abomasum in recycling of iodine in the bovine. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Proceedings of the Second International Symposium on Trace Elements in Man and Animals*. University Park Press, Baltimore, Maryland, pp. 638–640.

Mitchell, H.H. and McClure, F.J. (1937) *Mineral Nutrition of Farm Animals*. Bulletin No. 99, National Research Council, Washington, DC.

Mitchell, J.H., Nicol, F., Beckett, G.J. and Arthur, J.A. (1997) Selenium and iodine deficiencies: effects on brain and brown adipose tissue selenoenzyme activity and expression. *Journal of Endocrinology* 155, 255–263.

Mostyn, A., Sebert, S., Litten, J.C., Perkins, K.S., Laws, J., Symonds, M.E. and Clarke, L. (2006) Influence of porcine genotype on the abundance of thyroid hormones and leptin in sow milk and its impact on growth, metabolism and expression of key adipose tissue genes in offspring. *Journal of Endocrinology* 190, 631–639.

Nazifi, S., Saeb, M., Rowghani, E. and Kaveh, K. (2003) The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T_3), thyroxine (T_4) and cortisol concentrations. *Comparative Clinical Pathology* 12, 135–139.

Newton, G.L., Barrick, E.R., Harvey, R.W. and Wise, M.B. (1974) Iodine toxicity, physiological effects of elevated dietary iodine on calves. *Journal of Animal Science* 38, 449–455.

Nicol, F., Lefrane, H., Arthur, J.R. and Trayhurn, P. (1994) Characterisation and postnatal development of 5′-deiodinase activity in goat perirenal fat. *American Journal of Physiology* 267, R144–R149.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2005) *Mineral Tolerance of Animals*, 2nd edn. National Academy of Sciences, Washington, DC.

NRC (2012) *Nutrient Requirements of Swine*, 11th rev. edn. NRC Press, Washington,DC.

O'Connor, D.M., Blache, D., Hoggard, N., Brookes, E., Wooding, F.B.P., Fowden, A.L. and Forhead, A.J. (2007) Developmental control of plasma leptin messenger ribonucleic acid in the ovine foetus during late gestation: role of glucocorticoids and thyroid hormones. *Endocrinology* 148(8), 3750–3757.

Orr, J.B. and Leitch, I. (1929) *Iodine in Nutrition: A Review of Existing Information*. Special Report Series, Medical Research Council, UK, No. 123.

Pattanaik, A.K., Khan, S.A., Varshney, V.P. and Bedi, S.P.S. (2001) Effect of iodine level in mustard (*Brassica juncea)* cake-based concentrate supplement on nutrient utilisation and serum thyroid hormones of goats. *Small Ruminant Research* 41, 51–59.

Pattanaik, A.K., Khan, S.A. and Goswami, T.K. (2011) Iodine supplementation to a diet containing *Leucaena leucocephala* leaf meal: consequences on nutrient metabolism, clinical chemistry and immunity of goats. *Animal Production Science* 51, 541–548.

Pedersen, I.B., Laurberg, P., Knudsen, N., Jorgensen, T., Perrild, H., Ovesen, L. and Rasmussen, L.B. (2007) An increased incidence of overt hypothyroidism after iodine fortification of salt in Denmark: a prospective population study. *Journal of Clinical Endocrinology and Metabolism* 92, 3122–3127.

Pezzi, C., Accorsi, P.A., Vigo, D., Govoni, N. and Gaiani, R. (2003) 5′-Deiodinase activity and circulating thyronines in lactating cows. *Journal of Dairy Science* 86, 152–158.

Phillips, D.I.W., Nelson, M., Barker, D.J.P., Morris, J.A. and Wood, T.J. (1988) Iodine in milk and the incidence of thyrotoxicosis in England. *Clinical Endocrinology* 28, 61–66.

Potter, B.J., McIntosh, G.H. and Hetzel, B.S. (1981) The effect of iodine deficiency on foetal brain development in the sheep. In: Hetzel, B.S. and Smith, R.M. (eds) *Fetal Brain Disorders – Recent Approaches to the Problem of Mental Deficiency*. Elsevier/North Holland Biomedical Press, Amsterdam, pp. 119–147.

Potter, B.J., Mano, M.T., Belling, G.B., McIntosh, G.H., Hua, C., Cragy, B.G., Marshall, J., Wellby, M.L. and Hetzel, B.S. (1982) Retarded fetal brain development resulting from severe iodine deficiency in sheep. *Applied Neurobiology* 8, 303–313.

Prichard, R.K., Hennessy, D.R. and Griffiths, D.A. (1974) Endocrine responses of sheep to infection with *Trichostrongylus colubriformis. Research in Veterinary Science* 17, 182–187.

Rey-Crespo, F., López-Alonso, M. and Miranda, M. (2014) The use of seaweed from the Galician coast as a mineral supplement in organic dairy cattle. *Animal* 8, 580–586.

Rhind, S.M. and McMillen, S.R. (1996) Effects of methylthiouracil treatment on the growth and moult of cashmere fibre in goats. *Animal Science* 62, 513–520.

Rogers, P.A.M., Lynch, P.J., Porter, W.L. and Bell, G.D (1998) A slow-release iodine, selenium and cobalt cattle bolus. *Cattle Practice* 6, 129–132.

Rogler, J.C. (1958) Effects of iodine on hatchability and embryonic development. *Dissertation Abstracts* 18, 1925–1926.

Rose, M., Wolf, B. and Haresign, W. (2007) Effect of the level of iodine in the diet of pregnant ewes on the

concentration of immunoglobulin G in the plasma of neonatal lambs following the consumption of colostrum. *British Journal of Nutrition* 97, 315–320.

Rowntree, J.E., Hill, G.M., Hawkins, D.R., Link, J.E., Rincker, M.J., Bednar, G.W. and Kreft, R.A. Jr (2004) Effect of selenium on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. *Journal of Animal Science* 82, 2995–3005.

Sahin, K. and Kocuk, O. (2007) Selenium supplementation in heat-stressed poultry. *CAB Reviews: Perspectives in Agriculture, Veterinary Science and Natural Resources* 2, 1–8.

Sargison, N.D., West, D.M. and Clark, R.G. (1998) Effects of iodine deficiency on ewe fertility and perinatal lamb mortality. *New Zealand Veterinary Journal* 46, 72–75.

Schöne, F., Lüdke, H., Hennig, A. and Jahreis, G. (1988) Copper and iodine in pig diets with high glucosidolate rapeseed meal. II. Influence of iodine supplements for rations with rapeseed meal untreated or treated with copper ions on performance and thyroid hormone status of growing pigs. *Animal Feed Science and Technology* 22, 45–59.

Schöne, F., Jahreis, G., Richter, G. and Lange, R. (1992) Evaluation of rapeseed meals: effects of iodine supply and glucosinolate degradation by myrosinase or copper. *Journal of the Science of Food and Agriculture* 61, 245–252.

Scott, M.L., van Tienhoven, A., Holm, E.R. and Reynolds, R.E. (1960) Studies on the sodium, chlorine and iodine requirements of pheasants and quail. *Journal of Nutrition* 71, 282–288.

Siebert, B.D. and Hunter, R.A. (1982) In: Hacker, C.B. (ed.) *Nutritional Limits to Animal Production from Pasture.* Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 409–425.

Sihombing, D.T.H., Cromwell, G.L. and Hays, V.W. (1974) Effects of protein source, goitrogens and iodine level on performance and thyroid status of pigs. *Journal of Animal Science* 39, 1106–1112.

Sinclair, D.P. and Andrews, E.D. (1958) Prevention of goitre in new-born lambs from kale-fed ewes. *New Zealand Veterinary Journal* 6, 87–95.

Sinclair, D.P. and Andrews, E.D. (1961) Deaths due to goitre in new-born lambs prevented bv iodized poppyseed oil. *New Zealand Veterinary Journal* 9, 96–100.

Singh, J.L., Sharma, M.C., Kumar, M., Gupta, G.C. and Kumar, S. (2005) Immune status of goats in endemic goitre and its therapeutic treatment. *Small Ruminant Research* 63, 249–255.

Slebodzinski, A.B., Brzezinska-Slebodzinska, E., Nowak, J. and Kowalska, K. (1998) Triiodothyronine (T3), insulin and characteristics of 5′-monodeiodinase (5′-MD) in mare's milk from parturition to 21 days post-partum. *Reproduction Nutrition and Development* 38, 235–244.

Smyth, J.A., McNamee, P.T., Kennedy, D.G., McCullough, S.J., Logan, E.F. and Ellis, W.A. (1992) Stillbirth/perinatal weak calf syndrome: preliminary pathological, microbiological and biochemical findings. *Veterinary Record* 130, 237–240.

Spiegel, C., Bestetti, G.E., Rossi, G.L. and Blum, J.W. (1993) Normal circulating tri-iodothyronine concentrations are maintained despite severe hypothyroidism in growing pigs fed rapeseed presscake meal. *Journal of Nutrition* 123, 1554–1561.

Statham, M. and Bray, A.C. (1975) Congenital goitre in sheep in southern Tasmania. *Australian Journal of Agricultural Research* 26, 751–768.

Statham, M. and Koen, T.B. (1981) Control of goitre in lambs by injection of ewes with iodized poppy seed oil. *Australian Journal of Experimental Agriculture and Animal Husbandry* 22, 29–34.

Stewart, A.G. and Pharaoh, P.O.D. (1996) Clinical and epidemiological correlates of iodine deficiency disorders. In: Appleton, J.D., Fuge, R. and McCall, G.J.H. (eds) *Environmental Geochemistry and Health*. Geological Society Special Publication No.113, London, pp. 223–230.

Stoewsand, G.S. (1995) Bioactive organosulfur phytochemicals in Brassica oleracea – a review. *Food Chemistry and Toxicology* 33, 537–543.

Sullivan, K.M., May, W., Nordenberg, D., Houston, R. and Maberly, G.F. (1997) Use of thyroid stimulating hormone testing in newborns to identify iodine deficiency. *Journal of Nutrition* 127, 55–58.

Sutcliffe, E. (1990) Iodine in New Zealand milk. *Food Technology in New Zealand* 25, 32–38.

Symonds, M.E. and Clarke, L. (1996) Influence of thyroid hormones and temperature on adipose tissue development and lung maturation. *Proceedings of the Nutrition Society* 55, 561–569.

Taraz, Z., Jalali, S.M.A. and Rafeie, F. (2006) Effects of replacement of soybean meal with rapeseed meal on organ weight, some blood biochemical parameters and performance of broiler chicks. *International Journal of Poultry Science* 5, 1110–1115.

Todini, L. (2007) Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. *Animal* 1, 997–1008.

Todini, L., Malfatti, A., Valbonesi, A., Trabalza-Marinucci, M. and Debenedetti, A. (2007) Plasma total T3 and T4 concentrations in goats at different physiological stages as affected by energy intake. *Small Ruminant Research* 68, 285–290.

Underwood, E.J. and Suttle, N.F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Van der Reijden, O., Galetti, V., Herter-Aeberli, I., Zimmermann, M., Zeder, C., Krzystek, A. *et al.* (2019) Effects of feed iodine concentrations and milk processing on iodine concentrations of cows' milk and dairy products, and potential impact on iodine

intake in Swiss adults. *British Journal of Nutrition* 122, 172–185.

Villar, D., Rhind, S.M., Dicks, P., McMillen, S.R., Nicol, F. and Arthur, J.R. (1998) Effect of propylthiouracil-induced hypothyroidism on thyroid hormone profiles and tissue deiodinase activity in cashmere goats. *Small Ruminant Research* 29, 317–324.

Villar, D., Nicol, F., Arthur, J.R., Dicks, P., Cannavan, A., Kennedy, D.G. and Rhind, S.M. (2000) Type II and type III monodeiodinases in the skin of untreated and propylthiouracil-treated cashmere goats. *Research in Veterinary Science* 68, 119–123.

Voudouri, A., Chadio, S.E., Menegatos, J.G., Zervas, G.P., Nicol, F. and Arthur, J.R. (2003) Selenoenzyme activities in selenium- and iodine-deficient sheep. *Biological Trace Element Research* 94, 213–224.

Wainwright, P. and Cook, P. (2019) The assessment of iodine status – populations, individuals and limitations. *Annals of Clinical Biochemistry* 56, 7–14.

Wallace, A.L.C., Gleeson, A.R., Hopkins, P.S., Mason. R.W. and White, R.R. (1978) Plasma thyroxine concentrations in grazing sheep in several areas of Australia. *Australian Journal of Biological Science* 31, 39–41.

Walton, E.A. and Humphrey, J.D. (1979) Endemic goitre of sheep in the highlands of Papua New Guinea. *Australian Veterinary Journal* 55, 43–44.

Wassen, F.W., Klootwijk, W., Kaptein, E., Duncker, D.J., Visser, T.J. and Kulper, G.G. (2004) Characteristics and thyroid state-dependent regulation of iodothyronine deiodinases in pigs. *Endocrinology* 145, 4251–4263.

Wells, N.H., Halford, D.M. and Hernandez, J.A. (2003) Serum thyroid hormones and reproductive characteristics of Rambouillet ewe lambs treated with propylthiouracil before puberty. *Theriogenology* 59, 1403–1413.

Wheeler, J.L. and Vickery, P.J. (1989) Variation in HCN potential among cultivars of white clover (*Trifolium repens* L). *Grass and Forage Science,* 44, 107–109.

Whitehead, D.C. (1975) Uptake by perennial ryegrass of iodide, elemental iodine and iodate added to soil as influenced by various amendments. *Journal of the Science of Food and Agriculture* 26, 361–367.

Wichtel, J.J., Craigie, A.L., Freeman, D.A., Varela-Alvarez, H. and Williamson, N.B. (1996) Effect of selenium and iodine supplementation on growth rate and on thyroid and somatotropic function in dairy calves at pasture. *Journal of Dairy Science* 79, 1865–1872.

Wilson, J.G. (1975) Hypothyroidism in ruminants with special reference to foetal goitre. *Veterinary Record* 97, 161–164.

Zagrodzki, P., Nicol, F., McCoy, M.A., Smyth, J.A., Kennedy, D.G., Beckett, G.J. and Arthur, J.R. (1998) Iodine deficiency in cattle: compensatory changes in thyroidal selenoenzymes. *Research in Veterinary Science* 64, 209–211.

Zimmermann, M.B., Jooste, P.L. and Pandav, C.S. (2008) Iodine-deficiency disorders. *Lancet* 372, 1251–1262.

13 Iron

Iron is by far the most abundant trace element in the body and its value as a dietary constituent has been appreciated for over 2000 years. A connection between iron intake and blood disorders was proposed in the 16th century but a physiological link did not become apparent until horse haemoglobin (Hb) was shown to contain 0.335% Fe (Zinoffsky, 1886). A causal link was confirmed when blood Hb in anaemic women was rapidly increased by supplementation with diverse Fe compounds (Stockman (1893). Approximately 60% of body Fe is present as Hb in the bloodstream and rapid expansion of red cell mass greatly increased demands for Fe and risk of anaemia in the newborn, as first demonstrated in chicks (Elvehjem and Hart, 1929). For many years, nutritional interest was focused on the role of Fe in haematopoiesis but widened when Fe was found in the ubiquitous cytochrome oxidase (CCO) enzyme group (Gubler *et al*., 1957). Iron is now known to affect cell growth, development and gene expression (Wang and Pantopoulos, 2011). Iron-deficiency affects roughly 30% of the human population (Zimmerman and Hurrell, 2007), severely so in 0.9% of infants and 1.5% of pregnant women (WHO, 2015). Most research on Fe nutrition is now conducted with human needs in mind, often using piglets as models for the young infant (Antonides *et al*., 2015; Valcarcel *et al*., 2018). Investigations are driven by indications that early deprivation has long-term, detrimental effects on cognitive behaviour but the pig model may exaggerate such risks (p. 327): implications for livestock behaviour have yet to be considered. Fortunately, Fe supplies are rarely inadequate for older farm livestock and problems are far more likely to be presented by the pro-oxidant capacity of excess ferric (Fe^{3+}) Fe in diet and tissues.

Functions of Iron

Flexible co-ordination chemistry and redox reactivity allow Fe to form clusters with sulfur (S), which contribute to many cell pathways in mammals and microbes, including maintenance of DNA integrity, regulation of gene expression and protein translation, energy production and antiviral responses (Cardenas-Rodriguez *et al*., 2018). Iron is incorporated by mitochondria into Fe-S clusters, metal cofactors that comprise the largest class of metalloproteins. Redox activity allows Fe to influence cell-signalling, which can be disrupted *in vitro* by Fe chelation (Watson *et al*., 2016).

Early interests focused on the haem molecule, with its four Fe atoms, one in the centre of each of four linked porphyrin rings. A complex of haem and globin, Hb, is packaged in erythrocytes and allows tissues to 'breathe' by carrying O_2 from the lungs as oxyHb via arterial blood and returning carbon dioxide as carboxyHb via the venous circulation. Myoglobin is a simpler, less abundant Fe-porphyrin found in muscle, where high affinity for O_2 completes the transfer from oxyHb into the cell.

Although Fe is mostly present in erythropoietic tissues, significant amounts are found in macrophages and muscle, where ability to change between divalent (Fe^{2+}) and trivalent (Fe^{3+}) states allows other haem-containing proteins, including cytochromes a, b and c, to participate in the electron-transfer chain, on which cell respiration depends. By activating or assisting flavoprotein enzymes such as succinate dehydrogenase, Fe facilitates energy metabolism at every stage of the tricarboxylic acid (Krebs) cycle. Iron-containing catalases and peroxidases remove potentially dangerous products of metabolism. Connective tissue development is influenced by Fe-activated hydroxylases (O'Dell, 1981).

Clinical Signs that Diets Lack Iron

Anaemia

Lack of dietary Fe causes such marked reductions in blood Hb and haematocrit (packed cell volume,

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PCV): appetite decreases, animals become lethargic, visible mucous membranes are blanched and respiration rate increases. Piglets are particularly susceptible but anaemia becomes slower to develop with increasing age at onset of Fe deprivation. The anaemia is microcytic and hypochromic, newly released erythrocytes being smaller and containing less Hb than normal, compounding effects of a decrease in erythrocyte (rbc) count. Mortality in piglets is high but survivors slowly recover at about 6–7 weeks of age. Anaemic piglets are listless and flabby, appetite is poor, growth subnormal, skins become wrinkled, hair rough and incidence of diarrhoea is high. *Post-mortem* examination reveals a normoblastic, hyperplastic bone marrow containing little haemosiderin and a dilated heart, surrounded by excess pericardial fluid: lungs are oedematous (Seamer, 1956). Cardiac hypertrophy is found in all livestock species deprived of Fe, including the chick (Aoyagi and Baker, 1995), due to a combination of increased heart rate and defective cellular metabolism. Severe, debilitating anaemia, with similar characteristics to piglet anaemia, was produced experimentally in calves within 8–10 weeks by rearing them exclusively on cow's milk (Blaxter *et al*., 1957).

Cognitive dysfunction

Iron-deficiency anaemia in children was associated with loss of cognitive function but could not be unequivocally dissociated from impaired motor activity, i.e. lethargy (McCann and Ames, 2007). A 'marginal' iron intake lowered brain Fe and diminished startle responses and maze performance in mice, defects that were not completely reversed by subsequent Fe supplementation (Kwik-Uribe *et al*., 2000). In piglets, used as a surrogate for human infants, growth, acquisitive skills and memory were impaired at 7–10 weeks of age, having been allowed to become severely anaemic for the first three weeks of life (Antonides *et al*., 2015). Certain regions of the hippocampus had fewer Fe-staining cells. Anaemic piglets had increased in birthweight almost 30-fold by 12 weeks but brain weight, relative to body weight, was higher than that in Fe-replete controls (0.20 ± 0.02 versus 0.16 ± 0.005). Infants take five months to double birth weight and longer to double brain size, when well nourished.

- Coupled with greater competition for Fe from a rapidly expanding erythropoietic system, the piglet is arguably an unsuitable model for studying possible cognitive impact of Fe deprivation in children.

Hypothyroidism was reported in severely Fe-deprived rats and thermoregulation was impaired under cold-stress (Beard *et al*., 1989). It is possible that slow learning and poor memory in anaemic piglets is partially due to hypothyroidism. Simultaneous treatment of infants with Fe and I is more effective in treating infant goitre than treatment with I alone (Zimmerman *et al*., 2000).

- Low environmental temperatures may exacerbate clinical effects of Fe deprivation in young farm animals, born and reared in cold conditions.
- Transitory anaemia is seen in many newborn lambs, kids and calves shortly after birth but is less likely to impair cognitive dysfunction than in the faster-growing piglet.

Susceptibility to infection

Impaired immune responses in iron deprivation have been widely studied and the complex underlying mechanisms reviewed (Cherayil, 2011). Iron-deprived piglets were more susceptible to *Escherichia coli* endotoxin than healthy animals (Osborne and Davis, 1968). Both innate and cellular immunity can be compromised but such changes do not necessarily translate to increased susceptibility to infection. Indeed, systemic Fe deprivation is an immune defence mechanism (p. 331) and mild deprivation can occasionally improve resistance to infection. In piglets infected with porcine reproductive and respiratory syndrome virus, acute Fe deprivation (10 versus 100mg Fe kg^{-1}DM in milk replacer from 2 d.o.) decreased peripheral immune function leading to increased viraemia: immune activity in the brain was protected in the face of severe anaemia (final Hb <30g l^{-1} after 28 days; Leyshon *et al*., 2019). Decreased villus height, impaired mucosal integrity and reduced expression of immuno-associated factors in Fe-deprived piglets (Pu *et al*., 2018) may weaken resistance to gut infection but causal links are awaited. Where outcome of infection has been affected, impact varied from pathogen to pathogen. Anaemic calves, reared on a milk replacer containing 10mg Fe kg^{-1}DM, were more susceptible to infections (mostly pneumonias) than calves given five times more Fe: cellular immunity rather than humoral immunity was

compromised.With gut parasites, Fe deprivation in piglets increased susceptibility to *Trichuris suis* but not *Ascaris suis* (Pederson *et al*., 2001). The rapidity and extent with which anaemia develops in newborn piglets exceeds that found in other species and older animals.

- Using piglets as models for humans and other species may exaggerate respective susceptibilities to infection.

Occurrence of Dietary Iron Insufficiency

Farm livestock, other than piglets, rarely lack dietary Fe, especially grazing animals, whose intakes of contaminant soil Fe are often high. Confinement increases the possibility of Fe deprivation in young sucking animals and those artificially reared on milk or milk products, when extraneous sources of Fe are minimal. High egg production in poultry is sometimes accompanied by an anaemia that is not iron-responsive.

Neonatal anaemia

Piglets often became anaemic within 2–4 weeks of birth, by which time Hb may have fallen to 30–40 g l^{-1} and PCV to 10%, one third of normal values. The condition acquired the name 'thumps' because of laboured, spasmodic breathing (McGowan and Crichton, 1924; Hart *et al*., 1930) and occurs wherever pigs are housed in concrete pens and denied access to soil or pasture, unless preventive measures are taken: piglets reared outdoors were rarely affected. Newborn piglets absorb colostrum proteins intact into the bloodstream and plasma volume increases markedly during the first 12 hours of nursing without much change in red blood cell volume, causing a physiological anaemia (Underwood and Suttle, 1999): this is compounded by relatively low body iron stores at birth, large litter size and rapid early growth rate compared with other species. Piglets can increase their birth weight (about 1.5 kg) fourfold by 3 weeks (Fig. 13.1), requiring retention of 7–11 mg Fe day^{-1}, whereas sow's milk provides only 1 mg day^{-1} (Venn *et al*., 1947). Early weaning accelerates recovery

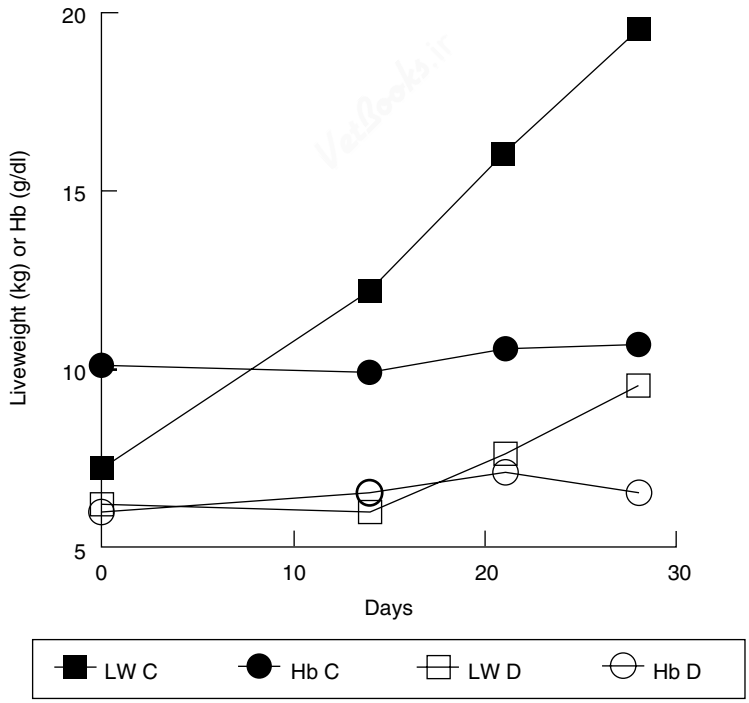

Fig. 13.1. Effect of reducing a conventional injection of iron dextran at birth from 180 mg (Group D) to 30 mg (Group C) on liveweight (LW) and blood haemoglobin (Hb) after weaning onto a cereal-based diet, containing 109 mg (D) or 229 mg (C) Fe kg^{-1}DM at 21 days of age: piglets deprived of Fe at birth are anaemic and stunted when weaned and recover body weight before Hb afterwards. (Data from Valcarcel *et al*., 2018)

from anaemia, while late weaning in organic rearing systems increases susceptibility (Heidbüchel *et al.*, 2019). Older pigs do not develop Fe-responsive anaemia unless dietary Cu is raised to stimulate growth.

Anaemia in newborn lambs and calves is far less prevalent and severe than in piglets. Transient, mild anaemia was reported in young, naturally reared calves (30% with <90 g Hb l^{-1}) (Hibbs *et al.*, 1963; Atyabi *et al.*, 2006) and in lambs reared indoors (Ullrey *et al.*, 1965; Green *et al.*, 1993, 1997), responding to Fe supplementation in both species (Carleson *et al.*, 1961; Ullrey *et al.*, 1965; Kume and Tanabe, 1994). Rearing suckled calves on pucca floors rather bare soil paddocks induced anaemia (Bhardwaj *et al.*, 2010). Twin calves are more susceptible than single calves because they compete for a limited maternal supply of Fe (Kume and Tanabe, 1994) and the same may be true of twin lambs. Short periods of anaemia may not affect performance. Growth was not improved by raising dietary Fe in mildly anaemic lambs (minimum Hb value 81 g l^{-1}over four weeks) (Bassett *et al.*, 1995). Lamb weaning weight was improved by 1 kg in a large study with 525 lambs, some of which received injections of Fe-dextran (Green *et al.*, 1997). Responsiveness to Fe may reflect the small proportion of clinically affected individuals (e.g. 4–14%) (Green *et al.*, 1993). Iron supplementation has improved calf growth (Carleson *et al.*, 1961). Use of vegetable protein concentrates in milk replacers may lower availability of dietary Fe and use of urea in rations for weaned ruminants will lower dietary Fe.

Abomasal ulceration in veal calves

Induction of anaemia was a requisite for 'white veal' production in which energy-rich milk substitutes, low in Fe, were fed to closely confined calves in dark buildings. Apparent tolerance of anaemia was demonstrated by rapid growth rates sustained with blood Hb 50% below the norm. However, the presence of abomasal ulcers in the majority of veal calves inspected at abattoirs added to already serious welfare concerns. Many causative factors have been postulated, foremost of which is pressure ischaemia in the pyloric mucosa after large meals (NFACC, 2016). A role of iron deficiency was discounted (Bus *et al.*, 2019) but abomasal ulceration occurred less frequently in the production of 'pink' or 'rose' veal, an alternative system involving less severe Fe deprivation and freedom of movement (Moran, 1990).

- It is plausible that flow of blood with diminished O_2-carrying capacity through a stretched abomasal musosa hastens onset of ischaemia and cell death.

Physiochemical Changes on Diets Lacking Iron

Normality

The metabolism of iron in livestock was reviewed by Harvey (2008). Iron is absorbed primarily from the duodenum by a two-stage process involving mucosal uptake and serosal transfer. At the mucosal stage, haem and non-haem Fe are processed and regulated differently[i] but both become bound to a specific transport protein, ferroportin (Wang and Pantopoulos, 2011). Serosal efflux is completed by binding to hephaestin (a Cu-dependent homologue of caeruloplasmin, Cp, p. 258) and possibly to Cp itself, which oxidizes Fe^{2+} to Fe^{3+}. Ferroportin-mediated efflux is negatively regulated by hepcidin, allowing Fe to be absorbed according to need (Fig. 13.2) and become affected by age and iron storage as well as dietary source. Efficiency of iron absorption (A_{Fe}) is maximal in young, iron-deprived animals such as the anaemic baby pig (Matrone *et al.*, 1960; Ullrey *et al.*, 1965) and during pregnancy. The influence of body Fe stores was confirmed by a negative exponential relationship between ^{59}Fe retention and serum ferritin, which reflected how much iron lies stored (Fig. 13.3). Superior availability of haem Fe over non-haem Fe in non-ruminants was also confirmed: both were influenced by body Fe status but an increase in dietary Fe only decreased A_{Fe} of non-haem Fe. At high Fe intakes, non-haem iron is trapped in the mucosa as ferritin.

On entering plasma, Fe^{3+} binds to apotransferrin and receptors for transferrin to carry Fe across cell membranes by endocytosis: Fe is then reduced and incorporated into functional forms, chiefly in mitochondria (Wang and Pantopoulos, 2011), where DMT1 facilitates uptake (Wolff *et al.*, 2018). Transferrin also recycles Fe from aged erythrocytes via the reticuloendothelial system (Liebold and Guo, 1992; Wang and Pantopoulos, 2011), where hepcidin, synthesized mainly in liver and regulated by free Fe^{2+} concentration, receives Fe from transferrin and partitions it between functional and storage pathways. (Wang and Pantopoulos, 2011).[ii] Ferritin and haemosiderin are the main iron-storage compounds of the

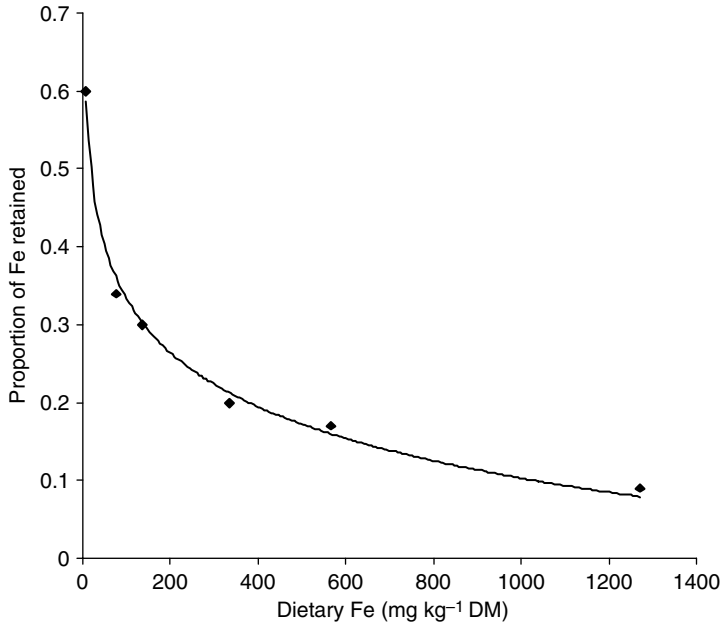

Fig. 13.2. Iron is absorbed according to need and retention of ^{59}Fe from a labelled wheat meal fell exponentially as previous dietary iron concentration increased in rats. (Data from Fairweather-Tait and Wright, 1984)

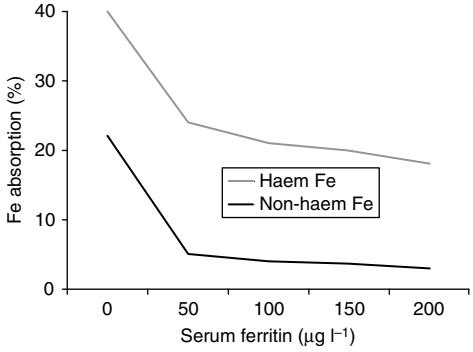

Fig. 13.3. Effect of body iron stores, as indicated by serum ferritin concentration, on retention of haem and non-haem iron in humans. Whole-body retention of iron was measured after dual labelling with ^{55}Fe and ^{59}Fe in volunteers: those with lowest iron stores absorbed and retained iron with highest efficiency, regardless of Fe source, but haem Fe was preferentially absorbed regardless of body stores. (Data from Roughead and Hunt, 2000)

body.[iii] Ferritin synthesis is facilitated by chaperones, abundant in gut and liver, and can act as a ferroxidase. Ferritin Fe remains bioavailable and is mobilized by lysosomal and proteasomal pathways when ferroportin is induced, thus playing a key role in homeostasis (Wang and Pantopoulos, 2011). Serum ferritin is highly correlated with body iron stores in humans. Haemosiderin accumulates once a high Fe status is attained and contains 35% Fe, primarily as ferritin degradation products and iron oxide clusters. Neither urinary nor endogenous faecal excretion play a significant part in iron homeostasis (Kreutzer and Kirchgessner, 1991).

Depletion

The sequence of changes when Fe requirements are not met is typical of a Type 1 mineral (Fig. 3.2). Iron stored as ferritin and haemosiderin in liver, kidneys, spleen and mucosa is depleted and serum ferritin decreases (e.g. piglets; Valcarcel *et al.*, 2018). Duration of depletion is determined by size of the initial iron reserve – which can vary from 20 to 540 mg Fe in newborn calves (Charpentier *et al.*, 1966, cited by ARC, 1980) – and shortfall in daily dietary Fe supply. In calves depleted with a milk diet containing only 5mg Fe kg^{-1}DM, the blood Fe pool *increased* by 750 mg and the muscle iron pool by 250 mg in three months, liver and diet contributing Fe in roughly equal measure: body Fe is redistributed. Hepcidin and DMT1 are widely upregulated during depletion, enhancing efficiency of iron utilization.

Deficiency

Serum Fe decreases, while both total iron-binding capacity (TIBC) and unsaturated iron-binding capacity (UIBC) can increase as transferrin receptors are upregulated in a concerted drive to maintain Fe status (Baynes, 1997). However, raising dietary Fe from 42 to 142mg kg^{-1}DM did not affect Fe distribution in plasma in 21–42 d.o. chicks that were neither anaemic nor growth-retarded (Liao *et al.*, 2017). Changes in serum Fe distribution in piglets were less clear-cut at weaning than those in haematological parameters (Fig. 13.4), due partly to two- to tenfold variation in parameters within the Fe-supplemented group (not shown): serum ferritin was uniformly low in piglets deprived of Fe (around 20g/l). Reticulocytes contain more metallothionein (Robertson *et al.*, 1989) and less Hb (Valcarcel *et al.*, 2018), reflecting changed erythropoietic activity. In pigs and calves, blood catalase activity decreases before blood Hb (Grassmann and Kirchgessner, 1973). Myoglobin concentrations and cytochrome c oxidase (Cox) activities were much reduced (Gubler *et al.*, 1957). The order and extent of biochemical changes vary between species. In iron-deprived chicks, blood Hb decreased earlier than either muscle myoglobin or liver Cox and SDH activities (Davis *et al.*, 1962). In calves, myoglobin has priority over Hb for scarce supplies of iron: surplus activities of such enzymes as catalase and Cox may explain why they decrease before growth is retarded in calves (ARC, 1980). Mild anaemia (70 g Hb l^{-1}) neither impaired O_2 transport and consumption nor increased heart rate in penned calves during unaccustomed exercise (Bremner *et al.*, 1976). A similar degree of iron deprivation induced biochemical and histological evidence of inflammation in the duodenum (Li *et al.*, 2016). Mildly anaemic animals may absorb increased amounts of potentially toxic metals (e.g. Pb and Cd and essential elements (e.g. Cu and Mn) as secondary consequences of increased mucosal DMT1 (Jiang *et al.*, 2013).

Dysfunction

Dysfunction is confirmed when growth becomes retarded but onset varies between species. Piglets become severely anaemic before growth is retarded (Antonides *et al.*, 2015) but anaemia is a later consequence of Fe deprivation in lambs (Bassett *et al.*, 1995) and calves (Bremner *et al.*, 1976). The haematological and biochemical changes of piglets are illustrated in Figs 13.1 and 13.4. Activities of Fe-dependent

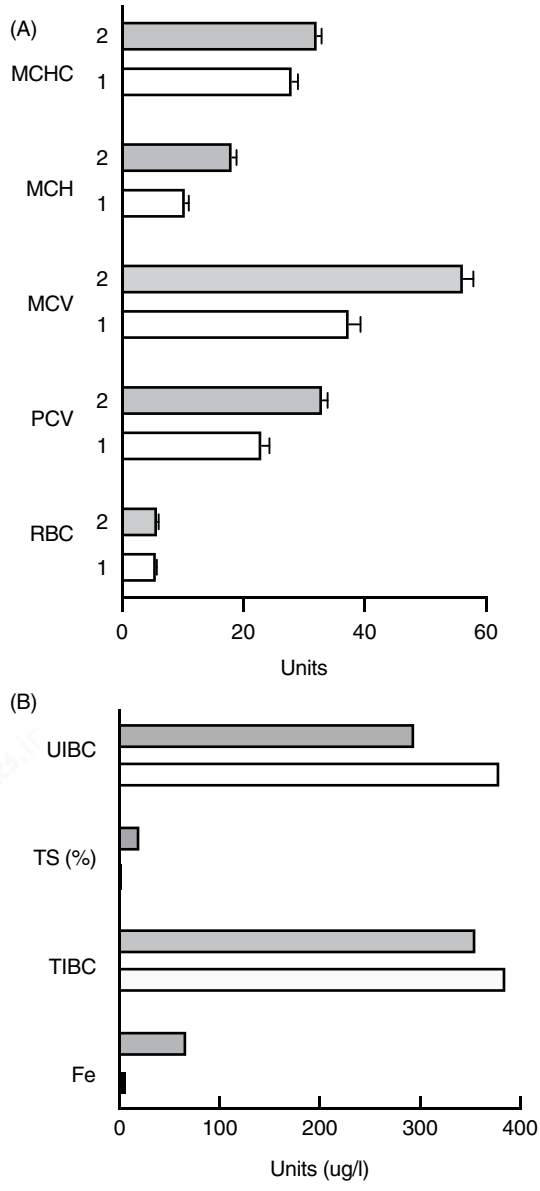

Fig. 13.4. (A) Anaemia in piglets deprived of iron (open columns) is characterized by reductions in red blood cell size (MCV, fl) but not count (rbc, 10^6cells/µl) and reductions in haemoglobin (Hb) content of each rbc (MCH, µg), the volume of packed cells (PCV, %) and Hb concentration in the PCV (g/dl). (B) Corresponding differences in iron status were relatively small and there was overlap between groups. (From Valcarcel *et al.*, 2018; the different treatments of control (solid columns) and deprived piglets were given in Fig. 13.1)

enzymes become so diminished that anaerobic glycolysis and lactate–glucose recycling increase, leading to inefficient glucose utilization (Hostettler-Allen *et al*., 1993). Anaemic calves are prone to oxidative stress (Rajabian *et al*., 2017). Mechanistic *in vitro* studies, in which single-cell cultures are acutely depleted of Fe by an iron chelator such as desferrioxamine reveal hitherto unknown consequences of deprivation, such as disruption of rapamycin-related cell-signalling (Watson *et al*., 2016).

- The point and site at which cell-signalling is disrupted during Fe deprivation *in vivo* is currently unknown and may provide a novel indicator of dysfunction.

Diagnosis of Iron Insufficiency

Haematology

Haematological criteria are non-specific indicators of Fe-responsive disorder and require careful interpretation (Roland *et al*., 2014). Most haematological values are low at birth, subsequently increase in lambs (Ullrey *et al*., 1965) and piglets (Miller, 1961; Antonides *et al*., 2015), and are not influenced by dietary Fe during pregnancy, even in piglets. In calves, most haematological parameters decline during the first month of life from the upper to lower limit of the broad ranges quoted, mean cell Hb concentration (MCHC) being the exception (Knowles *et al*., 2000: Mohri *et al*., 2007; Roland *et al*., 2014). Other factors, notably infection, can cause anaemia. Departure from normality is interpreted differently in livestock and humans, indicated in Table 13.1. The high incidence of 'iron deficiency' in humans (WHO, 2015) is based on the proportion of the global population with blood Hb <120 g Hb l^{-1}. Veterinarians are more circumspect because benefits of intervention supervene at much lower thresholds. Reductions in Hb (and PCV) can be tolerated: in veal calves, blood Hb <90 g l^{-1} was considered 'borderline' (Welchman *et al*., 1988); in piglets <70 g Hb l^{-1} indicated dysfunction (Ishaya, 2012); in lambs <50–60 g Hb l^{-1} produced clinically obvious pallor (Green *et al*., 1993). Diagnosis of Fe insufficiency is greatly strengthened by presence of small erythrocytes (low mean cell volume, MCV) with normal Hb concentrations (MCH). (Fig. 13.4): MCHC declined relatively late in piglets, causing a 'microcytic' anaemia to become 'hypochromic' (Valcarcel *et al*., 2018).

Serum and liver iron

Biochemical abnormalities are used in conjunction with haematological criteria to confirm lack of dietary Fe and diagnostic guidelines are given in Table 13.2. Plasma contains Fe bound to a glycoprotein, transferrin measured as TIBC and apotransferrin provides UIBC; the proportion of TIBC:UIBC (total saturation, TS, %) is a measure of Fe status. Low serum Fe and ferritin, accompanied by increased TIBC and UIBC, confirm iron insufficiency but normal values vary widely. There are significant differences between cows and newborn calves, calves having a higher TIBC that can remain elevated for up to two months. Serum Fe in dam and calf are similar at birth but normally decline in the calf thereafter (Atyabi *et al*., 2006). Iron deprivation would also be indicated by low liver Fe, a marginal band of 75–100mg Fe kg^{-1}DM distinguishing Fe-deprived from normal piglets (Ullrey *et al*., 1960): similar bands are given for other species in Table 13.2. Serum ferritin is of predictive rather than diagnostic value in all species because values become minimal before anaemia develops. Serum Fe in poultry varied with age and egg laying, averaging 2.25mg l^{-1} (range 1.73–2.90) in immature pullets and rising to 6.7–9.4 mg l^{-1} at onset of lay (Ramsay and Campbell 1954); diagnostic limits must change accordingly (Table 13.2).

Table 13.1. Reference ranges for haematological parameters in domestic livestock: values below the bold figures indicate anaemia. (From Fielder, 2015)

Class	Hb (g l^{-1})	PCV (%)	RBC (x10^6µl^{-1})	MCH (fl)	MCHC (g l^{-1})	MCV **(fl)**
Cattle	**80**–150	**21**–38	**5**–10	**11–17**	**300**–360	**40**–60
Sheep	**90**–150	**27**–45	**9**–15	**8**–12	**310**–340	**28**–40
Goats	**100**–160	**36**–43	**8**–1	**5.2**–8	**300**–360	**16**–25
Pigs	**100**–160	36–43	**5**–8	**17**–21	**310**–340	**50**–68
Horses	**100**–160	**27**–43	**6**–10	**14**–18	**359**–390	**37**–49

Table 13.2. Marginal bands for assessing risk of iron deprivation from biochemical criteria in newborn (N) or adult (A) domestic livestock. (Data from Puls, 1994)

Class	Diet Fe (mg kg^{-1} DM)	Liver Fe (mmol kg^{-1} DM)[b]	Serum Fe (μmol l^{-1})[b]	TIBC UIBC (g l^{-1} %)	Serum ferritin (μg l^{-1})
Cattle	**40**–60	**1.79**–2.68	**8.9**–17.9	**< 40 < 74**	**10**–30
Pigs (N)	**60**–80	**0.54**–0.89	**2.7**–10.7	<22 < 57	**5**–10
(A)	**30**–50	**1.79**–2.68	**14.3**–17.9		**35**–55
Poultry	**35**–45	**1.79**–2.68	**10**–15	–	–
Sheep	**30**–50	**1.25**–1.79	**15**–20	<60 <49	–
Horses			**13**–16	<59 <66	

A, adult; N, neonate. Marginal values are probably lower for neonates than for adults of other species.
The presence of individual values close to the **bold** limit in a sampled group with a normal mean value may justify intervention.
[b]Multiply by 55.9 to obtain liver values in mg kg–1DM and serum values in μg l–1.

Anaemia of infection

It is important to distinguish anaemias caused by lack of dietary Fe and those associated with infection and malnutrition. Microbial pathogens also need Fe, which is procured by Fe-responsive binding proteins (IRBP) in outer cell membranes. Hosts produce antibodies to microbial IRBP to protect from pathogens such as *Pasteurella haemolytica* (Confer *et al*., 1995). Inflammatory responses to chronic infections alter Fe distribution in plasma and between plasma and tissues, notably liver. Serum ferritin increases and plasma Fe decreases during the acute-phase response to infection, through the actions of cytokines that upregulate hepcidin and downregulate ferroportin, two host IRBP. Balance between pro- and anti-inflammatory cytokines is changed, plasma interleukin-6 (IL-6) increasing while IL-10 and interferon-δ decrease (Kuvibidi and Warrier, 2004; Wang and Pantopoulos, 2011; Cherayil, 2011). Effects on Fe and Hb status depend partly on the degree to which a particular pathogen stimulates an inflammatory response at a particular site. Secondary, systemic anaemia develops when erythropoietic tissues are deprived of Fe. Infections such as mastitis in cows raise milk ferritin but not serum ferritin and milk may become more bactericidal (Wang *et al*., 2006). Parenteral administration of complexed Fe enhanced survival in chicks infected with *Salmonella gallinarum* (Smith and Hill, 1978).

- Anaemia of microbial infection is indicated by high serum ferritin, normal MCHC and normal MCV, and increased serum acute-phase proteins (haptoglobin, fibrinogen, caeruloplasmin): in parasitic infections, faeces samples have high oocysts and egg counts.

High plasma Cu coupled with high coccidial oocyst counts in anaemic lambs suggested an infectious cause (Green *et al*., 1993). Parasites that cause severe blood loss can induce anaemia due to secondary iron-insufficiency. Infection of the abomasum by blood-sucking *Haemonchus contortus* can remove up to 24 ml erythrocytes, daily, in lambs (Abbot *et al*., 1988). An associated increase in abomasal pH may lower the release of complexed Fe, in ingested milk, herbage and creep feed.

Dietary Sources of Iron

Grains, seeds and cereal by-products

Cereal Fe has been reported to vary widely between species and countries (Table 13.3). Relatively high values in historical UK data may be attributable to contamination during analysis but maize generally contains less Fe than oats and wheat. Cereal by-products are richer in Fe than grains, reflecting both uneven distribution of iron in the grain and contamination during processing. Iron is present in feeds as organic complexes, with some bound to phytate and some stored in phytosiderophores as ferritin. Wheat contains iron mostly as monoferric phytate (Morris and Ellis, 1976) and soybeans contain substantial amounts as ferritin (Lonnerdal, 2009).

Milk, milk by-products and other animal sources

Cow's milk, in mid-lactation, averages about 0.5 mg Fe kg^{-1}FW, equivalent to only 4mg kg^{-1}DM: similar values are found in milks from most other species, although sow's milk contains three times as much, as does bovine colostrum (Tables 2.4 and

Table 13.3. Mean iron concentrations (mg kg^{-1}DM) in cereals, cereal by-products, soybean meal (SBM) and linseed meal (LSM) from the UK (MAFF, 1990) and the USA (Kerr *et al*., 2008): standard deviations, in parentheses, are for sample sizes of at least 5.

Country*	Maize	Barley	Wheat	Oats	Rice	Rice bran	Wheat bran	Maize GM	SBM	LSM
USA	28	71	56	69	39	-	184	125	210	70
	(7.6)	(-)	(9.1)	(6.8)	(47)	-	(41)	(13.5)	(153)	(16)
UK	100	100	140	120	-	2600	220	140	180	140
	(0)	(0)	(50)	(50)	(-)	(1000)	(5.0)	(6.1)	(110)	(6)

Leguminous seeds and oilseed meals are richer in Fe than cereals and commonly contain 100–200mg Fe kg^{-1}DM.

2.5). Iron is chiefly secreted as the glycoprotein lactoferrin but some, including adventitious Fe, becomes bound to casein. Dried skimmed milk, whey and buttermilk powders once varied greatly in iron content, due to contamination during processing, but are now usually low in Fe. Other feeds of animal origin were far richer sources (e.g blood meal, 3108; fish meal, 381; meat meal, 439, compared with dried skimmed milk, 52mg Fe kg^{-1}DM; Kolb, 1963). Inclusion of non-dairy products in cereal-based rations as protein supplements substantially increased iron intakes but their use has been restricted to prevent the spread of zoonotic diseases and antibiotic resistance.

Forages

Pasture Fe has shown marked seasonal fluctuations with peaks in spring and autumn and in New Zealand (Campbell *et al*., 1974) but a recent survey showed far less variation (mean 131 ± 6 (s.e.)mg kg^{-1}DM; Knowles and Grace, 2014). Egyptian green-cut clover and clover hay contained between 90 and 110 mg (Abou-Hussein *et al*., 1970) and grasses on poor sandy soils in Australia <30mg Fe kg^{-1}DM (Underwood, unpublished data). High values are attributable to soil contamination, confirmed by correlated high values for titanium and most likely to occur on soils prone to waterlogging. Soil contamination also affects conserved forage Fe. Corn silages, unaffected by flooding in north-eastern states of the USA in autumn 2011 had a mean value of 241mg Fe kg^{-1}DM but values ranged from 57 to 1544 (Kung *et al*., 2015).

Nutritive Value of Iron in Feeds

Methodology

The potential value or absorbability (A_{Fe}; p. 10) of a given Fe source will exceed the measured value (A_{Fe}) if, as is often the case, dietary Fe exceeds need. The major factors affecting availability are chemical form, presence of antagonistic minerals, organic constituents that reduce or enhance iron absorption and animal species (Fly *et al*., 1996). Attention of human nutritionists focused on contrasts between haem- and non-haem Fe (Fig. 13.3), A_{Fe} of the latter being greatly enhanced by ascorbic acid (e.g. Biesegel *et al*., 2007) but decreased by calcium (e.g. Minihane and Fairweather-Tait, 1998), phytate and polyphenols (e.g. Hurrell *et al*., 2003). Measurement of relative biological value (RBV) by comparative rates of Hb regeneration (p. 28) was assumed to obviate effects of host Fe status but

- rates of absorption may decrease as repletion proceeds because animals respond within 24 hours to changes in dietary Fe (Fig. 13.2), putting highly available sources at a disadvantage.
- instantaneous rates of repletion may reflect availability better than average rates for whole repletion periods.

Weaknesses of RBV assessments, using growth and Hb as indices of availability, are illustrated in Fig. 13.5, which shows results for an attempted comparison of Fe-rich hemicellulose sources for chicks by the slopes ratio method. Sugarbeet pulp stood out as the poorest source for growth but all sources were of similar availability to $FeSO_4$ for Hb synthesis. The RBV of iron ascorbate exceeds that of $FeSO_4$ and is sometimes used as the preferred standard of high availability. In whole-body ^{59}Fe retention studies, little isotope is lost from the body, once unabsorbed Fe from a bolus dose has passed through the digestive tract (Roughead and Hunt, 2000). Negligible endogenous losses of Fe (FEL_{Fe}) allow retention to be equated with absorption (Figs 13.2 and 13.3) at moderate Fe intakes. *In vitro* tests are immune from animal influences and have been developed to provide closer simulation of digestion *in vivo* (Hemalatha

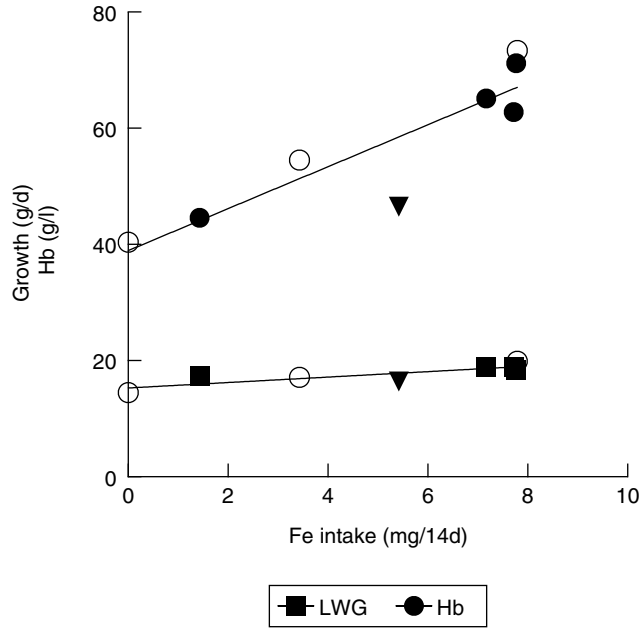

Fig. 13.5. Responses in growth and blood haemoglobin (Hb) in chicks to supplementary Fe as $FeSO_4$ (open symbols) or Fe-rich hemicellulose sources (solid symbol): growth was the more sensitive index of response but sugarbeet pulp (inverted triangle) probably contained a growth-inhibiting factor, falsely indicating low availability not evident from responses in Hb. (Data from Fly *et al.*,1996)

et al., 2007; p.26) but results must be calibrated against those from animal tests. Iron chelates have been compared by 'hybrid' duodenal loop methodology, using genomic markers (Zhang *et al.*, 2017), but physiological relevance and interpretation are questionable, as with Mn chelates (p. 30).

Milk iron

Iron is well absorbed from human milk by infants due to the high absorbability of lactoferrin and presence of two enhancers of iron absorption, citrate and lactose, but less available from milk replacers based on bovine milk (Lonnerdal, 1988). With inorganic Fe (Fe_i as $FeSO_4$) added to cow's milk, 53–59% was utilized for Hb formation in anaemic baby pigs fed less Fe than they required (Matrone *et al.*, 1960). In similar circumstances, 72–82% of ingested Fe was apparently absorbed (Ullrey *et al.*, 1965). Pasteurization and dehydration of bovine milk may lower A_{Fe} in milk replacers by destroying vitamin C. High vitamin C in milks of grazing animals may enhance A_{Fe}. Anaemia is rarely seen in suckled offspring at pasture. Young animals gradually eat more solid feed and consume less milk with the approach of weaning but nothing is known of the effects of transition on A_{Fe}. In humans, A_{Fe} is far lower in humans from dephytinized cereal porridges made with bovine milk than those made with water (Hurrell *et al.*, 2003). Could the 'culprit' be lactoferrin? Dosing calves with Fe-saturated bovine lactoferrin failed to prevent a post-natal fall in blood Hb, whereas the same amount of Fe_i was effective (Kume and Tanabe, 1994). By contrast, ferric iron salts readily form complexes with casein hydrolysates (Sugiarto *et al.*, 2009) and are being investigated as novel iron supplements (Kupczyński *et al.*, 2017).

Non-haem iron for chicks

Low RBV of 0.39 and 0.56 were reported for Fe in soybean protein meal (SBM) (Biehl *et al.*, 1997) and cottonseed meal (Boling *et al.*, 1998), respectively, compared to $FeSO_4$. Addition of phytase did not improve RBV for soybean concentrate (Biehl *et al.*, 1997) and may not hydrolyse preformed ferric phytates and/or ferritin. Availability of cereal Fe

may vary between plant species. Wheat Fe was of equal value to $FeCl_3$ for chicks and attributed to the presence of highly available monoferric phytate (Morris and Ellis, 1976). Rats retained 60% of Fe from a wheat meal (Fig. 13.2). By contrast, ground maize had an RBV of only 0.20 for chicks (Chausow and Czarnecki-Maulden, 1988), a result consistent with data for maize-based diets in humans. Although A_{Fe} was higher from genetically modified (GM), low-phytate maize than from wild-type maize (Mendoza *et al.*, 1998), A_{Fe} from Fe_i supplements was not improved (Mendoza *et al.*, 2001).

- Maize is a poor source of available Fe for non-ruminants.

Non-haem iron for pigs and ruminants

Little is known about the nutritive value of Fe in pig feeds. The effects of adding phytase to rations have been inconsistent. Adding phytase to a corn/SBM diet containing no non-phytate P improved blood Hb in young pigs (Stahl *et al.*, 1999). Phytase supplements also improved AA_{Fe} in sows on diets containing a mix of cereals other than corn and no inorganic P (Czech and Grela, 2004). However, addition of phytase did not raise AA_{Fe} from a value of 0.21 in corn/SBM rations for growing pigs in two more recent studies, one with and one without added P (Revy *et al.*, 2004 and Arredondo *et al.*, 2019, respectively). Release of P and Fe from SBM during *in vitro* incubation with phytase showed different dose-dependencies (Stahl *et al.*, 1999) and release of Zn and Fe from heat-treated feeds was also dissimilar (Hemalatha *et al.*, 2007), suggesting different effects of phytate on A_{Fe} compared with Zn. The effect of phytase may depend on the feed source of phytate and non-phytate P. Pre-formed, tetra-ferric phytate was poorly available to milk-fed calves (Bremner *et al.*, 1976) having presumably escaped rumen degradation. Availability of pasture Fe may be raised by a plentiful supply of vitamin C.

Haem iron for non-ruminants

Haem Fe is better absorbed than Fe_i or non-haem Fe complexes by humans (Fig. 13.3) but the margin (three- to fivefold) increases as body Fe increases and may be overestimated if recent Fe intake exceeded requirement, because absorption of non-haem Fe is inhibited (Fig. 13.3). Low RBV for chick growth in three blood meals fed to chicks (0.22–0.35; Henry and Miller, 1995) may be partly attributable to denaturation of haem and accompanying blood proteins by heat treatment (Fly *et al.*, 1996) and/or the high dietary Ca, then provided for chicks (10g kg^{-1}DM). Availability of haem Fe to humans is reduced by supplementary Ca (Roughead *et al.*, 2005). Higher RBV (0.40 and 0.70) for Fe were reported for Hb regeneration (Henry and Miller, 1995). Haem Fe now makes little contribution to livestock rations.

Iron Requirements

Influences of basal diet, age and criterion of Fe status on the need for Fe are strong here and three ground rules should be applied, for reasons given below:

- Requirements should be determined on practical diets and management regimens.
- Requirements for growth should be scaled to decrease as animals grow.
- Requirements should be for optimum health and performance rather than a particular biochemical or molecular marker, unless the two are correlated.

Requirements are lower on semi-purified than natural diets, particularly those based on corn and SBM, which contain poorly available Fe (p. 334). Requirements for prevention of anaemia are higher than those to avoid growth retardation because mild anaemia develops first. Since net growth requirement for Fe is predominantly for Hb and daily increments in red cell mass decrease as animals approach maturity and eat more food, needs decline with age in all species when expressed as dietary Fe concentrations. Carcase Fe decreased from 36 to 30mg Fe kg^{-1}FW as pigs grew from 20 kg to 140 kg LW (Ruiz-Ascacibar *et al.*, 2019). Spontaneous recoveries in growth in piglets (Fig. 13.1) and blood Hb in calves (Fig. 13.6), lambs (Bassett *et al.*, 1995) and chicks occur on diets initially low enough to halt growth and induce anaemia.

Pigs

Baby pigs needed between 40 and 50 mg added Fe_i for normal growth and 100 mg Fe kg^{-1}DM for full Hb repletion when reared on bovine, synthetic and condensed milks, containing 1.4 (Matrone *et al.*, 1960), 25 (Ullrey *et al.*, 1965) and 2mg Fe kg^{-1}DM

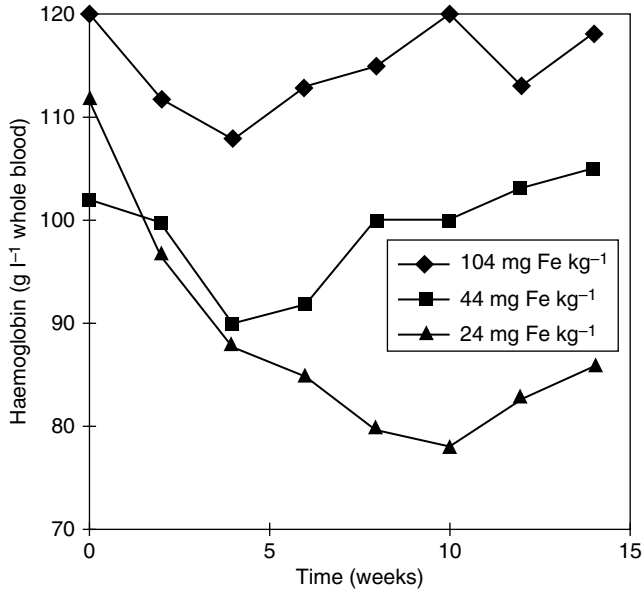

Fig. 13.6. Young calves given initially inadequate dietary iron concentrations show spontaneous recoveries from anaemia because iron requirements for each unit of weight gain decrease, while appetite increases as animals grow. (Data from Bremner *et al*., 1976)

(Hitchcock *et al*., 1974), respectively. Requirements on dry solid diets were similar but reduced by soaking (Hitchcock *et al*., 1974). A corn/SBM diet containing 52mg Fe kg^{-1}DM failed to restore blood Hb in baby pigs but an additional 50 mg Fe_i did (Stahl *et al*. 1999). A similar Fe supply (109mg kg^{-1}DM) was inadequate in piglets deliberately given less than the recommended amount of parenteral Fe at birth (Fig. 13.1). With Fe glycinate as the source, growth was maximal with 90 mg added Fe kg^{-1}DM (Feng *et al*., 2007). A simplified factorial model suggests that needs fall by 50% between 20 kg and 100 kg LW (Table 13.4): the estimates are close to those suggested by NRC (2012).[iv] Needs for lactation are relatively high because sow's milk is relatively rich in Fe (Table 2.5) and NRC (2012) recommended 80mg Fe kg^{-1}DM for gilts and sows.

Poultry growth

Early estimates of requirements for growth (up to 28 d.o.) were 20–40mg Fe kg^{-1}DM on semi-purified diets (Hill and Matrone, 1961; Aoyagi and Baker, 1995) and 85–95mg Fe kg^{-1}DM on cereal-based diets (Davis *et al*., 1962; Aoyagi and Baker, 1995): blood Hb was the common arbiter of need.

Table 13.4. Factorial estimates[a] of dietary iron requirements of growing pigs indicate that needs probably decrease by 60% between weaning and marketing.

Liveweight (kg)	DMI (kg day^{-1})	LWG (kg day^{-1})	Net need[a] (mg day^{-1})	Gross need[b] (mg kg^{-1}DM)
20	1.0	0.60	22	110
40	1.6	0.80	28	88
60	2.3	0.90	31	67
80	2.7	0.93	31	57
100	3.0	0.88	28	47

[a] Components of a simplified model were net needs of 32–36 mg Fe LWG^{-1}, declining as pigs grow (Ruiz-Ascacibar *et al*., 2019) and at weaning, negligible endogenous losses and an A_{Fe} of 0.20 to calculate gross needs[b].

Requirements are high on diets based on corn and SBM (p. 334), which contain Fe of low availability.

- Scaled reductions from 80 to 50mg Fe kg^{-1}DM for broilers up to 8 weeks and turkey poults between 4 and 16 weeks of age are more realistic than constant provision of 80mg Fe kg^{-1}DM (NRC, 1994).

Although growth rate increases substantially in broilers between 21 and 42 days, so too does food consumption. When optimal mRNA and activity of Cox

in heart and liver were used to delineate minimum need, requirements of chicks between 22 and 42 days were 104–110 and 47–67mg Fe kg^{-1}DM, respectively, but ≤47mg Fe kg^{-1}DM was sufficient to maximize growth and haematological measures of iron status (Fig. 13.7) (Liao *et al*., 2017). Since activity of Cox might indicate changes in intracellular redox status that have no adverse holistic consequences, new biochemical criteria of iron requirement must be shown to impact either broiler health or productivity.

Egg production

Requirements may increase prior to lay, since A_{Fe} may be lowered by increases in dietary Ca. An average hen's egg contains about 1 mg Fe and daily needs may decrease towards the end of lay when eggs may be laid every other day yet food intake remains high. More dietary Fe was recommended for egg production from energy-rich than from energy-lean diets (56 and 38mg Fe kg^{-1}DM, respectively; NRC, 1994). Commercial laying rations should cover overall requirements, especially under free-range conditions, where soil, rich in Fe, is ingested and where ground limestone or oyster shell is on offer, as these frequently contain 0.2–0.5% Fe, but requirements for intensive egg production remain ill-defined. Breeder hens required over twice the NRC (1994) recommendation for optimum eggshell strength and PCV (Fig. 13.8): results for tibia strength and hatchability of live embryos showed similar quadratic responses (not shown). Since improved hatchability, shell and bone strength all have cost benefits, breeder diets should be supplemented with 50mg Fe kg^{-1}DM; any more could be detrimental.

Sheep and cattle

Normal growth and blood Hb values were maintained in milk-fed calves given 30 mg supplemental Fe day^{-1} for 40 weeks from birth (Matrone *et al*.,

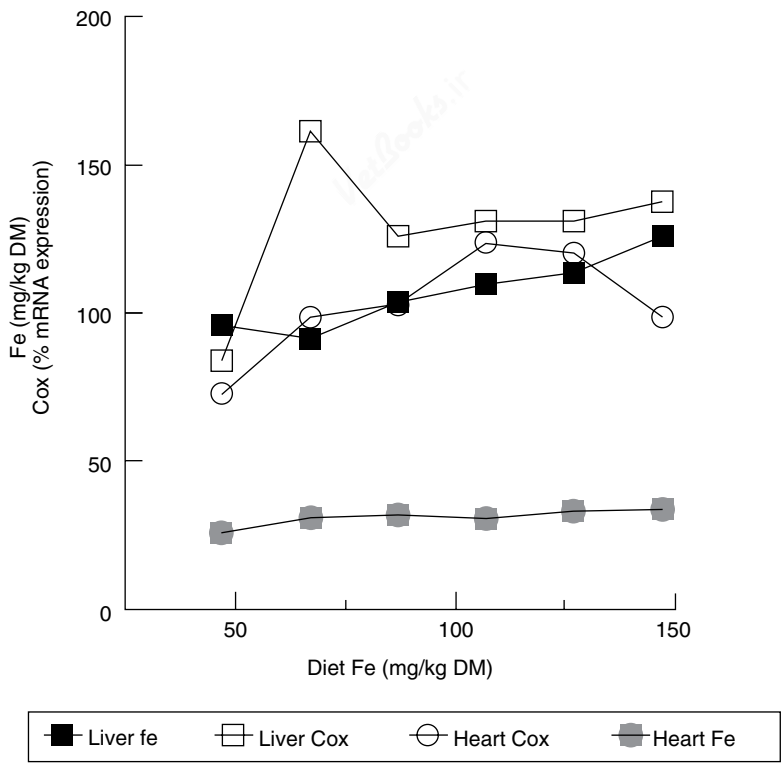

Fig. 13.7. Estimates of iron requirements for broiler chicks vary between indices of need: as dietary Fe increased, heart Fe and liver cytochrome c oxidas activity (Cox) reached early peaks but far more was needed to optimise heart Cox and liver Fe: growth (not shown) was not increased by raising dietary Fe (data from Liao *et al*., 2017)

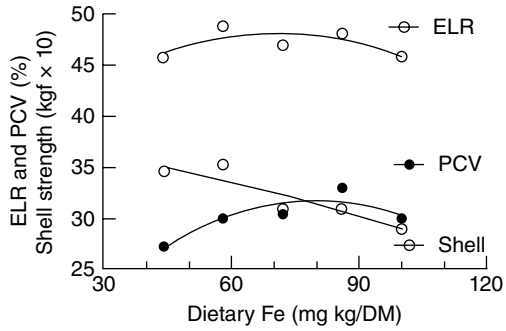

Fig. 13.8. Responses in egg-laying rate (ELR, %), haematocrit (PCV, %) and eggshell strength (kgf) of Yellow broiler breeder hens to increases in dietary iron: differences in ELR were not significant. (Data from Gou *et al.*, 2020)

1957). In veal calves, 40 mg Fe kg^{-1}DM prevented all but a mild anaemia (Bremner and Dalgarno, 1973). With weaned growing-finishing lambs, 10mg Fe kg^{-1}DM was inadequate and minimum requirement lay between 25 and 40 mg kg^{-1}DM (Lawlor *et al.*, 1965). Definitive studies with adult animals have not been reported. Net requirements for maintenance are low. Factorial estimates of gross requirements, assuming net needs of 28.5mg Fe kg^{-1} LWG for growth, 0.5mg Fe kg^{-1} for milk production and a low A_{Fe} of 0.1 put dietary requirements at 80 and 140mg Fe kg^{-1}DM for young weanling lambs and calves, respectively (van den Trop, 2005): these are gross overestimates because A_{Fe} is probably >0.2. Requirements are low during lactation, because relatively little Fe is secreted in milk. With A_{Fe} at 0.2, the additional need of a cow to produce 40 kg milk day^{-1} is met by just 10mg Fe kg^{-1}DM when an additional 10 kg DM is consumed.

- Weaned ruminants do not need iron supplements.

Treatment of Iron Insufficiency in the Young

Intramuscular (i.m.) injections of Fe-dextran (Patterson and Allen, 1972) are still widely used to treat piglet anaemia but the subcutaneous route is equally effective (Svoboda and Drabek, 2007). Injecting 200 mg Fe, in 1–2 ml volume at 3 d.o. restores and maintains blood Hb. A smaller dose (100 mg) may lessen harmful side-effects (reduced growth rate, appetite and antibody responses to vaccination) but Hb recovery may be delayed and incomplete (Schrama *et al.*, 1997; Svoboda and Pistkova, 2018). A second injection at 21 days conferred no benefits when the diet contained 80mg Fe kg^{-1}DM (Bruininx *et al.*, 2000). Combinations of low parenteral doses and dietary Fe supplements limit the downregulation of hepcidin (Starzyński *et al.*, 2013). Repeated injections may be needed in organic, indoor rearing systems, where lactation is prolonged (Heidbüchel *et al.*, 2019).

Anaemia in newborn lambs (Ullrey *et al.*, 1965) and calves (Carleson *et al.*, 1961) was controlled with i.m. doses of 200 mg (Green *et al.*, 1993, 1997) and 500 mg Fe (Hibbs *et al.*, 1963), respectively. In calves, growth as well as haematological parameters were improved by injecting 1g Fe-dextran at two d.o. (Heidarpour Bami *et al.*, 2008). Iron dextran injections have several disadvantages, including risk of infection, abscess formation at injection sites and high labour costs. Oral treatment with 20–40 mg Fe d^{-1} as $FeSO_4$ was effective in newborn calves (Kume and Tanabe, 1994). An Fe-casein hydrolysate (10 g d^{-1}) improved antioxidant as well as Fe status but 20 g d^{-1} caused Fe overload (Kupczyński *et al.*, 2017). Treatment of systemic iron deprivation due to loss of blood to blood-sucking parasites may aid recovery. Parenteral administration of iron in lambs with *H. contortus* infections and calves with *Fasciola hepatica* infections improved subsequent growth.

Prevention of Dietary Iron Insufficiency

Piglet anaemia was first prevented by simply giving piglets access to small amounts of soil or pasture sods (McGowan and Crichton, 1924). Disadvantanges of parenteral Fe injections led to investigations of prevention by more refined oral routes (Svoboda and Pistkova, 2018). Neither Fe_i (ARC, 1981) nor chelated Fe given to sows during pregnancy and lactation increased milk Fe and the increase in neonatal Fe stores was insufficient to prevent piglet anaemia (Peters and Mahan, 2008b). Massive supplements of 2g Fe kg^{-1}DM as $FeSO_4$ for sows controlled anaemia when piglets had access to sows' faeces (Gleed and Sansom, 1982). The anaemia of weaned pigs given copper as a growth stimulant is preventable by co-supplementation with iron and zinc (p. 286).

Inorganic iron supplements

Inorganic Fe_i sources vary in availability according to solubility, valency and the species used for evaluation (Henry and Miller, 1995). In chicks, insoluble ferric

carbonates and orthophosphates had RBV <20, compared to $FeSO_4$, but more soluble ferric salts such as the chloride and sulfate had RBV 44 and 65, respectively (Fritz *et al*., 1970). Ferric oxide (Fe_2O_3), used as a colouring agent in mineral mixes, had a poor but variable RBV for chicks (4–67) (Henry and Miller, 1995): adventitious Fe_2O_3 that colours limestones and defluorinated phosphates was more uniform (RBV 34–52) (Deming and Czarnecki-Maulden, 1989). Adventitious Fe in coarse particles of limestones, magnesites and phosphates may become trapped in the acid environment of the avian gizzard and availability may be more variable to chicks than pigs (Henry and Miller, 1995). Adventitious Fe, ingested as soil, may become trapped in the rumen, partially solubilize and impair absorption of Cu (A_{Cu}) in sheep (Suttle and Peter, 1985). Ferric citrate is being used to prevent sulfide toxicity in feedlot cattle consuming excess S in dried distillers grain solubles (DDGS) (p. 222) by trapping sulfide as insoluble Fe_2S. Since sulfide is probably released from Fe_2S in the acid abomasum and is the mechanism whereby Fe_2O_3 inhibits A_{Cu} (p. 274),

- feedlot cattle on DGGS should be given additional Cu as well as Fe.

Soluble Fe_i sources promote lipid oxidation and can generate unwanted sensory changes to a feed (Hurrell, 2002). Lipid oxidation was minimized in milk replacers for children by using micro-encapsulated $FeSO_4$ (Lysionek *et al*., 2001) but high costs may prohibit use for livestock.

- Soluble Fe_i need not and should not be added to compound feeds beyond the 'starter' stage in most livestock-rearing programmes, particularly in hot and/or humid environments.

Chelated iron supplements

Early comparisons in chicks (p. 22) indicated that ferric salts of citric, succinic, fumaric and tartaric acids had RBV (p. 27) of 119, 107, 97 and 72%, respectively (Fritz *et al*., 1970). In human volunteers, ferrous ascorbate had a high RBV (Bovell-Benjamin *et al*., 2000; Hurrell, 2002) but the fumarate, of similar RBV to $FeSO_4$, may be less likely to taint food (Hurrell, 2002). Complexation of iron with polysaccharide lowers availability. Chelates such as desferoxamine and desferiprone are used to treat iron-overload disorders in humans but NaFeEDTA is far superior to $FeSO_4$ as an Fe source (Mendoza *et al*., 2001). Chelates of specific amino acid and mixed peptides have been widely tested in livestock but were generally no more effective than $FeSO_4$.[v] A large-scale repletion rate comparison of 20 iron chelates, differing widely in chelation strength, in broiler chicks on corn/SBM diets was particularly revealing (Zhang *et al*., 2016). Using >10 different indices of Fe status, only one source had superior RBV when judged by one indice, mRNA for succinic dehydrogenase.

- Superiority may arise because less soluble chelates avoid anti-nutritional effects of excessive and unnecessary supplements of Fe_i as sulfates.

The single exception may be Fe glycinate (Fe-Gly), depending on formulation (Hertrampf and Olivares, 2004). Iron from ferrous bisglycinate was absorbed more efficiently than Fe from $FeSO_4$ from a maize porridge by human volunteers but iron from ferric trisglycinate was not (Bovell-Benjamin *et al*., 2000). Moreover, absorption from ferrous bisglycinate was negatively correlated with serum ferritin, an indication that it did not by-pass regulatory mechanisms in the gut. Studies with pigs indicated that superior absorption of Fe-Gly is accompanied by greater availability for Hb synthesis (Ettle *et al*., 2008) and tissue storage: a peptide transporter was up-regulated but performance was not enhanced (Fang *et al*., 2013). Supplementing pregnant sows with 80mg Fe kg^{-1} DM as ferrous N-carbamylglycinate rather than $FeSO_4$ raised placental Fe and increased litter weight but reduced TIBC and Fe stores in piglets (Wan *et al*., 2018). An alternative way of enhancing A_{Fe} is to attack organic antagonists with supplements of citric acid.

Toxicity of Iron

Tolerance towards dietary Fe has always appeared to be relatively high in all species. Protection is afforded by downregulation of hepcidin, which blocks Fe export from the mucosal cell and indirectly downregulates Fe uptake by DMT1 (Hansen *et al*., 2009). Tolerance of excess Fe will probably be reduced if the diet is low in Cu or Mn because DMT1 is upregulated through reciprocal interactions described earlier (p. 330). At excessive Fe intakes, shedding of Fe-loaded mucosal cells increases faecal loss as ferritin. Calves reared on milk substitute tolerated $FeSO_4$, providing 2g Fe kg^{-1}DM for six weeks, despite spleen and liver Fe reaching 1.30 and 1.55g kg^{-1}DM, respectively: a further increase to 5g Fe kg^{-1} diet DM depressed appetite and growth (Jenkins and Hidiroglou, 1987). Tolerance was

exploited in pigs by giving up to 3.2g Fe kg^{-1}DM for four months to combat gossypol poisoning, without causing Fe toxicity (Buitrago *et al*., 1970). Similarly, massive daily doses of up to 0.1g Fe kg^{-1}LW were used in sheep to combat sporidesmin intoxication (Munday and Manns, 1989) but 1g Fe kg^{-1}DM (as citrate) depressed LWG in lambs (Rosa *et al*., 1986). Tolerable dietary limits for livestock, based on short-term studies, were set at 1, 1 and 3g Fe kg^{-1}DM in cattle, poultry and pigs, respectively, by NRC (2005) but are misleading. In newly hatched chicks, 0.8g Fe kg^{-1}DM depressed appetite (Cao *et al*., 1996), while 0.52g Fe kg^{-1}DM induced inflammation in the piglet duodenum (Li *et al*., 2016) and may have inhibited A_{Mn} in cattle (Hansen *et al*., 2009). As with all cumulative poisons, cumulative absorption of ingested Fe rather than dietary concentration determines risk but,

- Marginal bands that separate acceptable and potentially harmful concentrations for iron sources that are soluble or become soluble at acid pH are more realistic than single tolerable limits (Table 13.5).

Lower liver Fe and dietary Fe concentrations may cause injury in the longer term, particularly if vitamin E status is low or if there are endogenous sources of excess Fe (NRC, 2005). Where exogenous Fe_i sources of low RBV such as oxides, hydroxides, carbonate or soil provide most of the ingested Fe, tolerable levels will be relatively high. A new hazard of oversupplementation with Fe has become apparent as a side effect of treatments for iron deficiency anaemia in children (Paganini and Zimmermann, 2017).

- Iron supplements raise colonic digesta Fe sufficiently to change the microbiome, favouring species that induce diarrhoea.
- High soil Fe ingestion from pasture in spring and autumn may exacerbate seasonal diarrhoeas in grazing ruminants.

Mechanisms of toxicity

Free iron is cytotoxic because of a high reduction–oxidation potential and ability to generate free oxygen radicals via Haber–Weiss and Fenton reactions (Jenkins and Kramer, 1988). For this reason, iron is transported and stored tightly bound to protein. When tissue stores become excessive during chronic Fe overload, peroxidative damage is caused at sites such as the liver (Sousa *et al*., 2020; Li *et al*., 2016): overload may arise either directly from excessive Fe intakes (Li *et al*., 2016) or indirectly and susceptibility increases with age (Wu *et al*., 1990). Indirect exposure can result from poisoning by plant toxins (e.g. lupinosis) (Gardiner, 1961), from the accelerated breakdown of erythrocytes after haemolytic crises (e.g. in Cu and brassica poisoning) and presumably in conditions such as vitamin E deficiency, in which erythrocyte lifespan is shortened. Copper deprivation also increases hepatic Fe. The underlying pathogenic mechanism is peroxidative damage to lipid membranes (Gordeuk *et al*., 1987) and is influenced by antioxidant status, particularly vitamin E status (Ibrahim *et al*., 1997). Vitamin E deficiency increases susceptibility to Fe toxicity, following the intramuscular injection of Fe in piglets (Patterson and Allen, 1972): conversely, Fe overload reduces liver vitamin E (Omara and Blakley, 1993). Ingestion of readily peroxidized lipid sources such as polyunsaturated fatty acids (PUFA) (Muntare *et al*., 1995; Ibrahim *et al*., 1997) is also a predisposing factor. In calves on milk replacer, peroxidative changes occur in heart and muscle after Fe exposure (Jenkins and Kramer,

Table 13.5. Marginal bands[a] for assessing risk of iron toxicity from biochemical criteria in domestic livestock.

Livestock	Dietary Fe[c] (mg kg^{-1}DM)	Liver Fe (mmol kg^{-1}DM)[b]	Serum Fe (μmol l^{-1})[b]	Serum ferritin (μg l^{-1})
Cattle	750–**1250**	>**17.9**	10.7–**32.2**	>**80**
Pigs	500–**2500**	>**17.9**	>**26.7**	–
Poultry	800–**3500**	17.9–**107.2**	>**53.6**	–
Sheep	750–**1250**	>**17.9**	>**39**	–

[a]Marginal values are probably higher for neonates than for adults.
[b]Multiply by 55.9 to obtain liver values in mg kg^{-1}DM and serum values in ug l^{-1}.
[c]Tolerable values will be far higher if contaminant soil iron contributes largely to the dietary iron level.
Presence of individual values close to the bold limit in a group with normal mean value may justify intervention.

1988). Pasture Fe can rise to high levels in spring and autumn and ingestion of Fe-rich soil with PUFA-rich grass in spring may present a hazard for grazing animals.

Diagnosis

Clinical signs for chronic and acute Fe toxicity differ: acute iron toxicity causes anorexia, vascular congestion and irritation in the gastrointestinal tract. With chronic toxicity, no abnormalities were recorded, *post mortem* and the primary cause of poor growth was poor appetite and feed conversion efficiency (Jenkins and Hidiroglou, 1987). In calves, blood indices such as serum Fe, TIBC and UIBC did not reflect excessive iron intakes (1g kg^{-1}DM) well (McGuire *et al*., 1985) and reliance must be placed on high liver Fe in association with hepatopathy.

Adverse subclinical interactions

In a recent study with broiler chicks, reared initially on a conventional corn/SBM blend without added Mn, addition of 100mg Fe kg^{-1}DM retarded growth, however much Mn was added (Bai *et al*., 2020). Was this a consequence of oxidative stress caused by excess Fe (p. 336)? Similar supplements lowered eggshell strength in breeder hens (Fig. 13.8). Excess Fe downregulates DMT1 expression in duodenal mucosa (Hansen *et al*., 2009) and inhibits absorption of Cu and Mn, which are, respectively, partly and wholly dependent on DMT1 for absorption (Hansen and Spears, 2008). Parenteral injection of Fe in piglets may impair adaptation to low Mn intakes (p. 354). Grazing animals cannot avoid ingesting excess Fe in the soil that contaminates relatively bare pasture in both wet and dry conditions. Diarrhoea developed in cattle grazing pastures irrigated with Fe-rich bore water in New Zealand and was associated with copper depletion (Campbell *et al*., 1974). The trapping of sulfide as Fe_2S and subsequent release of S^{2-} may impair Cu absorption (p. 274) Although ingested soil Fe is in a predominantly inert and insoluble form, sufficient Fe can be retained in weaned lambs to raise liver Fe to 1g Fe kg^{-1}DM (Suttle, unpublished data), a potentially hepatotoxic level. Systemic Fe overload may impair placental transfer of Mn and cause birth defects in calves (p. 350).

- The subclinical effects of Fe overload merit further investigation.

Notes

[i] Haem is probably taken up by a haem carrier protein, HCP1, and Fe^{2+} released by haem oxygenase into the intracellular pool (West and Oates, 2008). Non-haem iron in the Fe^{3+} state is reduced by cytochrome b in the apical membrane and conveyed by a divalent metal transporter (DMT1) (Garrick *et al*., 2003).

[ii] Hepcidin is also induced by anoxia and inflammatory cytokines, complicating assessment of F status (p. fe). DMT1 is found at sites beyond the gut mucosa, including isoforms in the human placenta and embryo (Chong *et al*., 2005).

[iii] Ferritin is a polyprotein with 24 heavy and light subunits: heavy subunits can store up to 4500 Fe^{3+} ions in a molecular core as ferric oxy-hydroxide phosphate. Tissues vary in subunit composition, heart containing predominantly 'heavy' and liver mostly 'light' chains, which serve as nucleation centres.

[iv] Addition of 150–250mg Cu kg^{-1}DM to stimulate growth increases Fe (and Zn) requirements (p. 286) by an unknown margin.

[v] In chicks, iron methionine was accorded a low RBV of 0.88 (Cao *et al*., 1996). A novel iron amino acid complex was claimed to have a high RBV for growing pigs (Yu *et al*., 2000) but design of the experiment made comparison difficult: other studies showed no advantages for chelated sources (Peters and Mahan, 2008a; Papadopoulos *et al*., 2009) Prolonged feeding of iron with other trace elements as proteinates to gilts through six parities improved litter size (Peters and Mahan, 2008b) in groups fed to excessive industrial standards but did not do so in groups fed to NRC standards: growth and other reproductive traits were unaffected.

References

Abbott, E.M., Parkins, J.J. and Holmes, P.J. (1988) Influence of dietary protein on the pathophysiology of haemonchosis in lambs given continuous infections. *Research in Veterinary Science* 45, 41–49.

Abou-Hussein, E.R.M., Raafat, M.A., Abou-Raya, A.K. and Shalaby, A.S. (1970) Manganese, iron and cobalt content in common Egyptian feedstuffs. *United Arab Republic Journal of Animal Production* 10, 245–254.

Antonides, A., Schoonderwoerd, A.C., Scholz, G., Berg, B.M., Nordquist, R.E. and Van Der Staay, F.J. (2015) Preweaning dietary iron deficiency impairs spatial learning and memory in the cognitive hole board task in piglets. *Frontiers of Behavioural Neuroscience* 30, 291.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

ARC (1981) *The Nutrient Requirements of Pigs*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 215–248.

Aoyagi, S. and Baker, D.H. (1995) Iron requirements of chicks fed a semi-purified diet based on casein and soy protein concentrate. *Poultry Science* 74, 412–415.

Arredondo, M.A., Casas, G.A. and. Stein, H.H. (2019) Increasing levels of microbial phytase increases the digestibility of energy and minerals in diets fed to pigs. *Animal Feed Science and Technology* 248, 27–36.

Atyabi, N., Gharagozloo, F. and Nassiri, S. (2006) The necessity of iron supplementation for normal development of commercially reared suckling calves. *Comparative Clinical Pathology* 15, 165–168.

Bai, S., Peng, J., Zhang, K., Ding, X., Wang, J. *et al*. (2020) Effects of dietary iron on manganese utilization in broilers fed with corn-soybean meal diet. *Biological Trace Element Research* 194, 514–524.

Bassett, J.M., Burrett, R.A., Hanson, C., Parsons, R. and Wolfensohn, S.E. (1995) Anaemia in housed lambs. *Veterinary Record* 136, 137–140.

Baynes, R.D. (1997) The soluble form of the transferrin receptor and iron status. In: Fisher, P.W.F., L'Abbé, M.R., Cockell, K.A. and Gibson, R.S. (eds) *Trace Elements in Man and Animals – 9 – Proceedings of the Ninth International Symposium*. NRC Research Press, Ottawa, pp. 471–475.

Beard, J., Tobin, B. and Green, W. (1989) Evidence for thyroid hormone deficiency in iron-deficient, anaemic rats. *Journal of Nutrition* 119, 772–778.

Bhardwaj, R.K., Randhawa, C.S. and Randhawa, S.S. (2010) Incidence of iron deficiency in crossbred cow calves reared on pucca floor. *Indian Journal of Animal Sciences* 80, 1037–1040.

Biehl, R.R., Emmert, J.L. and Baker, D.H. (1997) Iron bioavailability in soybean meal as affected by supplemental phytase and 1 alpha-hydoxycholecalciferol. *Poultry Science* 76, 1424–1427.

Biesegel, J.M., Hunt, J.R., Glahn, R.P., Welch, R.M., Menkir, A. and Maziya-Dixon, B.B. (2007) Iron bioavailability from maize and beans: a comparison of human measurements with Caco-2 cell and algorithm predictions. *American Journal of Clinical Nutrition* 86, 388–396.

Blaxter, K.L., Sharman, K.L. and MacDonald, A.M. (1957) Iron deficiency anaemia in calves. *British Journal of Nutrition* 11, 234–246.

Boling, S.D., Edwards, H.M. III, Emmert, J.L., Biehl, R.R. and Bafer, D.H. (1998) Bioavailability of iron in cottonseed meal, ferric sulfate and two ferrous sulfate by-products of the galvanising industry. *Poultry Science* 77, 1388–1392.

Bovell-Benjamin, A.C., Viteri, F.E. and Allen,]L.H. (2000) Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. *American Journal of Clinical Nutrition*, 71, 1563–1569.

Bremner, I. and Dalgarno, A.C. (1973) Iron metabolism in the veal calf: the availability of different iron compounds. *British Journal of Nutrition* 29, 229–243.

Bremner, I., Brockway, J.M., Donnelly, H.T. and Webster, A.J.F. (1976) Anaemia and veal calf production. *Veterinary Record* 99, 203–205.

Bruininx, E.M.A.M., Swinkels, J.W.G.M., Parmentier, H.K., Jetten, C.W.J., Gentry, J.L. and Schrama, J.W. (2000) Effects of an additional iron injection on growth and humoral immunity of weanling pigs. *Livestock Production Science* 67, 31–39.

Buitrago, J.A., Clawson, A.J. and Smith, F.H. (1970) Effects of dietary iron on gossypol accumulation in and elimination from porcine liver. *Journal of Animal Science* 31, 554–558.

Bus, J.D., Stockhofe, N. and Webb, L.E, (2019) *Invited review:* Abomasal damage in veal calves. *Journal of Dairy Science* 102, 943–960.

Campbell, A.G., Coup, M.R., Bishop, W.H. and Wright, D.E. (1974) Effect of elevated iron intake on the copper status of grazing cattle. *New Zealand Journal of Agricultural Research* 17, 393–399.

Cao, J., Lo, X.J., Henry, P.R., Ammerman, C.B., Littell, R.C. and Miles, D. (1996) Effect of dietary iron concentration, age and length of iron feeding on feed intake and tissue iron concentrations of broiler chicks for use as a bioassay of supplemental iron sources. *Poultry Science* 75, 495–504.

Cardenas-Rodriguez, M., Chatzi, A. and Tokatlidis, K. (2018) Iron–sulfur clusters: from metals through mitochondria biogenesis to disease. *Journal of Biological Inorganic Chemistry* 23, 509–520.

Carleson, R.H., Swenson, M.J., Ward, G.M. and Booth, N.H. (1961) Effects of intramuscular iron dextran in newborn lambs and calves. *Journal of the American Veterinary Medical Association* 139, 457–461.

Chausow, D.G. and Czarnecki-Maulden, G.L. (1988) The relative bioavailability of iron from feedstuffs of plant and animal origin to the chick. *Nutrition Research* 8, 175–179.

Cherayil, B.J. (2011) The role of iron in the immune response to bacterial infection. *Immunological Research* 50, 1–9.

Chong, W.S., Kwan, P.C., Chan, P.Y., Chiu, T.K., Cheung, T.K. and Lau, T.K. (2005) Expression of divalent metal transporter1 (DMT1) isoforms in first trimester human placenta and embryonic tissues. *Human Reproduction* 20, 3532–3538.

Confer, A.W., McGraw, R.D., Dierham, J.A., Morton, R.J. and Panciera, R.J. (1995) Serum antibody responses of cattle to iron-regulated outer membrane proteins of *Pasteurella haemolytica. Veterinary Immunology and Immunopathology* 47, 101–110.

Czech, A., and Grela, E.R. (2004) Biochemical and haematological blood parameters of sows during pregnancy and lactation fed the diet with different source

and activity of phytase. *Animal Feed Science and Technology* 116, 211–223.
Davis, P.N., Norris, L.C. and Kratzer, F.H. (1962) Iron deficiency studies in chicks using treated isolated soybean protein diets. *Journal of Nutrition* 78, 445–453.
Deming, J.G. and Czarnecki-Maulden, G.L. (1989) Iron bioavailability in calcium and phosphorus sources. *Journal of Animal Science* 67 (Suppl. 1), 253 (abstract).
Elvehjem, C.A. and Hart, E.B. (1929) The relation of iron and copper to haemoglobin synthesis in the chick. *Journal Biological Chemistry 84*, *131–141.*
Ettle, T., Schlegel, P. and Roth, F.X. (2008) Investigations on iron bioavailability of different sources and supply levels in piglets. *Journal of Animal Physiology and Animal Nutrition* 92, 35–43.
Fairweather-Tait, S.J. and Wright, A.J.A. (1984) The influence of previous iron intake on the estimation of bioavailability of iron from a test meal given to rats. *British Journal of Nutrition* 51, 185–191.
Fang, C.L., Zhuo, Z., Fang, S.L., Yue, M. and Feng, J. (2013) Iron sources on iron status and gene expression of iron related transporters in iron-deficient piglets. *Animal Feed Science and Technology* 182, 121–125.
Feng, J., Ma, W.Q., Xu, Z.R., Wang, Y.Z. and Liu, J.X. (2007) Effects of iron glycine chelate on growth, haematological and immunological characteristics in weanling pigs. *Animal Feed Science and Technology* 134, 261–272.
Fielder, S.E. (2015) Haematalogic reference ranges. *MSD Veterinary Manual*.
Fly, A.D., Czarnecki-Maulden, G.L., Fahey, G.C. Jr and Titgemeyer E.C.(1996) Hemicellulose does not affect iron bioavailability in chicks. *Journal of Nutrition 126*, *308–316.*
Fritz, J.C., Pla, G.W., Roberts, T., Boehne, J.W. and Hove, E.L. (1970) Biological availability in animals of iron from common dietary sources. *Journal of Agricultural and Food Chemistry* 18, 647–652.
Gardiner, M.R. (1961) Lupinosis – an iron storage disease of sheep. *Australian Veterinary Journal* 37, 135–140.
Garrick, M.D., Dolan, K.G., Horbinski, C., Ghoio, A.J., Higgins, D. *et al.* (2003) DMT 1: a mammalian transporter for multiple metals. *Biometals* 16, 41–54.
Gleed, P.T. and Sansom, B.F. (1982) Ingestion of iron in sows faeces by piglets reared in farrowing crates with slatted floors. *British Journal of Nutrition* 47, 113–117.
Gordeuk, V.R., Bacon, B.R. and Brittenham, G.M. (1987) Iron overload: cause and consequences. *Annual Reviews of Nutrition* 7, 485–508.
Gou, Z., Fan, Q., Li, L., Jiang, Z., Lin, X. *et al.* (2020) Effects of dietary iron on reproductive performance of Chinese Yellow broiler breeder hens during the egg-laying period. *Poultry Science* 99, 3921–3929.
Grassmann, E. and Kirchgessner, M. (1973) Katalase Aktivitat des Blutes von Saugferkeln und Mastkalbern bei mangelnder Eisenversorgung. *Zentralblatt fur Veterinarmedizin A* 20, 481–486.
Green, L.E., Berriatua, E. and Morgan, K.L. (1993) Anaemia in housed lambs. *Research in Veterinary Science* 54, 306–311.
Green, L.E., Graham, M. and Morgan, K.L. (1997) Preliminary study of the effect of iron dextran on a non-regenerative anaemia of housed lambs. *Veterinary Record* 140, 219–222.
Gubler, C.J., Cartwright, G.E. and Wintrobe, M.M. (1957) Studies on copper metabolism. 20. Enzyme activities and iron metabolism in copper and iron deficiencies. *Journal of Biological Chemistry* 224, 533–546.
Hansen, S.L. and Spears, J.W. (2008) Impact of copper deficiency in cattle on proteins involved in iron metabolism. *FASEB Journal* 22, 443–445.
Hansen, S.L., Trakooljul, N., Liu, H.-C., Moeser, A.J. and Spears, J.W. (2009) Iron transporters are differentially regulated by dietary iron, and modifications are associated with changes in manganese metabolism in young pigs, *Journal of Nutrition* 139, 1474–1479.
Hart, E.B., Elvehjem, C.A. and Steenbock, H. (1930) A study of the anemia of young pigs and its prevention. *Journal of Nutrition* 2, 277–294.
Harvey, J.W. (2008) Iron metabolism and its disorders. In: Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (eds) *Clinical Biochemistry of Domestic Animals*, 6th edn. Aademic Press, New York.
Heidarpour Bami, M., Mohri, M., Seifi, H.A. and Alavi Tabatabaee, A.A. (2008) Effects of parenteral supply of iron and copper on hematology, weight gain, and health in neonatal dairy calves. *Veterinary Research Communications* 32, 553–561.
Heidbüchel, K., Raabe, J., Baldinger, L., Hagmüller, W. and Bussemas, R. (2019) One iron injection is not enough: iron status and growth of suckling piglets on an organic farm. *Animals* 9, 651–663.
Hemalatha, S., Platel, K. and Srinivasin, K. (2007) Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *Journal of Trace Elements in Medicine and Biology* 21, 1–7.
Henry, P.R. and Miller, E.R. (1995) Iron bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 169–200.
Hertrampf, E. and Olivares, M. (2004) Iron amino acid chelates. *International Journal for Vitamin and Nutrition Research* 74, 435–443.
Hibbs, J.R., Conrad, H.R., Vandersall, J.H. and Gale, C. (1963) Occurrence of iron deficiency anaemia in dairy calves at birth and its alleviation by iron dextran injection. *Journal of Dairy Science* 46, 1118–1124.

Hill, C.H. and Matrone, G. (1961) Studies on copper and iron deficiencies in growing chickens. *Journal of Nutrition* 73, 425–431.

Hitchcock, J.P., Ku, P.K. and Miller, E.R. (1974) Factors influencing iron utilization by the baby pig. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Trace Element Metabolism in Animals – 2*. University Park Press, Baltimore, Maryland, pp. 598–600.

Hostettler-Allen, R., Tappy, L. and Blum, J.W. (1993) Enhanced insulin-dependent glucose utilization in iron-deficient veal calves. *Journal of Nutrition* 123, 1656–1667.

Hurrell, R. (2002) How to ensure adequate iron absorption from iron-fortified food. *Nutrition Reviews* 60, S7–S15.

Hurrell, R.F., Reddy, M.B., Juillerat, M.-A. and Cook, J.D. (2003) Degradation of phytic acid in cereal porridges by human subjects. *American Journal of Clinical Nutrition* 77, 1213–1219.

Ibrahim, W., Lee, V.-S., Ye, C.-C., Szabo, J., Bruckner, G. and Chow, C.K. (1997) Oxidative stress and antioxidant status in mouse liver: effects of dietary lipid, vitamin E and iron. *Journal of Nutrition* 127, 1401–1406.

Ishaya, V. (2012) Iron nutrition and anaemia in piglets: a review. *Journal of Veterinary Advances* 2, 261–265.

Jenkins, K. and Hidiroglou, M. (1987) Effect of excess iron in milk replacer on calf performance. *Journal of Dairy Science* 70, 2349–2354.

Jenkins, K. and Kramer, J.K.G. (1988) Effect of excess dietary iron on lipid composition of calf liver, heart and skeletal muscle. *Journal of Dairy Science* 71, 435–441.

Jiang, L., Garrick, M.D., Garrick, L.M., Zhao, L., and Collins, J.F. (2013) Divalent metal transporter 1 (Dmt1) mediates copper transport in the duodenum of iron-deficient rats and when overexpressed in iron-deprived HEK-293 cells. *Journal of Nutrition* 143, 1927–1933.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S.L., Bearson, B.L., Shurson, G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–310.

Knowles, T.G., Edwards, J.E., Bazeley, K.J., Brown, S.N., Butterworth, A. and Warriss, R.D. (2000) Changes in the blood biochemical and haematological profile of neonatal calves with age. *Veterinary Record* 147, 593–598.

Kolb, E. (1963) The metabolism of iron in farm animals under normal and pathologic conditions. *Advances in Veterinary Science and Comparative Medicine* 8, 49–114.

Kreutzer, M. and Kirchgessner, M. (1991) Endogenous iron excretion: a quantitative means to control iron metabolism. *Biological Trace Element Research* 29, 77–92.

Kume, S.-E. and Tanabe, S. (1994) Effect of twining and supplemental iron-saturated lactoferrin on iron status of newborn calves. *Journal of Dairy Science* 77, 3118–3123.

Kung, L., Lim, J.M., Hudson, D.J., Smith, J.M. and Joerger, R.D. (2015) Chemical composition and nutritive value of corn silage harvested in the northeastern United States after Tropical Storm Irene. *Journal of Dairy Science* 98, 2055–2062.

Kupczyñski, R., Bednarski, M., Œpitalniak, K. and Pogoda-Sewerniak, K. (2017) Effects of protein-iron complex concentrate supplementation on iron metabolism, oxidative and immune status in preweaning calves. *International Journal of Molecular Science* 18, 1501.

Kuvibidi, S. and Warrier, R.P. (2004) Differential effects of iron deficiency and underfeeding on serum levels of interleukin-10, interleukin-12 p40 and interferon-gamma in mice. *Cytokine* 26, 73–81.

Kwik-Uribe, C.L., Golub, M.S. and Keen, C.L. (2000) Chronic marginal iron intakes during early development in mice alter brain iron concentrations and behaviour despite postnatal iron supplementation. *Journal of Nutrition* 130, 2040–2048.

Lawlor, M.J., Smith, W.H. and Beeson, W.M. (1965) Iron requirement of the growing lamb. *Journal of Animal Science* 24, 742–747.

Leyshon, B.J., Peng, J., Caputo, M.P., Matt, S.M. and Johnson, R.W. (2019) Dietary iron deficiency impaired peripheral immunity but did not alter brain microglia in PRRSV-infected neonatal piglets. *Frontiers in Immunology* 9, 3150–3162.

Li, Y., Hansen, S.L., Borst, L., Spears, J.W. and Moeser, A.J. (2016) Dietary iron deficiency and oversupplementation increase intestinal permeability, ion transport, and inflammation in pigs. *Journal of Nutrition* 146, 1499–1505.

Liao, X., Ma, C., Lu, L., Zhang, L. and Luo, X. (2017) Determination of dietary iron requirements by full expression of iron-containing cytochrome c oxidase in the heart of broilers from 22 to 42 d of age. *British Journal of Nutrition* 118, 493–499.

Liebold, E.A. and Guo, B. (1992) Iron-dependent regulation of ferritin and transferrin receptor expression by the iron-responsive element binding protein. *Annual Review of Nutrition* 12, 345–368.

Lonnerdal, B. (1988) Trace elements in infancy: a supply/demand perspective. In: Hurley, L.S., Keen, C.L., Lonnerdal, B. and Rucker, R.B. (eds) *Proceedings of Sixth International Symposium on Trace Elements in*

Man and Animals. Plenum Press, New York, pp. 189–195.

Lonnerdal, B. (2009) Soybean ferritin: implications for iron status of vegetarians. *American Journal of Clinical Nutrition* 89, 1680S–1685S.

Lysionek, A.E., Zubilliga, M.B., Saigueiro, M.J., Caro, R.A., Weill, R. and Boccio, J.R. (2001) Bioavailability study of dried microencapsulated ferrous sulfate – SFE 171 – by means of a prophylactic-preventive method. *Journal of Trace Elements in Medicine and Biology* 15, 255–259.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Given, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Matrone, G., Conley, C., Wise, G.H. and Waugh, R.K. (1957) A study of iron and copper requirements of dairy calves. *Journal of Dairy Science* 40, 1437–1447.

Matrone, G., Thomason, E.L. Jr and Bunn, C.R. (1960) Requirement and utilization of iron by the baby pig. *Journal of Nutrition* 72, 459–465.

McCann, J.C. and Ames, B.N. (2007) An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive behaviour. *American Journal of Clinical Nutrition* 85, 931–945.

McGowan, J.P. and Crichton, A. (1924) Iron deficiency in pigs. *Biochemical Journal* 18, 265–272.

McGuire, S.O., Miller, W.J., Gentry, R.P., Neathery, M.W., Ho, S.Y. and Blackmon, D.M. (1985) Influence of high dietary iron as ferrous carbonate and ferrous sulfate on iron metabolism in young calves. *Journal of Dairy Science* 68, 2621–2628.

Mendoza, C., Viteri, F.E., Lonnerdahl, B., Young, K.A., Raboy, V. and Brown, K.H. (1998) Effect of genetically modified low-phytic acid maize on absorption of iron from tortillas. *American Journal of Clinical Nutrition* 68, 1123–1127.

Mendoza, C., Viteri, F.E., Lonnerdahl, B., Raboy, V., Young, K.A. and Brown, K.H. (2001) Absorption of iron from unmodified maize and genetically altered low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. *American Journal of Clinical Nutrition* 73, 80–85.

Miller, E.R. (1961) Sheep haematology from birth to maturity. *Journal of Animal Science* 20, 890–897.

Minihane, A.M. and Fairweather-Tait, S.J. (1998) Effect of calcium supplementation on daily nonheme-iron absorption and long-term iron status. *American Journal of Clinical Nutrition* 68, 96–102.

Mohri, M., Sharifi, K. and Eidi, S. (2007) Hematology and serum biochemistry of Holstein dairy calves: age-related changes and comparison with blood composition in adults, *Research in Veterinary Science* 83, 30–39.

Moran, J. (1990) Growing calves for pink veal: a guide to rearing, feeding and managing calves in Victoria. *Department of Agriculture Technical Report* 176, Melbourne, Australia.

Morris, E.R. and Ellis, R. (1976) Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. *Journal of Nutrition* 106, 753–760.

Munday, R. and Manns, E. (1989) Protection by iron salts against sporidesmin intoxication in sheep. *New Zealand Veterinary Journal* 37, 65–68.

Muntare, J., Mitjavila, M.T., Rodriguez, M.C., Puig-Parellada, P., Fernandez, Y. and Mitjavila, S. (1995) Dietary lipid and iron status modulate lipid peroxidation in rats with induced adjuvant arthritis. *Journal of Nutrition* 125, 1930–1937.

NFACC (2016) *Code of Practice for the Care & Handling of Veal Cattle: Review of Scientific Research on Priority Issues*. National Farm Animal Care Council, Lacombe, Alberta, Canada.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2005) *Mineral Tolerance of Animals*, 2nd edn. National Academy of Sciences, Washington, DC.

NRC (2012) *Nutrient Requirements of Swine*, Eleventh Revised Edition. NRC Press, Washington, DC.

O'Dell, B.L. (1981) Roles for iron and copper to connective tissue biosynthesis. *Philosophical Transactions of the Royal Society of London Series B* 294, 91–104.

Omara, F.O. and Blakley, B.R. (1993) Vitamin E is protective against iron toxicity and iron-induced hepatic vitamin E depletion in mice. *Journal of Nutrition* 123, 1649–1655.

Osborne, J.C. and Davis, J.W. (1968) Increased susceptibility to bacterial endotoxin of pigs with iron-deficiency anemia. *Journal of the American Veterinary Medical Association* 152, 1630–1632.

Paganini, D. and Zimmermann, M.B. (2017) The effects of iron fortification and supplementation on the gut microbiome and diarrhea in infants and children: a review. *American Journal of Clinical Nutrition 106*, *1688S–1693S.*

Papadopoulos, G.A., Maes, D.G.D. and Janssens, G.P.J. (2009) Mineral accretion in nursing piglets in relation to sow performance and mineral source. *Veterinarni Medicina* 54, 41–46.

Patterson, D.S.P. and Allen, W.M. (1972) Biochemical aspects of some pig muscle disorders. *British Veterinary Journal* 128, 101–111.

Pedersen, S., Saeed, I., Friis, H. and Michaelsen, K. (2001) Effect of iron deficiency on *Trichuris suis* and *Ascaris suum* infections in pigs. *Parasitology* 122, 589–598.

Peters, J.C. and Mahan, D.C. (2008a) Effects of neonatal iron status, iron injections at birth, and weaning in young pigs from sows fed either organic or inorganic trace minerals. *Journal of Animal Science* 86, 2261–2269.

Peters, J.C. and Mahan D.C. (2008b) Effects of dietary organic and inorganic trace mineral levels on sow reproductive performances and daily mineral intakes over six parities. *Journal of Animal Science* 86, 2247–2260.

Pu, Y., Li, S., Xiong, H., Zhang, X., Wang, Y. and Du, H. (2018) Iron promotes intestinal development in neonatal piglets. *Nutrients* 10, 726–737.

Puls, R. (1994) *Mineral Levels in Animal Health*, 2nd edn. Sherpa International, Clearbrook, British Columbia.

Rajabian, F., Mohri, M. and Heidarpour, M. (2017) Relationships between oxidative stress, haematology and iron profile in anaemic and non-anaemic calves. *Veterinary Record* 181, 265.

Ramsay, W.N.M. and Campbell, E.A. (1954) Iron metabolism in the laying hen. *Biochemical Journal* 58, 313–317.

Revy, P.S., Jondreville, C., Dourmad, J.Y. and Nys, Y. (2004) Effect of zinc supplemented as either an organic or an inorganic source and of microbial phytase on zinc and other minerals utilisation by weanling pigs. *Animal Feed Science and Technology* 116, 93–112.

Robertson, A., Morrison, J.N., Wood, A.M. and Bremner, I. (1989) Effects of iron deficiency on metallothionein I concentrations in blood and tissues of rats. *Journal of Nutrition* 119, 439–445.

Roland, L., Drillich, M. and Iwersen, M. (2014) Hematology as a diagnostic tool in bovine medicine. *Journal of Veterinary Diagnostic Investigation* 26, 592–598.

Rosa, I.V., Ammerman, C.B. and Henry, P.R. (1986) Interrelationships between dietary copper, zinc and iron on performance and tissue mineral concentration in sheep. *Nutrition Reports International* 34, 893–902.

Roughead, Z.K. and Hunt, J.R. (2000) Adaptation in iron absorption: iron supplementation reduces nonhaem Fe but not haem-Fe absorption. *American Journal of Clinical Nutrition* 72, 982–989.

Roughead, Z.K., Zito, C.A. and Hunt, J.R. (2005) Inhibitory effects of dietary calcium on the initial uptake and subsequent retention of haem and nonhaem Fe in humans: comparisons using an intestinal lavage method. *American Journal of Clinical Nutrition* 82, 589–597.

Ruiz-Ascacibar, I., Stoll, P., Bee, G. and Schlegel, P. (2019) Dynamics of the mineral composition and deposition rates in the empty body of entire males, castrates and female pigs. *Animal* 13, 50–58.

Schrama, J.W., Schouten, J.M., Swinkels, J.W.G.M., Gentry, J.L., Reiling, G. de V. and Parmentier, M.K. (1997) Effect of haemoglobin status on humoral immune response of weanling pigs of different coping styles. *Journal of Animal Science* 75, 2588–2596.

Seamer, J. (1956) Piglet anaemia: a review of the literature. *Veterinary Reviews and Annotations* 2, 79–93.

Smith, I.M. and Hill, R. (1978) The effect on experimental mouse typhoid of chelated iron preparations in the diet. In: Kirchgessner, M. (ed.) *Proceedings of Third International Symposium on Trace Elements in Man and Animals*. Arbitskreis Tierernährungsforchung, Weihenstephan, Germany, pp. 383–386.

Sousa, L., Oliveira, M.M., Túlio, C., Pessôa, C. and Barbosa, L.A. (2020) Iron overload: effects on cellular biochemistry. *Clinica Chimica Acta* 504, 180–189.

Stahl, C.H., Han, Y.M., Roneker, R.R., House, W.A. and Lei, X.G. (1999) Phytase improves iron bioavailability for haemoglobin synthesis in young pigs. *Journal of Animal Science* 77, 2135–2142.

Starzyński, R.R., Laarakkers, C.M.M., Tjalsma, H., Swinkels, D.W., Pieszka, M., Styś, A., Mickiewicz, M. and Lipinski, P. (2013) Iron supplementation in suckling piglets: how to correct iron deficiency anemia without affecting plasma hepcidin levels. *PLOS ONE* 8, e64022.

Stockman, R. (1893) The treatment of chlorosis by iron and some other drugs. *British Medical Journal* 1, 881–885, 942–944.

Sugiarto, M., Ye, A. and Singh, H. (2009) Characterisation of binding of iron to sodium caseinate and whey protein isolate. *Food Chemistry* 114, 1007–1013.

Suttle, N.F. and Peter, D.W. (1985) Rumen sulphide metabolism as a major determinant of the availability of copper to ruminants. In: Mills, C.F., Bremner, I. and Chesters J.K. (eds) *Proceedings of the Fifth International Symposium on Trace Elements in Man and Animals, Aberdeen*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 367–370.

Svoboda, M. and Drabek, J. (2007) Intramuscular versus subcutaneous administration of iron dextran in suckling piglets. *Acta Veterinaria Brno* 76, 11S–15S.

Svoboda, M. and Pistkova, K. (2018) Oral iron administration in suckling piglets – a review. *Acta Veterinaria Brno* 87, 77–83.

Ullrey, D.E., Miller, E.R., Thompson, O.A., Ackerman, I.M., Schmidt, D.A., Hoefer, J.A. and Luecke, R.W. (1960) The requirement of the baby pig for orally administered iron. *Journal of Nutrition* 70, 187–192.

Ullrey, D.E., Miller, E.R., Long, C.H. and Vincent, B.H. (1965) Sheep haematology from birth to maturity. 1. Erythrocyte population, size and haemoglobin concentration. *Journal of Animal Science* 24, 141–145.

Underwood, E.J. and Suttle, N.F. (1999) Iron. In: *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK, pp. 375–396.

Valcarcel, A.M.C., Gracia, C.M., Miro, S.M., Sanchez, J.M., Bermudez, C.A.G. *et al*. (2018) Iron bioavailability of four iron sources used to fortify infant cereals, using anemic weaning pigs as a model. *European Journal of Nutrition* 58, 1911–1922.

Van den Trop, A.M. (2005) *Reviews on the Mineral Provision in Ruminants (VIII): Iron Metabolism and Requirements*

in Ruminants. CVB Documentation Report No. 40. Centraal Veevoederbureau, Lelystad, The Netherlands.
Venn, J.A.J., McCance, R.A. and Widdowson, E.M. (1947) Iron metabolism in piglet anemia. *Journal of Comparative Pathology and Therapeutics* 57, 314–325.
Wan, D., Zhang, Y., Wu, X., Lin, X., Shu, X. *et al.* (2018) Maternal dietary supplementation with ferrous N-carbamylglycinate chelate affects sow reproductive performance and iron status of neonatal piglets. *Animal* 12, 1372–1379.
Wang, J. and Pantopoulos, K. (2011) Regulation of cellular iron metabolism. *Biochemical Journal* 434, 365–381.
Wang,Y.-Z., Shan, T.-Z., Xu, Z.-R., Feng, J. and Wang, Z.Q. (2006) Effects of lactoferrin (LF) on performance, intestinal microflora and morphology of weanling pigs. *Animal Feed Science and Technology* 135, 263.
Watson, A., Lipina, C., McArdle, H.J., Taylor, P.M. and Hundal. H.S. (2016) Iron depletion suppresses mTORC1-directed signalling in intestinal Caco-2 cells *via* induction of REDD1. *Cellular Signalling* 28, 412–424.
Welchman, D.D., Whelehan, O.P. and Webster, A.J. (1988) Haematology of veal calves reared in different husbandry systems and the assessment of iron deficiency. *Veterinary Record* 123.
West, A.R. and Oates, P.S. (2008) Mechanisms of heme iron absorption: current questions and controversies. *World Journal of Gastroenterology* 14, 4101–4110.
WHO (2015) *The Global Prevalence of Anemia in 2011*. WHO Press, Geneva.
Wolff, N.A., Garrick, M.D., Zhao, L., Garrick, L.M., Ghio, A.J. and Thevenod, F. (2018) A role for divalent metal transporter (DMT1) in mitochondrial uptake of iron and manganese. *Science Reports B, Article* 211.
Wu, W.-H., Meydani, M., Meydani, S.N., Burklund, P.M., Blumbery, J.B. and Munro, H.N. (1990) Effect of dietary iron overload on lipid peroxidation, prostaglandin synthesis and lymphocyte proliferation in young and old rats. *Journal of Nutrition* 120, 280–289.
Yu, B., Huang, W.-J. and Chiou, P.W.-S. (2000) Bioavailability of iron from amino acid complex in weanling pigs. *Animal Feed Science and Technology* 86, 39–52.
Zhang, L.Y., Lu, L. and Luo, X.G. (2016) The chemical characteristics of organic iron sources and their relative bioavailabilities for broilers fed a conventional corn–soybean meal diet. *Journal of Animal Science* 94, 2378–2396.
Zhang, L.Y., Li, X.F. Liao, X.D., Lu, L. and Luo, X.G. (2017) Effect of iron source on iron absorption and gene expression of iron transporters in the ligated duodenal loops of broilers. *Journal of Animal Science* 95, 1587–1597.
Zimmerman, M.B., Adou, P., Torresani, T. and Hurrell, R. (2000) Iron supplementation in goitrous children improves their response to oral iodized oil. *European Journal of Endocrinology* 142, 217–223.
Zimmermann, M.B. and Hurrell, R.F. (2007) Nutritional iron deficiency. *Lancet* 370, 511–520.
Zinoffsky, O. (1886) Ueber die Grosse des Hamoglobinmoleculs. *Hoppe-Seylers Zeitschriftur Physiologische Chemie* 10, 16.

14 Manganese

Manganese is the fifth most abundant metal on earth and known to be essential for growth and fertility in laboratory animals since the 1930s. The need for manganese in farmed livestock became evident when breeder hens, deprived of manganese, produced embryos with malformed skeletons (Lyons and Insko, 1937) and two chick diseases, perosis ('slipped tendon') and nutritional chondrodystrophy, were prevented by adding Mn to diets (Schaible *et al*., 1938). Bone development was also severely affected in pigs deprived of Mn (Johnson, 1943) but some years elapsed before adverse effects were demonstrated in cattle (Bentley and Phillips, 1951) and sheep (Lassiter and Morton, 1968). By this time, manganese had been found widely distributed at low concentrations in mammalian tissues and necessary for reproduction in both sexes. Further studies with small rodents showed that severe Mn deprivation could impair central nervous system function (Hurley, 1981) and immunity (Hurley and Keen, 1987). Interest among ruminant nutritionists was rekindled by three developments: induction of neonatal chondrodystrophy by feeding pregnant heifers a diet low in Mn (Hansen *et al*., 2006b); suggestions, albeit ill-founded (p. 360), that NRC (2001) requirements for dairy cattle were gross underestimates (Weiss and Socha, 2005) and by the discovery that excess manganese exacerbated lack of dietary Cu in cattle (Hansen *et al*., 2006a). Unusually amongst trace elements, manganese metabolism is not regulated by a specific transporter. Instead metabolism is facilitated by shared transporters, leaving manganese open to antagonism from other elements (e.g. Aydemir and Cousins, 2009; Felber *et al*., 2019). The emphasis in this chapter is on poultry, the species most likely to lack dietary Mn on farms and to which the latest molecular and genomic techniques are being applied, with questionable validity, to elucidate requirements and evaluate sources.

Functions of Manganese

Intermediary metabolism

Carbohydrate and lipid metabolism are Mn-dependent because pyruvate carboxylase is activated by Mn and generates oxaloaceteate, thus keeping the TCA cycle turning (Scrutton *et al*., 1966, 1972). The enzyme is most active in gluconeogenic (liver and kidney) and lipogenic tissues (adipose tissue and mammary gland). A diet low in Mn reduced fat deposition in pigs (Plumlee *et al*., 1956). Biotin also activates pyruvate carboxylase and lack of biotin can produce similar anomalies of energy metabolism. Manganese also activates arginase and glutamine synthase: the former detoxifies ammonia and affects the relaxation of endothelial tissue, lining blood vessels; the latter is involved in elaborating DNA and other proteins and therefore essential for cell growth and replication (Horning *et al*., 2015).

Anti-oxidant defences

A superoxide dismutase (MnSOD) was first isolated from chicken liver mitochondria (Gregory and Fridovich, 1974), which were later found to contain 2mg Mn mol^{-1}. Oxidative phosphorylation in mitochondria accounts for 60% of cellular O_2 consumption (Leach and Harris, 1997) and generates potentially toxic, reactive oxygen species (ROS) as a by-product. The capacity of Mn to change valency in MnSOD allows the enzyme to become the principal defender against ROS and intracellular oxidative stress. Tissue activities of MnSOD are low in lambs at birth (Paynter and Caple, 1984), probably reflecting the low oxidative stress of life *in utero*, but rise in the placentome at parturition for the opposite reason (Garrel *et al*., 2010). The subsequent need for neonates to respire is associated with a marked rise in MnSOD activity

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in lung by four weeks of age. Most Mn in ovine heart is present as MnSOD (Paynter and Caple, 1984). Other SODs depend on copper for redox activity but MnSOD is by far the most dominant dismutase in all tissues. Manganese deprivation of broiler breeder hens lowered MnSOD in heart and increased the expression of heat shock proteins during heat stress (Zhu *et al*., 2015) (p. 360). Capacity to remove ROS allows manganese to perform other functions.

Cell regulation and inflammation

At low concentrations, ROS participate in vital intracellular signalling pathways. By regulating ROS concentrations, MnSOD regulates cell division, development and death (Garrel *et al*., 2010). Recent studies with mice confirm findings in broilers (Zhang *et al*., 2020a) that lack of Mn *per se* restricted development of the intestinal mucosa, weakening tight junctions, increasing the expression of inflammatory cytokines and increasing peroxidative damage under pharmacological inflammatory challenge (Choi *et al*., 2020). The gene for MnSOD is induced by cytokines from the immune cascade, such as tumour necrosis factor α (Wong and Goeddel, 1988) and is 'stress-responsive' (St Clair, 2004). Manganese-deprived chicks have raised concentrations of TNF α, which may trigger secondary changes in bone and regulatory hormones (PTH and calcitonin (Wang *et al*., 2013)). Mitochondria are particularly vulnerable to damage from ROS at times of high metabolic activity. In women, mRNA for mitochondrial MnSOD is expressed during the intense activities of labour, inflammation and fever (Than *et al*., 2009).

Immunity

Evidence has emerged of the involvement of manganese in resisting viral and microbial infections. Adding Mn to diets containing 23–26mg Mn kg^{-1} DM improved antibody titres against Newcastle virus in broilers, vaccinated after only six days of treatment, while also eliminating leg abnormalities but not improving performance (Ghosh *et al*., 2016): this suggests that humoral immunity is very sensitive to dietary Mn. Under similar dietary conditions, permeability of the small intestine, inflammatory responses to oral inoculation with *S. typhimurium* and pathogen count in the digesta were all reduced by supplementary Mn in one-week-old chicks (Zhang *et al*., 2020b). Furthermore, infection changed the body distribution of Mn, lowering plasma Mn but increasing spleen, thymus and bursa Mn, tissues involved in developing immunity.

Connective tissue development

Manganese promotes the synthesis of mucopolysaccharides in cartilage by activating glycosyltransferase. Manganese deprivation reduces synthesis of glycosaminoglycan and oligosaccharide side chains, which strengthen cartilage (Leach and Harris, 1997). Manganese-deprived chicks had less proteoglycan, of abnormal composition, in tibial growth plate cartilage than Mn-replete chicks (Liu *et al*., 1994). Lack of Mn in laying hens caused subnormal egg production and poor shell formation, due to impaired mucopolysaccharide synthesis (Hill and Mathers, 1968): hexosamine content of the shell matrix was reduced (Longstaff and Hill, 1972). Shell ultrastructure is more sensitive to lack of dietary Mn than egg production, weakened by reduced density of mammillary knobs and decreased expression of genes encoding glucuronosyltransferase (Xiao *et al*., 2014), proteoglycans and glycoproteins in the eggshell gland (Zhang *et al*., 2018),

Blood clotting

Manganese is involved in the synthesis of prothrombin, a glycoprotein that facilitates blood-clot formation, through activation of glycosyltransferases. The clotting response to vitamin K is reduced in manganese-deprived chicks (Doisey, 1973).

Reproduction

Differences in Mn distribution among reproductive tissues from normal and anoestrous ewes suggested a possible role in corpus luteum function (Hidiroglou, 1975). A hypothesis that lack of Mn inhibited synthesis of cholesterol and certain sex hormones, thus causing infertility (Doisey, 1973), has not been confirmed. Plasma cholesterol and conception rate were unaffected in heifers, sufficiently deprived of Mn to give birth to deformed calves (Hansen *et al*., 2006a, b). Lack of Mn restricted testicular growth relative to body growth in ram lambs without reducing testicular Mn and may act indirectly by altering gonadotrophin activity (Masters *et al*., 1988). Sperm mass and motility were improved by raising dietary Mn from 16 to 26mg kg^{-1}DM in cockerels (Attia *et al*., 2010).

Clinical Consequences of Manganese Insufficiency

When diets lack Mn, the clinical consequences vary with species, degree and duration of deprivation and age and productivity at the onset of deprivation.

Leg abnormalities

Manganese deprivation causes 'perosis' or 'slipped tendon' in hatched chicks (Gallup and Norris, 1939a), poults and ducklings, due to enlargement and malformation of tibio-metatarsal joints. The tibia becomes twisted, bent, thickened and shortened, causing the gastrocnemius tendon to slip from its condyles. Affected chicks become reluctant to move, squat on their hocks and will die unless the tendon is manipulated back into position and remains there. Similar tibial abnormalities, resulting in lameness but not slipped tendons, were found in Mn-deprived pigs (Johnson, 1943; Plumlee *et al*., 1956), lambs (Lassiter and Morton, 1968) and goat kids (Anke *et al*., 1973a, b). Lack of Mn in breeder hens decreased endochondral growth (chondrodystrophy) in chick embryos (Liu *et al*., 1994), manifested as shortened, thickened limbs and a 'parrot beak'. The latter was due to shortening of the lower mandible and globular contour of the head: mortality was high. When heifers were deprived of Mn, their calves were small but showed disproportionate dwarfism, superior brachygnathism ('undershot' lower mandible), swollen joints and an unsteady gait (Hansen *et al*., 2006b). Ataxia was reported in Mn-deprived chicks (Caskey and Norris, 1940), lambs (Lassiter and Morton, 1968) and goat kids, deprived during pregnancy (Anke *et al*., 1973a): intensive studies in rodents[i] showed ataxia to be a secondary consequence of skeletal defects.

Reproductive disorders

Defective ovulation and testicular degeneration were observed in early experiments with severely Mn-deprived rodents and similar reproductive anomalies were encountered in cockerels and hens (Gallup and Norris, 1939b). Depressed or delayed oestrus and poor conception rates were associated with experimental Mn-deprivation in cows, goats and ewes (Underwood and Suttle, 1999) but were not reported in Mn-deprived heifers (Hansen *et al*., 2006a). In rams, Mn deprivation reduced testicular growth relative to body growth (Masters *et al*., 1988). A recent study in breeding ducks suggested that reproductive effects were associated with high (180) rather than low (18mg kg^{-1}DM) dietary Mn: follicle-stimulating hormone and progesterone concentrations were reduced in serum (Zhang *et al*., 2018).

Natural occurrence

Avian species are most at risk because of poor absorption of dietary Mn but incidents are rare on farms (Leeson and Caston, 2008). Short periods of deprivation, sufficient to retard growth immediately after hatching had residual effects (Mwangi *et al*., 2019) but may not occur on farms. The most vulnerable periods are the fattening stage for broilers (Ghosh *et al*., 2016) and the transition to lay, when net need is high and availability low, because increased dietary Ca lowers Mn availability further. High dietary Fe lowers Mn status in broilers (Bai *et al*., 2012) and

- high dietary Fe may increase risk of Mn insufficiency in all species.

Naturally occurring congenital chondrodystrophies in calves have been associated with low tissue Mn (e.g. Hidiroglou *et al*., 1990; Valero *et al*., 1990; Staley *et al*., 1994) and are still a cause for concern (White and Windsor, 2012; SRUC, 2011) but unequivocal responses to Mn have yet to be demonstrated. Outbreaks have been associated with drought conditions and exclusive feeding of silage during winter.

- Deprivation may be systemic rather than dietary in origin and a secondary consequence of iron overload, which disrupts placental transfer of Mn.

Placental transfer of Mn is affected by the same non-specific carrier proteins that facilitate absorption, which is downregulated during iron overload (p. 354). Ingestion of soil Fe can be high during droughts, during winter and from some silages. Ensilage raises the availability of soil Fe (Hansen and Spears, 2009). In ruminants, Mn insufficiency is most likely to occur in livestock grazing pastures on alkaline soils (Suttle, 2000) or reared on maize silage, with maize or barley supplements, all of which can be low in Mn. Heifers bearing their first progeny and still growing are particularly vulnerable under experimental conditions (Hansen *et al*., 2006b) and this may apply on farms. However, Mn-responsive disorders are rarely reported in the field. Grazing sheep and cattle can develop and

reproduce normally even in areas where application of Mn-containing fertilizers improves plant growth. Ewes in south Australia (Egan, 1972) and cows in south-west England (Wilson, 1966) on low-Mn pastures occasionally showed improved conception rates when given supplementary Mn. Recent claims of improved conception rate in cows given Mn in multi-element injections are unconvincing (p. 49).

Biochemical Changes when Diets Lack Manganese

Manganese is one of the least-abundant trace elements in the body and, in sheep, most Mn is found outside the carcase, in the gastrointestinal tract and pelt (Fig. 14.1).[ii] The sequence of biochemical changes that precedes development of clinical signs has to be inferred from end-point values of studies in which dietary Mn was increased from very low values, something achieved more easily in pigs and chicks than in ruminants. In pigs (Fig. 14.2), a logarithmic scale cannot hide the fact that tissue Mn decreased most in liver and kidney, organs that secrete and excrete Mn, while blood Mn was preserved as dietary Mn decreased: the largest pools (muscle and bone, the latter not shown) were always lowest in Mn and relatively unresponsive to change in dietary Mn; heart Mn was intermediate. Responses are characteristic of a Group 2 element (Fig. 3.2), a fourfold increase in dietary Mn from 8 to 32mg kg^{-1}DM producing little increase in tissue Mn: with little propensity to store Mn, depletion and deficiency phases are not easily distinguished. Decreased dietary Mn affects enzyme activities and tissue Mn quite differently (Fig. 14.2). Heart MnSOD is ultra-responsive and liver MnSOD completely unresponsive to decreases in dietary Mn. In muscle, as in liver, MnSOD activities were maintained during the most severe Mn deprivation. Similar results were obtained in broiler chicks (Lu *et al*., 2007, 2016). Earlier, reductions in heart MnSOD activity had been associated with structural changes in mitochondria in broiler chicks (Luo *et al*., 1993), suggesting transition from a state of deficiency to dysfunction (Fig. 3.2). Activities of arginase in liver decreased linearly as dietary Mn decreased in pigs (Pallauf *et al*., 2012).

In Mn-deprived lambs, MnSOD decreased in heart and lungs (Fig. 14.3): susceptibility to oxidant stress (e.g. from PUFA or insufficiencies of other dietary antioxidants, such as Cu, Se and vitamin E) may increase on diets lacking Mn (Masters *et al*., 1988).

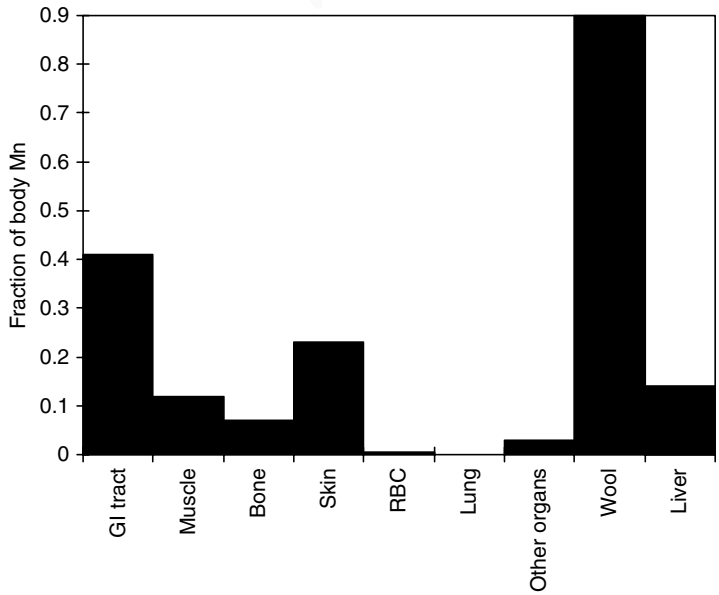

Fig. 14.1. Distribution of manganese in empty body of mature sheep: manganese content of the shorn fleece was relatively large. (Data from Grace, 1983). GI, gastrointestinal; RBC, red blood cells

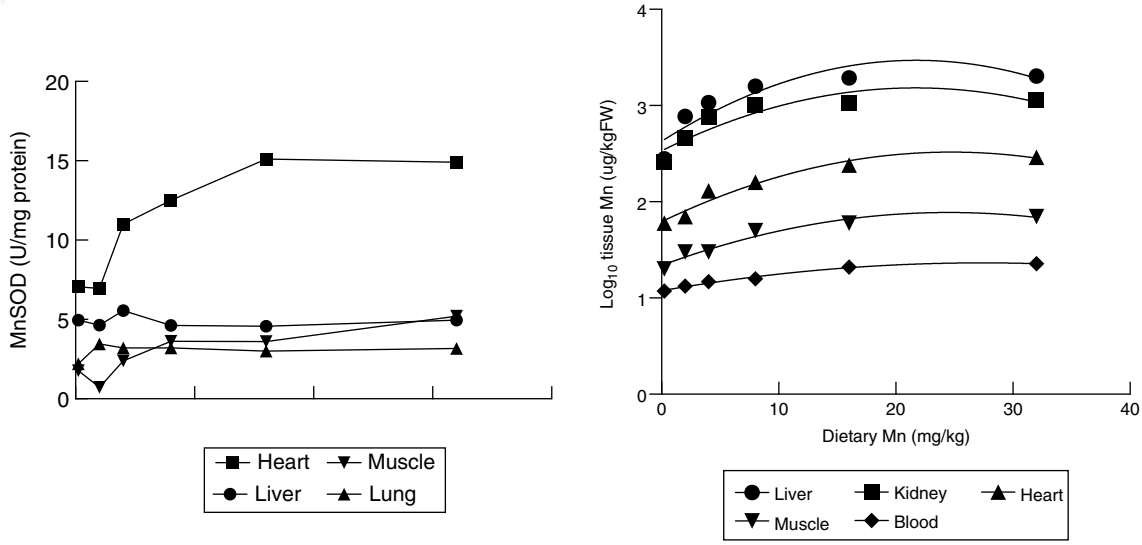

Fig. 14.2. Responses in manganese concentration and superoxide dismutase activity MnSOD; in tissues to increases in dietary Mn in young pigs given a semi-purified diet. (Data from Pallauf *et al*., 2012)

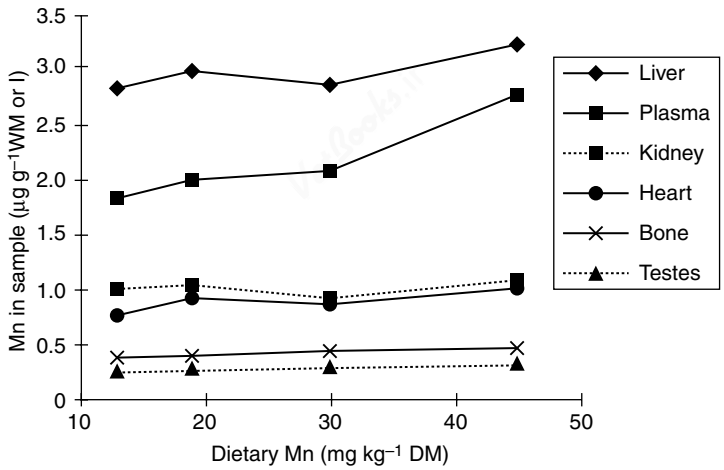

Fig. 14.3. In lambs, manganese concentrations in heart tissue are relatively more responsive to Mn deprivation than those in other soft tissues. (Data from Masters *et al*., 1988)

Diagnosis of Manganese Insufficiency

There are no agreed biochemical criteria for supporting a diagnosis of manganese insufficiency and different standards may be required at different stages of development. Blood Mn is higher in the newborn calf than in the dam (23–28 versus 15–17 µg l^{-1}) (Weiss and Socha, 2005). There may be a step change in plasma Mn with the resumption of oestrus cycling in heifers, judging from the results of Hansen *et al*., 2006a, b and Legleiter *et al*., 2005. Plasma Mn <2 µg l^{-1} may be indicative of deprivation in young lambs but normal values are much lower than those in cattle and heart Mn appeared to have greater diagnostic merit (Masters *et al*., 1988): values <0.2mg kg^{-1}FW may indicate insufficiency. Hair Mn is an unreliable index of status but wool and feather Mn may be more useful. Wool Mn had, on average, only 6.1 mg Mn kg^{-1}, compared to 18.7mg kg^{-1}DM in control

Table 14.1. Marginal bands[a] for mean manganese concentrations (mg kg^{-1}DM) in diet, tissues and bloodstream for assessing risk of manganese disorders in farm livestock.

	Diet	Blood[b]	Serum[b]	Liver	Bone	Heart	Pancreas	Integument
Cattle	8–20	<20	<20	<9.0	**1.0**–1.4^{R}	–	**3.0**–5.0	
Sheep	8–20	**12**–20	**1.8**–2.0	**8**–9	**0.3**–0.4^{MF}	**0.6**–0.7	**4**–8	
Goat	10–20	–	–	**3**–6	**5.0**–6.0^{R}	**1.5**–2.0	–	
Pigs	6–20	**10–1**2	**3**–4	**6**–9	**2.0**–2.3^{RCT}	–	**2.0**–3.0	
Poultry	**30**–40	**30**–50	**5–1**0	**6**–12	**4.0**–6.0^{TF}	–	**10**–15	

C, cortical bone used for assay; F, fat-free; M, metatarsal; R, rib; T, tibia

[a]Mean values below bands or individual values close to the lower (**bold**) limit indicate the probability of a positive response to manganese supplements; mean values within bands indicate the possibility of such responses. Multiply by 0.0182 to obtain values in mmol and µmol, respectively.

[b]Values for blood and serum are in µg l^{-1}

lambs after 22 weeks on a low-Mn diet (Lassiter and Morton, 1968). Similarly, skin and feathers of pullets fed on a low-Mn diet for several months averaged 1.2mg Mn kg^{-1}DM, one tenth of the value for comparable birds on a Mn-rich diet (Mathers and Hill, 1968). Responses at the molecular level may ultimately be required to give unequivocal confirmation of disorder and the use of mRNA for heart MnSOD has been advocated in broiler chicks (Li *et al*., 2004; Lu *et al*., 2007). However, diagnostic limits cannot yet be suggested for genomic and enzymic indices and reliance must still be placed on the conventional measures summarized in Table 14.1, despite obvious limitations.

Dietary Sources of Manganese

Seeds and grains

Seeds and seed products can contain 5–50mg Mn kg^{-1}DM, due partly to inherent species differences, which rank maize < sorghum < barley < wheat < oats (Table 14.2): values >30 are rare and probably caused by contamination. Species differences among lupin seeds are striking: white lupin (*Lupinus albus*) seeds can contain 817–3397mg Mn kg^{-1}DM, some 10–15 times more than other species growing on the same sites (Gladstones and Drover, 1962; White *et al*., 1981). *Lupinus angustifolius* contained as little as 6 mg Mn kg^{-1}DM (White *et al*., 1981). Field beans (*Vicia faba*) are also low in manganese (7.8 ± 1.5 mg kg^{-1}DM) (MAFF, 1990). Manganese is highly concentrated in the outer layers of grains. Mill by-products are therefore much richer in Mn than grains (Table 14.2) and enrich rations of which they are part.

Protein supplements and sources

Protein concentrates of animal origin, such as blood meal, meat meal, feather meal and fish meal, contain less Mn (0.2–20mg kg^{-1}DM) than protein supplements of plant origin, such as linseed, rapeseed and soybean meal (SBM) (35–55mg kg^{-1}DM) (MAFF, 1990). The limited use of single 'book' values for feeds is illustrated by recent data for SBM from different countries: mean values were higher in samples from Argentina (46.5) and India (46.9) than Brazil (36.7) and USA (39.7) but varied by 10.8, 40.9, 12.5 and 2.9mg Mn kg-^{1}DM, respectively within countries (Ibanez *et al*., 2020). Manganese is one of the least-abundant elements in milk, rarely exceeding 0.1 mg l^{-1} in any species, except in colostrum. Bovine milk contains 20–40 µg l^{-1} and concentrations cannot be raised by increasing manganese intake (Underwood and Suttle, 1999). Milk by-products are also low in Mn (<1mg kg^{-1}DM). Manganese in bovine milk is mostly associated with low-molecular-weight proteins in the casein fraction, whereas in human milk, Mn is found mostly in the whey fraction, bound to lactoferrin (Lonnerdal *et al*., 1985).

Forages

Pastures vary markedly in Mn, a high global mean of 86 mg kg^{-1}DM being reported and only 3% of grass samples having <20 mg Mn kg^{-1}DM (Minson, 1990). A recent survey of New Zealand pastures (Knowles and Grace, 2014) reported a median value of 74 ± 2 mg kg^{-1}DM, far less than an earlier study (Grace, 1973). Differences due to species and state of maturity are generally small (Minson, 1990) and are probably masked by soil contamination. Soils in the UK contained 300–1100mg Mn kg^{-1}DM (e.g. Suttle *et al*., 2003), much more than

the pasture they support. Contamination can also occur during sample processing if mills with steel blades are used. Increases in soil pH markedly decrease manganese uptake by plants, whether they are grasses (Fig. 2.1), legumes (Mitchell, 1957) or cereals (Fig. 14.2). Low manganese concentrations have been reported for maize silages from Canada (approximately 22 mg kg^{-1}DM) (Miltimore *et al.*, 1970; Buchanan-Smith *et al.*, 1974), the UK (14.6 ± 7.2 mg kg^{-1}DM) (MAFF, 1990) and USA (17.1 ± 20.3mg kg^{-1}DM) (Berger, 1995). Lucerne hays from Canada and USA contained, on average, 43.7 and 22.7 mg Mn kg^{-1}DM, respectively, but values varied widely (see Table 2.2).

Availability of Manganese in Feeds

Measuring the availability of Mn as a feed attribute, rather than an attribute of a particular experiment, is not easy.

Antagonism from iron

Valid assessments of availability as a feed attribute require an understanding of the ways in which absorptive efficiency (A_{Mn}, p. 10) is affected by dietary Mn, dietary Fe and body Fe stores. There is no specific transporter for Mn. Absorption is effected at the mucosal surface of the small intestine by DMT1, as is that of Fe: A_{Mn} can be inversely related to Mn and Fe intakes, which can range widely (Finley, 1999)[iii] (Figs 14.5 and 14.6). The relationship between group mean faecal Mn (F_{Mn}) and intake of Mn (I_{Mn}) in young pigs, given a diet based on dried skimmed milk and containing 0.2–16mg kg^{-1}DM (Pallauf *et al.*, 2012) was:

$$F_{Mn}\,(mg/d) = 0.71 \pm 0.015\, I_{Mn} - 0.11 \pm 0.123;\ r^2 = 0.99$$

indicating that AA_{Mn} was a uniform 0.29 of ingested Mn. However, routine parenteral injection of Fe-dextran in the newborn piglet may have suppressed

Table 14.2. Mean manganese concentrations (mg kg^{-1}DM) in cereal grains and mill products from four countries indicate minimal geographic and historic variation for grains, 30 years separating the UK (MAFF, 1990) and Chinese (Huang *et al.*, 2017) data: values in parentheses are standard deviations, with much larger sample sizes for the latter.

Cereal Country	Maize	Barley	Wheat	Oats	Rice	Rice bran	Wheat bran	Maize GM
N America	5	14	31	36	–		108	
Australia	8	15	37	43	16		133	
UK	6 (3.0)	19 (3.6)	36 (15)	45 (15)	8 (0.5)		89 (33)	–
China	6 (0.4)	21 (1.4)	40 (1.6)	–	78 (4.3)	166 (12)	147 (6.8)	4.6 (0.7)

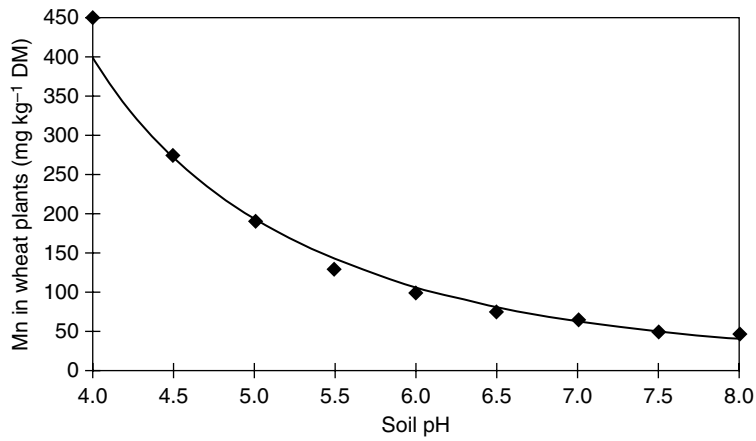

Fig. 14.4. Manganese concentrations in crops and pastures fall markedly when acid soils are limed. (Data for wheat plants in co-ordinated Food and Agriculture Organization trials) (Silanpaa, 1982)

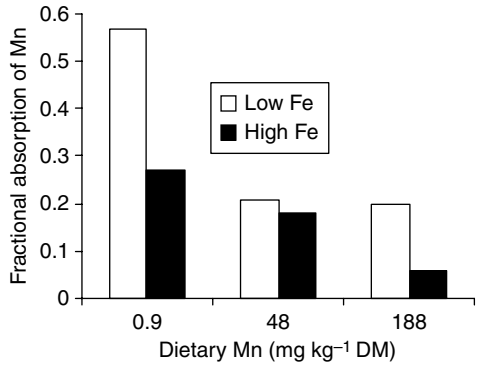

Fig. 14.5. Fractional absorption of manganese (Mn) in rats decreased non-linearly as dietary Mn increased, particularly if the diet was high rather than low in iron. (Data from Davis *et al.*, 1992)

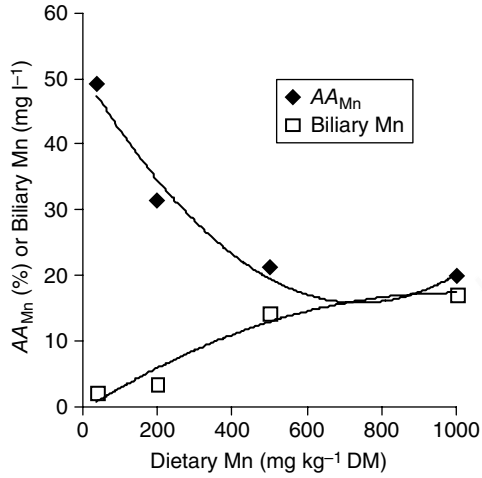

Fig. 14.6. Relationship between apparent absorption of manganese (AA_{Mn}), biliary Mn and dietary Mn concentration in calves fed milk replacers. (From Jenkins and Hidiroglou, 1991)

the ability to upregulate Mn absorption at the lowest Mn intakes. In humans, A_{Mn} is inversely related to serum ferritin, a measure of Fe storage (p. 329). Studies with broiler chicks suggest that high Fe status of the body reduces transfer of Fe and Mn across the gut mucosa by downregulating DMT1 (Bai *et al.*, 2020b). Antagonism between Fe and Mn was confirmed in calves (Hansen and Spears, 2008). Calves given a milk replacer containing 14.2 mg Mn and 91mg Fe kg^{-1}DM – far more of both elements than needed from such a source – retained only 4.9% of Mn intake (Suttle, 1979).

Interaction with zinc and vitamin D

Recent studies confirm that zinc transporters can also transport Mn (e.g. ZnTr4 and ZnTr10; Aydemir and Cousins, 2009; Felber *et al.*, 2019), with the help of a Ca^{+}-mediated process, indicating scope for a reciprocal Zn × Mn antagonism and influence from vitamin D. Retrospectively, there was evidence of such an antagonism in studies with pigs. Adding 100mg Zn kg^{-1}DM to a corn/soybean meal (SBM) diet containing 45mg Mn kg^{-1}DM from feed and inorganic sources, reduced $AA_{Mn,}$ from 0.5 to 0.3: without added Zn, phytase supplements reduced AA_{Mn} (Adeola *et al.*, 1995). Zinc may out-compete Mn for transport by DMT1.

Antagonism from phytate and fibre

Interactions with common constituents in less-digestible foodstuffs, such as phytate and fibre, may impair A_{Mn}, which rarely reaches the high values found with semi-purified (Fig. 14.5) and liquid diets (Fig. 14.6), which are naturally low in Fe. In chicks given a corn/SBM blend, AA_{Mn} was only 0.028 compared to 0.103 when $MnSO_4$ was added (Wedekind *et al.*, 1991). Similar contrasts between inherent feed Mn and inorganic Mn were found in human volunteers.[iv] Chicks retained <5% of an oral dose of ^{54}Mn when the diet contained 4mg Mn kg^{-1}DM and retention fell from 4.5% to 2.8% as chicks grew from two to five weeks of age (Mathers and Hill, 1967). Since a similar diet caused a low incidence of perosis, indicating a state of deprivation (Hill, 1967), maximum absorbability (A_{Mn}, p. 10) had probably been attained. A recent study comparing broiler chicks and turkey poults, given blends of wheat, SBM and rapeseed meal, containing over 100mg Mn kg^{-1}DM (mostly inorganic) and added phytase (500–3000 FU kg^{-1} DM), revealed a large reduction in AA_{Mn} in turkey poults from 0.21 to 0.05 ± 0.041 between 7 and 28 days of age: supra-supplementation with phytase did not prevent the decrease. In chicks, the corresponding reduction in AA_{Zn} was from 0.30 to 0.19 (Olokusi *et al.*, 2020). With one exception (Biehl *et al.*, 1995), microbial and vegetal phytases have not improved AA_{Mn} in chicks (Mohanna and Nys, 1999; Ghosh *et al.*, 2016; Olukosi *et al.*, 2020). In poultry, fibre may be more directly antagonistic towards Mn than phytate, which may become secondarily important by influencing absorbable Zn in the digesta. In pigs, additions of phytase of up to

2500FU kg^{-1}DM linearly increased AA_{Mn} from 0.34 to 0.50 ± 0.10 (Arredondo *et al.*, 2019) but dietary Zn was decreased in phytase supplemented diets.

Effects of calcium and phosphorus

Calcium and P supplements have long been known to lower availability of Mn in chicks but large excesses were given (e.g. Schaible *et al.*, 1938). When dietary P is optimal, the major impairment of Mn utilization from dry diets may come from interactions between Ca and organic feed constituents other than phytate. Lower AA_{Mn} in turkey poults than in broiler chicks may have been attributable to a higher dietary Ca (14.4 versus 9.1g kg^{-1}DM) (Olokusi *et al.*, 2020). However, in pigs, feeding diets low in Ca and P had no effect on AA_{Mn} (Arredondo *et al.*, 2019).

Experimental method

Measuring relative availability by comparing Mn retention from different sources (p. 28) is unsuitable for Group 2 minerals, like Mn, which are poorly stored. Liver and bone Mn did not increase in lambs when dietary Mn increased from 13 to 45mg Mn kg^{-1}DM (Masters *et al.*, 1988). Excess absorbed Mn is removed by increasing faecal endogenous loss (FEL_{Mn}). Biliary Mn increased linearly in chicks when a diet low in Mn was supplemented with 7 or 14mg Mn kg^{-1}DM (Halpin and Baker, 1986) but with negative curvilinearity in pre-ruminant calves (Fig. 14.6) and adult sheep (Grace and Gooden, 1980) as dietary Mn approached 1000mg kg^{-1}DM. Retention can be increased by overwhelming homeostatic control with vast increases in dietary Mn[v] but responses remain curvilinear (e.g. in chicks; Fig. 14.7): comparisons of linear slopes give unreliable values of availability (p. 28).

- Choices of diet type, dietary Mn, Fe, Zn, Ca and P, test animal and technique, made by the experimenter, can affect assessments of Mn availability in feed sources.[vi]

Milk and milk products

Manganese in milks becomes largely bound to lactoferrin receptors at the brush border in some species, transported across the mucosa by DMT1 and bound to ferritin in all, as does iron (Davidson and Lonnerdahl, 1989; Arrenondo and Munez, 2005). Manganese in milk and milk products is well absorbed by the suckling or artificially reared animal. All newborn animals may be able to absorb Mn bound to large molecules in solid as well as milk-based diets before gut closure. Calves reared on whole milk (Carter *et al.*, 1974) or milk substitute (Kirchgessner and Neese, 1976), containing 0.75mg Mn kg^{-1}DM, retained

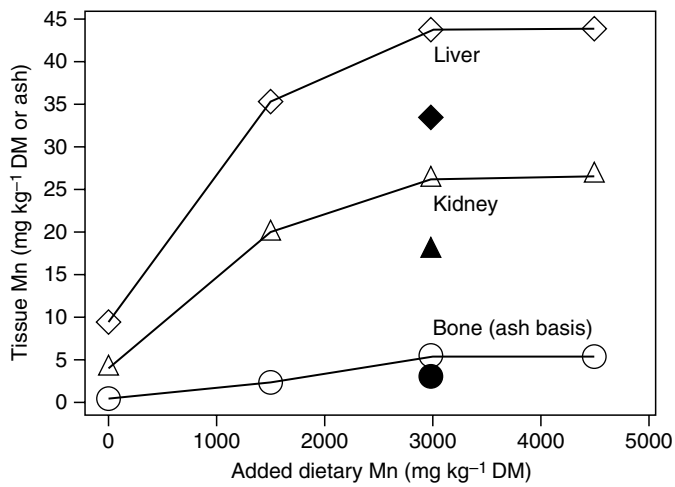

Fig. 14.7. Manganese accumulates in broiler tissues when dietary concentrations are very high but relationships are curvilinear and anomalous relative availabilities are derived by comparing linear slopes. (Data from two experiments (open and closed symbols) of Halpin and Baker, 1986)

60% and 40%, respectively, of ingested Mn, but a supplement of 15mg Mn kg^{-1}DM reduced that figure to 16.3% (Carter *et al*., 1974). Artificially reared piglets absorbed 35–68% of Mn from milk formula feeds low in Mn (Atkinson *et al*., 1993; Rutherfurd *et al*., 2006). The constant AA_{Mn} in the study of Pallauf *et al*., 2012) may represent the maximum availability of Mn_i added to diets based on dried skimmed milk. In calves reared on milk replacers containing 90–100mg Fe kg^{-1}DM, AA_{Mn} at seven weeks of age was 0.5 without added Mn (Fig. 14.6).

Availability of manganese in pig and poultry rations

In cereal and vegetable protein sources, <0.20 of the dietary Mn is available to older chicks and poults but more is available to pigs: supplementary Mn_i is marginally more available to both classes than feed Mn. In two corn/SBM blends with no added inorganic trace elements, AA_{Mn} was 0.25 and 0.27 ± 0.032 (Liu *et al*., 2014). With multi-element supplementation, AA_{Mn} in a similar diet was 0.35 (Arredondo *et al*., 2019).

- Manganese absorbability is normally low in pigs and poultry, but marginally higher for inorganic supplements (Fig. 14.8): the inhibitory factor in feeds is unlikely to be phytate.

Manganese availability in forages

There have been no reports relating to A_{Mn} in forages for ruminants, either as relative or absolute measurements. In a factorial model of Mn requirements of dairy cows, NRC (2001) assumed an A_{Mn} coefficient of 0.065–0.075 for feed Mn. Since two potential antagonists – phytate and fibre – are broken down in the rumen, A_{Mn} should be higher than that achievable on practical diets by non-ruminants. Linear regression of faecal Mn on Mn intake indicated an AA_{Mn} of 0.26 in beef cattle but probably applied to Mn_i provided in excess of need (Weiss and Socha, 2005). Unsupplemented blends of cereal concentrate with sugarcane, and sugarcane and soybean hulls or corn silage, low in Ca and P and containing 27–36mg Mn kg^{-1}DM, had AA_{Mn} of 0.23, 0.16 and 0.04, respectively (Zannetti *et al*., 2017).

- Availability of Mn to steers may be low when ground corn is fed with corn silage.

Soil Mn that often contaminates pasture may be partially available, since effluents of continuous

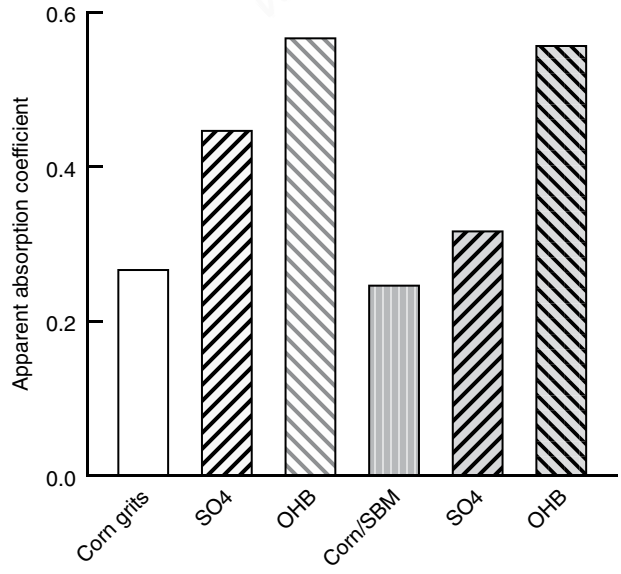

Fig. 14.8. Apparent availability of manganese in pigs given diets based on either corn grits or corn and soybean meal: availabilities of added $MnSO_4$ or 2-hydroxy-4-methylthio butanoic acid chelate (OHB), given with inorganic or chelated iron and zinc, were calculated by the method of additons (p. 11). (Data from Liu *et al*., 2014)

cultures of rumen microbes, supplemented with two soils, relatively low in Mn, were high in soluble Mn (Brebner *et al*., 1985).

Manganese Requirements: Common Problems

Estimates of Mn requirements vary with species, stage of development, dietary source and chemical composition of the whole diet. Criteria of adequacy are also important, in particular whether 'requirement' is for optimal performance, leg condition, tissue Mn or molecular indices. When functional or molecular indices were used to assess needs for Mn in broilers, requirement for the first 21 days of life in broiler chicks leapt to 130mg Mn kg^{-1}DM, close to the maximum fed, for optimal MnSOD activity or mRNA abundance (Li *et al*., 2011): similar methods put needs between 21 and 42 days close to 100mg kg^{-1}DM (Lu *et al*., 2016) but such levels are close to those labelled by others as 'excessive' (Zhang *et al*., 2018). Equally high estimates of needs in breeder hens were made but hens were depleted for a month at the beginning of lay by feeding a diet with only 22mg Mn kg^{-1}DM for a month: estimated need was reduced to 92mg kg^{-1}DM. simply by changing the mathematical model for assessing response curves (Noetzold *et al*., 2020). Manganese requirements are much lower in pigs but requirement for optimum growth rate was still only 25% of that for maximal arginase activity, MnSOD activity or tibia Mn (Pallauf *et al*., 2012). Responses of tissue Mn and enzyme activity to increases in dietary Mn differed (Fig. 14.2).

- The reason for differences in MnSOD responsiveness to dietary Mn must be elucidated before being used to define requirements.
- To date, neither maximal heart MnSOD activity nor mRNA abundance has been shown to confer health or production benefits upon broiler chicks.

Manganese absorbed in excess of need may either be partitioned to forms and sites with enzyme activity in the way that excess Cu accumulates in liver as CuZnSOD or cause oxidative stress and present a particular threat to physically active organs such as the heart and lung. Factorial models have rarely been used to derive requirements because of a dearth of reliable numerical values for model components. Regression of faecal Mn against Mn intake in young pigs indicated that faecal endogenous losses (FEL_{Mn}) can be reduced to zero on a highly digestible diet (p. 354).

Manganese Requirements for Poultry

Meat production

There is still little or no support for a widely promoted view (e.g. Bao *et al*., 2007) that modern broilers and turkey poults needs far more than the 60 and 80mg Mn kg^{-1}DM, respectively, recommended by NRC (1994). Adding Mn to diets already containing 60 (Sands and Smith, 1999; Yan and Waldroup, 2006; Lu *et al*., 2007), 34–38mg Mn kg^{-1}DM (Wedekind *et al*., 1991; Pachecho *et al*., 2017) or less (e.g. Fig. 14.2) has not improved performance. Gradual reduction in dietary Ca and P provision over the years may have raised A_{Mn} and countered the effect of increased FCE, which would otherwise be expected to increase needs, when expressed as dietary concentrations. Requirements did not decrease as birds grew (Fig. 14.9). No studies appear to have been made since in turkey poults since Kealy and Sullivan (1966) improved poult growth by adding 20–80mg Mn kg^{-1}DM to a corn/SBM diet. Incidence of leg abnormalities has often been reduced by Mn supplements (e.g. broilers, Li *et al*., 2004; turkey poults, Kealy and Sullivan, 1966) but presents problems as an indicator of need. In Fig. 14.9, requirement to minimize swelling of the tibiotarsal joint in broilers was higher than that for

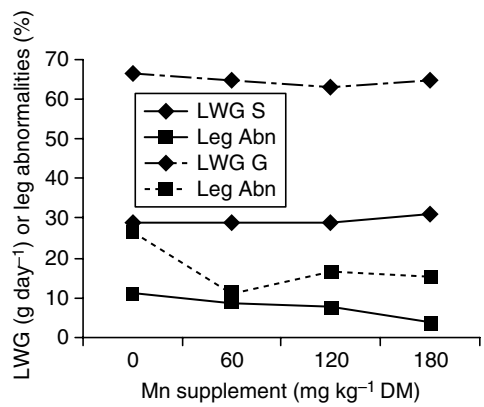

Fig. 14.9. Manganese supplementation of diets for starter (S) and grower (G) chicks, containing manganese at 23 and 21mg kg^{-1}DM, respectively, had no effect on liveweight gain (LWG) but incidence of leg abnormalities (Abn), higher in faster-growing G than S chicks, was reduced (from Li *et al*., 2004).

growth but Mn provision was never enough to eliminate swelling during the growth stage. Requirement for optimal leg score varied from 21–81 (Li *et al*., 2004) and 119–219mg Mn kg^{-1}DM (Lu *et al*., 2007), depending on growth stage, in two studies with the same broiler strain (Arbor Acres).

- Leg lesions, not sufficiently severe to impair performance, may be exacerbated by dietary factors (e,g polyunsaturated fatty acids; Lu *et al*., 2007).
- Lesions develop early in life but become progressively exacerbated by increased load-bearing and, hence, partly refractory to later Mn supplementation.

Susceptibility to tibial dyschondroplasia also increases with growth rate and/or body size (Whitehead *et al*., 2003; Dibner *et al*., 2007) but incidence is influenced by many factors other than manganese, both nutritional (e.g. choline deficiency; Jukes, 1940) and non-nutritional (p. 70).

Egg production and eggshell strength

NRC (1994) requirements were 17–25mg Mn kg^{-1}DM for white-egg-laying strains, depending on food intake, and 33mg kg^{-1}DM for breeding hens, but some 10% lower for brown strains because of a higher food intake. Raising dietary Mn from 14 to 40 and 116mg kg^{-1}DM did not improve egg production but the larger increment improved eggshell thickness and strength (Xiao *et al*., 2014). Follow-up studies confirmed that eggshells were thicker, stronger (Fig. 14.10) and contained elongated mammillary cones: glycosoaminoglycan and Ca-binding protein synthesis in the egg gland were upregulated by extra Mn (Zhang *et al*., 2018). However, in breeder hens, egg production, eggshell strength, settability and hatchability showed little further improvement when dietary Mn was increased to >49mg Mn kg^{-1}DM (Noetzold *et al*., 2020). Requirements given for breeding turkeys are provisionally increased from previous guidelines to 60mg Mn kg^{-1}DM. In breeder ducks, raising dietary Mn from 18 to 58mg kg^{-1}DM maximized egg production, fertility and shell strength, although more was recommended (Zhang *et al*., 2020c).

Manganese Requirements for Pigs

At birth, carcase Mn was high at 1.14mg kg^{-1}FW but values fell by two thirds as pigs grew to 20 kg, where values remained until 80 kg LW was reached

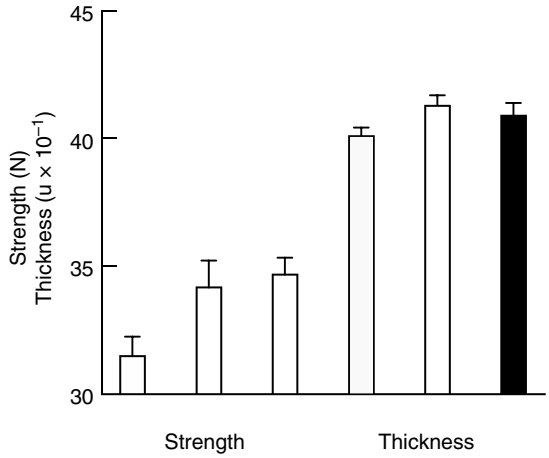

Fig. 14.10. Adding manganese to a corn/soybean meal blend containing 25mg Mn kg^{-1}DM (open) improved eggshell breaking strength and thickness, whether given in inorganic (shaded) or organic (solid column) to Hy-line Brown hens. (From Zhang *et al*., 2018).

(Ruiz-Ascacibar *et al*., 2019): values as low as 0.05mg Mn kg^{-1}FW were found in entire and castrate males and females >100 kg LW. With pigs consuming 3 kg feed, containing 1mg Mn kg^{-1}DM to gain 1 kg LW, daily, availability can fall to 0.33 and approach a net growth requirement of 0.4mg Mn kg^{-1}FW, as early experiments implied (Johnson, 1943; Plumlee *et al*., 1956). Modern pigs grew well with only 2.2mg Mn kg^{-1}DM, and an AA_{Mn} of 0.29 (Pallauf *et al*., 2012). Arguments that five times more Mn should be fed to maximize manganoenzyme activities are highly dubious, as in broiler chicks (p. 358). Requirements will be higher on fibrous diets but the NRC (2012) recommendation of 4mg kg^{-1}DM would theoretically suffice if AA_{Mn} was only 0.10. With sow's main milk low in Mn, requirements will be maximal in late gestation rather than lactation and met by the vast majority of commercial rations.

Manganese Requirements for Cattle

Requirements of sucklings are obviously met by normal milk and are therefore <1mg Mn kg^{-1}DM. Early results for weaned cattle, indicating substantially lower requirements for growth than for reproduction (Bentley and Phillips, 1951; Rojas *et al*., 1965; Howes and Dyer, 1971) have been

confirmed and extended: 8mg Mn kg^{-1}DM was adequate for the growth of finishing steers (Legleiter *et al*., 2005) and 16mg kg^{-1}DM for normal growth and fertility in growing heifers (Hansen *et al*., 2006a) but not for development of the unborn calf (Hansen *et al*., 2006b). This last finding appears to contradict some earlier work (Hartmans, 1974).[vii] Combined requirements for growth and pregnancy in the heifer clearly represent a period of maximum demand: the outcome may depend on the timing of conception in relation to manganese depletion and the growth achieved by the pregnant heifer. A factorial estimate that dairy cows required 17–18mg Mn kg^{-1}DM (NRC, 2001) was raised to 28–49mg kg^{-1}DM, based on a different model with an unrealistically elevated maintenance requirement (Weiss and Socha, 2005). With minimal supplementation, factorial estimates based on comparative slaughter data were close to 20mg Mn kg^{-1}DM in feedlot, which is recommended for such stock and few pastures or forages will lack Mn (Zanetti *et al*., 2017)

Manganese Requirements for Sheep and Goats

Growing sheep required 13mg Mn kg^{-1}DM for optimal liveweight gain and wool growth, but slightly more (16mg kg^{-1}DM) for testicular growth (Masters *et al*., 1988). The similarity to requirements for cattle may seem surprising, given that there is as much Mn in the fleece of a mature sheep as in the rest of its body (Fig. 14.1) but wool growth rate is slow. Data on goats are meagre and requirements ill-defined. Female goats on diets containing 20 and 6mg Mn kg^{-1}DM in their first and second years grew as well as those receiving an extra 100mg Mn kg^{-1}DM, but reproductive performance was greatly impaired (Anke *et al*., 1973a). This confirms the lower requirement for growth than for fertility but leaves the minimum requirement for either purpose undefined. Requirements at the seasonal peak in cashmere growth may be relatively high, like those for zinc (p. 432).

Preventing Disorder with Inorganic Supplements

Manganese deprivation can easily be prevented by supplementing rations with Mn_i as salts or oxides, even meeting the needs of developing chick embryos by raising reserves in the egg yolk (Attia *et al*., 2010). The commonly used sources are $MnSO_4$, MnO and $MnCO_3$; the former had the highest relative biological value (RBV_{Mn}) (100%) and the latter RBV_{Mn} of 30% and 55%, respectively, for poultry, and 35% and 30%, respectively, for sheep (Henry, 1995). RBV_{Mn} can vary for a given source, depending on purity and generally increases with solubility but insoluble MnO can have a relatively high RBV (Henry *et al*., 1989). Insolubility may confer benefits in hot and humid environments, causing less caking and peroxidation in feeds than more soluble salts. Most practical rations supply adequate Mn without supplementation.

Chelated ('organic') sources of manganese

Numerous comparisons have been made of the merits of chelated ('organic', Mn_o) over Mn_i but none had shown unequivocal advantages when last reviewed (Suttle, 2010).[viii] Relatively insoluble and unreactive chelates may cause less auto-oxidation of feed ingredients under humid conditions (Miles *et al*., 2003) but so would MnO and $MnCO_3$, compared to $MnSO_4$. Organic Mn sources had generally failed to improve either growth rate or FCE when compared with Mn_i at the same concentration (Sands and Smith, 1999; Sawyer *et al*., 2006; Yan and Waldroup, 2006; Bao *et al*., 2007; Lu *et al*., 2007). Three Mn_o sources were no more effective than $MnSO_4$ in reducing swelling of the tibiotarsal joint in starter and grower chicks by 50%: a residual 14% of cases was unresponsive to Mn (Li *et al*., 2004). Differences in biochemical response and sensory criteria were also unconvincing.[ix]

Newer comparisons

Most subsequent comparisons have shown little advantage for Mn_o over Mn_i sources in terms of performance (e.g. broilers; Mwangi *et al*., 2019); indeed the opposite was true in laying hens (Attia *et al*., 2010). Mixed amino acid chelates and $MnSO_4$ reduced expression of heat shock proteins and increased soft tissue Mn and MnSOD in hearts of heat-stressed broiler breeder hens equally (Zhu *et al*., 2015): Mn source had no other effects on susceptibility to heat stress, confirming earlier findings (Sands and Smith (2002). A claim for superior availability for Mn_o was based solely on higher bone Mn_i. In lambs, increasing dietary Mn from 31 to 150mg kg^{-1}DM

with glycinate rather than sulfate produced greater increases in kidney cortex Mn but similar increases in plasma Mn, liver Mn, liver MnDOD and arginase in lambs (Gresakova *et al.*, 2016). Chelation with OHB is being explored but, in dairy cows, only a small proportion (11%) of ingested OHB escaped rumen degradation (Lapierre *et al.*, 2007). Undegraded MnOHMB may enter the methionine pool after absorption, like selenomethionine (p. 380) and be relatively unavailable for enzyme synthesis though available for renal clearance.

Differences between chelated sources

Five subsequent comparisons between Mn-proteinates (p. 48; Mn_{Pr}) and Mn_i sources showed no differences in performance of broiler chicks (Wang *et al.*, 2013; Mwangi *et al.*, 2019; Saldahna *et al.*, 2020) and laying hens (Attia *et al.*, 2010; Zhu *et al.*, 2015). Of numerous biochemical measures of Mn status, only one parameter, bone Mn, was affected by source in one study, in heat-stressed broiler breeder hens (Zhu *et al.*, 2015). MnPr and $MnSO_4$ both increased soft tissue Mn and heart MnSOD while reducing expression of heat shock proteins to similar extents, confirming earlier findings (Sands and Smith, 2002). Suggestions had already been made that chelation by methionine and protein hydrolysates produced weak, ineffective chelates (Li *et al.*, 2004). Strong chelation to lysine may produce a molecule that can de absorbed intact by a CAT pathway (Bai *et al.*, 2020b) but may remain intact and generally unreactive, despite raising plasma, duodenal and tibial Mn more than Mn_i when given in dietary excess. Mn-lysine had less impact on expression of genes controlling Fe uptake than Mn_i (Bai *et al.*, 2020a). Strong chelation to a methionine analogue, Mn OHB, enhanced AA_{Mn} for pigs when given with FeOHB and ZnOHB (Fig. 14.8) but may be less effective in ruminants. Adding either Mn glycinate or Mn_i to a corn/SBM blend, containing 21mg Mn kg^{-1}DM, failed to improve performance in broiler chicks between 7 and 28 days but 68% more Mn was taken up from everted jejunal segments *in vitro* from glycinate than $MnSO_4$ (Irani *et al.*, 2019): higher uptake is not synonymous with either higher absorption or subsequent availability for all Mn-dependent functions.

Interpreting newer indices of availability

The last decades has seen new and increasingly sophisticated methods applied to the evaluation of specific amino acid chelates. Performance and mortality remained unaffected by chelation when broiler diets containing as little as 12mg Mn kg^{-1}DM were supplemented (Bai *et al.*, 2012; Liao *et al.*, 2019) but differences emerged in tissue Mn, Mn-enzyme activities and expression of genes affecting Mn transport. As with the availability of Mn in feeds (p. 356),

- some differences between Mn chelates may be artefacts of technique.

Take for example the determination of plasma Mn in hepatic venous blood and expression of mRNA for DMT1 in duodenum as putative indicators of relative availability, when previously depleted broiler chicks were repleted with different Mn sources (Bai *et al.*, 2012; Liao *et al.*, 2019). Fig. 14.11a shows that plasma Mn was always highest for the strongest chelate and lowest in unsupplemented chicks but ***decreased*** as repletion continued, probably taking around 21 days to reach steady values: health and performance did not deteriorate in unsupplemented chicks, which were unjustifiably described as 'deficient'. Relative abundance of mRNA for DMT1, primarily an Fe-transporter in duodenal mucosa, was increased by Mn supplementation but groups did not rank in the same order as they did for plasma Mn, Mn_i and the strongest chelate, being equally stimulatory. Furthermore, abundance ***increased*** between 21 and 28 days in groups given moderate and strong chelates (not shown).

- Neither DMT1 nor plasma Mn was a stable indicator of relative, intrinsic availability of a source.

Ferroportin (FPN), which completes the mucosal transfer of Fe (p. 238), was also *up*regulated by dietary repletion with all sources but most by the stronger chelates (Fig. 14.11b). In an accompanying experiment, with ligated loops of intestine from chicks (Fig. 14.12), previously depleted of Mn for 28 days, perfused with Mn from the same sources, DMT1 was ***down***regulated, $MnSO_4$ (and OW, not shown) having the greatest effect and OS the least. Mucosal uptake of Mn was greatest for OS, which can be taken up, intact, by a CAT

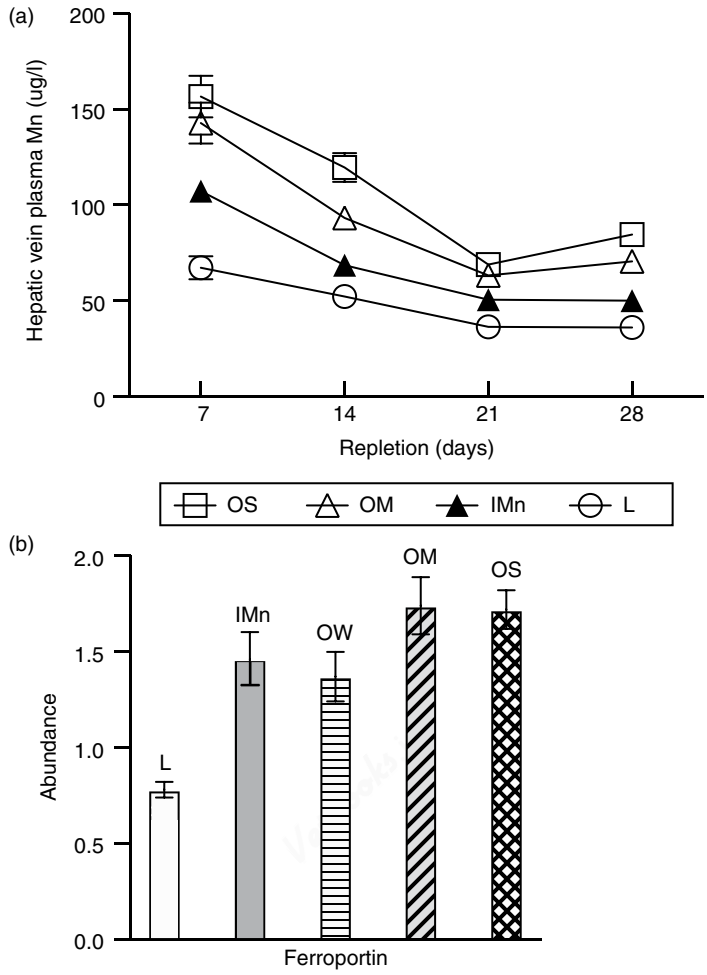

Fig. 14.11. (a) Plasma manganese (Mn) in hepatic vein samples from broiler chicks, previously given a diet low in Mn (13mg kg^{-1}DM) for 14 days and then repleted with 100mg Mn kg^{-1}DM as $MnSO_4$ (IMn), or chelated Mn in moderately (OM) and strongly bound (OS) complexes or left unsupplemented (L; 22mg Mn kg^{-1}DM) for 14 days (Bai *et al*., 2012) or 28 days (Liao *et al*., 2019). (b) Abundance of mRNA for ferroportin in the foregoing experiment, including results for an additional group, repleted with a weakly chelated source (OW).

transporter (P.mn) (Bai *et al*., 2012), but uptake is only part of the absorptive process. Taken together, the results do not constitute coherent evidence for maximal availability of the strongest chelate, as claimed by the authors. Other studies showed strong chelates to be impotent (Li *et al*., 2004), a conclusion supported by the perfusion experiment (Fig. 14.12). Perfusate Mn had been chosen to match the 113mg Mn kg^{-1}DM used for dietary repletion, which is at least five times the growth requirement, and likely to yield a higher free Mn concentration in the intestinal lumen than dietary repletion. Perfusion with $MnSO_4$ probably inhibited DMT1 synthesis instantly to protect cells from Mn toxicity but the strongest chelate did not, despite – or perhaps because of – being avidly taken up by the mucosa.

The contrary results of dietary repletion on mRNA DMT1 and FPN abundance may reflect effects on Fe status, amplified by feedback from body Fe stores, which may not happen in ligated loops. Induction of DMT1 synthesis may be triggered by a need to absorb more Fe, since excess Mn competes with Fe for transport by DMT1 (p. fe).

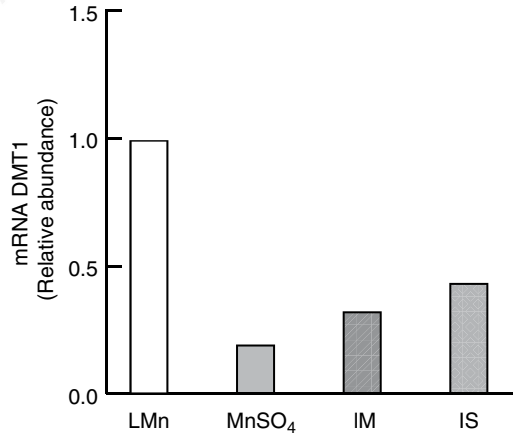

Fig. 14.12. Abundance of mRNA for DMT1 in duodenal mucosa from ligated loops of duodenum perfused with solutions containing 120mg Mn l^{-1} as IMn, OW (weak chelate), OM or OS, relative to initial abundance in L (open column, LMn) with no Mn in the perfusate (Bai *et al*., 2012).

Thus, progressive Fe depletion may have been responsible for increased mRNA abundance of DMT1 and FPN as repletion progressed. But why should plasma Mn decline with duration of repletion and at the slowest rate with the strong chelate? A strongly chelated source that is taken up independently of DMT1 is unlikely to be taken up by DMT1-dependent processes elsewhere, resulting in a slow turnover and high plasma Mn. However, turnover rate may increase as Mn status builds up, causing a gradual reduction in plasma Mn. The foregoing speculation is made necessary by the introduction of new indices of Mn availability, without prior validation by proven methods.

- Relative availabilities measured by hepatic vein Mn and mRNA abundances are time- and parameter-dependent and cannot predict appropriate inclusion rates for chelated Mn sources.

Requirements for comparing manganese sources

Rates of repletion can provide sensitive measures of RBV_{Mn}, provided that appropriate input ranges, antagonist concentrations and timescales are used: providing excess Mn and/or Fe for too long will mask real differences in availability. An experiment in which Mn_i and Mn-lysine were compared in the face of challenge with up 160mg added Fe kg^{-1}DM claimed evidence that the chelate had superior availability on the basis of differences in DMT 1 and FPN abundances and heart MnSOD (Bai *et al*., 2020a) but

- Indices should be chosen for specificity of function or physiological outcome e.g. arginase rather than total SOD activity and serum ferritin and biliary Mn rather than DMT1 and FPN.

Adaptation of the dietary repletion method of Bai *et al*. (2012) by comparing ≥3 increments of each source, none greater than 100mg Mn kg^{-1}DM, would allow valid linear slopes comparisons to be made. Until such comparisons reveal differences between availability between chelates,

- replacing Mn_i with chelated sources is unlikely to improve livestock performance unless adverse interactions in the feed bag are reduced.
- The epithet 'organic' is inappropriate for synthetic chelates with pharmaceutical properties and whose natural occurrence is unknown.

Toxicity

Estimates of crude tolerance in pigs and poultry have ranged from 0.5 to 4.0g kg^{-1}DM, presumably affected by dietary Cu, Fe, Ca and fibre, probably through effects on manganese absorption (Underwood and Suttle, 1999). Poultry are more tolerant than pigs, reflecting a relatively poor A_{Mn}, and the safe limit has been placed at 2g Mn kg^{-1}DM (NRC, 2005). However, 1.5g Mn kg^{-1}DM decreased growth rate but not food intake of broiler chicks on a corn/SBM diet (Miles *et al*., 2003): an iron supplement of 50mg kg^{-1}DM may have become insufficient and growth-limiting. Dietary Ca was lower than in earlier toxicity studies, although adequate for broilers. Neither appetite nor growth rate were decreased by adding 500mg Mn kg^{-1}DM to a calf milk replacer but anaemia was induced, despite the presence of 100mg added Fe kg^{-1}DM; 1000 mg Mn kg^{-1} DM was lethal (Jenkins and Hirdiroglou, 1991). No specific pathology was evident but liver Mn increased from 7.2 to 26.7mg kg^{-1}DM. Weaned ruminants are more tolerant: calves are said to tolerate 2600mg Mn kg^{-1}DM (NRC, 2005) and weaned lambs nearly twice as much (Wong-Valle *et al*., 1989). However, tolerance will probably decrease as available dietary Cu, Fe and Zn decrease and may be <1000mg Mn kg^{-1}DM in weaned ruminants. Given as $MnSO_4$, 500mg Mn

kg^{-1}DM lowered Cu status in cattle (Hansen *et al*., 2006a, b). Soil-contaminated pastures and silages can contain up to 400mg Mn kg^{-1}DM, enough to invariably lower Fe status in sheep when given as $MnSO_4$ and raise pancreatic Zn in one study but without consistently impairing performance.[x] Soils contain Mn as oxides rather than salts and sheep tolerated 3000mg Mn kg^{-1}DM for 21 days when given as MnO (Black *et al*., 1985). Although less reactive than soluble $MnSO_4$, soil Mn is surprisingly labile (Brebner *et al*., 1985) and may, like Fe, be partially solubilized when pasture is ensiled (p. 350). Soil-contaminated pastures are also high in Fe (1100–2200mg Fe kg^{-1}DM; Grace, 1973) and may present a dual challenge to copper absorption (p. 272): ingested soil Mn may contribute to the lowering of Cu status, previously attributed to antagonism from soil Fe (Suttle *et al*., 1984).

Mechanisms

Studies of manganese toxicity in farm livestock have been superficial. The only mechanistic studies of effects of high Mn intakes have been of impaired A_{Fe} and haemoglobin synthesis, caused by down-regulating DMT1 in the gastric mucosa. Given shared mechanisms for Mn, Fe, Zn and Cu transport (Felber *et al*., 2019), molecular studies may reveal adverse subclinical effects of manganese exposure that have yet to be recognized. As little as 52mg Mn kg^{-1}DM precipitated convulsive seizures in pigs, given a diet deficient in Mg, by unknown mechanisms (Miller *et al*., 2000). Manganese may be an important component of the redox interface between an organism and its environment and act as a mitochondrial life–death switch (Smith *et al*., 2017).

Notes

[i] In rats, Mn-deprivation during late pregnancy caused loss of balance in the neonate, due to irreversibly impaired vestibular function. Mucopolysaccharide synthesis was impaired, affecting skull development and causing a structural defect in otoliths of the inner ear (Underwood and Suttle, 1999).

[ii] Calf (Suttle, 1979) and sheep (Grace, 1983) carcases contained 0.6 to 3.9 mg kg^{-1} fresh weight (FW). In adult sheep, tissue Mn was highest in liver (4.2), followed by pancreas (1.7) and kidney (1.2); elsewhere, values were <0.3 mg kg^{-1}FW (Grace, 1983).

[iii] Expression of DMT1 at sites such as the kidney and lung provides another locus for interactions between Mn and Fe (Garrick *et al*., 2003).

[iv] Absorptive effeiciencies were 12.7, 2.2, 3.8, 5.2 and 6.9 for sunflower seeds, wheat, spinach, lettuce and $MgCl_2$, respectively (Johnson *et al*., 1991).

[v] In a grazing study, increasing dietary Mn from 123 to 473mg kg^{-1}DM (i.e. up to 30 times the requirement) increased tissue Mn by 25% in most organs and by 260% in the digestive tract (Grace and Lee, 1990).

[vi] Large supplements (8g P kg^{-1}DM) reduced A_{Mn} by about 50% in chicks, whether dietary Mn was suboptimal (< 12; Baker and Odoho, 1994) or excessive (1000mg kg^{-1}DM; Wedekind *et al*., 1991) in a maize/SBM diet. Excess Ca was added with P in both studies to stabilize Ca:P ratios, a common but questionable experimental strategy, although Ca alone had no effect on A_{Mn} (Wedekind *et al*., 1991).

[vii] Identical-twin heifers were given 'practical rations' containing 16 and 21mg Mn kg^{-1}DM for 2.5–3.5 years: they remained healthy and fertile, with or without added Mn (Hartmans, 1974). In other studies, 20mg Mn kg^{-1}DM was adequate for both the growth and reproduction of heifers (Rojas *et al*., 1965; Howes and Dyer, 1971).

[viii] Any effect of an Mn amino acid source was unlikely to reflect resistance to antagonisms occurring in or beyond the stomach. *In vitro* studies of 12 different Mn complexes, including five methionine complexes, five other specific amino acids and two 'proteinates', using the method of Brown and Zeringue (1994) (see Fig. 3.6), confirmed their general conclusion: none of the complexes was stable at pH 2 or 5 (Li *et al*., 2004). Any effects attributable to chelation in those sources must therefore occur after diet preparation but before reaching the acid stomach environment. The complexes provided far more than Mn (6.5–17.4%) and amino acids (1.5–51%): the remainder included Fe (up to 2.5%), Cu (1.1%) and Zn (5.3%). Any responses to such complexes may not attributable to Mn content. Using methionine-supplemented controls to allow for the effect of accompanying amino acids, Henry *et al*. (1989) reported increases of 8% and 33% for Mn–methionine over manganese when assessed by increases in tibia and kidney Mn, respectively, in chicks but dietary Mn reached 2.1g kg^{-1}DM. Kidneys are often a repository for unwanted or unusable trace elements and high kidney Mn from a supplement given at 'pharmacological' rather than nutritional levels is neither proof nor a quantitative test of superior availability. Experimental protocols generally addressed problems facing the poultry industry and conditions were set to maximize responsiveness to Mn. Reductions in carcase fat and peroxidation were sought with diets containing 7% polyunsarurated fatty acids (Li *et al*., 2004). Whatever the outcome, results may not be applicable to normal rations. Under heat stress, a manganese–proteinate complex had a relative availability of 142% for broilers when compared with $MnSO_4$, but only 120% under thermoneutral conditions; bone Mn was the response criterion with dietary Mn up to 3g kg^{-1}DM (Smith *et al*., 1995). However, the daily ration is also 'heat-stressed' and effects of source may reflect

interactions with feed and environment. A 16% advantage for Mn chelated by a methionine analogue (2-hydroxy-4-(methylthio)-butanoic acid) over $MnSO_4$ was claimed in a broiler study from slopes ratio comparisons of responses in tibia Mn, with 100–800 mg added Mn kg^{-1}DM (Yan and Waldroup, 2006), but there were no differences between manganese sources until the highest level was reached.

[ix] Serum insulin and non-esterified fatty acids (NEFA) were reduced by increasing dietary Mn from 60 to 240mg kg^{-1}DM in heat-stressed broilers, whether organic (proteinate) or inorganic Mn was added (Sands and Smith, 2002). Bone Mn, heart Mn, heart MnSOD and mRNA for the latter were compared for 15 organic Mn sources for broiler chicks: only one source showed enhanced availability by one of the three indices of status (MnSOD mRNA; Li *et al*., 2004). Differences in relative availability widened when a basal diet high in Ca (18.5g kg^{-1}DM) was used and reflected chelation strength, but a commonly used Mn–met complex, a weak chelate, showed no advantage with normal dietary Ca (Lu *et al*., 2007). Discolouration in pork chops on simulated retail display was reduced more by 350mg Mn kg^{-1}DM from an inorganic than from an organic source after 6–7 days: neither source had been effective after 4 days display (Sawyer *et al*., 2006).

[x] When sheep were given pellets providing 250 or 500 mg Mn day^{-1}, as $MnSO_4$, while grazing pastures high in Mn (140–200mg Mn kg^{-1}DM), growth rate decreased and heart and plasma Fe fell (Grace, 1973). A similar supplement lowered liver Fe and increased pancreatic Zn without retarding growth (Grace and Lee, 1990). An anorexic effect of manganese was less when ingested continuously in the diet rather in bolus doses, which briefly raise ruminal Mn to very high levels (Black *et al*., 1985).

References

Adeola, O., Lawrence, B.V., Sutton, A.C. and Cline, T.R. (1995) Phytase-induced changes in mineral utilisation in zinc-supplemented diets for pigs. *Journal of Animal Science* 73, 3384–3391.

Anke, M., Groppel, B., Reisseg, W., Ludke, H., Grun, M. and Dittrich, G. (1973a) Manganmangel beim Wiederkauer 3. Manganmangelbedingte Fortpflanzungs-, Skelett- und Nervenstorungen bei weiblichen Wiederkauern und ihren Nachkommen. *Archiv für Tierernahrung* 23, 197–211.

Anke, M., Hennig, A., Groppel, B., Dittrich, G. and Grun, M. (1973b) Manganmangel beim Wiederkauer. 4. Der Einfluss des Manganmangels auf den Gehalt neugeborener Lammer an Fett, Protein, Mangan, Asche, Kalzium, Phosphor, Zink und Kupfer. *Archiv für Tierernahrung* 23, 213–223.

Arredondo, M. and Munez, M.T. (2005) Iron and copper metabolism. *Molecular Aspects of Medicine* 26, 313–327.

Arredondo, M.A., Casas, G.A. and Stein, H.H. (2019) Increasing levels of microbial phytase increases the digestibility of energy and minerals in diets fed to pigs. *Animal Feed Science and Technology* 248, 27–36.

Atkinson, S.A., Shah, J.K., Webber, C.E., Gibson, I.L. and Gibson, R.S. (1993) A multi-element isotopic tracer assessment of true fractional absorption of minerals from formula with additives of calcium, phosphorus, zinc, copper and iron in young piglets. *Journal of Nutrition* 123, 1586–1593.

Attia, Y.A., Qota, E.M., Bovera, F., El-Din, A.E.T. and Mansour, S.A. (2010) Effect of amount and source of manganese and/or phytase supplementation on productive and reproductive performance and some physiological traits of dual purpose cross-bred hens in the tropics. *British Poultry Science* 51, 235–245.

Aydemir, T.B. and Cousins, R.J. (2009) The multiple faces of the metal transporter ZIP14 (SLC39A14). *Journal of Nutrition* 148, 174–184.

Bai, S., Lu, L., Wang, R., Xi, L., Zhang, L. and Luo, X. (2012) Manganese source affects manganese transport and gene expression of divalent metal transporter 1 in the small intestine of broilers. *British Journal of Nutrition* 108, 267–276.

Bai, S., Peng, J., Zhang, K., Ding, X., Wang, J. *et al.* (2020a) Effects of dietary iron concentration on manganese utilization in broilers fed with manganese-lysine chelate-supplemented diet. *Biological Trace Element Research* 198, 231–242.

Bai, S., Peng, J., Zhang, K., Ding, X., Wang, J. *et al.* (2020b) Effects of dietary iron on manganese utilization in broilers fed with corn-soybean meal diet. *Biological Trace Element Research* 194, 514–524.

Baker, D.H. and Odohu, G.W. (1994) Manganese utilisation in the chick: effects of excess phosphorus on chicks fed manganese deficient diets. *Poultry Science* 73, 1162–1165.

Bao, Y.M., Choct, M., Iji, P.A. and Bruerton, K. (2007) Effect of organically complexed copper, iron, manganese and zinc on broiler performance, mineral excretion and accumulation in tissues. *Journal of Applied Poultry Research* 16, 448–455.

Bentley, O.G. and Phillips, P.H. (1951) The effect of low manganese rations upon dairy cattle. *Journal of Dairy Science* 34, 396–403.

Berger, L.L. (1995) Why do we need a new NRC data base? *Animal Feed Science and Technology* 53, 99–107.

Biehl, R.R., Baker, D.H. and DeLuca, H.F. (1995) 1 α-Hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese utilization in chicks fed soy-based diets. *Journal of Nutrition* 125, 2407–2416.

Black, J.R., Ammerman, C.B. and Henry, P.R. (1985) Effect of quantity and route of manganese monoxide on feed intake and serum manganese of ruminants. *Journal of Dairy Science* 68, 433–436.

Brebner, J., Thornton, I., McDonald, P. and Suttle, N.F. (1985) The release of trace elements from soils under conditions of simulated rumenal and abomasal digestion. In: Mills, C.F., Bremner, I. and Chesters, J.K. (eds) *Proceedings of the Fifth International Symposium on Trace Elements in Man and Animals*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 850–852.

Brown, T.F. and Zeringue, L.K. (1994) Laboratory evaluations of solubility and structural integrity of complexed and chelated trace mineral supplements. *Journal of Dairy Science* 77, 181–189.

Buchanan-Smith, J.G., Evans, E. and Poluch, S.O. (1974) Mineral analyses of corn silage produced in Ontario. *Canadian Journal of Animal Science* 54, 253–256.

Carter, J.C., Miller, W.J., Neathery, M.W., Gentry, R.P., Stake, P.E. and Blackmon, D.M. (1974) Manganese metabolism with oral and intravenous ^{54}Mn in young calves as influenced by supplemental manganese. *Journal of Animal Science* 380, 1284–1290.

Caskey, C.D. and Norris, L.C. (1940) Micromelia in adult fowl caused by manganese deficiency during embryonic development. *Proceedings of the Society for Experimental Biology and Medicine* 44, 332–335.

Choi, E.-K., Aring, L., Das, N.K. *et al*. (2020) Impact of dietary manganese on experimental colitis in mice. *The FASEB Journal* 34, 2929–2943.

Davidson, L. and Lonnerdahl, B. (1989) Fe-saturation and proteolysis of human lactoferrin: effect on brush-border receptor-mediated uptake of Fe and Mn. *American Journal of Physiology–Gastrointestinal and Liver Physiology*, 257, G930–G934.

Davis, C.D., Wolf, T.L. and Greger, J.L. (1992) Varying levels of dietary manganese and iron affect absorption and gut endogenous losses of manganese by rats. *Journal of Nutrition* 122, 1300–1308.

Dibner, J., Richards, J.D., Kitchell, M.I. and Quiroz, M.A. (2007) Metabolic challenges and early bone development. *Journal of Applied Poultry Research* 16, 126–137.

Doisey, E.A. Jr (1973) Micronutrient controls on biosynthesis of clotting proteins and cholesterol. In: Hemphill, D.D. (ed.) *Trace Substances in Environmental Health – 6*. University of Missouri, Columbia, Missouri.

Egan, A.R. (1972) Reproductive responses to supplemental zinc and manganese in grazing Dorset Horn ewes. *Australian Journal of Experimental Agriculture and Animal Husbandry* 12, 131–135.

Felber, D.M., Wu, Y. and Zhao, N. (2019) Regulation of the metal transporters Zip14 and Znt10 by manganese intake in mice. *Nutrients* 11, 2099.

Finley, J.W. (1999) Manganese absorption and retention by young women is associated with serum ferritin concentration. *American Journal of Clinical Nutrition* 70, 37–43.

Gallup, W.D. and Norris, L.C. (1939a) The amount of manganese required to prevent perosis in the chick. *Poultry Science* 18, 76–82.

Gallup, W.D. and Norris, L.C. (1939b) The effect of a deficiency of manganese in the diet of the hen. *Poultry Science* 18, 83–88.

Garrel, C., Fowler, P.A. and Al-Gubory, K.H. (2010) Developmental changes in antioxidant enzymatic defences against oxidative stress in sheep placentomes. *Journal of Endocrinology* 205, 107–116.

Garrick, M.D., Dolan, K.G., Horbinski, C., Ghoio, A.J., Higgins, D. *et al.* (2003) DMT 1: a mammalian transporter for multiple metals. *Biometals* 16, 41–54.

Ghosh, A., Mandal, G.P., Roy, A. and Patra, A.K. (2016) Effects of supplementation of manganese with or without phytase on growth performance, carcass traits, muscle and tibia composition, and immunity in broiler chickens. *Livestock Science* 191, 80–85.

Gladstones, J.S. and Drover, D.P. (1962) The mineral composition of lupins. 1. A survey of the copper, molybdenum and manganese contents of lupins in the south west of Western Australia. *Australian Journal of Experimental Agriculture and Animal Husbandry* 2, 46–53.

Grace, N.D. (1973) Effect of high dietary Mn levels on the growth rate and the level of mineral elements in the plasma and soft tissues of sheep. *New Zealand Journal of Agricultural Research* 16, 177–180.

Grace, N.D. (1983) Amounts and distribution of mineral elements associated with fleece-free empty body weight gains in grazing sheep. *New Zealand Journal of Agricultural Research* 26, 59–70.

Grace, N.D. and Lee, J. (1990) Effect of Co, Cu, Fe, Mn, Mo, Se and Zn supplementation on the elemental content of soft tissues and bone in sheep grazing ryegrass/white clover pasture. *New Zealand Journal of Agricultural Research* 33, 635–647.

Grace, N.D. and Gooden, J.M. (1980) Effect of increasing intakes of Zn, Cu, and Mn on their secretion via bile and pancreatic juice and their excretion in faeces and urine in sheep fed lucerne pellets. *New Zealand Journal of Agricultural Research* 23, 293–298.

Gregory, E.M. and Fridovich, I. (1974) Superoxide dismutases: properties, distribution, and functions. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Trace Element Metabolism in Animals – 2*. University Park Press, Baltimore, Maryland, pp. 486–488.

Gresakova, L., Venglovska, K. and Cobanova, K. (2016) Dietary manganese source does not affect Mn, Zn and Cu tissue deposition and the activity of manganese-containing enzymes in lambs. *Journal of Trace Elements in Medicine and Biology* 38, 138–144.

Halpin, K.M. and Baker, D.H. (1986) Long-term effects of corn soyabean meal, wheat bran and fish meal on manganese utilisation in the chick. *Poultry Science* 65, 1371–1374.

Hansen, S.L. and Spears, J.W. (2008) Impact of copper deficiency in cattle on proteins involved in iron metabolism. *FASEB Journal* 22, 443–445.

Hansen, S.L. and Spears, J.W. (2009) Bioaccessibility of iron from soil is increased by silage fermentation. *Journal of Dairy Science* 92, 2896–2905.

Hansen, S.L., Spears, J.W., Lloyd, K.E. and Whisnant, C.S. (2006a) Growth, reproductive performance and manganese status of heifers fed varying concentrations of manganese. *Journal of Animal Science* 84, 3375–3380.

Hansen, S.L., Spears, J.W., Lloyd, K.E. and Whisnant, C.S. (2006b) Feeding a low manganese diet to heifers during gestation impairs foetal growth and development. *Journal of Dairy Science* 89, 4304–4311.

Hartmans, J. (1974) Tracing and treating mineral disorders in cattle under field conditions. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Trace Element Metabolism in Animals – 2*. University Park Press, Baltimore, Maryland, pp. 261–273.

Henry, P.R. (1995) Manganese bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 239–256.

Henry, P.R., Ammerman, C.B. and Miles, R.D. (1989) Relative bioavailability of manganese in a manganese–methionine complex for broiler chicks. *Poultry Science* 68, 107–112.

Hidiroglou, M. (1975) Mn uptake by the ovaries and reproductive tract of cycling and anestrous ewes. *Canadian Journal of Physiology and Pharmacology* 53, 969–972.

Hidiroglou, M., Ivan, M., Bryoa, M.K., Ribble, C.S., Janzen, E.D., Proulx, J.G. and Elliot, J.I. (1990) Assessment of the role of manganese in congenital joint laxity and dwarfism in calves. *Annales de Recherches Vétérinaires* 21, 281–284.

Hill, R. (1967) Vitamin D and manganese in the nutrition of the chick. *British Journal of Nutrition* 21, 507–512.

Hill, R. and Mathers, J.W. (1968) Manganese in the nutrition and metabolism of the pullet. 1. Shell thickness and manganese content of eggs from birds given a diet of low or high manganese content. *British Journal of Nutrition* 22, 635–643.

Horning, K.J., Caito, S.W., Tipps, K.G., Bowman, A.B. and Aschner, M. (2015) Manganese is essential for neuronal health. *Annual Reviews of Nutrition* 35, 71–108.

Howes, A.D. and Dyer, I.A. (1971) Diet and supplemental mineral effects on manganese metabolism in newborn calves. *Journal of Animal Science* 32, 141–145.

Huang, C.F., Stein, H.H., Zhang, L.Y., Defa Li and Lai, C.H. (2017) Concentrations of minerals in pig feed ingredients commonly used in China. *Translational Animal Science* 1, 126–136.

Hurley, L.S. (1981) Teratogenic effects of manganese, zinc and copper in nutrition. *Physiological Reviews* 61, 249–295.

Hurley, L.S. and Keen, C.L. (1987) Manganese. In: Mertz, W. (ed.) *Trace Elements in Human and Animal Nutrition*, Vol. II. Academic Press, New York.

Ibáñez, M.A., de Blas, C., Cámara, L. and Mateos, G.G. (2020) Chemical composition, protein quality and nutritive value of commercial soybean meals produced from beans from different countries: a meta-analytical study. *Animal Feed Science and Technology* 267, 114531.

Irani, F., Janmohammadi, H., Kianfar, R. and Sahraei, M. (2019) Evaluation of chemical characteristics and effects of different manganese sources on kinetics of manganese absorption and performance of broiler chickens. *Iranian Journal of Applied Animal Science* 9, 463–471.

Jenkins, K.J. and Hidiroglou, M. (1991) Tolerance of the pre-ruminant calf for excess manganese or zinc in milk replacer. *Journal of Dairy Science* 74, 1047–1053.

Johnson, P.E., Lykken, G.I. and Kortnta, E.D. (1991) Absorption and biological half-life in humans of intrinsic and extrinsic ^{54}Mn tracers from foods of plant origin. *Journal of Nutrition* 121, 711–717.

Johnson, S.R. (1943) Studies with swine on rations extremely low in manganese. *Journal of Animal Science* 2, 14–22.

Jukes, T.H. (1940) Effect of choline and other supplements on perosis. *Journal of Nutrition* 20, 445–458.

Kealy, K.D. and Sullivan, T.W. (1966) Studies on manganese requirement and interactions in the diet of young turkeys. *Poultry Science* 45, 1352–1358.

Kirchgessner, M. and Neese, K.R. (1976) Copper, manganese and zinc content of the whole body and in carcase cuts of veal calves at different weights. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* 161, 1–12.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–310.

Lapierre, H., Vazquez-Anon, M., Parker, D., Dubreuil, P. and Lobley, G.E. (2007) Absorption of 2-hydroxy-4-methylthiobutanoate in dairy cows. *Journal of Dairy Science* 90, 2937–2940.

Lassiter, J.W. and Morton, J.D. (1968) Effects of a low manganese diet on certain ovine characteristics. *Journal of Animal Science* 27, 776–779.

Leach, R.M. Jr and Harris, E.D. (1997) Manganese. In: O'Dell, B.L. and Sunde, R.A. (eds) *Handbook of Nutritionally Essential Mineral Elements*. Marcel Dekker, New York, pp. 335–356.

Leeson, S. and Caston, L. (2008) Using minimal supplements of trace minerals as a method of reducing trace mineral content of poultry manure. *Animal Feed Science and Technology* 142, 339–347.

Legleiter, L.R., Spears, J.W. and Lloyd, K.E. (2005) Influence of dietary manganese on performance, lipid

metabolism and carcass composition of growing and finishing steers. *Journal of Animal Science* 83, 2434–2439.

Li, S., Luo, X., Crenshaw, T.D., Kuang, X., Shao, G. and Yu, S. (2004) Use of chemical characteristics to predict the relative bioavailability of supplemental organic manganese sources for broilers. *Journal of Animal Science* 82, 2352–2363.

Li, S., Lin, Y., Lu, L., Lin, X., Wang, Z. *et al.* (2011) An estimation of the manganese requirement for broilers from 1 to 21 days of age. *Biological Trace Element Research* 143, 939–948.

Liao, X.D., Wang, G., Lu, L., Zhang, L.Y., Lan, Y.X., Li, S.F. and Luo, X.G. (2019) Effect of manganese source on manganese absorption and expression of related transporters in the small intestine of broilers. *Poultry Science* 98, 4994–5004.

Liu, A.C.-H., Heinrichs, B.S. and Leach, R.M. Jr (1994) Influence of manganese deficiency on the characteristics of proteoglycans of avian epiphyseal growth plate cartilage. *Poultry Science* 73, 663–669.

Liu, Y., Ma, Y.L, Zhao, J., Vazquez-Añón, M. and Stein, H.H. (2014) Digestibility and retention of zinc, copper, manganese, iron, calcium, and phosphorus in pigs fed diets containing inorganic or organic minerals. *Journal of Animal Science* 92, 3407–3415.

Longstaff, M. and Hill, R. (1972) The hexosamine and uronic acid contents of the matrix of shells of eggs from pullets fed on diets of different manganese content. *British Poultry Science* 13, 377–385.

Lonnerdal, B., Keen, C.L. and Hurley, L.S. (1985) Manganese-binding proteins in human and cow's milk. *American Journal of Clinical Nutrition* 41, 550–559.

Lu, L., Luo, X.G., Ji, C., Liu, B. and Yu, S.X. (2007) Effect of manganese supplementation on carcass traits, meat quality and lipid oxidation in broilers. *Journal of Animal Science* 85, 812–822.

Lu, L., Chang, B., Liao, X., Wang, R., Zhang, L. and Luo, X. (2016) Use of molecular biomarkers to estimate manganese requirements for broiler chickens from 22 to 42 d of age. *British Journal of Nutrition*, 116, 1512–1518.

Luo, X.G., Su, Q., Huang, J.C. and Liu, J.X. (1993) Effects on manganese (Mn) deficiency on tissue Mn-containing superoxide dismutase (MnSOD) activity and its mitochondrial ultrastructures in broiler chicks fed a practical diet. *Veterinaria et Zootechnica Sinica* 23, 97–101.

Lyons, M. and Insko, W.M. Jr (1937) *Chondrodystrophy in the Chick Embryo Produced by Manganese Deficiency in the Diet of the Hen*. Bulletin No. 371, Kentucky Agricultural Experiment Station.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Masters, D.G., Paynter, D.I., Briegel, J., Baker, S.K. and Purser, D.B. (1988) Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and the activity of manganese enzymes in tissues. *Australian Journal of Agricultural Research* 39, 517–524.

Mathers, J.W. and Hill, R. (1967) Factors affecting the retention of an oral dose of radioactive manganese by the chick. *British Journal of Nutrition* 21, 513–517.

Mathers, J.W. and Hill, R. (1968) Manganese in the nutrition and metabolism of the pullet. 2. The manganese contents of the tissues of pullets given diets of high or low manganese content. *British Journal of Nutrition* 22, 635–643.

Miles, R.D., Henry, P.R., Sampath, V.C., Shivazad, M. and Comer, C.W. (2003) Relative bioavailability of novel chelates of manganese and copper for chicks. *Journal of Applied Poultry Research* 12, 417–423.

Miller, K.B., Caton, J.S., Schafer, D.M., Smith, D.J. and Finley, J.W. (2000) High dietary manganese lowers heart magnesium in pigs fed a low-magnesium diet. *Journal of Nutrition* 130, 2032–2035.

Miltimore, J.E., Mason, J.L. and Ashby, D.L. (1970) Copper, zinc, manganese and iron variation in five feeds for ruminants. *Canadian Journal of Animal Science* 50, 293–300.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Mitchell, R.L. (1957) The trace element content of plants. *Research UK*, 10, 357–362.

Mohanna, C. and Nys, N. (1999) Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytase. *Animal Feed Science and Technology* 77, 241–253.

Mwangi, S., Timmons, J., Ao, T., Paul, M., Macalintal, L. *et al.* (2019) Effect of manganese preconditioning and replacing inorganic manganese with organic manganese on performance of male broiler chicks. *Poultry Science* 98, 2105–2113.

Noetzold, T.L., Vieira, S.L., Favero, A., Horn, R.M., Silva, C.M. and Martins, G.B. (2020) Manganese requirements of broiler breeder hens. *Poultry Science* 99, 5814–5826.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2001) *Nutrient Requirements of Dairy Cows*, 7th edn. National Academy of Sciences, Washington, DC.

NRC (2005) *Mineral Tolerance of Animals*, 2nd edn. National Academy of Sciences, Washington, DC.

NRC (2012) *Nutrient Requirements of Swine*, 11th edn. National Academy of Sciences, Washington, DC.

NRC (2016) *Nutrient Requirements of Beef Cattle*, 8th edn. National Academy of Sciences, Washington, DC.

Olukosi, O.A., González-Ortiz, G., Whitfield, H. and Bedford, M.R. (2020) Comparative aspects of phytase and xylanase effects on performance, mineral digestibility, and ileal phytate degradation

in broilers and turkeys. *Poultry Science* 99, 1528–1539.

Pacheco, B.H.C., Nakagi, V.S., Kobashigawa, E.H., Caniatto, A.R.M, Faria, D.E. and Faria Filho, D.E. (2017) Dietary levels of zinc and manganese on performance of broilers between 1 to 42 days of age. *Brazilian Journal of Poultry Science* 19, 171–178.

Pallauf, J., Kauer, C., Most, E., Habicht, S.D. and Moch, J. (2012) Impact of dietary manganese concentration on status criteria to determine manganese requirement in piglets. *Journal of Animal Physiology and Animal Nutrition* 96, 993–1002.

Paynter, D. and Caple, I.W. (1984) Age-related changes in activities of the superoxide dismutase enzymes in tissues of the sheep and the effect of dietary copper and manganese on these changes. *Journal of Nutrition* 114, 1909–1916.

Plumlee, M.P., Thrasher, D.M., Beeson, W.M., Andrews, F.N. and Parker, H.E. (1956) The effects of a manganese deficiency upon the growth, development, and reproduction of swine. *Journal of Animal Science* 15, 352–367.

Rojas, M.A., Dyer, I.A. and Cassatt, W.A. (1965) Manganese deficiency in the bovine. *Journal of Animal Science* 24, 664–667.

Ruiz-Ascacibar, I., Stoll, P., Bee, G. and Schlegel, P. (2019) Dynamics of the mineral composition and deposition rates in the empty body of entire males, castrates and female pigs. *Animal* 13, 50–58.

Rutherfurd, S.M., Darragh, A.J., Hendriks, W.H., Prosser, C.G. and Lowry, D. (2006) Mineral retention in three-week-old piglets fed goat and cow milk infant formulas. *Journal of Dairy Science* 89, 4520–4526.

Saldanha, M.M., Itallo, C.S., Araújo, M.V., Triguineli, D.P., Vaz, F.N.A. *et al.* (2020) Relative bioavailability of manganese in relation to proteinate and sulfate sources for broiler chickens from one to 20 d of age. *Poultry Science* 99, 5647–5652.

Sands, J.S. and Smith, M.O. (1999) Broilers in heat stress conditions: effects of dietary manganese proteinate or chromium picolinate supplementation. *Journal of Applied Poultry Research* 8, 280–287.

Sands, J.S. and Smith, M.O. (2002) Effects of dietary manganese proteinate or chromium picolinate supplementation on plasma insulin, glucagons, glucose and serum lipids in broiler chickens reared under thermoneutral or heat stress conditions. *International Journal of Poultry Science* 1, 145–149.

Sawyer, J.T., Tittor, A.W., Apple, J.K., Morgan, J.B., Maxwell, C.V., Rakes, L.K. and Fakler, T.M. (2006) Effects of supplemental manganese on performance of growing–finishing pigs and pork quality during retail display. *Journal of Animal Science* 85, 1046–1053.

Schaible, P.J., Bandemer, S.L. and Davidson, J.A. (1938) *The Manganese Content of Feedstuffs and its Relation to Poultry Nutrition*. Technical Bulletin No. 159, Michigan Agricultural Experiment Station.

Scrutton, M.C., Utter, M.F. and Mildvan, A.S. (1966) Pyruvate carboxylase. 6. The presence of tightly bound manganese. *Journal of Biological Chemistry* 241, 3480–3487.

Scrutton, M.C., Griminger, P. and Wallace, J.C. (1972) Pyruvate carboxylase: bound metal content of the vertebrate liver enzyme as a function of diet and species. *Journal of Biological Chemistry* 247, 3305–3313.

Silanpaa, M. (1982) *Micronutrients and the Nutrient Status of Soils: A Global Study*. Food and Agriculture Organization, Rome.

Smith, M.O., Sherman, I.L., Miller, L.C. and Robbins, K.R. (1995) Relative biological availability of manganese from manganese proteinate, manganese sulphate and manganese oxide in broilers reared at elevated environmental temperatures. *Poultry Science* 74, 702–707.

Smith, M.R., Fernandes, J., Go, Y.M. and Jones, D.P. (2017) Redox dynamics of manganese as a mitochondrial life-death switch. *Biochemical and Biophysical Research Communications* 482, 388–398.

SRUC (2011) Nutritional and metabolic disorders. *Veterinary Services Monthly Reports* 9 (September). Scottish Rural Universities and Colleges.

St Clair, D. (2004) Manganese superoxide dismutase: genetic variation and regulation. *Journal of Nutrition* 134, 3190S–3191S.

Staley, G.P., Van der Lugt, J.J., Axsel, G. and Loock, A.H. (1994) Congenital skeletal malformations in Holstein calves associated with putative manganese deficiency. *Journal of the South African Veterinary Association* 65, 73–78.

Suttle, N.F. (1979) Copper, iron, manganese and zinc concentrations in the carcases of lambs and calves and the relationship to trace element requirements for growth. *British Journal of Nutrition* 42, 89–96.

Suttle, N.F. (2000) Minerals in livestock production. *Asian-Australasian Journal of Animal Science* 13, 1–9.

Suttle, N.F. (2010) Manganese. In: *Mineral Nutrition of Livestock*, 4th edn. CAB Ineternational, Wallingford, UK.

Suttle, N.F., Abrahams, P. and Thornton, I. (1984) The role of a soil × dietary sulfur interaction in the impairment of copper absorption by soil ingestion in sheep. *Journal of Agricultural Science, Cambridge* 103, 81–86.

Suttle, N.F., Bell, J., Thornton, I. and Agyriaki, A. (2003) Predicting the risk of cobalt deprivation in grazing livestock from soil composition data. *Environmental Geochemistry and Health* 25, 33–39.

Than, N.G., Romero, R, Tarca, A.L., Draghici, S., Erez, O. *et al.* (2009) Mitochondrial manganese superoxide dismutase mRNA expression in human chorioamniotic membranes and its association with labor, inflammation, and infection. *Journal of Maternal, Fetal and Neonatal Medicine* 22, 1000–1013.

Underwood, E.J. and Suttle, F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Valero, G., Alley, M.R., Badcoe, L.M., Manktellow, B.W., Merral, M. and Lowes, G.S. (1990) Chondrodystrophy in calves associated with manganese deficiency. *New Zealand Veterinary Journal* 38, 161–167.

Wang, Z., Wang, L., Wang, Z., Wang, J. and Liu, R. (2013) Effects of manganese deficiency on serum hormones and biochemical markers of bone metabolism in chicks. *Journal of Bone and Mineral Metabolism* 31, 285–292.

Wedekind, K.J., Titgemeyer, E.C., Twardock, R. and Baker, D.H. (1991) Phosphorus but not calcium affects manganese absorption and turnover in chicks. *Journal of Nutrition* 121, 1776–1786.

Weiss, W.P. and Socha, M.T. (2005) Dietary manganese for dry and lactating cows. *Journal of Dairy Science* 88, 2517–2523.

White, C.L., Robson, A.D. and Fisher, H.M. (1981) Variation in the nitrogen, sulfur, selenium, cobalt, manganese, copper and zinc contents of grain from wheat and two lupin species grown in a Mediterranean climate. *Australian Journal of Agricultural Research* 32, 47–59.

White, P.J. and Windsor, P.A. (2012) Congenital chondrodystrophy of unknown origin in beef herds. *Veterinary Journal* 193, 336–343.

Whitehead, C.C., Fleming, R.H., Julian, R. and Sorensen, P. (2003) Skeletal problems associated with selection for increased production. In: Muir, W. and Aggrey, S. (eds) *Poultry Genetics, Breeding and Biotechnology*. CAB International, Wallingford, UK, pp. 29–52.

Wilson, J.G. (1966) Bovine functional infertility in Devon and Cornwall: response to manganese supplementation. *Veterinary Record* 79, 562–566.

Wong, G.H.W. and Goeddel, D.V. (1988) Induction of manganous superoxide dismutase by tumour necrosis factor: possible protective mechanism. *Science* 242, 941–944.

Wong-Valle, J., Henry, P.R., Ammerman, C.B. and Rao, P.V. (1989) Estimation of the relative bioavailability of manganese sources for sheep. *Journal of Animal Science* 67, 2409–2414.

Xiao, J.F, Zhang, Y.N., Wu, S.G., Zhang, H.J., Yue, H.Y. and Qi, G.H. (2014) Manganese supplementation enhances the synthesis of glycosaminoglycan in eggshell membrane: a strategy to improve eggshell quality in laying hens. *Poultry Science* 93.

Yan, F. and Waldroup, P.W. (2006) Evaluation of Mintrex manganese as a source of manganese for young broilers. *International Journal of Poultry Science 5*, 708–713.

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle, T.E., Silva, B.C. *et al.* (2017) Estimating mineral requirements of Nellore beef bulls fed with or without inorganic mineral supplementation and the influence on mineral balance. *Journal of Animal Science* 95, 1696–1706.

Zhang, Y.N., Zhang, H.J., Wu, S.G., Yue, H.Y., Wang, J. and Qi, G.H. (2018) Dietary manganese supplementation affects mammillary knobs of eggshell ultrastructure in laying hens. *Poultry Science* 97, 1253–1262.

Zhang, H., Pan, S., Zhang, K., Michiels, J., Zeng, Q. *et al.* (2020a) Impact of dietary manganese on intestinal barrier and inflammatory response in broilers challenged with *Salmonella typhimurium. Microorganisms* 8, 757–777.

Zhang, Y.N., Wang, S., Huang, X.B., Li, K.C., Chen, W., Ruan, D. *et al.* (2020b) Estimation of dietary manganese requirement for laying duck breeders: effects on productive and reproductive performance, egg quality, tibial characteristics, and serum biochemical and antioxidant indices. *Poultry Science* 99, 5752–5762.

Zhang, Y.N., Wang, S., Li, K.C., Ruan, D., Chen, W. *et al.* (2020c) Estimation of dietary zinc requirement for laying duck breeders: effects on productive and reproductive performance, egg quality, tibial characteristics, plasma biochemical and antioxidant indices, and zinc deposition. *Poultry Science* 99, 454–462.

Zhu, Y., Lu, L., Li, W., Zhang, L., Ji, C. *et al.* (2015) Effect of dietary manganese on antioxidant status and expression levels of heat-shock proteins and factors in tissues of laying broiler breeders under normal and high environmental temperatures. *British Journal of Nutrition* 114, 1965–1974.

15 Selenium

Introduction

For many years, nutritional interest in selenium (Se) was confined to toxic effects on livestock. Two naturally occurring diseases of cattle, 'blind staggers' and 'alkali disease', foraging on the Great Plains of North America, were attributed to acute and chronic selenium poisoning, respectively (see Moxon, 1937). Selenium was studied in soils, plants and animal tissues to determine minimum toxic intakes and effective control measures. Eventually, Se was shown to be an essential nutrient, with minute amounts in an inorganic form (selenite) preventing development of liver necrosis in rats (Schwarz and Foltz, 1957) and exudative diathesis (ED) in chicks on torula yeast diets (Patterson *et al*., 1957). Within two years, a muscular degeneration that occurred naturally in lambs and calves in parts of Oregon and New Zealand was found to respond to Se therapy. Subsequently, selenium deprivation was linked to impaired growth, health and fertility of livestock in areas of many countries and an interrelationship with vitamin E was discovered (Thompson and Scott, 1969). A specific biochemical role emerged with the discovery that glutathione peroxidase (GPX) was a selenoprotein (SeP) (Rotruck *et al*., 1973) and tissue activity correlated with Se intake. However, the essentiality of Se has a much broader base. Numerous biologically active SeP were identified (Behne and Kyriakopoulos, 2001), each with its own apparent Se requirement (Lei *et al*., 1998; Sunde *et al*., 2005), and some interacted with iodine to affect thyroid hormone function (p. 301) (Beckett and Arthur, 2005). For Se, more than any other element, dietary form determines metabolic fate and two myths were nurtured: that inorganic Se (Se_i) was poorly utilized by livestock and that a certain form of organic selenium (Se_o), selenomethionine (SeMet), was simultaneously hyperavailable yet relatively non-toxic. In reality, SeMet, had a lower and more variable availability for important functions than Se_i (Suttle, 2010). The subject of Se in nutrition and health has occupied whole conferences (Hatfield *et al*., 2006) and books (Surai, 2006) and inspired one country, Finland, to enforce the selenization of general fertilizers to boost the Se status of its citizens (Alfthan *et al*., 2015). The need for such comprehensive intervention in livestock nutrition will be critically reviewed here.

Functions of Selenium

Most of the functions of Se are effected by selenoproteins. The number of mammalian SeP encoded was 25 (Beckett and Arthur, 2005) and continues to grow: all have oxido-reductase properties (Lammi and Qu, 2018) and their nomenclature takes up much of the alphabet (Table 15.1). SeP expression is generally upregulated by Se supplementation (Dalia *et al*., 2017) and all SeP can protect tissues from reactive oxygen species (ROS), by-products of essential oxidative processes and primers of cell signalling and transcription.[i] Uncontrolled peroxidation can initiate chain reactions of ROS generation that damage tissues: capacity to terminate such reactions is shared with other enzymes (e.g. the superoxide dismutases CuZnSOD and MnSOD, catalase, glutathione-S-transferase) and scavengers such as vitamin E and metallothionein (MacPherson, 1994). Vitamin E functions as a lipid-soluble antioxidant in cell membranes while water-soluble GPX acts primarily as an intracellular antioxidant.

Glutathione peroxidases

Six different peroxidases reduce peroxide (H_2O_2) and hydroperoxide (ROOH), using glutathione (GSH) as substrate; multiplicity and ubiquity reflect the importance of controlling peroxidation. Hydroperoxides are generated by the oxidation of fatty acids and other substances in tissues.

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Table 15.1. Selenoproteins that have been purified and/or cloned, their location and possible functions. (After Beckett and Arthur, 2005)

Identifier	Selenoprotein	Principal location	Function
GPX1	Cytosolic glutathione peroxidases (GPX)	Tissue cytosol, red blood cells	Storage, antioxidant
GPX2	Phospholipid hyperoxide GPX	Intracellular membranes, particularly testes	Intracellular antioxidant
GPX3	Plasma GPX	Plasma, kidney, lung	Extracellular antioxidant
GPX4	Gastrointestinal GPX	Gut mucosa, kidney	Mucosal antioxidant
GPX5	Epididymal GPX	Epididymis	Weak antioxidant
SPS-2	Selenophosphate synthetase 2	Ubiquitous	SeCys biosynthesis
ID1 or DIO1	Iodothyronine 5′-deiodinase type I	Liver, kidney, muscle[a]	Conversion of T4 to T3
ID2 or DIO2	Iodothyronine 5′-deiodinase type II	BAT	Conversion of T4 to T3
ID3 or DIO3	Iodothyronine 5′-deiodinase type III	Placenta	Conversion of T4 to rT3
TR1, 2 & 3	Thioredoxin reductases 1, 2, and 3	Kidney, brain	Redox cycling
SelH	Selenoprotein H	Nerve tissue	Neuroprotection
SelN	Selenoprotein N	Muscle	Cell proliferation
SelP	Selenoprotein P	Plasma	Transport, metal detoxifier
SelR	Selenoprotein R	Liver, kidney	Methionine sulfoxide reductase
SelW	Selenoprotein W	Muscle	Antioxidant, calcium-binding
MCSeP	Mitochondrial capsular selenoprotein	Sperm mitochondrial capsule	Store for GPX4

BAT, brown adipose tissue; GPX, glutathione peroxidase; rT3, reverse T3; SeCys, selenocysteine; T3, triiodothyronine; T4, thyroxine

The general reactions, which generate oxidized GSH (GSSG) from either GSH or reduced GSH (GSSH) are:

$$H_2O_2 + 2GSSH \rightarrow GSSG + 2H_2O$$
$$ROOH + 2GSH \rightarrow ROH + GSSG + H_2O$$

Cytosolic GPX1, the first peroxidase to be identified, is the most abundant, accounts for most of the Se in erythrocytes and liver and contains four Se atoms per tetrameric molecule in selenocysteine (SeCys) residues (Flohe *et al.*, 1973). However, blood and tissue GPX1 activities can fall to almost undetectable levels without obvious pathological or clinical changes. Animals can survive without the gene for GPX1 but changes are triggered in other peroxidases and SeP that are functionally more important (Behne and Kyriakopoulos, 2001), including:

- GPX2, located in the intestinal mucosa where it may afford protection from without (dietary hydroperoxides) and within, when metabolic activity is intense;
- GPX3, an extracellular peroxidase, present in plasma and synthesized principally in kidney and lung, protecting delicate structures such as the proximal renal tubule and alveolar capillaries; and
- GPX4, a phospholipid hydroperoxidase monomer associated with intracellular membranes, 'spared' during Se deprivation (Weitzel *et al.*, 1990) and possibly responsible for the substitutive relationship between Se and vitamin E (Arthur and Beckett, 1994). Polymeric GPX4 can provide structural support to membranes (Behne and Kyriakopoulos, 2001).

Species and tissues differ in expression of GPX. In liver, GPX4 activity is tenfold higher and GPX1 activity is tenfold lower in turkeys than in rats (Sunde *et al.*, 2015). In turkeys, GPX3 expression was high in all tissues except kidney, where GPX1 expression was high. Substrate (GSH) concentrations in plasma are too low for GPX3 to have a major extracellular protective role but the mRNA for GPX3 is expressed in mammary tissue (Bruzelius *et al.*, 2007) and GPX3 provides some of the Se in milk. Co-operation between different GPX, non-Se-containing GSH transferases, superoxide dismutases and vitamin E constructs robust defences against tissue peroxidation (Fig. 15.1) and may be particularly important in thyrocytes, in which

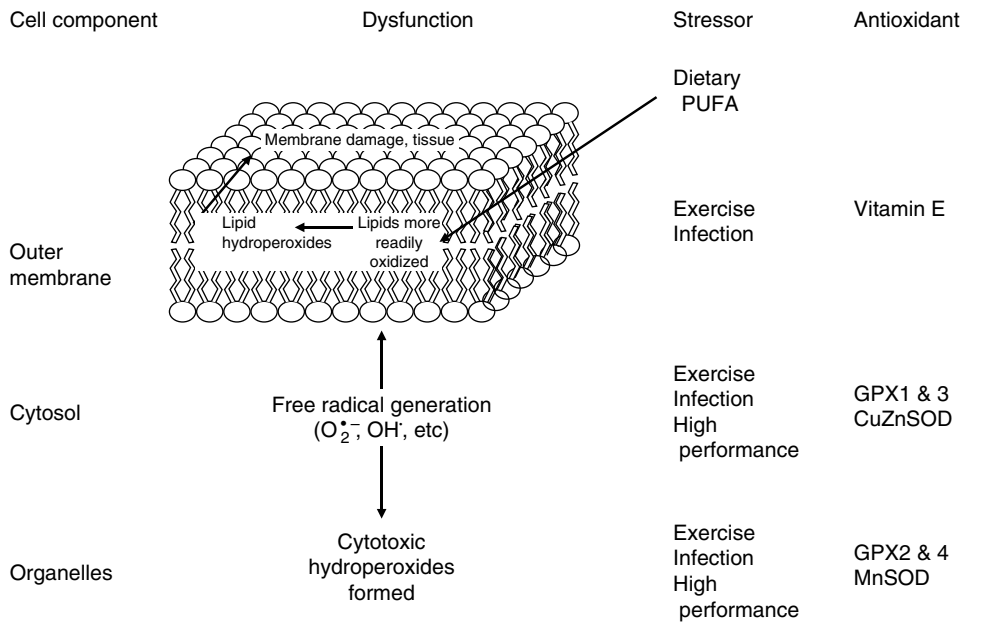

Fig. 15.1. Schematic of cell membrane and organelle protection from peroxidation: glutathione peroxidases (GPX1, 2 and 4) copper/zinc- and manganese-dependent superoxide dismutases (CuZnSOD and MnSOD, together, counter-challenge superoxide (O_2), hydroxyl radicals (OH-.) and polyunsaturated fatty acids (PUFA).

H_2O_2 is produced during thyroid hormone synthesis (Korhle *et al*., 2005).

Iodothyronine 5′-deiodinase

The membrane-bound, iodothyronine 5′-deiodinase type I (ID1), transforms thyroxine (T4) to physiologically active triiodothyronine (T3) (Arthur and Beckett, 1994, p. 301). Lack of Se can increase plasma T4:T3 ratios in cattle (Arthur *et al*., 1988; Awadeh *et al*., 1998; Contreras *et al*., 2005) and sheep (Donald *et al*., 1994b). A second deiodinase (type II or ID2) is under feedback control from T4 and more susceptible to Se deprivation. Species differ in the way T3 is generated: the predominant deiodinase is ID1 in ruminants and ID2 in non-ruminants (Nicol *et al*., 1994). The thyroid in ruminants contains no IDI and T3 is mostly generated outside the thyroid (for sheep, see Vouduri *et al*., 2003). Thermogenesis in the newborn depends heavily on T3 generation in brown adipose tissue (BAT) by ID2 (p. 301), which is also expressed in brain and bone cells. In non-ruminants, ID1 is located primarily in the thyroid, liver and kidneys.[ii] A third Se-containing deiodinase (ID3) was found in the placenta (Salvatore *et al*., 1995).

Thioredoxin reductases

Three ubiquitous SeP, thioredoxin reductases TR1, 2 and 3, have been identified, the TR3 isoform occurring predominantly in testis. These flavoproteins have diverse roles, including reduction–oxidation regulation, and thus regulate transcription (Korhle *et al*., 2005). These reductases also recycle vitamins C and E and influence absorption of minerals such as Ca (Moreno-Reyes *et al*. 2006). By maintaining muscle protein in an active, reduced form, TR may protect muscle from dystrophy (Surai, 2006). Bone turnover decreases in Se-deprived rats and TR may protect osteoblasts and osteoclasts from ROS, produced during bone remodelling (Moreno-Reyes *et al*., 2006).

Selenophosphate synthetase

Another enzyme, selenophosphate synthetase (SPS) -2, catalyses incorporation of selenide into SeCys to form, SelP, principal constituent of plasma Se. The SelP molecule has ten SeCys residues and serves as a transporter of Se, while possessing antioxidant properties. Deletion of the *SelP* gene has lethal consequences in mice, and local expression

in tissues such as the testes and brain is vital (Renko *et al.*, 2008). SelP has the potential to complex heavy metals and may incidentally protect tissues from toxic elements such as cadmium and mercury (Hill, 1974).

- Lack of dietary Se indirectly influences basic metabolic rate and a wide range of physiological processes, including parturition and survival during cold stress (Fig. 15.2), adversely affecting livestock health and production.

Clinical Abnormalities on Diets that Lack Selenium

Signs of Se insufficiency are generally non-specific and vary widely within and between species, this probably reflects cellular vulnerability to oxidant stress at critical sites and stages of development, as well as many factors, other than Se, that contribute to antioxidant defence (Fig. 15.1). Detailed descriptions of specific clinical disorders were given in a previous edition (Underwood and Suttle, 1999) and are summarized in Table 15.2. Many Se-responsive disorders (SeRD) resemble other conditions with different aetiologies and occur with or without the abnormalities listed in Table 15.2.

Reproductive disorders

Selenium deprivation reduced egg production in hens and conception rate, litter size and piglet survival in sows (Underwood and Suttle, 1999). In boars, sperm development was particularly sensitive to Se supply, 0.06mg Se kg^{-1}DM being adequate for growth but insufficient to prevent low sperm motility, 'tail abnormalities' and low fertilization rates (Marin-Guzman *et al.*, 1997). Similar observations were made in cockerels (Surai *et al.*, 1998). Sperm viability was reduced in semen from Se-deprived bulls (Slaweta *et al.*, 1988). Testes are rich in GPX4 (Lei *et al.*, 1998). In ewes, high embryonic mortality around implantation was attributed to lack of Se (Hartley, 1963; Wilkins and Kilgour, 1982). In a New Zealand study, some 20–50% of ewes were infertile unless additional Se was given before mating, but neither vitamin E nor a synthetic antioxidant afforded protection (Table 15.3). Fertility seems less vulnerable to Se insufficiency in grazing cattle (Wichtel *et al.*, 1998) but was improved by Se supplementation in housed heifers (MacPherson *et al.*, 1987). In cattle, delayed expulsion of afterbirth ('retained placenta') can be a SeRD (Underwood and Suttle, 1999). However, herd incidence can vary tremendously from year to year and be influenced by other dietary factors such as vitamin E supply (Bourne *et al.*, 2006). Supplementation with Se reduced incidence of endometritis and cystic ovaries (Harrison *et al.*, 1984). Fertility may be improved indirectly by decreasing retained placenta and other reproductive disorders.

Peri- and post-natal mortality

Supplementing pregnant ewes with Se can improve lamb viability (Table 15.3) (Kott *et al.*, 1983). Lack of Se may compromise survival by restricting the thermogenic response to cold stress, lowering ID2 activity in BAT (see p. 301) and be subject to interactions with iodine (I). Probability of perinatal survival was increased from 0.61 to 0.91 by Se in a study in which administration of an I antagonist

Table 15.2. A summary of specific clinical disorders that respond to selenium supplementation.

Disorder	Description and consequence	Predilection site	Affected species	Responsiveness to vitamin E
White muscle disease	Dystrophy of striated muscle: myopathy	Skeletal muscle, heart, gizzard	A, B, Ca, Ce, E, O, P	Rare in ruminants, ++ in A, P
Exudative diathesis	Increased permeability of capillaries: oedema, swelling and bruising	Thorax, neck, wing	A > P	++
Hepatosis	Necrosis	Liver	P	+
Mulberry heart disease	Microangiopathy	Heart > brain	P	++ on PUFA-rich diets

A, avian; B, bovine; Ca, caprine; Ce, cervine; E, equine; O, ovine; P, porcine; PUFA, polyunsaturated fatty acids

Table 15.3. Effects of pre-mating treatment with selenium, vitamin E or synthetic antioxidant on ewe fertility (200 ewes/group). (From Hartley, 1963)

	Untreated	Selenium	Vitamin E	Antioxidant
Barren ewes (%)	45	8	50	43
Lambs born/100 ewes lambing	105	120	109	112
Lamb mortality (%)	26	15	16	14
Lambs marked/100 ewes lambing	43	93	46	54

(thiocyanate) during pregnancy prevented the response: Se may have only been able to alleviate a mild I deficiency (Donald *et al*., 1994b; Moreno-Reyes *et al*., 2006). Survival of older lambs can also be compromised by lack of Se. Injecting five-month-old lambs with Se reduced mortality from 27% to 8% (Hartley, 1967) and treatment of Merino lambs regularly from marking time reduced mortality from 17.5% to 0% (McDonald, 1975). In Japanese quail, viability of newly hatched chicks was impaired by lack of dietary Se and hatchability was the most sensitive criterion of Se deprivation in poultry (Underwood and Suttle, 1999).

Lowered disease resistance

Claims that susceptibility to infection is an early consequence of Se insufficiency still far outnumber proven cases of cause and effect. Supplementation with Se has shortened episodes of bovine mastitis without reducing their frequency and was often given with vitamin E (e.g. Smith *et al*., 1997). Only one case of Se-responsive, infectious disease has been reported: mastitis in dairy goats (Sanchez *et al*., 2007). Adding Se-yeast (SeY) to the diet decreased episodes of diarrhoea in double-muscled, Belgian Blue calves (Guyot *et al*., 2007) but a probiotic response to yeast cannot be ruled out. Mean blood Se1 and herd incidence of *Staphylococcus aureus* infections of the udder were inversely related, values ranging 90–360μg Se l^{-1} (Jukola *et al*., 1996) but the relationship may not have been causal. Prolonged Se depletion did not impair resistance to viral infection in calves (Reffett *et al*., 1988) or nematode infection in lambs (Jelinek *et al*., 1988; McDonald *et al*., 1989). Marginal Se depletion lowered resistance of chicks to the protozoan parasite *Eimeria tenella* (Colnago *et al*., 1994). Deaths and lesions in chickens following *Escherichia coli* infection were reduced from 86% to 21% by raising dietary Se from 0.05 to 0.45mg Se kg^{-1}DM, one day before challenge, but was ineffective if the birds were cold-stressed (Larsen *et al*., 1997). Raising dietary Se from 0.15 to 0.3mg kg^{-1}DM for 14 days reduced cloacal and oro-pharyngeal shedding of low-pathogenicity avian flu virus, after artificial challenge, and stimulated expression of interferon-regulating genes (Shojadoost *et al*., 2019). Weekly drenching with Se, equivalent to 0.3 and 0.5mg kg^{-1}DM, for over a year did not reduce foot-rot incidence and severity in a flock whose diet contained about 0.05mg Se kg^{-1}DM (Hall *et al*., 2013). Any responses to such supra-nutritional Se exposures may be pharmacological rather than physiological in origin. Evidence of compromised immunocompetence is also far from convincing.

Blood disorders

Heinz-body anaemias have been reported in steers (Morris *et al*., 1984) and lambs (Suttle *et al*., 1987) of low Se status and attributed to peroxidative damage, due to low activities of GPX1 in erythrocytes. Post-parturient haemoglobinuria can develop in dairy cows turned out to graze spring pasture and low erythrocyte GPX1 activities have been implicated but the disease is not primarily attributable to lack of dietary Se (Ellison *et al*., 1986).

Occurrence of Selenium-responsive Disorders

Reduced inputs of anthropomorphic Se from atmospheric deposition and crude fertilizers may have reduced the Se status of soils, crops, pastures and people (Broadley *et al*., 2006) but routine Se supplementation of livestock feeds has probably arrested and even reversed that decline in developed countries. Continued heavy dressings with super-

phosphate (containing $CaSO_4$) to increase pasture yields may have contributed to cases of Se insufficiency in grazing sheep and cattle in parts of New Zealand (Andrews *et al.*, 1968) and Australia (McDonald, 1975). Such interventions, together with the multifactorial nature of the underlying dysfunctions, lead to poor correlations between local incidence of SeRD and Se status of soil, diet and animal. This is illustrated in Fig. 15.2 (see also Table 15.9 and the following text).

Selenium status of soils and feeds

Soil geochemistry and mapping have defined broad areas of high risk for SeRD in man and/or livestock (Combs, 2001; Fordyce 2005; Broadley *et al.*, 2006). These include soils of volcanic and granitic origin, such as the pumice soils of New Zealand, tablelands of NSW; mountainous countries of northern Europe, such as Finland, Sweden (Selinus, 1988) and Denmark; Siberia and much of China (Fordyce, 2005). Selenium uptake by crops and forages was higher in those growing on alkaline than on acid soils (Ullrey, 1974). Effects of soil origin can be confounded by those of climate and altitude. Forage Se was inversely related to altitude in western Kenya (p. 28), probably because of higher rainfall, soil leaching effect and the diluting effect of lusher growth. In cattle (Langlands *et al.*, 1981) and sheep (Langlands *et al.*, 1991a) in NSW, Se status was negatively correlated with rainfall. Prevalence of low blood Se in sheep (Anderson *et al.*, 1976) and cattle (Arthur *et al.*, 1979) in Scotland is attributable to a combination of granitic soils, high altitude, high rainfall and slightly acidic soils.

Vitamin E

Much evidence can be adduced to support the role of vitamin E, illustrated in Fig. 15.2. In pigs, white

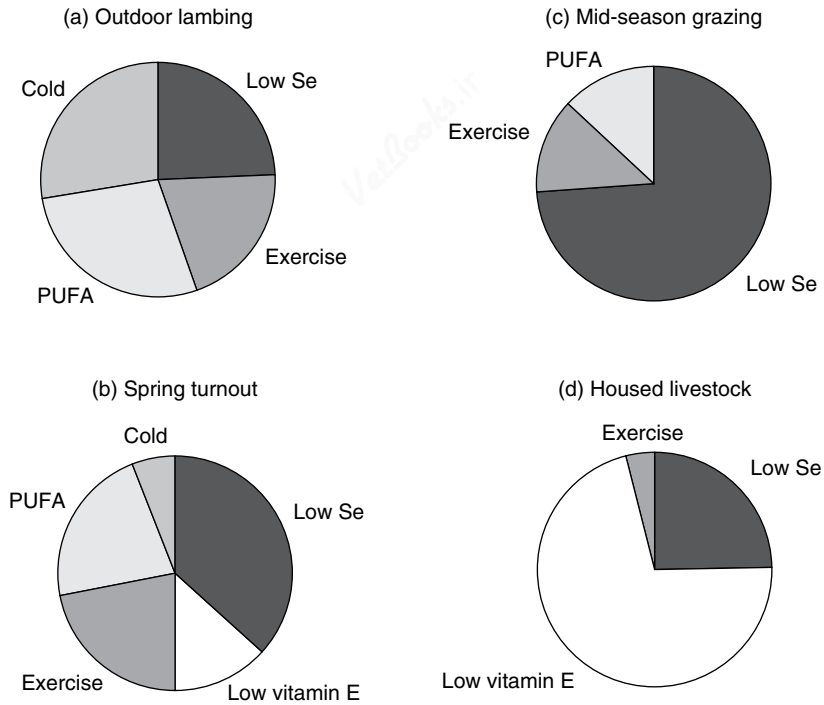

Fig. 15.2. Hypothetical illustration of relative contributions of selenium and vitamin E deprivation, oxidant stress (from cold exposure, muscular exercise or dietary polyunsaturated fatty acids (PUFA)) to risk of selenium-responsive disorders developing in sheep or cattle when housed or at pasture: (a) lambs born outdoors, green sward, cold spring; (b) growing cattle turned out on to lush spring pasture; (c) continuously grazed sheep or cattle, mild temperatures; (d) housed sheep or cattle.

muscle disease (WMD) was commonplace in the USA's midwest, with mortalities of 15–20% recorded in Michigan: 0.10–0.15mg Se kg^{-1}DM was needed to prevent disorder, because vitamin E deficiency was a contributory factor (Ullrey, 1974). Supplies of vitamin E to ruminants grazing green pastures are plentiful and may explain the tolerance of low pasture Se in NSW (Langlands *et al.*, 1989). Vitamin E supplies fall during the dry season and during conservation as hay, particularly if material is badly weathered (Miller *et al.*, 1995): values are also low in dried pasture, cereal stubbles and grains (Liu *et al.*, 2014). Treatment of grains and straws with alkali and storage of moist grain in silos with propionic acid as preservative can destroy vitamin E, predisposing livestock to SeRD (Allen *et al.*, 1975). Vitamin E is poorly transported via the placenta and offspring rely on colostrum for early supplies.

- The large quantities of vitamin E secreted in colostrum cause maternal plasma tocopherol concentrations to halve around parturition (Goff and Stabel, 1990) and may briefly increase the mother's need for Se.

Low colostrum and vitamin E intakes in early life may increase risk of SeRD in the newborn, leaving them slow to suckle. In the USA, incidence of mastitis in cows was reduced by massive supplements of vitamin E (2000–4000 IU day^{-1}) (Weiss *et al.*, 1997). Supplementation with Se may reduce somatic cell count (SCC) and udder infections caused by a primary lack of vitamin E. Inclusion rates for expensive vitamin E in supplements may often be too low to eliminate responses to Se (Swain *et al.*, 2000) but supra-nutritional supplementation with Se is a biologically inefficient solution.

Dietary oxidant stress in herbivores

After absorption, PUFA form unstable lipid hydroperoxides, a potential source of ROS in tissues, and high intakes of PUFA can induce myopathy (Rice and Kennedy, 1988). Most cases of WMD in calves and acute paralytic myoglobinuria in older cattle occur shortly after turnout onto spring pastures, which are rich in PUFA (Fig. 15.2) (McMurray *et al.*, 1983). Myopathy occurs in the face of a rapid rise in plasma tocopherol and is selenium-responsive (McMurray and McEldowney, 1977; Arthur, 1988). Supplementation of rations with PUFA can reduce plasma tocopherol (Wang *et al.*, 1996) and high PUFA intakes probably increase risk of SeRD in livestock, including WMD in goat kids (Rammell *et al.*, 1989). Although PUFA are hydrogenated in the rumen, increases in unsaturated linolenic acid in plasma at turnout show that, in the short term, hydrogenation is far from complete (McMurray *et al.*, 1983). Plasma tocopherol in the UK is low in spring and may reflect initially poor saturation of PUFA from spring grass (Small *et al.*, 1996). Higher risk of WMD on legume than on grass diets may be related to a higher rate of passage from the rumen, which allows more PUFA to escape hydrogenation. Horses differ from ruminants in that they do not saturate PUFA prior to absorption and may be more prone to lipid peroxidation. Foals develop WMD in areas where Se-responsive diseases occurred in grazing sheep and cattle (Andrews *et al.*, 1968; Caple *et al.*, 1978).

- PUFA-rich supplements, such as marine algae, and formulations that resist rumen degradation and decrease saturated fat in human foods may increase risk of SeRD.

Dietary oxidant stress in pigs and poultry

Unhatched chick embryos meet needs for energy largely by β-oxidation of fatty acids, while yolk lipids are used to synthesize membrane phospholipids. Oxidant stress may be high during embryo development and addition of PUFA to the hen's diet may increase peroxidation, leading to Se-responsive reductions in hatchability (Surai, 2006). Addition of PUFA (50 g fish oil kg^{-1}DM) to a cereal-based, broiler-breeder ration, containing 0.08mg Se kg^{-1}DM, reduced hatchability from 75% to 57%, chick birth weight by 1.1 g and increased four-day mortality. However, addition of 0.5 mg Se as selenium yeast (SeY) did not improve hatchability (Pappas *et al.*, 2006): responsiveness to Se_i, with higher functional availability (p. 380), should be studied.

Endogenous oxidants

Generation of ROS increases with metabolic rate and the need for protection from GPX (and vitamin E) increases with metabolic activity. In calves on a low-Se diet, change to a PUFA-rich grass diet alone did not induce myopathy, but grazing, with the inevitable increase in exercise, did (Fig. 15.3; Arthur, 1998). The mingling of piglet litters can precipitate WMD and the physical exertion of establishing a social order was implicated (Ullrey, 1974). The

importance of endogenous ROS is indicated by the uneven distribution of myopathic lesions amongst fibre types in cattle, with type I (slow-twitch, oxidative) fibres being more vulnerable than type II (fast-twitch, glycolytic). Lack of previous exercise may increase susceptibility to myopathy because there is less development of resistant type II muscle fibres in the confined animal (McMurray *et al.*, 1983). Sudden exercise and associated stressors can cause capture myopathy in deer (Montane *et al.*, 2002). High metabolic activity of spermatozoa may generate ROS and compromise male fertility in animals lacking Se (p. 374) (Liu *et al.*, 2014).

- Metabolic activity and the consequent generation of ROS is a risk factor for SeRD (Fig 15.2).

Prolonged heat stress in Japanese quail, exposed to 34°C for 8 h day^{-1} for 90 days, increased serum, liver and egg yolk MDA, indicating tissue peroxidation (Sahin *et al.*, 2008). Supplementation of the corn/SBM diet, containing 0.05mg Se and 25 IU added vitamin E kg^{-1}DM and also heat-exposed, with either selenite or SeMet (0.15 or 0.3mg Se kg^{-1}DM), significantly reduced MDA (Fig. 15.4) and improved egg production, but protection was far from complete. Cold stress did not increase tissue peroxidation (Ozkan *et al.*, 2007).

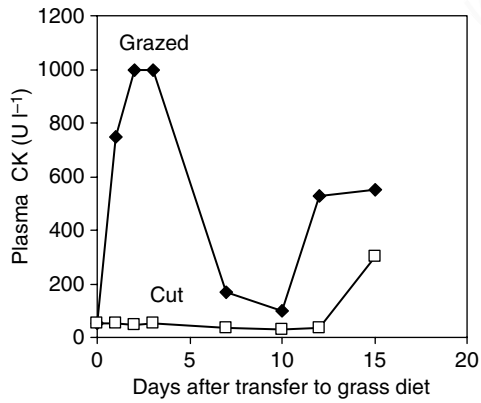

Fig. 15.3. Transference of calves of very low selenium status from a semi-purified diet to grass, either grazed or cut and fed indoors, only induced biochemical; evidence of myopathy (increased plasma creatine kinase (CK) activities) in the grazed group. When the indoor group was turned out after 12 days, a muted increase in CK was seen. Thus, a combination of low selenium status, transition to grass and exercise was necessary to precipitate myopathy. (Data for the worst pair of grazed calves from Arthur, 1988)

Toxins

Fungal toxins such as aflatoxin and ochratoxin often contaminate livestock feeds and harm livestock by increasing lipid peroxidation in tissues (Surai, 2006). Combined supplementation with Se and vitamin E decreased aflatoxin and ochratoxin toxicity to broiler chicks (Anil Kumar *et al.*, 2005). Specific protective effects of moderate Se supplements against mycotoxicoses should not be hard to demonstrate but remain conspicuous by their absence. An Se plus vitamin E-responsive myopathy occurred in sheep grazing lupin stubbles contaminated with the fungus *Diaporthe toxica* in Western Australia: however only certain forms (SeMet) and methods of administration (subcutaneous vitamin E) were protective (Smith and Allen, 1997).

Iodine status and cold stress

If the biochemical dysfunction is one of poor deiodination rather than peroxidation, a new set of interactants, including goitrogens, is introduced and these may influence the occurrence of 'ill-thrift' (Fig. 15.2). If oxidant stress is minimal, impairment of Se-dependent thyroid hormone function may become health-limiting through a reduction in ID1 or ID2 activity in BAT: this is most likely to occur when animals are born outdoors in cold, wet and

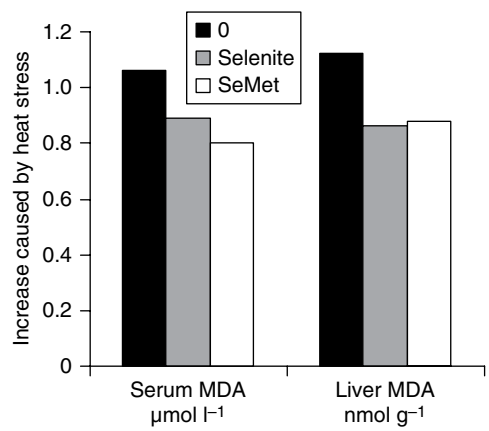

Fig. 15.4. Prolonged heat stress in Japanese quail increased two indices of peroxidation – malondialdehyde (MDA) concentrations in serum and liver – on a basal diet marginal in selenium and vitamin E (0). Addition of 0.3mg Se kg^{-1}DM as selenite or selenomethionine (SeMet) afforded similar partial protection. (From Sahin *et al.*, 2008)

windy conditions. Extremes of temperature and humidity increased WMD incidence in piglets (Ullrey, 1974). A hypothesis that wool growth was suppressed by Se-induced iodine depletion was tested and rejected when fortnightly injections of T4 or T3 did not improve wool production (Donald *et al*., 1994a) but irregular administration was unphysiological and plasma T3 was decreased by T3 supplementation. Cold stress increased blood T3 from 0.97 to 1.89 ng ml^{-1} in broilers on a diet with 0.15 mg added Se_i, but neither doubling the supplement nor change to an organic source affected the response (Ozkan *et al*., 2007). However, susceptibility to cold stress and the T3 response was compromised in Se-deprived rats and affected by a Se–I interaction (Fig.15.5) (Arthur *et al*., 1992).

High dietary sulfur

High dietary S has been implicated in the aetiology of WMD (Underwood and Suttle, 1999). A hypothesis that S-rich molasses might lower Se status was tested and supported, insofar as liver and plasma Se were higher in forage-fed steers supplemented with corn rather than sugarcane molasses (Arthington, 2008). However, raising dietary S from 1.6 to 3.7g kg^{-1}DM did not affect tissue or hair Se in fattening pigs (Kim *et al*., 2014). Inhalation of S_2^- from the rumen gas cap on high S diets (p. 209) may constitute a challenge to Se-dependent functions by generating reactive sulfide species, which are as potentially lethal as ROS (Fig. 9.1).

Subclinical Effects of Insufficiency in Ruminants

Mild 'subclinical' SeRD that had no distinctive pathological features were first identified in parts of Australia and New Zealand as 'ill-thrift', affecting pastured lambs, beef and dairy cattle of all ages (Hartley, 1967; McDonald, 1975). The underlying dysfunctions are unknown but, in economic terms, 'ill-thrift' may be the most important manifestation of selenium deprivation.

Wool production

Wool production was particularly affected by lack of dietary Se (Gabbedy, 1971) and often improved by supplementation (e.g. Slen *et al*., 1961; McDonald, 1975). In a four-year study in New South Wales (NSW), fleece yields from ewes were increased by 3.8–7.5% by annual Se supplementation (Langlands *et al*., 1991a) and their lambs produced 9.5% more wool, without themselves being supplemented (Langlands *et al*., 1990). Responses were greater in ewes that reared a lamb to weaning than in those that did not, reflecting the competing demands of pregnancy and lactation for Se. Greasy wool yield was increased by 22% at two successive shearings

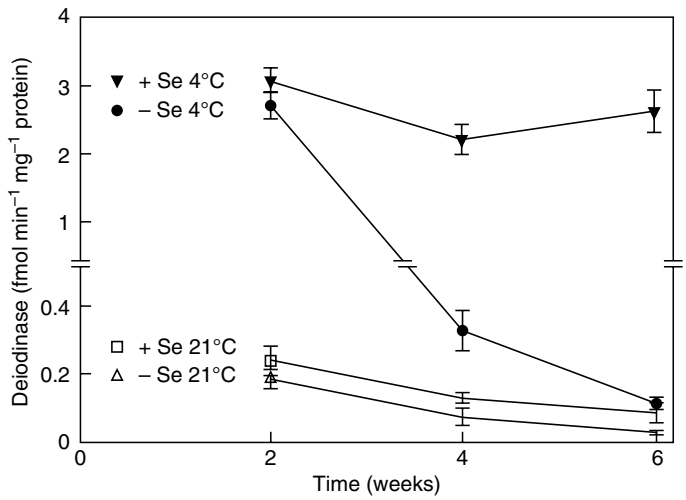

Fig. 15.5. Interaction between selenium metabolism and cold stress in rats. Exposure to low temperatures caused an early increase in deiodinase activity in brown adipose tissue to combat increased oxidative stress in the tissue, but adaptation was not sustained if the diet was low in Se. (From Arthur *et al*., 1992)

in Western Australia, following Se supplementation (Whelan *et al*., 1994b). Responses in wool yield may be partly attributable to increases in body size because the two parameters are correlated.

- Wool is rich in S-amino acids and may act as a sink for SeMet, depriving sheep of recyclable Se.

Growth retardation

Lamb growth has been improved by Se supplementation in many countries (Underwood and Suttle, 1999). A 10.8% improvement in weaned lamb weight was recorded in NSW flocks (Langlands *et al*., 1990), the greater in Merino than in crossbred lambs and at the higher of two widely spaced stocking rates: ewe fecundity was unaffected (Langlands *et al*., 1991b). Growth retardation in lamb flocks in New Zealand increased exponentially as mid-trial mean blood Se declined (Grace and Knowles, 2002). Vitamin E does not stimulate growth in these situations and the underlying defect may be lack of deiodinase activity rather than poor antioxidant defence. By contrast, cattle grew without restriction on pastures that provided insufficient Se for wool-growing sheep (Langlands *et al*., 1989). Growth responses to Se in cattle were recorded in herds showing concomitant hypocupraemia and maximal responses were obtained if Cu was also given (Gleed *et al*., 1983; Koh and Judson, 1987). Growth is also retarded by lack of Se in broiler chickens (Jianhua *et al*., 2000).

Loss of milk yield

Milk production from grazing dairy cows was only impaired when blood and pasture Se were exceedingly low (Tasker *et al*., 1987). The main effect was a reduction in milk fat yield (Fraser *et al*., 1987; Knowles *et al*., 1999), raising the possibility of an effect on lipid metabolism via pancreatic dysfunction. Pancreatic acinar cells are highly sensitive to Se insufficiency in the chick (p. 383). Effects on milk yield in sheep and goats have not been specifically studied, but growth retardation in the suckling (e.g. Langlands *et al*., 1990) could be a secondary effect of reduced milk yield in the nursing ewe.

Cumulative effects

The combined effects of improvements at different stages of production can be substantial. When highly significant weight gains in surviving lambs were multiplied by the increased number of lambs born and surviving, in early New Zealand trials, an improvement of 1513 kg in total weaned lamb weight was obtained by supplementing 201 ewes with selenium (Hartley, 1967). In eastern Australia, the increase in total annual fleece production of Se-treated groups (40 lambs) over untreated groups (33 surviving lambs) was 47 kg (39%) (McDonald, 1975).

Physiochemical Changes on Diets Lacking Selenium

Assessments of degree of Se deprivation (and availability) depend on proper assessments of normality and are affected by source of dietary Se.

Normal metabolism

Uptake and metabolism of Se by mammals differs for Se_i and Se_o sources (Fig. 15.6) (Burk *et al*., 2001; Sunde *et al*., 2016): SeMet and SeCys follow the respective routes of methionine and cysteine across the small intestinal mucosa and are taken up as intact molecules but their paths then diverge. A unique biosynthesis incorporates SeCys into SelP while SeMet continues to track methionine and enters protein pools. Selenite and selenate are reduced to selenide and incorporated into SelP in the gut mucosa, thereafter following the path of SeCys (Fig. 15.6) (Vendeland *et al*., 1992). The liver regulates retention and distribution of Se via the secretion of SelP into plasma. Plasma Selp is distributed to tissues in relation to their expression of a SelP1 receptor, creating a tissue Se hierarchy (Burk and Hill, 2015). As dietary Se_i increases, expression of each SelP increases until a plateau is reached: comparable studies with SeMet and SeCys would be most revealing. Responses in tissue and blood Se do not always correspond to changes in functional SeP. Young pigs given supplements of Se_i had higher tissue Se but lower serum GPX3 activity than those given SeY (Mahan *et al*., 1999, 2014). Giving cows SeY rather than Se_i lowered the slope of the relationship between blood GPX and blood Se but not that between liver and blood Se (Knowles *et al*., 1999). Inconsistencies are due to slow turnover of non-functional SeMet from Se_o sources in blood and tissues (Fig. 15.5) and have diagnostic implications.[iii] Incorporation of SeCys into erythrocyte GPX1 occurs at erythropoiesis and there is a

lag before newly 'packaged' GPX1 is released into the bloodstream: SeMet, on the other hand, can be incorporated into erythrocytes as methionine in haemoglobin.

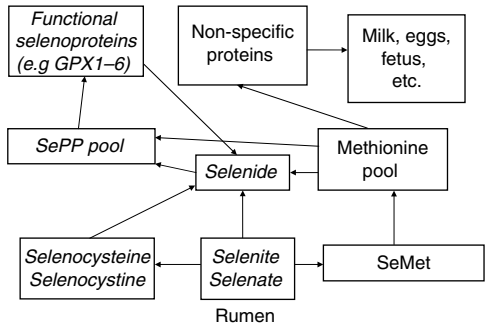

Fig. 15.6. A simplified illustration of contrasting movements of selenomethionine (SeMet), selenocysteine (SeCys) and inorganic selenium (Se_i) after ingestion in mammals (after Waschulewski and Sunde, 1988; Burk *et al*., 2001), adapted to include ruminants. SeCys and Se_i enter the Se-transport (SePP) pool and are incorporated into functional proteins such as glutathione peroxidases (GPX); SeMet follows a different pathway, entering the methionine pool and going where methionine rather than Se is required. Transsulfuration or transamination can partially convert SeMet to SeCys. In ruminants, Se_i can be incorporated into SeCys and SeMet by rumen microbes.

Effects of source, site and species

An investigation of the impact of natural variation in crop Se across the USA on Se status of fattened pigs indicated that hair Se increased faster than liver or loin Se with increases in inherent feed Se (Fig. 15.7). intercepts of the respective slopes represent effects of Se_i, included at the maximum permitted level of 0.3mg Se_o kg^{-1}DM, regardless of need. Equivalent intakes of Se from feed increased loin Se 3.3 times more, hair Se 1.7 times more but liver Se 0.67 times less than Se_i: liver processes all forms of Se from the portal blood flow. Loin presumably accumulated Se as SeMet in protein, hair because it is high in SAA. When methionine requirements are high, as in wool-bearing sheep, early lactation, and suckled offspring, wool, milk and newly deposited muscle will be enriched with SeMet from unsupplemented diets at the expense of functional forms, such as GPX1 in blood and GPX3 in plasma, in both dam and offspring. As dietary methionine increases, less SeMet is incorporated into general proteins and more into specific SeP, such as GPX1 (Butler *et al*., 1989).

Maternal transfer

In all species, effects of source maybe amplified in non-recipient offspring by differences between sources in their ability to cross placental and mammary

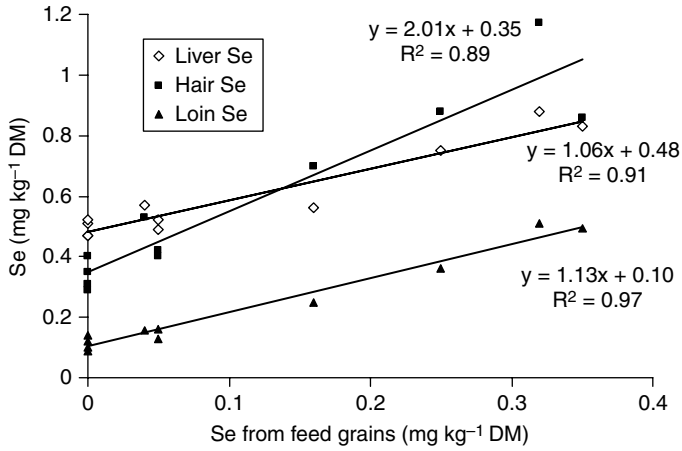

Fig. 15.7. Relationship between selenium concentrations in natural (organic) pig rations and in liver, loin, muscle and hair at commercial slaughter in groups given a standard supplement of 0.3 mg inorganic Se kg^{-1}DM in collaborative US trials (data from Mahan *et al*., 2005). Assuming that intercepts represent the contribution to Se accretion coming from the inorganic supplement, an equivalent increase in concentration of organic Se (0.3mg kg^{-1}DM) produces much larger increases in hair and muscle Se but not liver Se.

barriers. SeMet may cross placental and mammary barriers with methionine rather than as a SeP. Supplementation with Se during pregnancy increases Se status in the newborn lamb (Langlands *et al*., 1990; Rock *et al*., 2001), calf (Pehrson *et al*., 1999; Gunter *et al*., 2003) and piglet (Mahan and Peters, 2004; Yoon and McMillan, 2006) but the parenteral route is more effective than the oral route. Colostrum and main milk Se can both be raised by Se_i supplements in sheep (Meneses *et al*., 1994; Rock *et al*., 2001), goats (Zachara *et al*., 1993) and cattle (Conrad and Moxon, 1979; Rowntree *et al*., 2004) but SeMet is by far the more effective. Adding SeY to the diet raises milk Se in dairy cows linearly at a rate eight times that obtained with Se_i or a 'chelated' Se source (Givens *et al*., 2004), but the effect of source was only marginal in ewes given 0.3mg Se kg^{-1}DM. A crucial but as yet unanswered question is:

- To what extent does SeMet ingested in milk continue to behave distinctly and is the increase in milk Se matched by an increase in functional availability?

Biochemical Consequences of Insufficiency

The sequence of biochemical changes in Se-deprived livestock differs in many respects from the general model described earlier (Fig. 3.2) and is illustrated in Fig. 15.8.

Depletion

Selenium can be lost from the body by exhalation of methylselenol (CH_3SeH), urinary excretion and faecal endogenous loss (FEL_{Se}), the latter contributing significantly to faecal Se at low Se intakes in sheep (Langlands *et al*., 1986) and cattle (Koenig *et al*., 1991). Urinary losses decline as body status (Langlands *et al*., 1986) and dietary Se intake decline (Fig. 15.9; Pedrosa *et al*., 2012). Tissue Se, accumulated during times of plenty, is mobilized to replace losses at widely different rates, depending on speciation of tissue Se and metabolic activity of the tissue (Juniper *et al*., 2008). Selenium that has accumulated as SeMet in tissue proteins cannot be

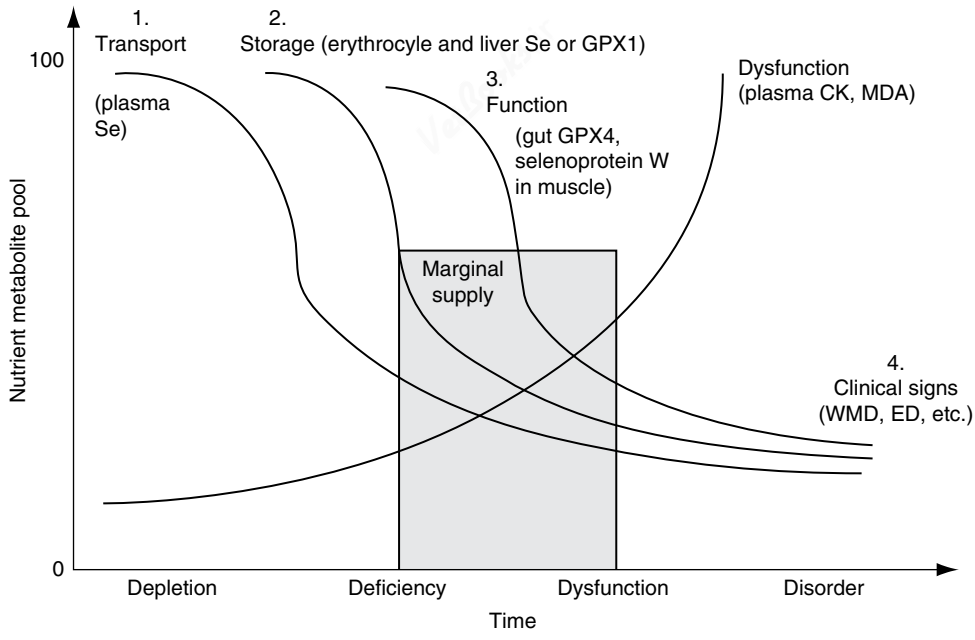

Fig. 15.8. General sequence of biochemical changes in livestock deprived of selenium: transition from one phase to another can be influenced by oxidant stress, status of other antioxidants (notably vitamin E) and selenium source. Glutathione peroxidase (GPX)1 and GPX4 are just two of several GPX with functional significance. Dysfunction is marked by increased concentrations of abnormal metabolites in plasma and tissues, such as creatine kinase (CK) and malondialdehyde (MDA). White muscle disease (WMD) and exudative diathesis (ED) are two of many possible clinical end-points in different species.

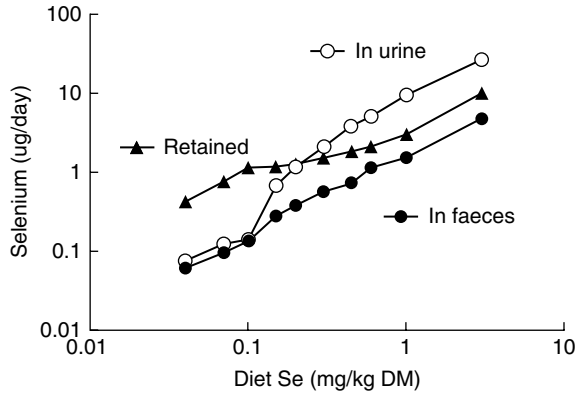

Fig. 15.9. Selenium balance data from growing rats, given a semi-purified diet supplemented with varying amounts of sodium selenite, indicated a high, unregulated absorptive efficiency (0.9), urine as the major route for excreting excess absorbed Se and unlimited Se retention. (From Kirchgessner *et al*., 1997)

mobilized without catabolism of protein (Fig. 15.5). Thus, muscle Se cannot be regarded as a 'store' in the same sense as liver Cu. Insufficient Se intakes reduce plasma, erythrocyte and tissue Se by reducing expression of GPX and SeP (Burk and Hill, 2015; Sunde *et al*., 2016) and reduce incorporation of SeMet into tissue proteins. The decline in erythrocyte Se and GPX1 activity is delayed by the time taken for newly released cells, low in Se, to reduce average values in a population of long-lived erythrocytes. The lag between decline in SeP and a function becoming rate-limiting can be interpreted as constituting a period of depletion, during which Se is recycled and redistributed: most of the Se in liver and blood can be viewed as that store in the form of GPX1. Changes in liver and blood Se or GPX1 activity reflect depletion more than deficiency. Milk and egg Se increase with Se intake and reductions provide opportunities for conserving Se when intakes fall. Cold stress reduced liver vitamin E but not liver Se in broilers (Ozkan *et al*., 2007).

Deficiency

The liver curtails Se excretion and secretes SelP into the plasma at the expense of intracellular SeP. Both GPX3 and SelP in plasma decline on diets lacking SeP synthesis. Reductions in serum Se occurred early during deprivation in Fig. 15.10 but were complete *before* disease (ED) was apparent in chicks. Pancreatic GPX 3 activity declined prior to the earliest signs of acinar cell atrophy (Bunk and Combs, 1980) but low enzyme activity may predispose to pancreatic dysfunction (Whitacre *et al*., 1987). Upregulation of enzymes that can compensate for loss of GPX activity, such as glutathione-S-transferase in liver and heart of Se-deprived pigs (Lei *et al*., 1998), may be a sign of imminent dysfunction. Dysfunction may only arise when GPX2 and GPX4 activities are reduced.

Dysfunctional antioxidant defences

Dysfunction is measured as end-products of lipid peroxidation, such as malondialdehyde (MDA) and other thiobarbiturate-active substances (McMurray *et al*., 1983). Another indicator of liver peroxidation, F_2-isoprostanes, increased in Se-deprived pigs, although growth was not impaired (Lei *et al*., 1998). Exhalation of ethane and pentane also indicate peroxidation. Hatching is a time of maximal ROS generation (Surai, 2006) and Fig. 15.10 shows the interactive effects in chicks of Se and vitamin E on peroxidation in liver mitochondria at that time. Initially, only groups given vitamin E showed full recovery from peroxidation but, as time passed and Se status declined, both antioxidant supplements were needed to eliminate peroxidation and associated disorder (ED). Selenium requirements to prevent hepatic microsomal peroxidation *in vitro* and pancreatic fibrosis in chicks were similar (Combs and Scott, 1974). There is high risk of lipid peroxidation in semen because spermatozoa are rich in PUFA and very active but vitamin E was far more protective than Se (Surai *et al*., 1998).

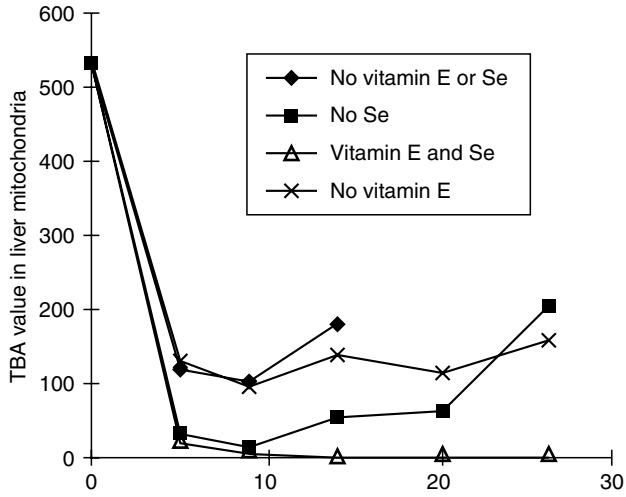

Fig. 15.10. Tissue peroxidation is influenced by interactions of selenium with other antioxidants, such as vitamin E, and with sources of oxidant stress: thus, thiobarbiturate (TBA)-like products of peroxidation were high in liver mitochondria of chicks deprived of vitamin E and Se at hatching (a stressor) and had only partially disappeared when disease (exudative diathesis) occurred after 13 days; combined supplementation with vitamin E and Se was needed to prevent peroxidation (data from Noguchi *et al*., 1973).

- The multifactorial causation of SeRD, illustrated for ruminants (Fig. 15.2), will also apply to pigs and poultry.

Lambs given a semi-purified diet low in Se (0.03mg kg^{-1}DM) developed subclinical myopathy but blood Se remained marginal (112 µg Se l^{-1}): oxidative stress may have been exacerbated by the PUFA-rich diet (3.6% corn oil; Vouduri *et al*., 2003). Reduced concentrations of Ca-binding SelW in muscle may cause mitochondrial Ca overload and calcification (Tripp *et al*., 1993).

Dysfunctional deiodination

Plasma T3 fell in periparturient cows of marginal Se status but showing no clinical response to Se supplementation (Awadeh *et al*., 1998; Contreras *et al*., 2005): both T3 and T4 declined in pregnant ewes on a low-Se diet (<0.02mg Se kg^{-1}DM) (Rock *et al*., 2001). In laying hens deprived of Se, reduction in plasma T3 was accompanied by a fall in T4 (Zuberbuehler *et al*., 2006): similar changes occurred in chicks and were accompanied by reduced hepatic ID1 activity decreased (Jianhua *et al*., 2000). Impaired IDI activity is clearly an early biochemical sign of deprivation in many species and may become the rate-limiting function in growth retardation.

In rats, Se deprivation induced thyrotrophin (TSH) and ID1 expression in an attempt to preserve thyroidal SeP (Bernamo *et al*., 1995), ahead of changes in T3:T4 ratio.

- Response to Se may depend on the concurrent supply of iodine.

Combined deficiencies of Se and I were required to decrease hepatic ID1 activity and increase thyroid weight in lambs; cerebellar ID3 activity, T3, T4 and TSH remained unaffected by either single or combined deficiencies (Vouduri *et al*., 2003). Although mild, the degree of I depletion was sufficient to increase erythrocyte and thyroidal cytosolic GPX markedly.

Kidney dysfunction

Broiler chicks developed clinical abnormalities after 20 days on a diet containing 0.03mg Se kg^{-1}DM and biochemical evidence of kidney dysfunction (increased plasma uric acid and creatine) was found: some SeP were downregulated (including ID1 and 2, GPX3) and others upregulated (including GPX1, 2 and 4 and thioreductases), while mRNA for several inflammatory factors also increased, suggesting an organ under stress (Zhang *et al*., 2016).

- Kidney dysfunction may be an unrecognized site of dysfunction in ruminants on diets low in Se.

Immunological Dysfunction

Components of the immune response can be compromised early in Se-deprivation (Stabel and Spears, 1993). Both vitamin E and Se improved antibody responses in non-ruminants and ruminants (Stabel and Spears, 1993; MacPherson, 1994) but sham injections of carrier were rarely used in controls and additivity varied. Raising dietary Se from 0.06 to 0.1mg Se kg^{-1}DM increased plasma interleukin-1 in weaned lambs (Qin *et al*., 2007). In chicks, Se supplementation raised antibody titres to injected sheep rbc (Larsen *et al*., 1997). In cows, Se supplements increased IgG in colostrum and blood of calves without improving survival (Swecker *et al*., 1995; Awadeh *et al*., 1998). Relationships between SCC and blood GPX1 have been inconsistent.[iv] Cellular immunity may appear to be compromised by Se deprivation *in vitro* (see MacPherson, 1994) but intensive, acute mitogenic challenge may overestimate dysfunction *in vivo* (Chesters and Arthur, 1988).

- Generation of ROS in neutrophils undergoing a respiratory burst after phagocytosis may explain both impaired phagocytic killing *in vitro* by cells from Se-depleted calves (Boyne and Arthur, 1979) and occasional reports of impaired immunocompetence to acute challenge *in vivo*.
- Selenium retained as SeMet in intracellular protein may afford relatively poor protection against such 'explosive' intracellular events.

Polymorphonuclear cells from sows supplemented with Se showed stronger responses to stimulation *in vitro* but vitamin E was more influential (Wuryastuti *et al*., 1993). Relative neutrophil expression of IL-1 and IL-8 in horses was increased by Se supplementation and SeMet increased IL-5 expression, whereas selenite did not: humoral immune responses were unaffected (Montgomery *et al*., 2012).

Diagnosis of Selenium-responsive Disorders

Diagnosis requires biochemical confirmation but is complicated by:

- presence of Se in diverse functional and non-functional forms in samples;
- 'critical' levels that depend on response criteria and productive state;
- uncertainty as to which of many Se-dependent functions is rate-limiting; and
- factors other than Se status that precipitate SeRD (Fig. 15.2).

The best diagnosis is often afforded by a positive response to Se supplementation but the numbers of animals required and/or precision of response measurement required to obtain significant responses is often high (p. 517).

Indices of Functional Selenium Status

GPX in blood and serum

Activity of GPX is a more reliable indicator of low status than blood and plasma Se, particularly in neonates, and plasma GPX3 a better indicator of current functional status than blood GPX1: this is because blood and plasma contain Se in non-functional forms. In a study with beef suckler calves (Fig. 15.11), blood GPX1 activity increased rapidly in all groups after birth, indicating an adequate Se supply from pasture that contained 0.1mg Se kg^{-1}DM but blood Se decreased, irrespective of treatment, falsely indicating marked deficiency. To derive diagnostic limits for blood GPX1 in sheep from a relationship with

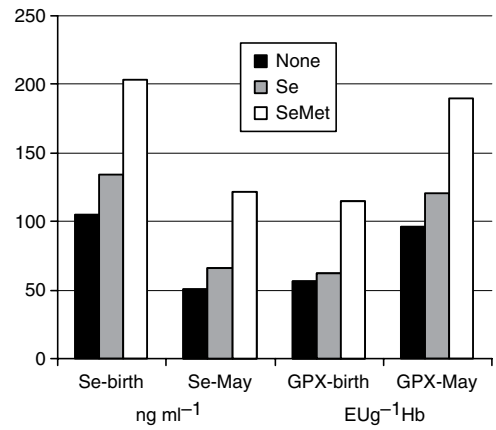

Fig. 15.11. Criterion of selenium (Se) status affects interpretation of responses in suckler calves to source of supplementary Se for dams during pregnancy. Blood Se declined on all treatments, while erythrocyte glutathione peroxidase (GPX)1 activity increased. The authors' (Gunter *et al*., 2003) inference that only selenomethionine (SeMet) prevented a decline in Se status applied to blood Se but not GPX1.

less reliable blood Se (e.g. Pavlata *et al*., 2018) weakens the diagnostic value of the enzyme assay: the same applies to assays of enzyme and elements in platelets (Dalir-Naghadeh *et al*., 2015). In dairy goats, mean GPX activities of 43–45 IU g^{-1}Hb were associated with susceptibility to mastitis (Sanchez *et al*., 2007). Activities of GPX rise to plateaus as dietary Se increases but plateau activities differ between species and between peroxidases (Sunde and Ranies, 2011, 2015).[v] A plateau for blood GPX1 was reached in lactating cows with <0.15mg Se kg^{-1}DM (Juniper *et al*., 2006). Plasma GPX3 responded to dietary Se up to 0.4mg kg^{-1}DM in chicks (Cantor and Tarino, 1982) but did not increase in mature geldings when Se was added to a grass-hay/concentrate diet containing 0.06mg Se kg^{-1}DM (Shellow *et al*., 1985).

- Enzyme assays in platelets may provide the best index of functional Se status from an easily procured sample.

The proportion of total plasma Se provided by GPX3 is relatively small and highly variable (Podoll *et al*., 1992), reflecting dietary influences, such as supplementation with SeMet, and physiological limits to the GPX3 response. Downregulation of GPX3 activity occurs during pregnancy and lactation in rats (Sunde *et al*., 2005) but was not evident in sows (Fig. 15.12). An apparent breakthrough was made when mRNA for tissue GPX was detected in plasma, allowing functional Se status in tissues to be predicted from an accessible sample (Evenson *et al*., 2004). Five of 24 SeP were markedly downregulated in rats when diets lacked Se, Gpx1, SePlW1, and SelH, and considered to have potential as molecular biomarkers for Se status (Sunde *et al*., 2011), but liver GPX1 and plasma GPX3 were considered to be robust markers of status (Sunde *et al*., 2015). However, species differ, GPX4 being conserved in rodent liver. Upregulation of tissue GPX3 activities in simple or goitrogen-induced iodine deprivation also complicates interpretation (p. 307).

Indices of dysfunction

Indices of peroxidation have been used experimentally but not diagnostically. Reductions in erythrocyte MDA in periparturient cows given vitamin E supplements indicate both potential and non-specificity of such tests, since Se supplements were without effect (Brzezinska-Slebodzinska *et al*., 1994). Where animals respond to Se because of secondary impairment of I metabolism, low plasma T3 (<2 nmol l^{-1}) may be present prior to treatment and rise afterwards, but T3:T4 ratios may not be helpful (Wichtel *et al*., 1996) and analytical costs are high. Furthermore, increases in plasma T3 associated with cold stress and changes associated with parturition complicate interpretation. In cold-stressed broilers, neither doubling a dietary Se_i supplement, providing 0.15mg Se kg^{-1}DM nor adding SeMet prevented a twofold increase in T3, but liver Se without supplementation was high at 0.45 μg kg^{-1}FM (Ozkan *et al*., 2007).

- Measurement of MDA in platelets might provide a measure of cellular dysfunction in an accessible sample.

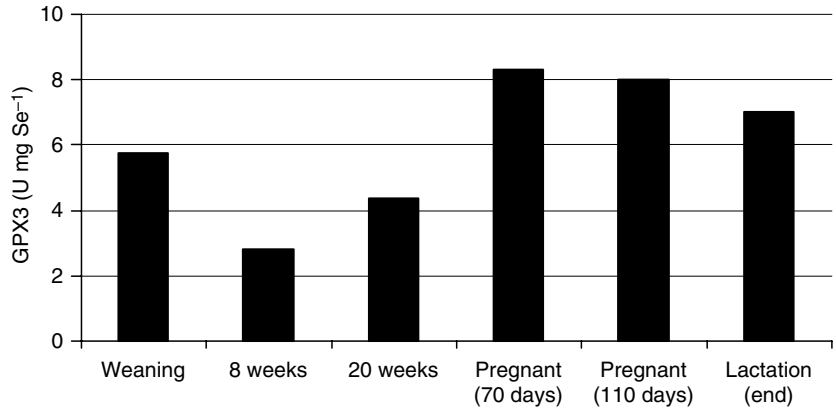

Fig. 15.12. Specific activity of serum glutathione peroxidase (GPX3) in pigs at different stages of development on diets with 0.06–0.08mg Se kg^{-1}DM. (Data from Kim and Mahan, 2001; Mahan and Peters, 2004)

Table 15.4. Mean (± standard error) blood selenium and erythrocyte glutathione peroxidase (GPX)1, plasma aspartate aminotransferase (AAT), lactate dehydrogenase (LDH) and creatine kinase (CK) activities in normal lambs and those with white muscle disease (WMD) on low-selenium hay plus oat diets, with and without selenium treatment, which gave only partial protection. (From Whanger *et al*., 1977)

Blood components or incidence	With Se		Without Se	
	Normal	WMD	Normal	WMD
WMD incidence (%)	–	44	–	56
CK (U μl^{-1})	0.1 ± 0.02[a]	3.4 ± 1.1[b]	0.1 ± 0.01[a]	5.0 ± 0.9[b]
LDH (U μl^{-1})	0.61 ± 0.07[a]	3.89 ± 1.10[b]	0.56 ± 0.06[a]	4.6 ± 1.04[b]
AAT (U ml^{-1})	53 ± 6[a]	748 ± 260[b]	62 ± 5[a]	635 ± 92[b]
GPX1 (U mg^{-1}Hb)	59 ± 7[a]	66 ± 5[a]	2.2 ± 0.5[b]	3.6 ± 1.0[b]
Blood selenium (mg l^{-1})	0.14 ± 0.03	0.15 ± 0.04	<0.02	<0.02

Hb, haemoglobin
Means within a row not sharing a common superscript letter are significantly different (P<0.01).

Indices of disorder

Plasma creatine kinase activities can be increased >100 times in WMD, due to leakage of intracellular enzyme through damaged muscle membranes. In subclinical cases, however, creatine kinase can decline markedly after the attainment of peak values (N.F. Suttle and D.W. Peter, unpublished data), cannot differentiate between lack of Se and lack of vitamin E as cause and cannot define the cause (Table 15.4). Assays of specific lactic dehydrogenase isoenzymes and troponin, released from cardiac myocytes, can differentiate damage to cardiac and skeletal muscle but not the specific disease (e.g. cardiac WMD or MHD). Raised plasma activities of enzymes found only in liver (e.g. glutamate dehydrogenase) can facilitate diagnosis of hepatosis dietetica (HD) but activities are raised in other hepatopathies (e.g. copper toxicity). Raised activity of the ubiquitous aspartate aminotransferase (AAT) cannot define site of damage and may not reflect severity of clinical symptoms in WMD (Oksanen, 1967). Furthermore, Se-responsiveness is not necessarily accompanied by raised AAT (Stevens *et al*., 1985).

Non-functional indices of selenium status

Blood, plasma and milk selenium in ruminants

In sheep, 'bent-stick' analysis of relationships with wool yield in individual grazing, pregnant Merino ewes indicated diagnostic limits of <0.89 (70 µg) and <0.51 µmol l^{-1} for blood and plasma Se, respectively (Langlands *et al*., 1991a). Corresponding threshold values were similar in weaned lambs (Whelan *et al*., 1994a). but much lower for barren ewes (<0.51 and <0.25 µmol Se l^{-1} for blood and plasma) (Langlands *et al*., 1991a). In New Zealand, likelihood and size of a positive growth response to Se supplementation in lambs increased exponentially as mean blood Se fell below 0.20 µmol l^{-1}, a relatively low threshold. Small but significant improvements in ewe fertility were obtained in pooled data from five large hill flocks with mean initial blood and plasma Se higher than all the above thresholds and subsequently rising in untreated ewes (Munoz *et al*., 2009).

- Responsiveness to Se will be more closely related to the proportion of a flock (or herd) whose Se status falls below the critical individual threshold for the most sensitive of recordable traits than mean values for the flock.

In grazing cattle, growth was rarely improved by Se treatment and the few herds that responded (three out of 21) were not distinguishable on the basis of mean blood Se, which was usually <0.25 µmol l^{-1} (Langlands *et al*., 1989). Dairy herds showed no reduction in milk-fat yield until mean blood Se fell below 0.15 µmol l^{-1} (Fraser *et al*., 1987; Tasker *et al*., 1987). However, in collated data from all trials, milk yield was improved by Se supplementation with blood Se >0.15 µmol l^{-1} (Wichtel, 1998). Interpretation of blood Se in lactating cows was affected by supplementing with SeMet as opposed to Se_i, which decreased GPX1 activity blood Se by 15% (from Knowles *et al*., 1999). Mean plasma Se was 0.15 µmol l^{-1} in calves from a herd with a low incidence of WMD

(10–14%) (Hidiroglou *et al.*, 1985) but plasma Se as low as 0.12 µmol l^{-1} was tolerated by grazing heifers (Wichtel *et al.*, 1996). Growing and lactating cattle at pasture in the Antipodes tolerate lower blood and plasma Se than sheep, as do deer (Mackintosh *et al.*, 1989). Marginal bands for blood and plasma Se in Table 15.5 were based on the above findings and apply to grazing situations where herbage SeMet is the major Se source.

- Higher marginal bands may be needed in intensively fed US herds, which yield twice as much milk fat as New Zealand herds and may face greater oxidant stress (Table 15.10).

A curvilinear relationship between milk Se and blood Se was reported in dairy cows and diagnostic limits for milk Se were extrapolated from that relationship (Grace *et al.*, 2001). However, the relationship changed when cows were given SeMet, which is preferentially transferred to milk (Knowles *et al.*, 1999).

- Blood, plasma and milk Se are flawed indices of Se status.
- Varied use of SeMet in compound feeds will affect conclusions drawn from bulk milk Se as an indicator of functional Se status in lactating cows.

Tissue selenium

Tissue Se is correlated with dietary Se but affected by Se source (Fig 15.7), but relationships to function and disease incidence have not been studied sufficiently to establish reliable diagnostic limits. Tissue Se increases with the proportion of dietary Se provided by SeMet but may decrease if the diet lacks protein. Tissue Se was low in pigs (Table 15.6) and foals (Caple *et al.*, 1978) with WMD. Tissue samples from pigs affected with MHD or HD were also low in Se (Table 15.6) (Van Vleet *et al.*, 1970; Simesen *et al.*, 1979). In a Canadian study, cardiac failure, myocardial necrosis and calcification in aborted calves were associated with low liver Se when compared with unaffected fetuses (mean 4.4–5.5 and 7.5 µmol kg^{-1}WM, respectively) (Orr and Blakley, 1997), but values were still high compared with marginal bands for neonates given in Table 15.5. Liver Se (0.07–0.4 µmol kg WM) was low in deer with WMD (Wilson and Grace, 2001).

Dietary Sources of Selenium

Provision of adequate dietary Se is complicated by wide variation in feed and forage Se, which are influenced by plant species (Table 15.7), season of sampling and soil Se status. The dominant form of

Table 15.5. Marginal limits[a] or bands[b] for non-functional indices of selenium deprivation in domestic livestock given diets adequate in vitamin E.

	Blood (µmol l^{-1})	Serum (µmol l^{-1})	Liver (µmol kg^{-1} FW)	Muscle (µmol kg^{-1} FW)	Diet (mg kg^{-1}DM)
Sheep, adult	**<0.50**	**<0.25**	**0.25**–0.45	**0.30**–0.40	**0.03**–0.05
Lamb or lambing	**<0.9**	**<0.5**	0.75–1.35	–	–
Cattle and deer, adult	**0.15**–0.25	**0.10**–0.12	**0.20**–0.30	**0.25**–0.30	**0.02**–0.04
Cattle, neonate	**0.30**–0.50	–	**0.80**–0.90	–	–
Pigs, sow	**1.00**–1.26	**0.90**–1.30	**0.8**–1.3	**0.63**–0.95	**0.05**–0.07
Pigs, piglet	–	**0.50**–0.71	–	–	–
Poultry, chick turkey poult	**0.20**–0.25	**0.90**–1.30	1.0–2.0	**0.80**–1.00	**0.06**–0.10
Horses, mature[c]	<2.3	**0.40**–0.70	–	–	**0.05**–0.08

Conversion factors: µmolar to elemental, × 79.0 (for µg); elemental to µmolar, × 12.665 (for µmol).
[a]Proximity of the mean for a population sample to the lower (**bold**) limit indicates increasing probability of benefits in health and production from selenium supplementation.
[b]The further an individual value falls below the 'bent stick' threshold or the higher the proportion of such values in a population sample, the greater the likelihood of selenium responsiveness.
[c]Derived from Calamari *et al*. (2009) and Montgomery *et al*., (2012).

Table 15.6. Selenium concentrations (μg kg^{-1} FW; mean ± standard deviation) in skeletal muscles, livers and hearts of pigs with three different selenium-responsive diseases compared with tissues from normal pigs (Pedersen and Simesen, 1977).

Se-responsive disease	Liver	Heart	Muscle
Nutritional muscular dystrophy	66 ± 17[ab]	38 ± 18[ab]	32 ± 16[b]
Hepatosis dietetica	68 ± 52	51 ± 41	–
Mulberry heart disease	141 ± 67	98 ± 44	–
Normal pigs	300 ± 100	164 ± 60	
	670 ± 53[ab]	210 ± 20[ab]	104 ± 12[ab]

[a]Data for normal pigs will reflect selenium intake; those for liver and heart from Lindberg (1968) are relatively high.
[b]Data converted from a dry weight basis, using a conversion factor of 0.2 for muscle and heart, 0.3 for liver.

Table 15.7. Variation in selenium concentrations in forages sampled throughout the year on a Louisiana farm (Kappel *et al*., 1984).

Feed (no. of samples)	Mean Se (μg kg^{-1}DM)	Standard deviation	Range
Bahia grass (15)	61	20	27–98
Bermuda grass (5)	173	158	71–448
Ryegrass/oats (9)	72	19	45–105
Corn silage (10)	59	25	27–100
Sorghum silage (6)	57	12	39–74
Alfalfa hay (9)[a]	295	197	51–954

[a]Some of the alfalfa hays were bought in.

Se in ordinary feeds and forages is protein-bound SeMet, which accounts for 55–65% of cereal Se and more in protein concentrates: there are smaller proportions of SeCys (5–15%) and other seleno-compounds (Whanger, 2002). In the UK, fresh grass contained 0.05 ± 0.02 (s. d., n = 71) mg Se kg^{-1}DM (MAFF, 1990) and a similar distribution has been reported in New Zealand (Knowles and Grace, 2014). Legumes tend to contain less Se than grasses but the difference diminishes as soil Se declines (Minson, 1990) and lucerne is an exception to the rule (Table 15.7). The importance of species is strikingly illustrated by 'accumulator' plants that survive in seleniferous areas where other species cannot, with some accumulating Se in macro- rather than micro-quantities (p. 397). Grain and seed Se varies widely between and within countries, due largely to geochemical factors (p. 376). Average values for wheat grown in UK, USA and Canada were 0.03, 0.37–0.46 and 0.76mg Se kg^{-1}DM, respectively (Adams *et al*., 2002). Differences between grains are relatively small, but ranked in the order wheat > rice > maize > barley > oats (Lyons *et al*., 2005). Linseed meal was generally richer in Se than extracted soybean meal (SBM) and rapeseed meal (0.82 ± 0.05, 0.30 ± 0.19 and 0.14 ± 0.09mg Se kg^{-1}DM, respectively) (MAFF, 1990). Lupin seed meals can be extremely low in Se (<0.02) (Moir and Masters, 1979). Cereal by-products such as maize gluten feed, rice-bran meal and distillers dark grains were good sources (0.24 ± 0.09, 0.16 ± 0.06 and 0.18 ± 0.18mg Se kg^{-1}DM, respectively) (MAFF, 1990). Mean values for large, geologically diverse countries like the USA give little indication of probable Se intakes on a particular farm. In large-scale trials, values in corn and soybean meal ranged ten-fold (0.026–0.283 mg Se/kg and 0.086–0.798 mg Se/kg, respectively; Mahan *et al*., 2014). Protein concentrates of marine origin are rich in Se (1.9–6.2mg kg^{-1}DM: Miltimore *et al*., 1975; Scott and Thompson, 1971). Animal protein concentrates of non-marine origin contain less Se but vary greatly (0.11–1.14mg Se kg^{-1}DM) (Moir and Masters, 1979). Two products, blood and feather meals, used in the UK, had consistently high values (0.6 ± 0.08 and 0.76 ± 0.06mg Se kg^{-1}DM, respectively) (MAFF, 1990).

Colostrum and milk

As with other minerals (see Table 2.4), colostrums are much richer in Se than main milks: respective mean values for unsupplemented groups were 75 and 40 µg Se l^{-1} for sows (Mahan and Peters, 2004), 70 and 40 µg Se l–1 for cows (Awadeh *et al*., 1998), 81 and 23 µg Se l^{-1} for ewes (Meneses *et al*., 1994). Colostral and milk Se can be doubled by parenteral supplementation of the ewe at lambing but the effect can be short-lived (14 days) (Meneses *et al*., 1994; Rock *et al*., 2001). Similarity of milk Se in different species masks the fact that, when expressed on a DM basis, they are higher in cows than in sows and ewes (approximately 0.32 and 0.2mg Se kg^{-1}DM, respectively).

Nutritional Value of Dietary Sources

Availability in non- and pre-ruminants

Selenium is well absorbed from feeds by all livestock species, though less efficiently than from inorganic sources (Fig. 15.13). In poultry, RBV was lower in 36 of 42 feeds and foods, when evaluated by functional (e.g blood GPX, egg production and hatchability) than non-functional tests: variability ranged widely about a mean of 0.62 with values as low as 0.09 recorded. In an early balance study in young pigs, given a corn/SBM diet with either selenite or seleniferous corn providing an extra 0.2mg Se kg^{-1}DM, AA_{Se} was 0.72 and 0.64, respectively, and differed significantly (Groce *et al*., 1973). The relatively low functional RBV of feed Se is attributable to the predominance of SeMet in feeds, SeMet also having a low value due to its unique metabolism (Fig. 15.6). Availability is largely unaffected by Se intake, rats apparently absorbing (AA_{Se}) 90% of ingested Se_i over a wide range in dietary Se (Fig. 15.9). Mice were able to regulate faecal endogenous loss (FEL_{Se}) when switched from a diet virtually devoid of Se from weaning to maturity to one containing up to 3mg Se kg^{-1}DM (Pedrosa *et al*., 2012) but such extremes have little or no practical relevance.

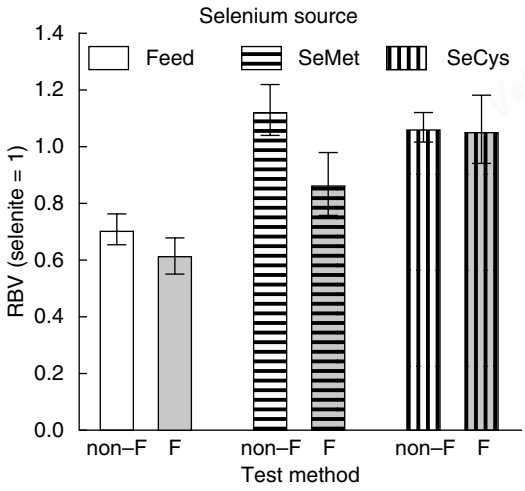

Fig. 15.13. Relative biological value (RBV) of organic selenium (Se) in feeds for poultry is low when compared with inorganic sources and not significantly affected by whether the test involves functional attributes (F – e.g. selenoenzyme activity, growth or health) or not (non-F – usually tissue Se): test method affected the RBV of selenomethionine (SeMet) but not selenocystine (SeCys), SeMet having a similar low value to feeds when assessed by function. (Data from Henry and Ammerman, 1995)

- There is no regulation of A_{Se} over the commonly encountered range of dietary Se.

The importance of functional tests of nutritive value for SeMet has long been known: SeMet was poorly utilized for GPX synthesis and resistance to exudative diathesis but not when measured as Se accretion (Fig. 15.15). Prior to weaning, the RBV of Se in milk and milk replacers is similar to that of dry feeds, though lowered by large Se_i supplements in calves (5-10mg Se kg^{-1}DM; Jenkins and Hidiroglu, 1986). A relative A_{Se} of 0.82 for cow's milk compared with selenite (1.0) was reported in rats (Mutanen *et al*., 1986).

Functional tests present their own problems of interpretation because requirements for different functions vary. The dose–response profile of each function must be known for valid assessments to be made. For example, plateaux for GPX3 in plasma and GPX1 and 4 in thyroid and pituitary glands were reached with 0.2mg Se kg^{-1}DM, but >0.3mg Se kg^{-1}DM was required to reach plateaux in GPX1 and 4 in liver, heart and lung tissue of young pigs (Lei *et al*., 1998). The majority of availability studies involved only two levels of Se supplementation, the higher being the maximum level permitted in the USA (0.3mg Se kg^{-1}DM), insufficient to accurately gauge functional availability (e.g. Mahan and Peters, 2004). Furthermore, downregulation of plasma GPX3 and both erythrocyte and liver GPX1 in pregnancy and lactation, reduced plateau

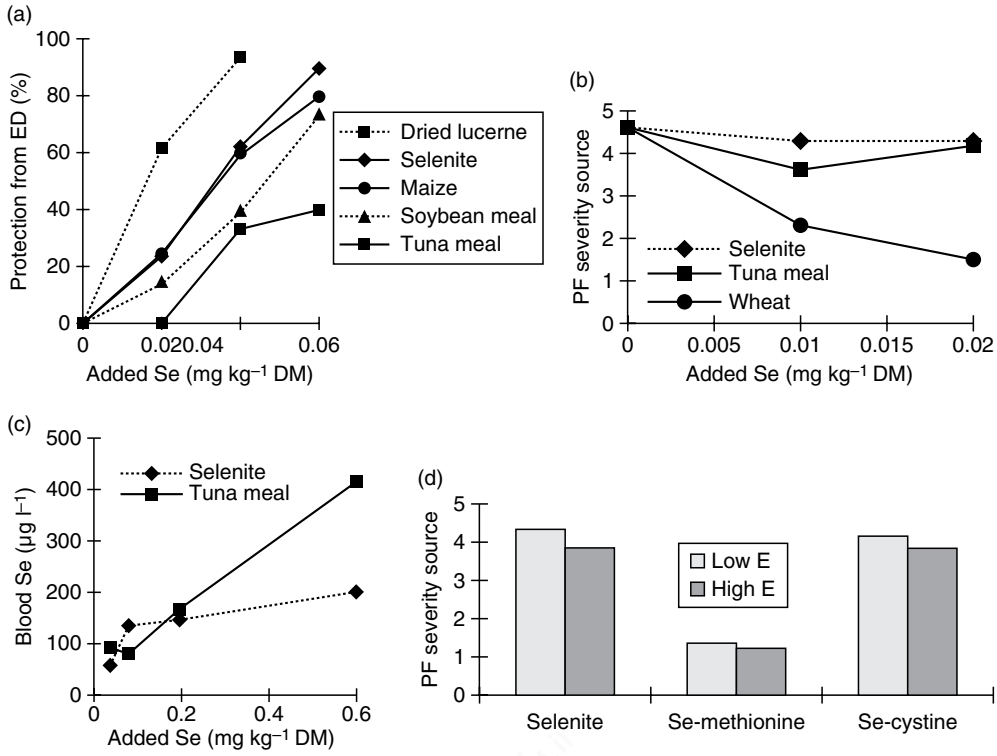

Fig. 15.14. Nutritive value of different selenium sources for chicks is determined by test system as well as source (data from Cantor *et al*., 1975a, b). (a) Protection against exudative diathesis (ED) was high from lucerne meal and low from fish meal, with selenite intermediate. (b) Protection against pancreatic fibrosis (PF) was equally poor with selenite and fish meal, but good with a cereal source. (c) The ability to raise blood selenium was higher for fish meal than for selenite. (d) Protection against PF was good with selenomethionine, the major source of selenium in cereals, and may explain the good performance of wheat in (b).

dietary Se from 0.075–0.1 to 0.05–0.075mg Se kg^{-1}DM in rats (Sunde *et al*., 2005), lowering the optimal range for dietary Se. Tests that involve protection from SeRD (e.g. ED incidence) of necessity involve the use of special diets, low in Se.

Availability in ruminants

With development of a functioning rumen come added complications. Microbes reduce selenite with reduced glutathione to yield selenodiglutathione, which is further reduced to glutathioselenol and finally hydrogen selenide for incorporation into Se- Cys and SeMet (Turner *et al*., 1998). Differential Se uptake of Se_i and Se_o by rumen microbes and the need for subsequent release of microbial Se at absorption sites may partly explain the relatively low AA_{Se} (0.30–0.59; Table 15.8) in ruminant diets. Uptake of Se by rumen microbes *in vitro* was four times greater from SeMet than Se_i but wrongly claimed to explain the higher Se status found in ewes given SeMet (Galbraith *et al*., 2016). It is hardly surprising that microbes supplied with ready-made SeMet will have retained more Se after 24 hours than those given Se_i, particularly when added at a supra-nutritional level (1.8mg kg^{-1}DM).

- Uptake of Se by cultured rumen microbes does not indicate value to the host.

Ingested Se leaves the rumen mostly in the bacterial fraction (Koenig *et al*., 1997), which contains Se that is marginally less available than Se_i to mice, when assessed by tissue accretion (Serra *et al*., 1997). Some feed seleno-amino acids escape undegraded from the rumen. In sheep given a pelleted diet, ^{75}Se-labelled selenite and ^{75}SeMet were

Table 15.8. Apparent absorption of selenium (AA_{Se}) in pre-ruminant calves and weaned sheep suggests that AA_{Se} decreases with development of a rumen but increases as dietary selenium concentration and roughage content increases.

Diet	Se source	Animal	Diet Se (mg kg^{-1} DM)	AA_{se}	Reference
Milk replacer	Selenite	Calf	10.0	0.61	Jenkins and Hidiroglou (1986)
	Inherent	Calf	0.20	0.90	Jenkins and Hidiroglou (1986)
Roughage	Inherent	Sheep	0.37	0.42	Koenig *et al*. (1997)
	Inherent	Sheep	0.16	0.50	Langlands *et al*. (1986) expt. 3[a]
	Inherent	Sheep	0.15	0.41	Krishnamurti *et al*. (1989)
	Inherent	Sheep	0.10	0.45	Krishnamurti *et al*. (1997)
	Inherent	Sheep	0.01	0.59	Krishnamurti *et al*. (1997)
Concentrate	Inherent	Sheep	0.27	0.53	Koenig *et al*. (1997)
	Mixed	Sheep	0.24	0.67	Langlands *et al*. (1986) expt. 1
	Inherent	Sheep	0.06	0.63	Alfaro *et al*. (1987)

[a]Large effects of previous selenium intake and selenium status on AA_{Se} here suggest possible errors in making comparisons between studies.

equally well absorbed (Ehlig *et al*., 1967) but less SeMet may be retained when given intravenously (Langlands *et al*., 1986). When a roughage and a concentrate diet were compared (Koenig *et al*., 1997), AA_{75Se} was lower from SeMet with the roughage diet. Low and even negative AA_{Se} values were obtained when feeds low in Se were fed to sheep of high initial Se status (Langlands *et al*., 1986). In lambs given a concentrate diet, relatively low in Se and S (0.07 and 1.6g kg^{-1}DM, respectively), ^{75}SeMet had a high AA_{Se} of 0.8 (White *et al*., 1998). Additions of sulfate lowered AA_{Se} in an Se_i -supplemented corn silage diet from 0.39 to 0.29 in lactating cows (Ivancic and Weiss, 2001) and reduced uptake of Se_i by rumen microbes in sheep (van Ryssen *et al*., 1998): Se_i may simply be out-competed by S for incorporation into microbial methionine. Tracers for dietary Se are likely to give false estimates of fluxes from gut to body, especially in ruminants, because the orally admimistered isotope is converted to organic forms by rumen microbes and can enter pools other than the one labelled by parenteral injection, invalidating the principle on which isotope dilution techniques rest (p. 27).[vi]

- To return to the opening myths (p. 371), low functional availability of SeMet, the predominant component of feed Se, is part of the problem rather than a panacea.

Selenium Requirements

Requirements for optimum growth shortly after birth were exceedingly low (<0.007mg kg^{-1}DM) in all five species studied, including lambs, chicks and turkeys (Sunde *et al*., 2016) but may have been influenced by slow growth rates on purified, torula yeast diets of high antioxidant content.

Weaned herbivores

Relationships between field and experimental disorder and dietary Se have been used to define requirements for sheep and cattle (ARC, 1980) but yield a three-fold range, from 0.02 to 0.06mg kg^{-1}DM.[vii] Factorial estimates such as those of the previous edition (Suttle, 2010) narrow the range but are misleading because they cannot, at the present state of knowledge, incorporate weighting for the factors known to influence need (Table 15.2). They can reassure users that factors thought to influence required dietary Se, such as growth rate and milk yield, are relatively unimportant but high digestibility increases the required concentration (Underwood and Suttle, 1999). Data for horses indicated a similar range in Se requirement. Mares grazing pastures containing 0.01–0.07mg Se kg^{-1}DM in New Zealand were no less healthy than reported in cohorts given supplementary Se (Wichtel *et al*., 1998) but vitamin E status would have been high. Stabled geldings remained healthy when dietary Se was reduced from 0.26 to 0.06mg kg^{-1}DM (Shellow *et al*., 1985). Erythrocyte Se, plasma Se and blood GPX1 activity all slowly declined in adult horses given hay and oats, containing 0.045mg Se kg^{-1}DM but gained no benefit from added Se (Montgomery *et al*., 2012).

Pigs

Piglets from sows on diets adequate in vitamin E (100 IU) and containing 0.06mg Se kg^{-1}DM had satisfactory growth rates, feed intakes and feed conversion efficiencies (Glienke and Ewan, 1977). That requirement confirmed by Mahan *et al.* (1999) but associated with high perinatal mortality elsewhere (Van Vleet *et al.*, 1973). Comparisons of responses of serum GPX3 activity to graded Se supplements at growing and finishing stages suggested that 30% less Se was required at the later stage (Mahan *et al.*, 1999). With purified diets for gilts, 0.03–0.05 mg Se (with 22 IU vitamin E) kg^{-1}DM was adequate for the first pregnancy (Piatkowski *et al.*, 1979) but animals can become progressively depleted. In sows, GPX1 and GPX3 activities in blood and plasma had fallen by the fourth parity on a diet containing 0.06–0.08mg Se kg^{-1}DM (Mahan and Peters, 2004). Supplementary Se improved litter size but not total weaned litter weight, suggesting that Se supply was marginal. In boars, 0.06mg Se kg^{-1}DM was insufficient for optimum male fertility (Marin-Guzman *et al.*, 1997): requirements are raised if the diet is high in PUFA (Nolan *et al.*, 1995). Thus, requirements for Se are generally higher in pigs (around 0.05mg kg^{-1}DM) than herbivores but probably subject to similar dietary and environmental influences.

Poultry

Influences of vitamin E and Se source on requirements were demonstrated in early studies.[viii] As much as 0.28mg Se kg^{-1}DM was required to prevent gizzard and heart myopathies in turkey poults on diets marginal in vitamin E *and* SAA (Scott *et al.*, 1967). In laying hens, 0.05mg Se kg^{-1}DM maintained production without added vitamin E or antioxidants but hatchability was suboptimal (Latshaw *et al.*, 1977). Hens given a diet containing 0.085mg Se kg^{-1}DM preferred an Se-supplemented diet, when given a choice, until they were repleted (Zuberbuehler *et al.*, 2006). Requirements were much higher for maximal liver GPX1 activity than growth in turkey poults for the first month of life, with a diet adequate in vitamin E and SAA (Hadley and Sunde, 1997). A recent re-evaluation concluded that minimum requirements of broiler chicks for growth, liver and gizzard GPX and pancreatic GPX activities were 0.025, 0.15 and 0.2mg Se kg^{-1}DM, respectively, when added as Se_i to a torula-yeast diet containing hardly any Se (Li and Sunde, 2016).

Genomic or antioxidant requirements?

Hierarchies of requirements for optimal SeP activity (Sunde *et al.*, 2005) and expression have been advocated (Sunde *et al.*, 2016). Each GPX has a different profile, with plasma GPX3 and liver GPX1 and 4 reaching plateau activity at lower dietary Se in chick than turkeys but lower dietary Se than laboratory animals. A similar genomic approach has been taken to the definition of Mn requirements for broilers (p. 358). The genomic approach yields far higher requirements than those for optimal growth in all species that vary between markers, even in a stress-free laboratory environment. Another hierarchy, based on the degree of oxidant stress imposed by a given combination of dietary, environmental and physiological circumstances is conceptualized in Table 15.9. In this author's view,

- nutritional requirements should be based on need for optimal productivity rather than maximal synthesis of specific metalloproteins;
- whatever functional criterion is used, requirements will be roughly one third higher for feeds and SeMet or SeY supplements than those established for Se_i, because availability is that much lower.

Requirements should be met for all stages of production in all species by diets containing 0.1 mg supplementary Se_i kg^{-1}DM and adequate vitamin E. Only the exercising racehorse may need more dietary Se. No upward adjustment for Se source is required because the least functionally available source, SeMet, is the predominant source in natural feeds, the basis for pragmatic estimates in Table 15.9. Genomic requirements for SeP expression could be of practical use if based on the most functionally demanding, cross-referenced to indices of dysfunction (p. 383 et seq) and/or coincided with threshold increases in urinary Se excretion (Fig. 15.9).

Prevention and Control of Insufficiency

Soil and foliar treatments

Selenite solutions can be applied to soil or foliage but fertilizer Se is poorly absorbed by most plants, especially from acid soils, and residual effects can be small (Underwood and Suttle, 1999). A less

Table 15.9. A suggested hierarchy of selenium requirements and critical blood glutathione peroxidase activities in sheep and cattle under field circumstances that provide different degrees of oxidant stress: such hierarchies will also influence needs of non-ruminants.

Vitamin E	PUFA	Temperature stress	Dietary Se (mg kg^{-1}DM)	Blood GPX1 (Ug^{-1} Hb)	Field circumstances
High	Low	Low	0.02–0.03*	20–30*	Mid-season grass pasture, vitamin E-supplemented
	Medium	Low	0.03–0.04	30–40	Spring grass, legume sward
Low	Low	Low	0.04–0.06	40–60	Brown sward, poor hay, grain fed turkey poults
	High	Low	0.1–0.2	80–100	PUFA-supplemented diets for production of 'healthy' foods
High	Medium	High	0.04–0.06	40–60	Outdoor spring calving, lambing or farrowing; pigs/poultry in hot weather
Medium	Medium	Medium	0.06–0.08	60–80	High-yielding cow with mastitis

GPX, glutathione peroxidase; PUFA, polyunsaturated fatty acids
*The 'high Vit E' class probably applies to pigs and chickens on commercial rations.

soluble form, barium selenite ($BaSeO_4$), at 10g Se ha^{-1} in prill (granular) form, raised herbage Se for three years but improvements in Se status of grazing sheep took six weeks to materialize (Whelan *et al*., 1994b). Admixture with the more soluble Na_2SeO_4 speeds the response and a range of products became available (White and Broadley, 2009). A single application raised blood, serum and milk Se in cattle for at least 12 months (Grace and West, 2006). Grain Se can also be increased by soil or foliar treatment, the latter resulting in seven- and ninefold increases in rice, when applied at 29 g Se ha^{-1} as selenite and selenate, respectively (Chen *et al*., 2002; cited by White and Broadley, 2009).

Dietary supplementation

Selenium intakes can be increased by importing grains and forages from high-Se areas but requires systematic analyses of ingredients. Addition of 0.15mg Se kg^{-1}DM to compound feeds is a long-established practice in New Zealand (Andrews *et al*., 1968). Supplementation to 0.3mg Se kg^{-1}DM is permitted in the USA and was highly effective in raising Se status of gilts and offspring (Mahan and Kim, 1996). Trace mineralized salt, fortified with 26–30 mg Se kg^{-1} Na_2SeO_3 and offered freely to ewes and lambs, decreased incidence of WMD (or plasma creatine kinase) without raising tissue Se above levels found naturally (Underwood and Suttle, 1999) but may not protect all individuals. A compressed, selenized salt block, containing 11.8 mg Se kg^{-1}, raised mean blood and plasma Se in ewes and lambs, but left 7–33% of ewes unprotected (Langlands *et al*., 1990). The problem of individual variations in block consumption cannot be overcome by adding more Se (Money *et al*., 1986), although average block consumption may be increased (Langlands *et al*., 1990).

- Selenite supplements have never failed to treat SeRD, including poultry diseases such as ED (Noguchi *et al*., 1973; Cantor *et al*., 1975b) and WMD (Cantor and Tarino, 1982; Cantor *et al*., 1982), a fact neglected by those advocating 'organic' selenium supplements.

Given the uncertainty and small scale of many production responses to Se, selenized mineral supplements given in loose or block form can be a cost-effective option.

Parenteral and oral dosage

Subcutaneous injections or oral drenching, usually with Na_2SeO_3 providing 0.1 mg Se kg^{-1}LW at one- to three-monthly intervals, have often prevented SeRD in grazing livestock (e.g. Meneses *et al*., 1994). Animals can be dosed when gathered for other purposes, such as anthelmintic dosing or vaccination, and Se can be combined with other drugs in a single product (e.g. Field *et al*., 1988). Frequency of anthelmintic dosing may exceed that necessary for Se but need not result in excessive edible tissue Se (e.g. Cooper *et al*., 1989). Dosage

must occur at responsive times (i.e. at mating, late pregnancy and prior to weaning) and optimum oral dose may be closer to 0.2 than 0.1mg kg^{-1}LW (Langlands *et al*., 1990). Pig diseases such as MHD and HD were prevented by injecting 0.06 mg Se kg^{-1}LW, together with vitamin E, into sows and baby pigs (Van Vleet *et al*., 1973). Parenteral administration of $BaSeO_4$ at a high but safe dose of 1 mg Se kg^{-1}LW was effective in lambs (Zachara *et al*., 1993), ewes (Munoz *et al*., 2009), pregnant cows (Contreras *et al*., 2005), horses (Wichtel *et al*., 1998) and dairy goats (Sanchez *et al*., 2007). In meat animals, recommended sites of injection and withdrawal periods must be observed to avoid residue problems (Archer and Judson, 1994). A micro-encapsulated product providing 60 mg Se, together with vitamin B_{12} maintained Se status of ewes and lambs on pastures low in Se for >300 days, following a pre-mating injection (see Grace *et al*., 2006).

Slow-release oral supplements

In early Australian studies, heavy ruminal pellets, consisting of 95% finely divided iron and 5% elemental Se, released sufficient Se to maintain adequate blood values for several months in sheep and years in beef cattle (Underwood and Suttle, 1999). The pellets could not be given to young lambs and sometimes provided limited and variable individual protection to older sheep (e.g. Whelan *et al*., 1994b) but formulations were improved (Donald *et al*., 1994c). In beef cattle, 7–56% of administered pellets were not retained in three out of 21 herds in one study (Langlands *et al*., 1989). Regurgitation occurred within minutes of dosing and often occurred again upon re-dosing. Such failures were neither observed nor suspected in dairy heifers, given two 30 g pellets containing 10% elemental Se, and growth was improved in a single-herd study (Wichtel *et al*., 1994). Regurgitation was not a problem when dosing sheep (Langlands *et al*., 1990). Controlled-release capsules delivering Se and anthelmintic simultaneously, protected lambs for at least 180 days (Grace *et al*., 1994). Sustained protection was also provided by soluble glass boluses in cattle (Koh and Judson, 1987), goats (Zervas, 1988) and sheep (Millar and Meads, 1988; Kendall *et al*., 2001).

- Slow-release devices are so effective that they should not be given with other sources of supplementary Se and dosing may not need to be repeated every year.

Organic Supplements

It should be noted from the outset that SeRD arise in the field on feeds and forages that contain SeMet as the major Se source and are successfully treated with Se_i. Three forms of Se_o are in commercial use as supplements for livestock: SeY, SeMet and Se-metalosates (mixtures of Se_i and amino acids). SeY and SeMet have similar properties because SeMet provides most of the Se in industrially cultured yeast. Functional tests show SeMet to be less available and more variable than Se_i (Fig. 15.13) and unlikely to provide an advantage over Se_i unless the latter is oversupplied. Non-functional criteria, such as blood, plasma, milk and tissue Se are higher after supplementation with SeMet and SeY than with Se_i but few health, production and enzymatic activity advantages have been reported (Mahan *et al*., 2014; Markovic *et al*., 2018). Supplementing the sow with SeY rather than Se_i increased Se but not GPX3 activities in sow and piglet serum at weaning (Fig. 15.15): since Se intakes were 20% higher in piglets nursed by sows given SeY, low functional availability of SeMet-enriched milk is indicated and in line with results for SeMet in solid diets (Fig. 15.13). Highly efficient conversion of milk protein to muscle protein in neonates may divert more Se from SeMet than Se_i from SeP synthesis to the muscle protein 'sink'.

- Accretion of tissue Se in the newborn and secretion in milk overestimate physiological availability of Se to the supplemented dam.

More efficient transfer of SeMet than inorganic Se across placental, mammary and oviduct barriers will only benefit offspring without corresponding increases in functional SeP status or health-related indices (Suttle, 2010).[ix] Source of Se should be less influential on health and performance in ruminants than non-ruminants and so it has proved. Inorganic Se is partially incorporated into selenoamino acids during anaerobic fermentation in the rumen, just as it is during yeast culture. The capacity of three rumen microbial sources to raise tissue GPX activities in broiler chicks when compared with Se_i (Dalia *et al*.,

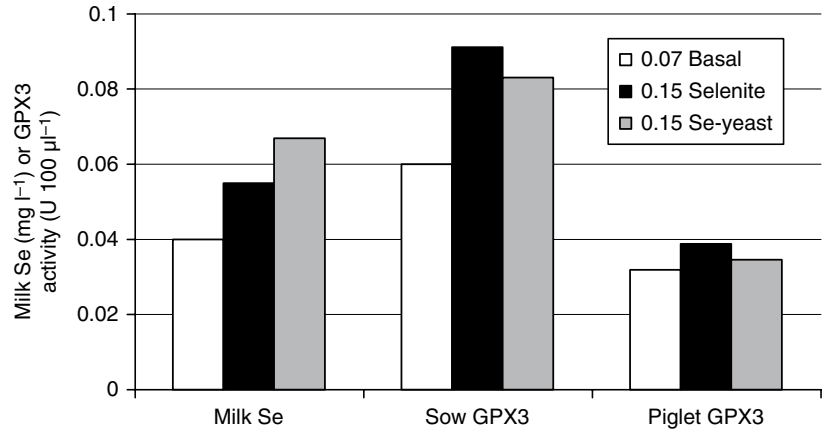

Fig. 15.15. Supplementing sows with 0.15mg Se kg^{-1}DM as selenium yeast (Se-yeast) rather than selenite gives greater increases in milk Se but smaller increases in serum glutathione peroxidase (GPX3) activity in sows and weaned offspring. (Data from Mahan and Peters, 2004)

2017) indicated superior availability. A hydoxy-analogue of SeMet improved plasma GPX as well as plasma Se but did not improve milk yield or lower plasma MDA when compared with selenite (Sun *et al*., 2017). Possible benefits from SeY supplementation to the consumer of milk, eggs and other edible products are discussed in Chapter 20. Effects of Se source on flock productivity with ewes given weekly drenches, equivalent to or above legal dietary limits, have no practical relevance (Stewart *et al*., 2012). Supplementation with 0.3mg Se kg^{-1}DM as SeY was no more effective in raising GPX activity than Se_i in horses and no health benefits were evident (Montgomery *et al*., 2012). The 'disadvantage' for SeMet is of the same order as the 'advantage' over Se_i in terms of relative transfer efficiencies of SeY and Se_i into cows' milk methionine (+10–20%) (Givens *et al*., 2004). Enhanced transfer of Se from SeMet to the newborn and mother's milk may provide Se of relatively low value.

Selenium Toxicity

Selenium is the most toxic essential trace element and toxicoses arise naturally and from careless administration of Se supplements (O'Toole and Raisbeck, 1995). Experimental stations (i.e. relatively well-informed establishments) continue to add Se to pig rations at the maximum permitted concentration (0.3mg Se kg^{-1}DM), even in seleniferous areas (Mahan *et al*., 2005, 2014).

Clinical signs

Lengthy exposure to seleniferous pasture can cause lack of vitality, stiffness and lameness in cattle due partly to sore and sloughing hooves and hair loss. Affected animals become unwilling to move and die from thirst and starvation in what became known as 'Alkali Disease' (Rosenfeld and Beath, 1964; O'Toole and Raisbeck, 1995). The chronic selenosis was reproduced in experiments but early lesions were confined to the integument. Lesions in the hoof were epidermal, tubules in the stratum medium becoming replaced by islands of parakeratotic cellular debris and the germinal epithelium becoming disorganized (O'Toole and Raisbeck, 1995). These features distinguish selenosis from laminitis, a common disorder of cattle and horses in which the lesions are dermal. Tail hair loss was associated with dyskeratosis and atrophic hair follicles. Calves and foals in seleniferous areas could be born with or develop deformed hooves. Animals may also collapse and die suddenly in a geographically and epidemiologically related disorder, 'Blind Staggers' (Rosenfeld and Beath, 1964; O'Toole and Raisbeck, 1995).[x] 'Blind Staggers' is an acute selenosis. Growing pigs exposed to high intakes of Se can develop similar hoof lesions. If Se intake is sufficiently high, pigs become anorexic and central nervous system lesions predominate, taking the form of bilateral malacia of grey matter in the spinal cord (Goehring *et al*., 1984b). Exposure to 10mg Se_i kg^{-1}DM in a corn/SBM

diet caused alopecia and hoof lesions within 12 weeks (Kim and Mahan, 2001). Similar exposure had reduced conception rate, impaired embryonic development and increased piglet mortality and morbidity (Wahlstrom and Olson, 1959). Growing chicks eat less food and grow slowly when given seleniferous diets. Egg production is reduced in laying hens and hatchability is particularly affected in breeder hens (Ort and Latshaw, 1978). A proportion of fertile eggs from hens on high-Se diets produced grossly deformed embryos, with missing eyes and beaks and distorted wings and feet.

Natural occurrence

Seleniferous areas are found in many countries, including the Great Plains of the USA, Canada and Russia and also parts of China and Israel, due to weathering of Se-rich rocks (Fordyce, 2005), and on shale outcrops in Ireland (McLaughlin *et al*., 1989). Risk can be broadly predicted by geochemical mapping (Combs, 2001) but vegetation also plays a part (Rosenfeld and Beath, 1964; Fordyce, 2005). Plant species can be divided into three classes, high (>1g), intermediate (0.5–1.0 g) and low in Se (< 0.2mg kg^{-1}DM).[xi] Accumulator plants play a dual role, absorbing Se from forms that are relatively unavailable to other species and returning Se to the soil in more widely available organic forms. Major forms of Se in accumulator plants are the soluble organic compounds, methylselenocysteine and selenocystathionine (Fordyce, 2005), while SeMet predominates in normal range species (Whanger, 2002). Accumulation of Se is favoured by low rainfall and implicated in outbreaks of selenosis in India (Underwood and Suttle, 1999). Ingestion of seleniferous soil may contribute to the Se burden, but has not been specifically investigated. Incidence and severity of hoof lesions is influenced by quality and quantity of diet and the related rate of hoof growth.

Physiochemical Abnormalities in Chronic Selenosis

Biochemical events follow the general scheme outlined in Fig. 3.7 (p. 51). During an initial accretion phase, tissue Se rises steadily over weeks or months depending on rate, source and continuity of Se intake. Injected Se_i accumulated principally in liver and was extensively secreted via bile (Archer and Judson, 1994) but losses of FEL_{Se} were twice as high when the body Se pool was labelled with ^{75}SeMet than with $^{75}Se_2O_3$ in sheep (Langlands *et al*., 1986). In Se-loaded pigs, biliary Se secretion increased as dietary Se increased until excretion in urine and faeces keep pace with absorption, irrespective of dietary source (selenite or SeY) (Kim and Mahan, 2001). Saturation may be reached with 20–30mg Se kg^{-1}DM in liver, kidney, hair/fleece and hooves of clinically affected animals but healthy animals can attain higher values during chronic exposure (Davis *et al*., 2006). At toxic selenium intakes, the relationship between blood Se and GPX1 activity breaks down. Blood Se continues to increase, particularly with Se_o sources (Kim and Mahan, 2001), while GPX1 and hair Se values plateau (Fig. 15.16) (Goehring *et al*., 1984a; Ullrey, 1987; Kim and Mahan, 2001). Where exposure has been discontinuous, separating hair or hoof into proximal, medial and distal portions may give a better measure of historic exposure than composite samples (J. Small and N.F. Suttle, unpublished data). Milk Se and egg Se are particularly sensitive to high Se intake, values ranging from 0.16 to 1.27 mg Se l^{-1} being reported for cow's milk from seleniferous areas in the USA (Rosenfeld and Beath, 1964). Egg Se increased several-fold, particularly in the egg white, when 8–10mg Se_i kg^{-1}DM was added to hens' diets (Moxon and Poley, 1938; Arnold *et al*., 1973).

The mechanisms that become dysfunctional in selenosis remain unknown and there are no specific biochemical changes that indicate the onset of dysfunction. Increased thiol oxidation, reduction–oxidation cycling (Stewart *et al*., 1999) and lipid peroxidation (Kaur *et al*., 2003) may occur. Sulfur

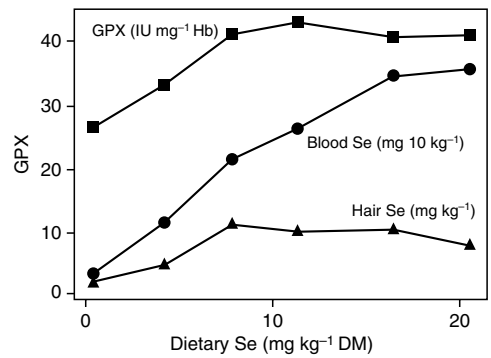

Fig. 15.16. Selenium concentrations in the hair and blood and glutathione peroxidase (GPX) activity in the blood of pigs exposed to excess Se via the diet. (Data from Goehring *et al*., 1984a)

may be replaced by Se, in sulfide bonds, including the disulphide bonds that give strength to keratin (NRC, 2005). Indices of tissue damage such as plasma AAT rise little and late (e.g. Kim and Mahan, 2001). In beef cows exposed during pregnancy, mitogenic responses of lymphocytes *in vitro* were diminished in the absence of clinical abnormalities (Yaeger *et al*., 1998). Genomic markers hold little promise for characterizing the development of selenosis (Sunde *et al*., 2016). Ewe lambs exposed to selenate or seleniferous wheat (3–15mg Se kg^{-1}DM) exhibited proliferation of jejunal mucosa and increased liver weight and protein content (Neville *et al*., 2008).

Selenium Tolerance in Non-ruminants

Tolerance in all species depends on source, duration and continuity of exposure, the criteria of tolerance, animal genotype and interactions between these factors. Elemental Se is insoluble, poorly absorbed and well tolerated. Selenides are less toxic than soluble selenites or selenates, regardless of species. Selenium occurs in feeds and forages largely in organic forms, such as SeMet, which can be diverted into tissue protein pools (Fig. 15.6), where it is generally less toxic, despite raising tissue Se more than equivalent intakes of Se_i (e.g. in poultry; Scott and Thompson, 1971): this means that:

- Selenosis cannot be diagnosed solely on the basis of tissue Se and is better interpreted using marginal bands rather than a single threshold.

High tolerances have been reported in pigs given seleniferous grain (Goehring *et al*., 1984a, b) or SeY (Kim and Mahan, 2001). With a corn/SBM diet, 8mg Se kg^{-1}DM as Se_i impaired appetite and growth during five weeks' exposure (Goehring *et al*., 1984a) but was harmless when given as seleniferous wheat and oats (Goehring *et al*., 1984b). In older pigs, hoof lesions developed with 15 mg Se as SeY compared to 5–10mg kg^{-1}DM as Se_i (Kim and Mahan, 2001). Tissue and erythrocyte Se were higher with SeY but accumulated in less toxic forms. The contrast in accretion from Se_i and Se_o varies widely between organs, being most marked in muscle and least in kidney in young growing pigs (Goehring *et al*., 1984a, b; Kim and Mahan, 2001), steers (Lawler *et al*., 2004) and mature sheep (Davis *et al*., 2006). Selenium accretion probably reflects different rates of methionine accretion in tissues. Raising dietary Se to the current legal limit in Europe, 0.5mg kg^{-1}DM, reduced food intake in fattening pigs (Schwarz *et al*., 2017). The biochemical changes, described above, are translated into diagnostic guidelines in Table 15.10.

In poultry and pre-ruminants exposed to Se_i, tolerances are similar to those reported above for pigs. Supplementation of chicks via drinking water, equivalent to 7mg Se kg^{-1}DM, impaired growth and appetite within seven days (Cantor *et al*., 1984) and in hens, 5mg Se kg^{-1}DM was borderline for hatchability (Ort and Latshaw, 1978). Tolerances depend partly on the rate at which Se can be 'deposited' in methionine-rich products such as muscle, milk and eggs. Hens tolerated 10mg Se kg^{-1}DM from seleniferous grain, but embryonic development was adversely affected (Poley and Moxon, 1938). In calves given Se_i in a milk replacer for 45 days, growth and FCE were impaired with 10 mg but not 5 mg added Se kg^{-1}DM (Jenkins and Hidiriglou, 1986).

Selenium Tolerance in Ruminants

Ruminants are more tolerant than non-ruminants because of a lower A_{se} (Table 15.8). Wethers tolerated 10 mg Se_i kg^{-1}DM for a year (Cristaldi *et al*., 2005), ewe lambs 15 mg for 84 days (Neville *et al*., 2008) and ewes 20mg Se_i kg^{-1}DM for 72 weeks (Davis *et al*., 2006), showing neither biochemical nor histological evidence of tissue damage. In steers given

Table 15.10. Marginal bands[a] for biochemical indices of selenium status for use in assessing risk of chronic selenosis.

Species	Diet (mg kg^{-1}DM)	Blood (mg l^{-1})	Serum/plasma (mg l^{-1})	Liver (mg kg^{-1} FW)	Hair/fleece (mg kg^{-1}DM)	Hoof (mg kg^{-1}DM)
Cattle	5–**10**	1.5–**2.0**	2.5–**3.5**	4.0–**9.0**	5–**10**	2–**5**
Sheep	5–**20**	1.0–**2.0**	2.0–**3.0**	15–**25**	4–**8**	4–**8**
Pigs	3–**5**	2–**3**	0.7–**1.0**	1.5–**3.0**	4–**6**	3–**4**
Poultry	3–**5**	–	0.15–**0.30**	2.0–**6.0**	–	–

[a]Values above the band indicate a definite risk of selenosis; values within bands indicate high previous selenium intakes that, if continued, will probably lead to selenosis the closer they are to the upper, **bold** limit.

the equivalent of 5, 10 and 25mg kg^{-1}DM Se_i for 120 days, only one out of four developed hoof lesions at the highest intake (O'Toole and Raisbeck, 1995). Pregnant beef cows tolerated 12mg Se kg^{-1}DM for three months (Yaeger *et al*., 1998). Tolerance of grazing cattle and horses on seleniferous rangeland is difficult to establish because forage Se, palatability and accessibility vary widely between plant species. Where consumption was restricted to non-accumulator plants, containing about 5mg Se kg^{-1}DM, clinical selenosis was induced after prolonged exposure. Exposure to 20mg kg^{-1}DM as SeMet induced hoof lesions in four out of five steers, indicating higher toxicity than for Se_i (O'Toole and Raisbeck, 1995). If the steers' diet allowed little growth, a high proportion of the SeMet, given in bolus doses, might have been deposited in the hoof. Diets high in protein reduce Se toxicity, probably through diluting effects. Tolerances of lactating animals have not been studied but are probably relatively high because large amounts of Se are secreted safely in milk, particularly from organic sources. In cows yielding 31 kg milk day^{-1}, 12% of Se ingested as SeY in a total mixed ration, containing 6.1 mg added Se_i kg^{-1}DM, was secreted and maximal blood Se was only 0.72 μg l^{-1} compared to 1.51 μg l^{-1} in beef cattle on a similar diet (Juniper *et al*., 2008).

Prevention and Control of Chronic Selenosis

Selenium poisoning is incurable and early preventive methods used substances that were themselves potentially toxic (e.g. bromobenzene, arsenic) (Underwood and Suttle, 1999). Preventive measures now rely on treatment of soil or diet but each has practical limitations. It may be simpler to utilize seleniferous land for grain production and export the grain to low-Se areas and countries (see Chapter 20).

- An obvious preventive measure is to reduce Se exposure by not automatically adding Se at the legal limit irrespective of inherent Se in the unsupplemented feed.

Supra-nutritional supplementation can impair performance and have no benefits in terms of meat storage quality (Schwarz *et al*., 2017). Raising the soil S:Se ratio can reduce Se uptake by plants in some circumstances. Applications of 1000 kg $CaSO_4$ ha^{-1} greatly reduced selenium concentrations in sugarcane in India (Dhillon and Dhillon, 1991). Addition of sulfur or gypsum ($CaSO_4$) to soils in a seleniferous area in North America did not reduce selenium uptake by cereals, possibly because the soils were already high in SO_4. Several heavy metals afford protection in chicks (Hill, 1974): in the case of copper, this may be attributable to formation of unavailable copper selenides in the gut. Daily doses of a high-SO_4 mixture (30 g) protected buffalo from Degnala disease, a selenosis (Arora *et al*., 1975). Sulfate supplements may afford protection to ruminants by increasing methionine synthesis in the rumen, 'trapping' Se_i in the less toxic form of SeMet. Provision of molasses–urea blocks may similarly lower Se toxicity by increasing microbial protein synthesis. Any protein supplement that increases growth rate or milk yield should 'dilute' the effect of high Se uptake.

Acute Selenium Toxicity

Acute poisoning is characterized by salivation, respiratory distress, oedema and pulmonary congestion, reflecting circulatory failure and degenerative changes in heart, liver and kidneys (Rosenfeld and Beath, 1964). Toxicity has occurred at injected doses of 0.4–0.6 mg Se_i kg^{-1}LW in sheep where there was no history of selenium insufficiency and in cattle, where other stressors (weaning and vaccination) were present (Underwood and Suttle, 1999). Selenite and selenate are more acutely toxic than elemental Se or selenides and careful scaling of parenteral therapeutic doses to body weight is recommended. Accumulator plants can contain >4g Se kg^{-1}DM and cause 'Blind Staggers', an acute selenosis. In a study with groups of five pigs, similar dietary exposure as selenate, SeMet or *Astragalus bisulcatus* caused poliomyelomalacia, but lesions were least likely and slowest to develop in the SeMet group (two cases after 9 and 24 days) and fastest in the group given the seleniferous plant (four cases in <5 days) (Panter *et al*., 1996).

Notes

[i] First to be isolated was SelP from heart and muscle, where concentrations declined during Se depletion, but SelW was conserved in brain. A Ca-binding domain in SelW may explain calcification that occurs in dystrophic muscles of animals deprived of Se. SelN may play a role in cell proliferation and early development (Surai, 2006). SeP expressions vary within and between species. In turkeys, SelW1 expression is high in heart, gizzard and

muscle, and SeIU expression high in liver. SeIP2, not found in mammals, was highly expressed in avian liver but not in other tissues (Sunde *et al*., 2015).

[ii] Depletion of ID2 in the pituitary of Se-deprived rats may have reduced growth by inhibiting release of growth hormones, but concurrent iodine deprivation attenuated growth inhibition by increasing ID2 activity (Moreno-Reyes *et al*., 2006). Adaptive processes in the brain protect that organ from functional Se deprivation, occurring elsewhere.

[iii] Past use of radioactive tracers did not accurately reflect either availability for absorption or kinetics of absorbed Se, distorted in the latter case by the complex forms of Se present in the body (Pedrosa *et al*., 2012). For example, megalin, a SeIP receptor in the renal proximal tubule, can take up specific forms of SeIP1 to regulate urinary excretion and some Se is excreted in urine as sugar selenols. However, clearance of ^{75}SeMet from the bloodstream was relatively slow and partition to muscle relatively high (White *et al*., 1998), whereas clearance of ^{75}SeCys or $^{75}Se_i$ was rapid and distribution more even (Davidson and Kennedy, 1993).

[iv] Inverse relationships between somatic cell counts (SCC) and GPX1 in blood have been reported (Erskine *et al*., 1989b) and Se supplementation lowered SCC after experimental udder infection (Erskine *et al*., 1989a). However, Se supplementation did not lower SCC in a grazing herd of exceedingly low Se status, despite improving milk-fat yield (Knowles *et al*., 1999). In Se-deprived cows, bactericidal activity towards *S. aureus* and GPX activity of neutrophil leucocytes isolated from the mammary gland were both reduced, but SCC was not correlated with blood Se (Grace *et al*., 1997).

[v] Results for GPX1 assays can vary between laboratories and should be standardized (Underwood and Suttle, 1999): assay temperatures may range from 25°C to 37°C and pH from 6.8 to 7.4, increasing recorded activity more than twofold, yet these details are rarely specified in papers.

[vi] An AA_{Se} coefficient of 0.65 for ^{75}Se, given orally as selenious acid, contrasted with AA_{Se} of 0.30 from stable selenium balance data on a hay plus concentrates diet (Alfaro *et al*., 1987). An A_{Se} of 0.2 for hay by isotope dilution after parenteral administration of labelled inorganic ^{75}Se, contrasted with an AA_{Se} of 0.41 from stable balance data (Krishmamurti *et al*., 1989). In cattle given a grass hay, A_{Se} was only 0.10–0.16 but the stable isotope study required preliminary tissue enrichment with 4 mg ^{76}Se as selenite, orally, for five days (Koenig *et al*., 1991).

[vii] Lambs in Michigan required 0.03–0.05 mg Se d^{-1} to avoid WMD on hay/grain diets (Ullrey, 1974). Indoor experiments, using a highly digestible semi-purified diet, gave higher needs for growth of 0.04–0.06mg Se kg^{-1}DM (Oh *et al*., 1976). Responses in fleece yield, the most sensitive index of need, indicated lower requirements for sheep grazing Australian pastures (<0.03mg Se kg^{-1}DM) (Langlands, 1991a; Whelan *et al*., 1994b), unless there was an additional need for fetal growth in the ewe lamb (Wilkins *et al*., 1982). Rations of hay and concentrate, containing 0.027 and 0.04 mg kg^{-1}DM, failed to maintain normal blood GPX activity and resistance to mastitis in five flocks of dairy goats (Sanchez *et al*., 2007). Field experience with grazing cattle in Australia and New Zealand suggested that 0.02–0.03mg Se kg^{-1}DM was marginal for growth (Langlands *et al*., 1981; Wichtel *et al*., 1994, 1996) and milk-fat yield (Knowles *et al*., 1999). In the latter study, a herd of 80 was screened for blood Se and 35 cows of lowest status were used in the trial. In five trials with steers newly introduced to feedlot conditions, given alfalfa hay, corn silage and a supplement providing approximately 0.05mg kg^{-1}DM, neither performance nor health were improved by parenteral injections of Se (25–50 mg), with or without vitamin E (340 IU) (Droke and Loerch, 1989). For higher-yielding cows, fed indoors on diets that required massive vitamin E supplements, Se responses may be obtained on diets containing around 0.05mg Se kg^{-1}DM (Weiss, 1998).

[viii] Chicks' requirements decreased from 0.05 to 0.02 and <0.01mg Se kg^{-1}DM when 10 and 100 mg vitamin E. respectively, were added to a purified diet containing no vitamin (Thompson and Scott, 1969). More than 0.06mg Se kg^{-1}DM was required from SeMet-rich natural feeds to give complete protection from ED, but less Se was required as SeMet than as Se_i for complete protection from pancreatic fibrosis (Thompson and Scott, 1970).

[ix] Serum, milk and piglet tissue Se were raised more by giving sows SeY rather than Se_i (Mahan, 2000; Mahan and Peters, 2004) but GPX3 activity in both dam and offspring were lower. Similar results were obtained in comparisons of Se_o and Se_i with blood GPX1 in the piglet at birth (Yoon and McMillan, 2006), IgG in colostrum and milk of sows (Quesnel *et al*., 2008) and IgG in serum of gilts and their offspring (Gelderman and Clapper, 2013). Addition of oxidized fat to the hen's diet neither harmed egg production nor increased need for Se (Leeson *et al*., 2008). Short-term benefit was evident when SeMet restored appetite and liver GPX activity of selenium-deprived chicks faster than selenite (Bunk and Combs, 1980). Hatchability and plasma GPX3 activity were lower with SeY rather than selenite providing an additional 0.1mg Se kg^{-1}DM in broiler breeder and laying hen diets (Leeson *et al*., 2008). In adult horses, supplementation with SeY rather than Se_i increased blood Se but not GPX1 activity (Calamari *et al*., 2009; Montgomery *et al*., 2012).

[x] Cardiac atrophy ('dish-rag' heart), liver cirrhosis and anaemia were reported in early studies of 'Blind Staggers' but were not reproducible, experimentally, and may have been attributable to confounding factors such as alkaloid poisoning and starvation (O'Toole and Raisbeck, 1995).

[xi] High Se ('accumulator') species include *Astragalus*, *Haplopappus* and *Stanleya* genera; intermediate ('secondary absorber') species include *Atriplex* and some *Brassica* species; 'low' species include normal forage species (e.g. *Pascopyrum*, *Poasecunda*, *Trifolium*) and cereals.

References

Adams, M.I., Lombi, E., Zhao, F.-J. and McGrath, S.P. (2002) Evidence for low selenium concentrations in bread-making wheat. *Journal of the Science of Food and Agriculture* 82, 1160–1165.

Alfaro, E., Neathery, M.W., Miller, W.J., Gentry, R.P., Crowe, C.T. *et al.* (1987) Effects of ranging amounts of dietary calcium on selenium metabolism in dairy calves. *Journal of Dairy Science* 70, 831–830.

Alfthan, G., Eurola, M., Ekholm, P., Venäläinen, E.R., Root, T. *et al.* (2015) Selenium Working Group. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: from deficiency to optimal selenium status of the population. *Journal of Trace Elements in Medicine and Biology* 31, 142–147.

Allen, W.M., Bradley, R., Berrett, S., Parr, W.H., Swannack, K., Barton, C.R.Q. and MacPhee, A. (1975) Degenerative myopathy with myoglobinuria in yearling cattle. *British Veterinary Journal* 131, 292–306.

Anderson, P.H., Barrett, S. and Patterson, D.S.P. (1976) Some observations on 'paralytic myoglobinuria' of cattle in Britain. *Veterinary Record* 99, 316–318.

Andrews, E.D., Hartley, W.J. and Grant, A.B. (1968) Selenium-responsive diseases of animals in New Zealand. *New Zealand Veterinary Journal* 16, 3–17.

Anil Kumar, P., Sathyanarayana, M.L., Vijayasarathi, S.K., Sreenivasa Gowda, R.N. and Rao, S. (2005) Effect of vitamin E and selenium on serum biochemical parameters in broiler chicken fed with aflatoxin and ochratoxin. *Indian Veterinary Journal* 82, 522–525.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

Archer, J.A. and Judson, G.J. (1994) Selenium concentrations in tissues of sheep given a subcutaneous injection of barium selenate or sodium selenate. *Australian Journal of Experimental Agriculture* 34, 581–588.

Arnold, R.I., Olson, O.E. and Carlson, C.W. (1973) Dietary selenium and arsenic additions and their effects on tissue and egg selenium. *Poultry Science* 52, 847–854.

Arora, S.P., Parvinder, K., Khirwar, S.S., Chopra, R.C. and Ludri, R.S. (1975) Selenium levels in fodders and its relationship with Degnala disease. *Indian Journal of Animal Science* 28, 249.

Arthington, J.D. (2008) Effects of supplement type on measures of growth and selenium status in yearling steers. *Journal of Animal Science* 86, 1472–1477.

Arthur, J.R. (1988) Effects of selenium and vitamin E status on plasma creatine kinase activity in calves. *Journal of Nutrition* 118, 747–755.

Arthur, J.R. (1998) Free radicals and diseases of animal muscle. In: Reznick, A.Z. (ed.) *Oxidative Stress in Skeletal Muscle*. Birkhauser Verlag, Basel, pp. 321–330.

Arthur, J.R. and Beckett, G.J. (1994) Roles of selenium in type I iodothyronine 5′-deiodinase and in thyroid hormone and iodine metabolism. In: Burk, R.F. (ed.) *Selenium in Biology and Human Health*. Springer-Verlag Inc., New York, pp. 93–115.

Arthur, J.R., Price, J. and Mills, C.F. (1979) Observations on the selenium status of cattle in the north-east of Scotland. *Veterinary Record* 104, 340–341.

Arthur, J.R., Morrice, P.C. and Beckett, G.J. (1988) Thyroid hormone concentrations in selenium deficient and selenium-sufficient cattle. *Research in Veterinary Science* 45, 122–123.

Arthur, J.R., Nicol, F., Guo, Y. and Trayhurn, P. (1992) Progressive effects of selenium deficiency on the acute, cold-induced stimulation of type II deiodinase activity in rat brown adipose tissue. *Proceedings of the Nutrition Society* 51, 63A.

Awadeh, F.T., Kincaid, R.L. and Johnson, K.A. (1998) Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. *Journal of Animal Science* 76, 1204–1215.

Beckett, G.J. and Arthur, J.R. (2005) Selenium and endocrine systems. *Journal of Endocrinology* 184, 455–465.

Behne, D. and Kyriakopoulos, A. (2001) Mammalian selenoproteins. *Annual Review of Nutrition* 21, 453–473.

Bernamo, G., Nicol, F., Dyer, J.A., Sunde, R.A., Beckett, G.J., Arthur, J.R. and Hesketh, J.E. (1995) Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats. *Biochemical Journal* 311, 425–430.

Bourne, N., Laven, R., Wathes, D.C., Martinez, T. and McGowan, M. (2006) A meta-analysis of the effects of vitamin E supplementation on the incidence of retained foetal membranes in dairy cows. *Theriogenology* 67, 494–501.

Boyne, R. and Arthur, J.R. (1979) Alterations of neutrophil function in selenium deficient cattle. *Journal of Comparative Pathology* 89, 151–158.

Broadley, M.R., White, P.J., Bryson R.J., Meacham, M.C., Bowen, H.C. and Johnson, S.E. (2006) Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society*, 65, 169–181.

Bruzelius, K., Hoac, T., Sundler, R., Onning, G. and Akesson, B. (2007) Occurrence of selenoprotein enzyme activities and mRNA in bovine mammary tissue. *Journal of Animal Science* 90, 918–927.

Brzezinska-Slebodzinska, E., Miller, J.K., Quigley, J.D. and Moore, J.R. (1994) Antioxidant status of dairy cows supplemented pre-partum with vitamin E and selenium. *Journal of Dairy Science*, 77, 3087–3095.

Bunk, M.J. and Combs, G.F. (1980) Effect of selenium on appetite in the selenium-deficient chick. *Journal of Nutrition* 110, 743–749.

Burk, R.F. and Hill, K.E. (2015) Regulation of selenium metabolism and transport. *Annual Reviews of Nutrition* 35, 109–134.

Burk, R.F., Hill, K.E. and Motley, A.K. (2001) Plasma selenium in specific and non-specific forms. *Biofactors* 14, 107–114.

Butler J.A., Beilstein, M.A. and Whanger, P.D. (1989) Influence of dietary methionine on the metabolism of selenomethionine in rats. *Journal of Nutrition* 119, 1001–1009.

Calamari, L., Ferrari, A. and Bertin, G. (2009) Effect of selenium source and dose on selenium status of horses. *Journal of Animal Science* 87, 167–178.

Cantor, A.H. and Tarino, J. (1982) Comparative effects of inorganic and organic dietary sources of selenium on selenium levels and selenium-dependent glutathione peroxidase activity in blood of young turkeys. *Journal of Nutrition* 112, 2187–2196.

Cantor, A.H., Langevin, M.L., Noguchi, T. and Scott, M.L. (1975a) Efficacy of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. *Journal of Nutrition* 105, 106–111.

Cantor, A.H., Scott, M.L. and Noguchi, T. (1975b) Biological availability of selenium in feedstuffs and selenium compounds for prevention of exudative diathesis in chicks. *Journal of Nutrition* 105, 96–105.

Cantor, A.H., Moorhead, P.D. and Musser, M.A. (1982) Comparative effects of selenite and selenomethionine upon nutritional muscular dystrophy, selenium-dependent glutathione peroxidase and tissue selenium concentrations of turkey poults. *Poultry Science* 61, 478–484.

Cantor, A.H., Nash, D. and Johnson, T.H. (1984) Toxicity of selenium in drinking water of poultry. *Nutrition Reports International* 29, 683–688.

Caple, I.W., Edwards, S.J.A., Forsyth, W.M., Whitely, P., Selth, R.H. and Fulton, L.J. (1978) Blood glutathione peroxidase activity in horses in relation to muscular dystrophy and selenium nutrition. *Australian Veterinary Journal* 54, 57–60.

Chesters, J.K. and Arthur, J.R. (1988) Early biochemical defects caused by dietary trace element deficiencies. *Nutrition Research Reviews* 1, 39–56.

Colnago, G.L., Jensen, L.S. and Long, P.L. (1994) Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens. *Poultry Science* 63, 1136–1143.

Combs, G.F. Jr (2001) Selenium in global food systems. *British Journal of Nutrition* 85, 517–547.

Combs, G.F. and Scott, M.L. (1974) Dietary requirements for vitamin E and selenium measured at the cellular level in the chick. *Journal of Nutrition* 104, 1292–1296.

Conrad, H.R. and Moxon, A.L. (1979) Transfer of dietary selenium to milk. *Journal of Animal Science* 62, 404–411.

Contreras, P.A., Wittwer, F., Matamoros, R., Mayorga, I.M. and van Schaik, G. (2005) Effect of grazing pasture with a low selenium content on the concentration of triiodothyronine and thyroxine in serum and glutathione peroxidase activity in erythrocytes in cows in Chile. *New Zealand Veterinary Journal* 53, 77–80.

Cooper, B.S., West, D.M. and Pauli, J.V. (1989) Effects of repeated oral doses of selenium in sheep. *New Zealand Veterinary Journal* 37, 37.

Cristaldi, L.A., McDowell, L.R., Buergelt, C.D., Davis, P.A., Wilkinson, N.S. and Martin, F.G. (2005) Tolerance of inorganic selenium by wether sheep. *Small Ruminant Research* 56, 205–213.

Dalia, A.M., Loh, T.C., Sazili, A.Q., Jahromi, M.F. and Samsudin, A.A. (2017) The effect of dietary bacterial organic selenium on growth performance, antioxidant capacity, and Selenoproteins gene expression in broiler chickens. *BMC Veterinary Research* 13, 254. doi:10.1186/s12917-017-1159-4

Dalir-Naghadeh, B., Bahrami, Y., Rezaei, S.A., Anassori, E., Janalipour, A. and Khosravi, V. (2015) Platelet indices of selenium status in healthy and selenium-deficient sheep: a comparison with selenium indices in plasma, whole blood, and red blood cells. *Biological Trace Element Research* 168, 74–81.

Davidson, W.B. and Kennedy, D.G. (1993) Synthesis of [^{75}Se] selenoproteins is greater in selenium-deficient sheep. *Journal of Nutrition* 123, 689–694.

Davis, P.A., McDowell, L.R., Wilkinson, N.S., Buergelt, C.D., Van Alstyne, R., Weldon, R.N. and Marshall, T.T. (2006) Tolerance of inorganic selenium by range-type ewes during gestation and lactation. *Journal of Animal Science* 84, 660–668.

Dhillon, K.S. and Dhillon, S.K. (1991) Accumulation of selenium in sugar cane (*Sacharum officinarum Linu*) in seleniferous areas of Punjab, India. *Environmental Geochemistry and Health* 13, 165–170.

Donald, G.E., Langlands, J.P., Bowles, J.E. and Smith, A.J. (1994a) Subclinical selenium deficiency. 5. Selenium status and the growth and wool production of sheep supplemented with thyroid hormones. *Australian Journal of Experimental Agriculture* 34, 13–18.

Donald, G.E., Langlands, J.P., Bowles, J.E. and Smith, A.J. (1994b) Subclinical selenium deficiency. 6. Thermoregulatory ability of perinatal lambs born to ewes supplemented with selenium and iodine. *Australian Journal of Experimental Agriculture* 34, 19–24.

Donald, G.E., Langlands, J.P., Bowles, J.E., Smith, A.J. and Burke, G.L. (1994c) Selenium supplements for grazing sheep. 3. Development of an intraruminal pellet with an extended life. *Animal Feed Science and Technology* 40, 295–308.

Droke, E.A. and Loerch, S.C. (1989) Effects of parenteral selenium and vitamin E on performance, health and humoral immune response of steers new to the feedlot environment. *Journal of Animal Science* 67, 1350–1359.

Ehlig, C.F., Hogue, D.E., Allaway, W.H. and Ham, D.J. (1967) Fate of selenium from selenite or selenomethionine, with or without vitamin E, in lambs. *Journal of Nutrition* 92, 121–126.

Ellison, R.S., Young, B.J. and Read, D.H. (1986) Bovine postparturient haemoglobinuria: two distinct entities in New Zealand. *New Zealand Veterinary Journal* 34, 7–10.

Erskine, R.J., Eberhart, R.J., Grasso, P.J. and Scholz, R.W. (1989a) Induction of *Escherichia coli* mastitis in

cows fed selenium-deficient or selenium-supplemented diets. *American Journal of Veterinary Research* 50, 2093–2100.

Erskine, R.J., Eberhart, R.J. and Hutchinson, L.J. (1989b) Blood selenium concentrations and glutathionine peroxidase activities in dairy herds with high and low somatic cell counts. *Journal of American Veterinary Medical Association* 190, 1417–1421.

Evenson, J.K., Wheeler, A.D., Blake, S.M. and Sunde, R.A. (2004) Selenoprotein mRNA is expressed in blood at levels comparable to major tissues in rats. *Journal of Nutrition* 134, 2640–2645.

Field, A.C., Suttle, N.F., Brebner, J. and Gunn, G. (1988) An assessment of the efficacy and safety of selenium and cobalt included in an anthelmintic for sheep. *Veterinary Record* 123, 97–100.

Flohe, L., Gunzler, W.A. and Schock, H.H. (1973) Glutathione peroxidase: a selenoenzyme. *FEBS Letters* 32, 132–134.

Fordyce, F. (2005) Selenium deficiency and toxicity in the environment. In: Selinus, O. (ed.) *Essentials of Medical Geology: Impacts of the Natural Environment on Public Health*. British Geological Survey, pp. 373–415.

Fraser, A.J., Ryan, T.J., Sproule, R., Clark, R.G., Anderson, D. and Pederson, E.O. (1987) The effect of selenium on milk production in dairy cattle. *Proceedings of the New Zealand Society of Animal Production* 47, 61–64.

Gabbedy, B.J. (1971) Effect of selenium on wool production, body weight and mortality of young sheep in Western Australia. *Australian Veterinary Journal* 47, 318–322.

Galbraith, M.L., Vorachek, W.R., Estill, C.T., Whanger, P.D., Bobe, G., Davis, T.Z. and Hall, J.A. (2016) Rumen microorganisms decrease bioavailability of inorganic selenium supplements. *Biological Trace Element Research* 171, 338–343.

Gelderman, A. and Clapper, J. (2013) Effects of inorganic or organic selenium on immunoglobulins in swine. *Journal of Animal Science and Biotechnology* 4, art. 47.

Givens, D.I., Allison, R., Cottrill, B. and Blake, J.S. (2004) Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. *Journal of Science in Food and Agriculture* 84, 811–817.

Gleed, P.T., Allen, W.M., Mallenson, C.B., Rowlands, G.J., Sanson, B.F., Vagg, M.J. and Caswell, R.D. (1983) Effects of selenium and copper supplementation in the growth of beef steers. *Veterinary Record* 113, 388–392.

Glienke, L.R. and Ewan, R.C. (1977) Selenium deficiency in the young pig. *Journal of Animal Science* 45, 1334–1340.

Goehring, T.B., Palmer, I.S., Olson, O.E., Libal, G.W. and Wahlstrom, R.C. (1984a) Effects of seleniferous grains and inorganic selenium on tissue and blood composition and growth performance of rats and swine. *Journal of Animal Science* 59, 725–732.

Goehring, T.B., Palmer, I.S., Olson, O.E., Libal, G.W. and Wahlstrom, R.C. (1984b) Toxic effects of selenium on growing swine fed corn–soya bean meal diets. *Journal of Animal Science* 59, 733–737.

Goff, J.P. and Stabel, J.R. (1990) Decreased plasma retinol, α-tocopherol and zinc concentrations during the periparturient period: effect of milk fever. *Journal of Dairy Science* 73, 3195–3199.

Grace, N.D. and Knowles, S.O. (2002) A reference curve using blood selenium concentration to diagnose selenium deficiency and predict growth responses in lambs. *New Zealand Veterinary Journal* 50, 163–165.

Grace, N.D. and West, D.M. (2006) Effect of Se-amended fertilisers on the Se status of grazing dairy cattle. *Proceedings of New Zealand Society of Animal Production* 66, 182–186.

Grace, N.D., Venning, M. and Vincent, G. (1994) An evaluation of a controlled release system for selenium in lambs. *New Zealand Veterinary Journal* 42, 63–65.

Grace, N.D., Knowles, S.O. and Lee, J. (1997) Relationships between blood selenium concentrations and milk somatic cell counts in dairy cows. *New Zealand Veterinary Journal* 45, 171–172.

Grace, N.D., Ankenbauer-Perkins, K., Alexander, A.M. and Marchant, R.M. (2001) Relationship between selenium concentration or glutathione peroxidase activity and milk selenium concentrations in dairy cows. *New Zealand Veterinary Journal* 49, 24–28.

Grace, N.D., Knowles, S.O. and West, D.M. (2006) Dose response effects of long-acting injectable vitamin B_{12} plus selenium (Se) on the vitamin B_{12} and Se status of ewes and their lambs. *New Zealand Veterinary Journal* 54, 67–72.

Groce, A.W., Miller, E.R., Hitchcock, J.P., Ullrey, D.E. and Magee, W.T. (1973) Selenium balance in the pig as affected by selenium source and vitamin E. *Journal of Animal Science* 37, 942–947.

Gunter, S.A., Beck, P.A. and Phillips, J.M. (2003) Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. *Journal of Animal Science* 81, 856–864.

Guyot, H., Spring, P., Andrieu, S. and Rollin, F. (2007) Comparative responses to sodium selenite and organic selenium supplements in Belgian Blue cows and calves. *Livestock Science* 111, 259–263.

Hadley, K.B. and Sunde, R.A. (1997) Determination of dietary selenium requirement in female turkey poults using glutathione peroxidase. In: *Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals (TEMA 9)*. NRC Research Press, Ottawa, pp. 59–60.

Hall, J.A., Vorachek, W.R., Stewart, W.C., Gorman, M.E., Mosher, W.D., Pirelli, G.J. and Bobe, G. (2013) Selenium supplementation restores innate and humoral immune responses in footrot-affected sheep. *PLOS ONE* 8, e82572.

Harrison, J.H., Hancock, D.D. and Conrad, H.R. (1984) Vitamin E and selenium for reproduction in the dairy cow. *Journal of Dairy Science* 67, 123–132.

Hartley, W.J. (1963) Selenium and ewe fertility. *Proceedings of the New Zealand Society of Animal Production* 23, 20–27.

Hartley, W.J. (1967) Levels of selenium in animal tissues and methods of selenium administration. In: Muth, O.H. (ed.) *Selenium in Biomedicine*. Avi Publishing, Westport, Connecticut, pp. 79–96.

Hatfield, D.L., Berry, M.J. and Gladyshev, V.N. (2006) *Selenium: Its Molecular Biology and Role in Human Health*. Springer, New York.

Henry, P.R. and Ammerman, C.B. (1995) Selenium bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 303–331.

Hidiroglou, M., Proulx, J. and Jolette, J. (1985) Intraruminal selenium for control of nutritional muscular dystrophy in the dairy cow. *Journal of Dairy Science* 68, 57–66.

Hill, C.H. (1974) Reversal of selenium toxicity in chicks by mercury, copper, and cadmium. *Journal of Nutrition* 104, 593–598.

Ivancic, J. and Weiss, W.P. (2001) Effect of dietary sulfur and selenium concentrations on selenium balance in lactating Holstein cows. *Journal of Dairy Science* 84, 225–232.

Jelinek, P.D., Ellis, T., Wroth, R.H., Sutherland, S.S., Masters, H.G. and Pettersen, D.S. (1988) The effect of selenium supplementation on immunity, and the establishment of an experimental *Haemonchus contortus* infection, in weaner Merino sheep fed a low selenium diet. *Australian Veterinary Journal* 65, 214.

Jenkins, K.J. and Hidiriglou, M. (1986) Tolerance of the pre-ruminant calf for selenium in milk replacer. *Journal of Dairy Science* 69, 1865–1870.

Jianhua, H., Ohtsuka, A. and Hayashi, K. (2000) Selenium influnces growth via thyroid hormone status in broiler chickens. *British Journal of Nutrition* 84, 727–732.

Jukola, E., Hakkarainan, J., Saloniemi, H. and Sankari, S. (1996) Blood selenium, vitamin E, vitamin A and β carotene and udder health, fertility treatments and fertility. *Journal of Dairy Science* 79, 838–845.

Juniper, D.T., Phipps, R.H., Jones, A.K. and Bertin, G. (2006) Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood, milk, urine and feces. *Journal of Dairy Science* 89, 3544–3551.

Juniper, D.T., Phipps, R.H., Ramos-Morales, E. and Bertin, G. (2008) Selenium persistency and speciation in the tissues of lambs following the withdrawal of a high-dose selenium-enriched yeast. *Animal* 2, 375–380.

Kappel, L.C., Ingraham, R.H., Morgan, E.B., Dixon, J.M., Zeringue, L., Wilson, D. and Babcock, D.K. (1984) Selenium concentrations in feeds and effects of treating pregnant Holstein cows with selenium and vitamin E on blood selenium values and reproductive performance. *American Journal of Veterinary Research* 45, 691–694.

Kaur, R.S., Sharma, S. and Rampal, S. (2003) Effect of subchronic selenium toxicosis on lipid peroxidation, glutathione redox cycle and antioxidant enzymes in calves. *Veterinary and Human Toxicology* 45, 190–192.

Kendall, N.R., Jackson, D.W., Mackenzie, A.M., Illingworth, D.V., Gill, I.M. and Telfer, S.B. (2001) The effect of a zinc, cobalt and selenium soluble glass bolus on the trace element status of extensively grazed sheep over winter. *Animal Science* 73, 163–169.

Kim, B.G., Kil, D.Y., Mahan, D.C, Hill, G.M. and Stein, H.H. (2014) Effects of dietary sulfur and distillers dried grains with solubles on carcass characteristics, loin quality, and tissue concentrations of sulfur, selenium, and copper in growing- finishing pigs. *Journal of Animal Science* 92, 4486–4493.

Kim, Y.Y. and Mahan, D.C. (2001) Comparative effects of high dietary levels of organic and inorganic selenium on selenium toxicity of growing-finishing pigs. *Journal of Animal Science* 79, 942–948.

Kirchgessner, M., Gabler, S. and Windisch, W. (1997) Homeostatic adjustments of selenium metabolism and tissue selenium to widely varying selenium supply in ^{75}Se labeled rats. *Journal of Animal Physiology and Animal Nutrition* 78, 20–30.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–310,

Knowles, S.O., Grace, N.D., Wurms, K. and Lee, J. (1999) Significance of amount and form of dietary selenium on blood milk and casein selenium concentrations in grazing cows. *Journal of Dairy Science* 82, 429–437.

Koenig, K.M., Buckley, W.T. and Shelford, J.A. (1991) Measurement of endogenous faecal excretion and true absorption of selenium in dairy cows. *Canadian Journal of Animal Science* 71, 167–174.

Koenig, K.M., Rode, L.M., Cohen, R.D.H. and Buckley, W.T. (1997) Effects of diet and chemical form of selenium on selenium metabolism in sheep. *Journal of Animal Science* 75, 817–127.

Koh, T.-S. and Judson, G.J. (1987) Copper and selenium deficiency in cattle: an evaluation of methods of oral therapy and an observation of a copper–selenium interaction. *Veterinary Research Communications* 11, 133–148.

Korhle, J., Jakob, F., Contempre, B. and Dumont, J.E. (2005) Selenium, the thyroid and the endocrine system. *Endocrine Reviews* 26, 944–984.

Kott, R.W., Ruttie, J.L. and Southward, G.M. (1983) Effects of vitamin E and selenium injections on reproduction and pre-weaning lamb survival in ewes consuming diets marginally deficient in selenium. *Journal of Animal Science* 57, 553–558.

Krishnamurti, C.R., Ramberg, C.F. and Shariff, M.A. (1989) Kinetic modelling of selenium metabolism in non-pregnant ewes. *Journal of Nutrition* 119, 1146–1155.

Krishnamurti, C.R., Ramberg, C.F., Shariff, M.A. and Boston, R.C. (1997) A compartmental model depicting short-term kinetic changes in selenium metabolism in ewes fed hay containing normal or inadequate levels of selenium. *Journal of Nutrition* 127, 95–102.

Lammi, M.J. and Qu, C. (2018) Selenium-related transcriptional regulation of gene expression. *International Journal of Molecular Science* 19, 2665–2681.

Langlands, J.P., Wilkins, J.F., Bowles, J.E., Smith, A.J. and Webb, R.F. (1981) Selenium concentration in the blood of ruminants grazing in northern New South Wales. 1. Analysis of samples collected in the National Brucellosis Eradication Scheme. *Australian Journal of Agricultural Research* 32, 511–521.

Langlands, J.P., Donald, G.E., Bowles, J.E. and Smith, A.J. (1986) Selenium excretion in sheep. *Australian Journal of Agricultural Research* 37, 201–209.

Langlands, J.P., Donald, G.E., Bowles, J.E. and Smith, A.J. (1989) Selenium concentration in the blood of ruminants grazing in northern New South Wales. 3. Relationship between blood concentration and the response in liveweight of grazing cattle given a selenium supplement. *Australian Journal of Agricultural Research* 40, 1075–1083.

Langlands, J.P., Donald, G.E., Bowles, J.E. and Smith, A.J. (1990) Selenium supplements for grazing sheep. 1. A comparison between soluble salts and other forms of supplement. *Animal Feed Science and Technology* 28, 1–13.

Langlands, J.P., Donald, G.E., Bowles, J.E. and Smith, A.J. (1991a) Subclinical selenium insufficiency. 1. Selenium status and the response in liveweight and wool production of grazing ewes supplemented with selenium. *Australian Journal of Experimental Agriculture* 31, 25–31.

Langlands, J.P., Donald, G.E., Bowles, J.E. and Smith, A.J. (1991b) Subclinical selenium insufficiency. 2. The response in reproductive performance of grazing ewes supplemented with selenium. *Australian Journal of Experimental Agriculture* 31, 33–35.

Larsen, C.T., Pierson, F.W. and Gross, W.B. (1997) Effect of dietary selenium on the response of stressed and unstressed chickens to *Escherichia coli* challenge and antigen. *Biological Trace Element Research* 58, 169–176.

Latshaw, J.D., Ort, J.F. and Diesem, C.D. (1977) The selenium requirements of the hen and effects of a deficiency. *Poultry Science* 56, 1876–1881.

Lawler, T.L., Taylor, P., Filey, J.W. and Canton, J.S. (2004) Effects of supranutritional and organically bound selenium on performance, carcase characteristics and selenium distribution in finishing beef steers. *Journal of Animal Science* 82, 1488–1493.

Leeson, S., Namkung, H., Caston, L., Durosoy, S. and Schlegel, P. (2008) Comparison of selenium levels and sources and dietary fat quality in diets for broiler breeders and layer hens. *Poultry Science* 87, 2605–2612.

Lei, X.G., Dann, H.M., Ross, D.A., Cheng, W.-S., Combs, G.F. and Roneker, K.R. (1998) Dietary selenium supplementation is required to support full expression of three selenium-dependent glutathione peroxidases in various tissues of weanling pigs. *Journal of Nutrition* 128, 130–135.

Li, J.-L. and Sunde, R.A. (2016) Selenoprotein transcript level and enzyme activity as biomarkers for selenium status and selenium requirements of chickens (*Gallus gallus*). *PLOS ONE* 11, e0152392. DOI: 10.1371/journal.pone.0152392

Lindberg, P. (1968) Selenium determination in plant and animal material, and in water. *Acta Veterinaria Scandinavica* 9, .

Liu, S., Masters, D., Ferguson, M.B. and Thompson, A.N. (2014) Vitamin E status and reproduction in sheep: potential implications for Australian sheep production. *Animal Production Science* 54, 694–714.

Lyons, G., Oritz-Monasterio, I., Stangoulis, J. and Graham, R. (2005) Selenium concentrations in wheat grain: is there sufficient genotypic variation to use in breeding? *Plant and Soil* 269, 369–380.

Mackintosh, C.G., Gill, J. and Turner, K. (1989) Selenium supplementation of young deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 137, 143–145.

MacPherson, A. (1994) Selenium, vitamin E and biological oxidation. In: Garnsworthy, P.C. and Cole, D.J.A. (eds) *Recent Advances in Nutrition*. Nottingham University Press, Nottingham, UK, pp. 3–30.

MacPherson, A., Kelly, E.F., Chalmers, J.S. and Roberts, D.J. (1987) The effect of selenium deficiency on fertility in heifers. In: Hemphill, D.D. (ed.) *Proceedings of the 21st Annual Conference on Trace Substances in Environmental Health*. University of Missouri, Columbia, Missouri, pp. 551–555.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Mahan, D.C. (2000) Effects of organic and inorganic selenium sources on sow colostrum and milk selenium content. *Journal of Animal Science* 78, 100–105.

Mahan, D.C. and Kim, Y.Y. (1996) Effect of inorganic and organic selenium at two dietary levels on reproductive performance and tissue selenium concentrations in first parity gilts and their progeny. *Journal of Animal Science* 74, 2711–2718.

Mahan, D.C. and Peters, J.C. (2004) Long-term effects of dietary organic and inorganic selenium sources and levels on reproducing sows and their progeny. *Journal of Animal Science* 82, 1343–1358.

Mahan, D.C., Cline, T.R. and Richert, B. (1999) Effects of dietary levels of selenium-enriched yeast and

sodium selenite as selenium sources fed to growing-finishing pigs on performance, tissue selenium, serum glutathione peroxidase activity, carcass characteristics and loin quality. *Journal of Animal Science* 7, 2172–2179.

Mahan, D.C., Brendemuhl, J.H., Carter, S.D., Chiba, L.I., Crenshaw, T.D. *et al.* (2005) Comparison of dietary selenium fed to grower-finisher pigs from various regions of the United States on resulting tissue Se and loin mineral concentrations. *Journal of Animal Science* 83, 852–857.

Mahan, D.C., Azain, M., Crenshaw, T.D., Cromwell, G.L, Dove, C.R. *et al.* (2014) Supplementation of organic and inorganic selenium to diets using grains grown in various regions of the United States with differing natural Se concentrations and fed to grower-finisher swine. *Journal of Animal Science* 92, 4991–4997.

Marin-Guzman, J., Mahan, D.C., Chung, Y.K., Pate, J.L. and Pope, W.F. (1997) Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality and subsequent fertilization rates in mature gilts. *Journal of Animal Science* 75, 2994–3003.

Marković, R., Ćirić, J., Starčević, M., Šefer, D. and Baltić, M. (2018) Effects of selenium source and level in diet on glutathione peroxidase activity, tissue selenium distribution, and growth performance in poultry. *Animal Health Research Reviews* 19, 166–176.

McDonald, J.W. (1975) Seleinium-responsive unthriftiness of young Merino sheep in Central Victoria. *Australian Veterinary Journal* 51, 433–435.

McDonald, J.W., Overend, D.J. and Paynter, D.I. (1989) Influence of selenium status in Merino weaners on resistance to trichostrongylid infection. *Research in Veterinary Science* 47, 319–322.

McLaughlin, J.G., Cullen, J. and Forristal, T. (1989) Blood selenium concentrations in cattle on seleniferous pastures. *Veterinary Record* 124, 426–427.

McMurray, C.H. and McEldowney, P.K. (1977) A possible prophylaxis and model for nutritional degenerative myopathy in young cattle. *British Veterinary Journal* 133, 535–542.

McMurray, C.H., Rice, D.A. and Kennedy, S. (1983) Nutritional myopathy in cattle: from a clinical problem to experimental models for studying selenium, vitamin E and polyunsaturated fatty acid interactions. In: Suttle, N.F., Gunn, R.G., Allen, W.M., Linklater, K.A. and Wiener, G. (eds) *Trace Elements in Animal Production and Veterinary Practice*. Occasional Publication No. 7, British Society of Animal Production, Edinburgh, pp. 61–76.

Meneses, A., Batra, T.R. and Hidiroglou, M. (1994) Vitamin E and selenium in milk of ewes. *Canadian Journal of Animal Science* 71, 567–569.

Millar, K.R. and Meads, W.J. (1988) Selenium levels in the blood, liver, kidney and muscle of sheep after the administration of iron/selenium pellets or soluble-glass boluses. *New Zealand Veterinary Journal* 36, 8–10.

Miller, G.Y., Bartlett, P.C., Erskine, R.J. and Smith, K.L. (1995) Factors affecting serum selenium and vitamin E concentrations in dairy cows. *Journal of American Veterinary Medicine Association* 206, 1369–1373.

Miltimore, J.E., van Ryswyk, A.L., Pringle, W.L., Chapman, F.M. and Kalnin, C.M. (1975) Selenium concentrations in British Columbia forages, grains and processed feeds. *Canadian Journal of Animal Science* 55, 101–111.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Moir, D.C. and Masters, H.G. (1979) Hepatosis dietetica, nutritional myopathy, mulberry heart disease and associated hepatic selenium levels in pigs. *Australian Veterinary Journal* 55, 360–364.

Money, D.F.L., Meads, W.J. and Morrison, L. (1986) Selenised compressed salt blocks for selenium deficient sheep. *New Zealand Veterinary Journal* 34, 81–84.

Montane, J., Marco, I., Manteca, X., Lopez, J. and Laving, S. (2002) Delayed acute capture myopathy in three roe deer. *Journal of Veterinary Medicine. Series A* 49, 93–98.

Montgomery, J.B., Wichtel, J.J., Wichtel, M.G., McNiven, M.A., McClure, J.T., Markham, F. and Horohov, D.W. (2012) Effects of selenium source on measures of selenium status and immune function in horses. *Canadian Journal of Veterinary Research* 76, 281–291.

Moreno-Reyes, R., Egrise, D., Boelaert, M., Goldman, S. and Meuris, S. (2006) Iodine deficiency mitigates growth retardation and osteopenia in selenium-deficient rats. *Journal of Nutrition* 136, 595–600.

Morris, J.G., Chapman, H.C., Walker, D.F., Armstrong, J.B., Alexander, J.D. *et al.* (1984) Selenium deficiency in cattle associated with Heinz body anaemia. *Science* 223, 291–293.

Moxon, A.L. (1937) *Alkali Disease or Selenium Poisoning*. Bulletin No. 311, South Dakota Agricultural Experiment Station.

Moxon, A.L. and Poley, W.E. (1938) The relation of selenium content of grains in the ration to the selenium content of poultry carcass and eggs. *Poultry Science* 17, 77–80.

Munoz, C., Carson, A.F., McCoy, M.A., Dawson, L.E.R., Irwin, D., Gordon, A.G. and Kilpatrick, D.J. (2009) Effects of supplementation with barium selenate on the fertility, prolificacy and lambing performance of hill sheep. *Veterinary Record* 164, 265–271.

Mutanen, M.P., Aspila, P. and Mykkanen, H.M. (1986) Bioavailability to rats of selenium in milk of cows fed sodium selenite or selenited barley. *Annals of Nutrition and Metabolism* 30, 183–188.

Neville, T.L., Ward, M.A., Reed, J.J., Soto-Navarro, S.A., Julius, S.L. *et al*. (2008) Effects of level and source of dietary selenium on maternal and fetal body weight,

visceral organ mass, cellularity estimates, and jejunal vascularity in pregnant ewe lambs. *Journal of Animal Science* 86, 890–901.

Nicol, F., Lefrane, H., Arthur, J.R. and Trayhurn, P. (1994) Characterisation and post-natal development of 5′-deiodinase activity in goat perinatal fat. *American Journal of Physiology* 267, R144–R149.

Noguchi, T., Cantor, A.H. and Scott, M.L. (1973) Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. *Journal of Nutrition* 103, 1502–1511.

Nolan, M.R., Kennedy, S., Blanchflower, W.J. and Kennedy, D.G. (1995) Lipid peroxidation, prostacyclin and thromboxane A2 in pigs depleted of vitamin E and selenium and supplemented with linseed oil. *British Journal of Nutrition* 74, 369–380.

NRC (2005) *Mineral Tolerances of Animals*, 2nd edn. National Academy of Sciences, Washington, DC.

Oh, S.H., Pope, A.L. and Hoekstra, W.G. (1976) Dietary selenium requirement of sheep fed a practical-type diet as assessed by tissue glutathione peroxidase and other criteria. *Journal of Animal Science* 42, 984–992.

Oksanen, H.E. (1967) Selenium deficiency: clinical aspects and physiological responses in farm animals. In: Muth, O.H. (ed.) *Selenium in Biomedicine*. Avi Publishing, Westport, Connecticut, pp. 215–229.

Orr, J.P. and Blakley, B.R. (1997) Investigation of the selenium status of aborted calves with cardiac failure and myocardial necrosis. *Journal of Veterinary Diagnostic Investigation* 9, 172–179.

Ort, J.F. and Latshaw, J.D. (1978) The toxic level of sodium selenite in the diet of laying chickens. *Journal of Nutrition* 1114–1120.

O'Toole, D. and Raisbeck, M.F. (1995) Pathology of experimentally induced chronic selenosis (alkali disease) in yearling cattle. *Journal of Veterinary Diagnostic Investigation* 7, 364–373.

Ozkan, S., Malayoglu, H.B., Yalçin, S., Karadas, F., Koçtürk, S. *et al.* (2007) Dietary vitamin E (α-tocopherol acetate) and selenium supplementation from different sources: performance, ascites-related variables and antioxidant status in broilers reared at low and optimum temperatures. *British Poultry Science* 48, 580–593.

Panter, K.E., Hartley, W.J., James, L.F., Mayland, H.F., Stegelmeier, B.L. and Kechele, P.O. (1996) Comparative toxicity of selenium from seleno-DL-methionine, sodium selenate and *Astragalus bisulcatus* in pigs. *Fundamental and Applied Toxicology* 32, 217–223.

Pappas, A.C., Acamovic, T., Sparks, A.C., Surai, P.F. and McDevitt, R.M. (2006) Effects of supplementing broiler breeder diets with organoselenium compounds and polyunsaturated fatty acids on hatchability. *Poultry Science* 85, 1584–1593.

Patterson, E.L., Milstrey, R. and Stokstad, E.L.R. (1957) Effect of selenium in preventing exudative diathesis in chicks. *Proceedings of the Society for Experimental Biology and Medicine* 95, 617–620.

Pavlata, L., Misurova, L., Pechov, A., Hus, T. and Dvorak, R. (2018) Direct and indirect assessment of selenium status in sheep. *Veterinarni Medicina* 57, 219–223.

Pedersen, K.B. and Simesen, M.G. (1977) Om tilskud of selen og vitamin E-selen mangelsyndromet hos svin. *Nordisk Veterinaermedicin* 29, 161–165.

Pedrosa, L.F., Motley, A.K., Stevenson, T.D., Hill, K.E. and Burk, R.F. (2012) Fecal selenium excretion is regulated by dietary selenium intake. *Biological Trace Element Research* 149, 377–381.

Pehrson, B., Ortman, K., Madjid, N. and Trafikowska, U. (1999) The influence of dietary selenium as selenium yeast or sodium selenite on the concentration of selenium in the milk of suckler cows and on the selenium status of their calves. *Journal of Animal Science*, 77, 3371–3376.

Piatkowski, T.L., Mahan, D.C., Cantor, A.H., Moxon, A.L., Cline, J.H. and Grifo, A.P. Jr (1979) Selenium and vitamin E in semi-purified diets for gravid and nongravid gilts. *Journal of Animal Science* 48, 1357–1365.

Podoll, K.L., Bernard, J.B., Ullrey, D.B., DeBar, S.R., Ku, P.K. and Magee, W.T. (1992) Dietary selenate versus selenite for cattle, sheep and horses. *Journal of Animal Science* 70, 1965–1970.

Poley, W.E. and Moxon, A.L. (1938) Tolerance levels of seleniferous grains in laying rations. *Poultry Science* 17, 72–76.

Qin, S., Gao, J. and Huang, K. (2007) Effects of different selenium sources on tissue selenium concentrations, blood GSH-Px activities and plasma interleukin levels in finishing lambs. *Biological Trace Element Research* 116, 91–102.

Quesnel, H., Renaudin, A., Le Foc'h, N., Jondreville, C., Pere, M.C., Taylor-Pickard, J.A. and Le Dividich, J. (2008) Effect of organic and inorganic selenium sources in sow diets on colostrum production and piglet response to a poor sanitary environment after weaning. *Animal* 2, 859–866.l

Rammell, C.G., Thompson, K.G., Bentley, G.R. and Gibbons, M.W. (1989) Selenium, vitamin E and polyunsaturated fatty acid concentrations in goat kids with and without nutritional myodegeneration. *New Zealand Veterinary Journal* 37, 4–6.

Reffett, J.K., Spears, J.W. and Brown, T.T. (1988) Effect of dietary selenium and vitamin E on the primary and secondary immune response in lambs challenged with parainfluenza virus. *Journal of Animal Science* 66, 1520–1528.

Renko, R., Werner, M., Renner-Muller, I., Cooper, T.G., Yeung, C.H. *et al.* (2008) Hepatic selenoprotein P (SePP) expression restores selenium transport and prevents infertility and motor-incoordination in *Sepp*-knockout mice. *Biochemical Journal* 409, 741–749.

Rice, D.A. and Kennedy, S. (1988) Assessment of vitamin E, selenium and polyunsaturated fatty acid

interactions in the aetiology of disease in the bovine. *Proceedings of the Nutrition Society* 47, 177–184.

Rock, M.J., Kincaid, R.L and Carstens, C. (2001) Effects of prenatal source and level of dietary selenium on passive immunity and thermometabolism of newborn lambs. *Small Ruminant Research* 40, 129–138.

Rosenfeld, I. and Beath, O.A. (1964) *Selenium: Geobotany, Biochemistry, Toxicity and Nutrition*. Academic Press, New York.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman. D.G. and Hoekstra, W.G. (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science, USA* 179, 588–590.

Rowntree, J.E., Hill, G.M., Hawkins, D.R., Link, J.E., Rincker, M.J., Bednar, G.W. and Kreft, R.A. Jr (2004) Effect of selenium on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. *Journal of Animal Science* 82, 2995–3005.

Sahin, N., Onderci, M., Sahin, K. and Kucuk, O. (2008) Supplementation with organic or inorganic selenium in heat-stressed quail. *Biological Trace Element Research* 122, 229–237.

Salvatore, D., Low, S.C., Berry, M., Maia, A.C., Harney, J.W., Croteau, W., St. Germain, D.L. and Larsen, P.J. (1995) Type 3 iodothyronine deiodinase cloning *in vitro:* expression and functional analysis of the selenoprotein. *Journal of Clinical Investigation* 96, 2421–2430.

Sanchez, J., Montes, P., Jimenez, A. and Andres, S. (2007) Prevention of clinical mastitis with barium selenate in dairy goats from a selenium-deficient area. *Journal of Dairy Science* 90, 2350–2354.

Schwarz, C., Ebner, K., Furtner, F., Duller, S., Wetscherek, W. *et al.* (2017) Influence of high inorganic selenium and manganese diets for fattening pigs on oxidative stability and pork quality parameters. *Animal* 11, 345–353.

Schwarz, K. and Foltz, C.M. (1957) Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Journal of the American Chemical Society* 79, 3292–3293.

Scott, M.L. and Thompson, J.N. (1971) Selenium content of feedstuffs and effects of dietary selenium levels upon tissue selenium in chick and poults. *Poultry Science* 50, 1742–1748.

Scott, M.L., Olson, G., Krook, L. and Brown, W.R. (1967) Selenium-responsive myopathies of myocardium and of smooth muscle in the young poult. *Journal of Nutrition* 91, 573–583.

Selinus, O. (1988) Biogeochemical mapping of Sweden for geomedical and environmental research. In: Thornton, I. (ed.) *Proceedings of the Second International Symposium on Geochemistry and Health*. Science Reviews, Norwood, UK, pp. 13–20.

Serra, A.B., Serra, S.D., Shinchi, K. and Fujihara, T. (1997) Bioavailability of rumen bacterial selenium in mice using tissue uptake technique. *Biological Trace Element Research* 58, 255–261.

Shellow, J.S., Jackson, S.G., Baker, J.P. and Cantor, A.H. (1985) The influence of dietary selenium levels on blood levels of selenium and glutathione peroxidase activity in the horse. *Journal of Animal Science* 61, 590–594.

Shojadoost, B., Kulkarni, R.R., Yitbarek, A., Laursen, A.,Taha-Abdelaziz, K. *et al.* (2019) Dietary selenium supplementation enhances antiviral immunity in chickens challenged with low pathogenic avian influenza virus subtype H9N2. *Veterinary Immunology and Immunopathology* 207, 62–68.

Simesen, M.G., Nielsen, H.E., Danielsen, V., Gissel-Nielsen, G., Hjarde, W., Leth, T. and Basse, A. (1979) Selenium and vitamin E deficiency in pigs 2. Influence on plasma selenium, vitamin E, ASAT and ALAT and on tissue selenium. *Acta Veterinaria Scandinavica* 20, 289–305.

Slaweta, R., Wasowiez, W. and Laskowska, T. (1988) Selenium content, glutathione peroxidase activity and lipid peroxide level in fresh bull semen and its relationship to motility of spermatozoa after freezing and thawing. *Journal of Veterinary Medicine, Animal Physiology, Pathology and Clinical Veterinary Medicine* 35, 455–460.

Slen, S.B., Demuriren, A.S. and Smith, A.D. (1961) A note on the effects of selenium on wool growth and body gains in sheep. *Canadian Journal of Animal Science* 41, 263–265.

Small, J.N.W., Burke, L., Suttle, N.F., Bain, M.S., Edwards, J.G. and Lewis, C.J. (1996) Seasonal fluctuations in subnormal tocopherol concentrations detected in bovine and ovine samples submitted by Veterinary Investigation Cenres (VIC) throughout Great Britain during 1995. In: *Proceedings of the XIX World Buiatrics Congress, Edinburgh, Volume 2.* British Cattle Veterinary Association, pp. 413–416.

Smith, G.M. and Allen, J.G. (1997) Effectiveness of α-tocopherol and selenium supplements in preventing lupinosis-associated myopathy in sheep. *Australian Veterinary Journal* 75, 341–437.

Smith, K.L., Hogan, J.S. and Weiss, W.P. (1997) Dietary vitamin E and selenium affect mastitis and milk quality. *Journal of Animal Science* 75, 1659–1665.

Stabel, J.R. and Spears, J.W. (1993) Role of selenium in immune responsiveness and disease resistance. In: Kurfield, D.M. (ed.) *Human Nutrition – A Comprehensive Treatise, Vol. 8. Nutrition and Immunology*. Plenum Press, New York, pp. 333–355.

Stevens, J.B., Olsen, W.C., Kraemer, R. and Archaublau, J. (1985) Serum selenium concentrations and glutathione peroxidase activities in cattle grazing forages of various selenium concentrations. *American Journal of Veterinary Research* 46, 1556–1560.

Stewart, M.S., Spallholz, J.E., Neldner, K.H. and Pence, B.C. (1999) Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. *Free Radical Biology and Medicine* 26, 42–48.

Stewart,W.C., Bobe, G., Pirelli, G.J., Mosher, W.D. and Hall, J.A. (2012) Organic and inorganic selenium. III. Ewe and progeny performance. *Journal of Animal Science* 90, 4536–4543.

Sun, P., Wang, J., Liu, W., Bu, D.P., Liu, S.J. and Zhang, K.Z. (2017) Hydroxy-selenomethionine: a novel organic selenium source that improves antioxidant status and selenium concentrations in milk and plasma of mid-lactation dairy cows. *Journal of Dairy Science* 100, 9602–9610.

Sunde, R.A. and Raines, A.M. (2011) Selenium regulation of the selenoprotein and nonselenoprotein transcriptomes in rodents. *Advances in Nutrition* 2, 138–150.

Sunde, R.A., Evenson, J.K., Thompson, K.M. and Sachdev, S.W. (2005) Dietary selenium requirements based on glutathione peroxidase-1 activity and mRNA levels and other selenium-dependent parameters are not increased by pregnancy in rats. *Journal of Nutrition* 135, 2144–2150.

Sunde, R.A., Sunde, G.R., Sunde, C.M., Sunde, M.L. and Evenson, J.K. (2015) Cloning, sequencing, and expression of selenoprotein transcripts in the turkey (Meleagris gallopavo). *PLOS ONE* 10(6), e0129801. DOI: 10.1371/journal.pone.0129801

Sunde, R.A., Li, J.-L. and Taylor, R.M. (2016) Insights for setting of nutrient requirements, gleaned by comparison of selenium status biomarkers in turkeys and chickens versus rats, mice, and lambs. *Advances in Nutrition* 7, 1129–1138.

Surai, P.F. (2006) *Selenium in Animal and Human Health*. Nottingham University Press, Nottingham, UK.

Surai, P.F., Kostjuk, I., Wishart, A.M., McPherson, A., Speak, B.K. *et al*. (1998) Effect of vitamin E and selenium supplementation of cockerel diets on glutathione peroxidase activity, and lipid peroxidation susceptibility in sperm, testes and liver. *Biological Trace Element Research* 64, 119–132.

Suttle, N.F. (2010) Selenium. In: *Mineral Nutrition of Livestock*, 4th edn. CAB International, Wallingford, UK, pp.389–392.

Suttle, N.F., Jones, D.G., Woolliams, C. and Woolliams, J.A. (1987) Heinz body anaemia in copper and selenium deficient lambs grazing improved hill pastures. *British Journal of Nutrition* 58, 539–548.

Swain, B.K., Johri, T.S. and Majumdar, S. (2000) Effect of supplementation of vitamin E, selenium and their different combinations on the performance and immune response of broilers. *British Poultry Science* 41, 287–292.

Swecker, W.S., Thatcher, C.D., Eversole, D.E., Blodgett, D.J. and Schurig, G.G. (1995) Effect of selenium supplementation on colostral IgG concentration in cows grazing selenium deficient pastures and on post-suckle serum IgG concentration in their calves. *American Journal of Veterinary Research* 56, 450–453.

Tasker, J.B., Bewick, T.D., Clark, R.G. and Fraser, A.J. (1987) Selenium response in dairy cattle. *New Zealand Veterinary Journal* 35, 139–140.

Thompson, J.N. and Scott, M.L. (1969) Role of selenium in the nutrition of the chick. *Journal of Nutrition* 97, 335–342.

Thompson. J.N. and Scott, M.L. (1970) Impaired lipid and vitamin E absorption related to atrophy of the pancreas in selenium deficient chicks. *Journal of Nutrition* 100, 797–809.

Tripp, M.J., Whanger, P.D. and Schmitz, J.A. (1993) Calcium uptake and ATPase activity of sarcoplasmic reticulum vesicles isolated from control and selenium deficient lambs. *Journal of Trace Elements and Electrolytes in Health and Disease* 7, 75–82.

Turner, R.J., Weiner, J.H. and Taylor, D.E. (1998) Selenium metabolism in Escherichia coli. *Biometals* 11, 223–227.

Ullrey, D.J. (1974) The selenium deficiency pattern in animal agriculture. In: Hoekstra, W.G. and Ganther, H.E. (eds) *Proceedings of the Second International Symposium on Trace Elements in Man and Animals, Wisconsin*. University Park Press, Baltimore, Maryland, pp. 275–294.

Ullrey, D.J. (1987) Biochemical and physiological indicators of selenium status in animals. *Journal of Animal Science* 65, 1712–1726.

Underwood, E.J. and Suttle, N.F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Van Ryssen, J.B.J., van Malsen, P.M. and Hartman, F. (1998) Contribution of dietary suphur to the interaction between selenium and copper in sheep. *Journal of Agricultural Science, Cambridge* 130, 107–114.

Van Vleet, J.F., Carlton, W. and Olander, H.J. (1970) Hepatosis dietetica and mulberry heart disease associated with selenium deficiency in Indiana swine. *Journal of the American Veterinary Medicine Association* 157, 1208–1219.

Van Vleet, J.F., Meyer, K.B. and Olander, H.J. (1973) Control of selenium-vitamin E deficiency in growing swine by parenteral administration of selenium: vitamin E preparations to baby pigs or to pregnant sows and their baby pigs. *Journal of the American Veterinary Medical Association* 163, 452–456.

Vendeland, S.C., Butler, J.A. and Whanger, P.D. (1992) Intestinal absorption of selenite, selenate and selenomethionine in the rat. *Journal of Nutritional Biochemistry* 3, 359–365.

Vouduri, A., Chadio, S.E., Mengatos, J.G., Zervas, G.P., Nicol, F. and Arthur, J.R. (2003) Selenoenzyme activities in selenium- and iodine-deficient sheep. *Biological Trace Element Research* 94, 213–224.

Wahlstrom, R.C. and Olson, O.E. (1959) The effect of selenium on reproduction in swine. *Journal of Animal Science* 18, 141–145.

Wang, Y.H., Leibholz, J., Bryden, W.L. and Fraser, D.R. (1996) Lipid peroxidation status as an index to evalu-

ate the influence of dietary fats on vitamin E requirements of young pigs. *British Journal of Nutrition* 75, 81–95.

Waschulewski, I.H. and Sunde, R.A. (1988) Effect of dietary methionine on utilisation of dietary selenium from dietary selenomethionine for glutathione peroxidase in the rat. *Journal of Nutrition* 118, 367–374.

Weiss, W.P. (1998) Requirements of fat-soluble vitamins for dairy cows: a review. *Journal of Dairy Science* 81, 2493–2501.

Weiss, W.P., Hogan, J.S., Todhunter, D.A. and Smith, K.L. (1997) Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. *Journal of Dairy Science* 80, 1728–1737.

Weitzel, F., Ursini, F. and Wendel, A. (1990) Phospholipid hydroperoxide glutathione peroxidase in various mouse organs during selenium deficiency and repletion. *Biochemica et Biophysica Acta* 1036, 88–94.

Whanger, P.D. (2002) Selenocompounds in plants and animals and their biological significance. *Journal of the American College of Nutrition* 21, 223–232.

Whanger, P.D., Weswig, P.H., Schmitz, J.A. and Oldfield, J.E. (1977) Effects of selenium and vitamin E on blood selenium levels, tissue glutathione peroxidase activities and white muscle disease in sheep fed purified or hay diets. *Journal of Nutrition* 107, 1298–1307.

Whelan, B.R., Barrow, N.J. and Peter, D.W. (1994a) Selenium fertilizers for pastures grazed by sheep. I. Selenium concentrations in whole blood and plasma. *Australian Journal of Agricultural Research* 45, 863–875.

Whelan, B.R., Barrow, N.J. and Peter, D.W. (1994b) Selenium fertilizers for pastures grazed by sheep. II. Wool and liveweight responses to selenium. *Australian Journal of Agricultural Research* 45, 875–886.

White, C.L., Cadwalader, T.K., Hoekstra, W. and Pope, A.L. (1998) The metabolism of ^{75}Se-selenomethionine in sheep given supplementary copper and molybdenum. *Journal of Animal Science* 67, 2400–2408.

White, P.J. and Broadley, M.R. (2009) Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist* 182, 49–84.

Whitacre, M.E., Combs, G.F., Combs, S.B. and Parker, R.S. (1987) Influence of dietary vitamin E on nutritional pancreatic atrophy in selenium-deficient chicks. *Journal of Nutrition* 117, 460–467.

Wichtel, J.J. (1998) A review of selenium deficiency in grazing ruminants 2: towards a more rational approach to diagnosis and prevention. *New Zealand Veterinary Journal* 46, 54–58.

Wichtel, J.J., Craigie, A.L., Varela-Alvarez, H. and Williamson, N.B. (1994) The effect of intra-ruminal selenium pellets on growth rate, lactation and reproductive efficiency in dairy cattle. *New Zealand Veterinary Journal* 42, 205–210.

Wichtel, J.J., Craigie, A.L., Freeman, D.A., Varela-Alvarez, H. and Williamson, N.B. (1996) Effect of selenium and iodine supplementation on growth rate and on thyroid and somatotropic function in dairy calves at pasture. *Journal of Dairy Science* 79, 1865–1872.

Wichtel, J.J., Grace, N.D. and Firth, E.C. (1998) The effect of injectable barium selenate on the selenium status of horses on pasture. *New Zealand Veterinary Journal* 42, 205–210.

Wilkins, J.F. and Kilgour, R.J. (1982) Production responses to selenium in northern New South Wales. 1. Infertility in ewes and associated production. *Australian Journal of Experimental Agriculture and Animal Husbandry* 22, 18–23.

Wilkins, J.F., Kilgour, R.J., Gleeson, A.C., Cox, R.J., Geddes, S.J. and Simpson, I.H. (1982) Production responses to selenium in northern New South Wales. 2. Liveweight gain, wool production and reproductive performance in young Merino ewes given selenium and copper supplements. *Australian Journal of Experimental Agriculture and Animal Husbandry* 22, 24–28.

Wilson, P.R. and Grace, N.D. (2001) A review of tissue reference values used to assess the trace element status of farmed deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 49, 126–132.

Wuryastuti, H., Stowe, H.D., Bull, R.W. and Miller, E.R. (1993) Effects of vitamin E and selenium on immune responses of peripheral blood, colostrum and milk leukocytes of sows. *Journal of Animal Science* 71, 2464–2472.

Yaeger, M.J., Neiger, R.D., Holler, L., Fraser, T.L., Hurley, D.J. and Palmer, I.S. (1998) The effect of selenium toxicosis on pregnant beef cattle. *Journal of Veterinary Diagnostic Investigation* 10, 268–273.

Yoon, I. and McMillan, E. (2006) Comparative effects of inorganic and organic selenium on selenium transfer from sows to nursing pigs. *Journal of Animal Science* 84, 1729–1733.

Zachara, B.A., Frafikowska, V., Lejman, H., Kimber, C. and Kaptur, M. (1993) Selenium and glutathione peroxidase in blood of lambs born to ewes injected with barium selenate. *Small Ruminant Research* 11, 135–141.

Zervas, G.P. (1988) Use of soluble glass boluses containing Cu, Co and Se in the prevention of trace-element deficiencies in goats. *Journal of Agricultural Science, Cambridge* 110, 155–158.

Zhang, J.L., Xu, B., Huang, X.D., Gao, Y.H., Chen, Y. and Shan, A.S. (2016) Selenium deficiency affects the mRNA expression of inflammatory factors and selenoprotein genes in the kidneys of broiler chicks. *Biological Trace Element Research* 171, 201–207.

Zuberbuehler, C.A., Messelkommer, R.E., Arnold, M.M., Forrer, R.S. and Wenk, C. (2006) Effects of selenium depletion and selenium repletion by choice feeding on selenium status of young and old laying hens. *Physiology and Behavior* 87, 430–440.

16 Zinc

Introduction

Sometime after the discovery that zinc was essential for growth and health in rats (Todd *et al.*, 1934) and present in an enzyme, carbonic anhydrase (CA) (Keilin and Mann, 1940), found in blood, studies were made in livestock. Experimental deprivation in chicks (O'Dell and Savage, 1957), pigs (Stevenson and Earle, 1956), lambs (Ott *et al.*, 1964) and calves (Mills *et al.*, 1967) had similar consequences: loss of appetite, growth depression, skin abnormalities and reproductive failure. Skin disorders were being encountered in the field as parakeratosis, a thickening and hardening of the skin, in pigs (Tucker and Salmon, 1955) and poultry (Ziegler *et al.*, 1961), given grain-based diets, rich in Ca. The disorders were cured and prevented by Zn supplements, which countered an antagonism from phytate, the major source of Phosphorous (P) in feed grains (Humer *et al.*, 2015). Parakeratosis was rarely seen in ruminants, which degrade phytate in the rumen. Subsequent biochemical studies revealed >300 Zn-dependent enzymes of diverse structure and function (Vallee and Falchuk, 1993) and a far greater number of functional Zn proteins (Coleman, 2002). The biochemical Zn armoury enables animals to break homeostatic conventions and increase Zn in and within cells when dietary Zn is lacking. Even plasma Zn can buck convention, unless there are offspring to raise (McCormick *et al.*, 2015), making it difficult to identify the rate-limiting factor in Zn insufficiency. The quest for an ideal marker for zinc status is ongoing, spurred by fears that many malnourished children around the globe are susceptible to infections because their diets lack zinc (King *et al.*, 2016). Recent studies of zinc in livestock nutrition have lacked such impetus. Chelated Zn supplements have been investigated in the vain hope of subverting the intricacies of homeostatic control. Therapeutic properties, known since ancient times, were exploited by adding Zn at near-molar concentrations to the diets of recently weaned piglets to prevent diarrhoea and improve health (Hill *et al.*, 2001): microbicidal side-effects, which generated antiobiotic-resistant strains of bacteria, have halted that line of investigation (Vahjen *et al.*, 2015). The tide is turning, with genomic studies of marginal lack of dietary Zn in pigs (Brugger and Windisch, 2019a, b), which may have relevance to the zinc nutrition of infants.

Functions of Zinc

Zinc is arguably the most influential of minerals, known to catalyse, co-catalyse or provide structural integrity to around 300 enzymes in all major categories, from hydrolases to transferases (Choi *et al.*, 2018): ten times as many transcription factors require Zn for structural integrity (King *et al.*, 2016). Tetrahedral co-ordination of Zn to cysteine and histidine residues creates 'zinc-finger' domains that stabilize protein folds (Berg, 1990). Most communication and metabolic pathways in cells are Zn-dependent (Beattie and Kwun, 2004; Cousins *et al.*, 2006; Choi *et al.*, 2018). Amongst new functions that require zinc is the calcification of bone matrix (Alcantara *et al.*, 2011). The long-established function of zinc as catalyst of angiotensin-converting enzyme 2 (ACE 2) has a broader facet: ACE 2 hydrolyses other peptides, including some involved in humoral and innate immunity (Bernstein *et al.*, 2018). The list of critical functions that might limit health and production in livestock deprived of zinc is growing.

Gene expression

Fetal growth is especially affected by lack of zinc (Hurley, 1981), reflecting roles in DNA synthesis and nucleic acid metabolism that are most influential when cells are rapidly dividing (Chesters, 1992).

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Zinc insufficiency affects membrane signalling systems and intracellular second messengers that coordinate cell proliferation in response to insulin-dependent growth factor (IGF-I) (McDonald, 2000). Zinc regulates genes involved in reduction–oxidation (redox) reactions, growth and energy utilization, responses to stress and infection, such as expression of the T-cell cytokine receptor, uroguanylin (Cousins *et al.*, 2003). Metal responsive transcription factor (MTF-1) is a zinc-finger protein that activates metallothein and other target genes: without MTF-1, mice die from liver degeneration (King *et al.*, 2016). Ionic Zn^{2+} controls numerous cell-signalling pathways by modulating kinase and phosphorylase activities.

Appetite control

Extreme sensitivity of appetite is unique to zinc insufficiency and emerged from studies with rats and mice (O'Dell and Reeves, 1989). Pattern, preference and rate of food intake were all affected in acute zinc deprivation on diets almost devoid of zinc. Acutely deprived rats nibble at food rather than 'meal eat', avoid carbohydrate and prefer protein and fat (Kennedy *et al.*, 1998). Force-feeding a low-Zn diet exacerbates the harm caused (Flanagan, 1984). Deprivation on diets containing 3–6mg Zn kg^{-1}DM avoided such complications but the description of such diets as 'marginal' (e.g. Kwun *et al.*, 2007) is misleading because growth and development are unequivocally retarded in the longer term. Such deprivation has multiple effects: expressions of cholecystokinin, the appetite-regulating hormone (Cousins *et al.*, 2003) and leptin, a cytokine whose secretion from adipocytes acts as a satiety signal, are increased (Kwun *et al.*, 2007); expression of pyruvate kinase, highly regulated by insulin, is reduced (Beattie *et al.*, 2008) and concentrations of neurotransmitters in the brain are changed (Kwun *et al.*, 2007). Orally administered Zn can change receptiveness of the hypothalamus to leptin and another appetite-regulating peptide, grehlin, via the vagus nerve that innervates the stomach: intraperitoneal Zn has no such effect (Suzuki *et al.*, 2011). However, impaired digestibility may also lower feed intake in the pig (Brugger and Windisch, 2016).

Fat and protein absorption

Low plasma and liver concentrations of fat-soluble vitamins, A and E in Zn-deprived animals suggested a role in fat absorption. The pancreas secretes Zn-dependent phospholipase A_2 (Kim *et al.*, 1998), which hydrolyses phosphatidylcholine, and duodenal infusion of hydrolysed phosphatidylcholine greatly increased absorption of fats and fat-soluble vitamins in rats, 'marginally deprived' of Zn (Noh and Koo, 2001). Zinc deprivation may also restrict synthesis of retinol-binding protein, which transports vitamin A (King *et al.*, 2016). Adding 50 or 100mg Zn kg^{-1}DM to a corn/soybean meal (SBM) diet increased serum vitamin A and E in broilers (Kakhki *et al.*, 2016). Eight days zinc deprivation of young piglets was sufficient to decrease activities of carboxypeptidases A and B, elastase and α-amylase and decrease concentrations of trypsin and chymotrpsinin in pancreatic tissue (Brugger and Windisch, 2016).

Antioxidant and anti-inflammatory defences

Zinc acts as a 'pro-antioxidant' in several ways. Zinc gives structural stability to a superoxide dismutase (CuZnSOD), thus indirectly protecting cell membranes from oxidative damage by superoxide radicals. Zinc also protects cell surfaces against lipid peroxidation by redox-active elements, blocking binding sites for pro-oxidant iron (Zago and Oteiza, 2001) and copper, acting synergistically with vitamin E. Zinc deprivation increases susceptibility of endothelial cells to oxidant stress (Beattie and Kwun, 2004; Choi *et al.*, 2018). Zinc may also enhance antioxidant defences by inducing metallothionein (MT), a zinc- and thiol-rich metalloprotein that scavenges free radicals. Metallothionein may act as a redox sensor and signalling switch in cells (Beattie and Trayhurn, 2002). Such mechanisms have been invoked to explain protective effects of zinc against apoptosis in a diabetic rat model (Tunçdemir *et al.*, 2017) and may affect livestock health (see p. ff). Zinc supplementation of heat-stressed broilers reduced serum malondialdehyde, indicator of oxidative stress, and lipid oxidation in liver, breast and thigh muscle, while performance was not affected (Akhavan-Salamat and Ghasemi, 2019). Zinc supplementation had similar antioxidant effects in unstressed broilers (Kakhki *et al.* 2016) and turkeys (Jankowskia *et al.*, 2019). In humans and small rodents, zinc deprivation decreases expression of anti-inflammatory cytokines following acute challenge with inflammatory agents such as lipopolysaccharide (LPS) (Prasad,

2007; King *et al*., 2016: Choi *et al*., 2018) but it is unclear whether such effects impact health and performance (Brugger and Windisch, 2017).

Immune defence

Zinc is believed to regulate virtually all aspects of innate and adaptive immunity via a network based on transport proteins (ZnT and ZIP), metallothionein, free Zn^{2+} ion concentrations (Haase and Rink, 2014), ACE 2 (Bernstein *et al*., 2018) and other zinc enzymes (Read *et al*., 2019). Thus, zinc influences the immune cascade (King *et al*., 2016). Zinc supplementation improved cellular and humoral immune responses in broiler chicks (Gajula *et al*., 2011). Zinc also influences responses to viral infection but is needed to sustain some of the same enzymes in viruses and animals (Read *et al*., 2019). However, direct evidence of increased susceptibility to infection remains rare in zinc-deprived subjects, animal or human. Zinc supplementation aids infant recovery from infections, including those causing diarrhoea and pneumonia (King *et al*., 2016), but gross malnutrition and growth spurts during recovery make it unlikely that these responses are wholly attributable to previous, uncomplicated lack of dietary Zn. Reports that bolus doses of zinc control diarrhoea in new born calves (e.g Chang *et al*., 2020) have a pharmacological rather than immuno-stimulatory explanation (p. 434).

Bone remodelling, cardio-respiratory and kidney function

Zinc is needed for all these processes through its presence in CA, an enzyme that converts CO_2 to HCO_3^-, while generating protons: thus, CA controls intracellular pH. Most of the Zn in erythrocytes is present as CA. Of 16 isomeric forms, five are found in membranes, where they preserve acic-base and electrolyte (Na^+/K^+) balances. The presence of CA in osteoclasts allows Zn to influence the remodelling of trabecular bone by controlling pH (O'Connor *et al*., 2020). Wherever CA is found in the body, metabolic rate is high (Purkerson and Schwartz, 2007). Human volunteers experienced cardio-respiratory problems when exercising after a period of zinc depletion (Lukaski, 2005), the first sign of any dysfunction. Ubiquitous CA is particularly abundant in the nephron, where Na and K retention are controlled. Furthermore, CA collaborates with the membrane protein aquaporin to control water retention (Devishree *et al*., 2018). Marginal Zn deprivation increased urine volume and urinary Na:K in steers (Engle *et al*., 1997).

- The importance of CA in livestock lacking dietary Zn may have been overlooked (Brugger and Windisch, 2017).

Clinical Consequences of Zinc Deprivation

Anorexia

Loss of appetite is an early sign of zinc deprivation in lambs, which begin nibbling at food (Droke *et al*., 1993a) but impaired digestibility may be the driving force, at least in pigs (Brugger and Windisch, 2016). Pair-feeding studies show that many adverse effects of severe zinc deprivation were secondary to loss of appetite (e.g. Table 16.1), including those on male fertility (Neathery *et al*., 1973b; Martin and White, 1992).

Table 16.1. Pair-fed baby pigs deprived of zinc showed that poor growth was due largely to poor appetite, while poor food conversion, parakeratosis and raised white cell count were due solely to lack of zinc. (From Miller *et al*., 1968)

Dietary Zn (mg kg^{-1}DM)	12		100		100
Food intake	*Ad lib*		Restricted		*Ad lib*
Liveweight gain (g d^{-1})	25	<	88	<	288
Food consumption (g d^{-1})	170	=	170	<	430
Gain/food	0.14	<	0.57	<	0.67
Parakeratosis (%)	100	>	0	=	0
Serum Zn (mg l^{-1})	0.22	<	0.57	<	0.67
Alkaline phosphatase (U l^{-1})	0.4	<	5.5	<	7.0
Leucocyte count ($10^3/mm^3$)	17.0	>	12.9	=	11.8
Liver alcohol dehydrogenase (Δ OD $min^{-1}mg^{-1}$protein)	0.060	=	0.061	=	0.063

Abnormalities of skin and appendages

The parakeratotic lesions that affect stratified epithelia of the skin, tongue, oesophagus and genitalia in animals lacking dietary Zn (e.g. Demertsis, 1973) resembled those induced by vitamin A deficiency (Smith *et al.*, 1976; Chhabra and Arora, 1993). Evidence that rate of healing of artificially inflicted skin wounds is retarded in zinc-deprived animals is conflicting (Kogan *et al.*, 2017) and may depend on vitamin A status:

- Skin infections may be slower to heal in animals grazing brown rather than green pastures or given grain without added vitamins as a drought supplement.

Excessive salivation was an early sign, peculiar to severely deprived ruminants (Mills *et al.*, 1967; Apgar *et al.*, 1993) and may reflect a combination of copious saliva production and difficulty in swallowing. In horned lambs, normal ring structure disappeared and horns were ultimately shed, leaving soft, haemorrhagic, spongy outgrowths that continually haemorrhaged: hoof structure was also affected by severe deprivation (Mills *et al.*, 1967). Wool fibres lose crimp, become thin and loose and the whole fleece may be shed: Hoof, horn and wool growth fail because of impaired keratin synthesis but such abnormalities can have other causes, such as lack of dietary protein.

Skeletal disorders

Size and strength of the femur was reduced in baby pigs deprived of zinc but comparisons with pair-fed controls indicated that reduced feed intake was responsible (Table 16.1) (Miller *et al.*, 1968). Thickening and shortening of long bones also occurred in chicks (O'Dell and Savage, 1957; O'Dell *et al.*, 1958). Severe abnormalities of head, limbs and vertebrae developed in chick embryos in eggs of severely deprived hens (Kienholz *et al.*, 1961). Zinc deprivation caused bowing of hind limbs, joint stiffness and swelling of hocks in calves (Miller and Miller, 1962). Such defects may arise from impaired calcification of bone matrix (p. 414).

Reproductive disorders

Lack of dietary zinc reduced litter size in early studies with pigs (Hoekstra *et al.*, 1967) and impaired hatchability in eggs from marginally deprived hens (Kienholz *et al.*, 1961). Hypogonadism occurs in zinc-deprived bull calves (Pitts *et al.*, 1966), kids (Miller *et al.*, 1964) and ram lambs (Underwood and Somers, 1969; Martin and White, 1992).[i] Testicular size and libido were reduced in male goats that were sexually mature when severely deprived of zinc (Neathery *et al.*, 1973b). However, when chronic deficiencies of zinc and/or vitamin A were compared in goats, spermatogenesis was improved by supplements of vitamin A but not zinc, in the presence of zinc-responsive skin lesions (Chhabra and Arora, 1993). Mild zinc deprivation in pregnant ewes does not cause congenital malformations, although numbers born and birth weights may be slightly reduced (Egan, 1972; Masters and Fels, 1980; Mahmoud *et al.*, 1983). Severe zinc deprivation during pregnancy increases mortality in newborn lambs and pregnancy toxaemia may occur as a secondary consequence of anorexia in the ewe (Apgar *et al.*, 1993).

Disease resistance

Increased susceptibility to infection is considered to be an early consequence of zinc deprivation in humans (Prasad, 2007), based partly on improved recoveries from infection in Zn-supplemented children (Fischer-Walker and Black, 2004; King *et al.*, 2016), but Zn was usually given with other nutrients and rehydration agents. Supporting evidence from studies involving specific infections in experiments is scant. In marginally depleted, mature mice, immunopathology was *secondary* to loss of appetite (Beach *et al.*, 1981). Severely or marginally Zn-deprived lambs lost appetite, grew poorly and developed skin lesions either before (Droke and Spears, 1993) or without (Droke *et al.*, 1993b) increases in susceptibility to infection. Supplementation of a diet marginal in Zn did not decrease the impact of coccicidial infection of broiler chicks: markers for cellular immunity were not enhanced but faecal oocyst excretion was reduced (Bun *et al.*, 2011). Mixed parasitic and microbial infections of the gut in chicks lowered serum Zn and selectively affected expression of transporters involved in zinc trafficking in the jejunum and caecal tonsils (He *et al.*, 2019). The hypozincaemic effect of infection has long been known and raises an important question:

- Is the alleged high prevalence of zinc deficiency in the human population exaggerated by ubiquitous infections?

Zinc supplementation did not improve humoral immune responses of marginally Zn-deficient pigs (Sun *et al.*, 2009). Chick growth was improved by Zn supplementation but antibody responses to sheep red blood cells (SRBC) were not (Mohanna and Nys, 1999). Responses of heifers to subcutaneous injection of phytohaemagglutinin (PHA) were impaired by Zn deprivation *before* loss of appetite and fall in plasma Zn (Engle *et al.*, 1997) but zinc proteinate improved growth without affecting most *in vitro* or *in vivo* immune responses in finishing steers: secretory immunoglobulin (IgA) was increased independently by infection and zinc supplementation (Spears and Kegley, 2002). Zinc supplementation improved plasma Ig in turkey poults but mortality was unaffected (Jankowskia *et al.*, 2019).

- The immune system is not consistently ultrasensitive to Zn deprivation under acute challenges (e.g. from SRBC, PHA and LPS).
- There is no evidence that reduced immune responses to acute antigen challenge indicate increased susceptibility to natural infections.
- Sensitivity of immune responses to lack of dietary Zn could indicate that they are expendable and sacrificed to conserve Zn for functions more essential to survival.

Accumulation of heavy metals

Zinc deprivation may enhance the risks of heavy metal toxicity. Liver and kidney mercury levels increased two- to threefold in rats when dietary Zn was reduced from 40 to 6mg kg^{-1}DM with 10mg Hg kg^{-1}DM present (Kul'kova *et al.*, 1993). The interaction may be mediated by decreases in the protective MTF-1, which is induced by cadmium (Gunther *et al.*, 2012). Marginal zinc deprivation may therefore be important in grazing livestock in areas of heavy metal pollution.

Subclinical and Secondary Consequences

Poor growth is attributable primarily to failure of appetite and reduced digestibility of carbohydrates, lipids and proteins (Brugger and Windisch, 2016). Poor capacity to absorb essential fatty acids and fat-soluble vitamins may contribute to clinical abnormalities, particularly those affecting the skin. Low plasma vitamin A status may cause night blindness in goats (Chhabra and Arora, 1993). Male infertility may be compromised because oxidant stress is uniquely high in spermatozoa.

- Support and confirmation of the above subclinical disorders as consequences of lack of Zn requires assays of a biochemical marker and a predetermined relationship between marker and disorder.

Occurrence of Zinc Insufficiency

Ruminants

While lack of Zn frequently limits yields of crops and pastures (Alloway, 2004; Cakmak, 2008), ruminant production is rarely constrained. Early reports of Zn-responsive abnormalities in cattle, some with relatively high pasture Zn,[ii] were not confirmed and analytical accuracy was questioned (ARC, 1980). Zinc-responsive foot lesions in housed young bulls (Demertzis, 1973) and broilers (p. 8) receiving adequate dietary Zn (30–50mg kg^{-1}DM) may have been attributable to anti-bacterial effects of impacted, Zn-enriched faeces. Forages in Idaho contained insufficient Zn (often <17mg kg^{-1}DM) for suckler cows and calves (Mayland *et al.*, 1980) but pastures of similar zinc status caused no problems in Scotland (Price and Humphries, 1980). It is possible that on clay soils, adsorption of Zn by ingested clay minerals induces a deficit in herbage Zn (Schlattl *et al.*, 2019). Heavy ewe and lamb mortality in a flock given hay from irrigated Rhodes grass pastures, containing 20mg Zn kg^{-1}DM and low in crude protein (95g kg^{-1}DM), was controlled by injecting Zn (Mahmoud *et al.*, 1983). Fertility was inconsistently improved by Zn supplementation in southern (Egan, 1972) and Western Australia (Masters and Fels, 1980, 1985) on pastures containing less Zn. Plentiful reserves of vitamins A and E in livestock grazing green swards may offset reductions in fat-soluble vitamin absorption, caused by marginal lack of Zn. Exposure to excess Cu and Cd may increase risk of disorder by increasing the mucosal binding of zinc by MT (Bremner, 1993). Adding Zn to cereal-based diets, containing around 20mg Zn kg^{-1}DM, has occasionally improved feed-conversion efficiency (FCE) in calves (Engle *et al.*, 1997) and growth rate in lambs (Fadayifar *et al.*, 2012). Growth rate and FCE were improved in cashmere goats by raising dietary Zn from 22mg Zn kg^{-1}DM during the cashmere growing period (Jia *et al.*, 2008). Wool yield, fibre

length, plasma Zn and plasma testosterone were improved in male cashmere goats when Zn was added to a diet containing 46mg kg^{-1}DM (Liu *et al.*, 2015): high rates of fibre growth coupled with high need for sperm development may have created uniquely high needs for Zn.

Non-ruminants

Risks of disorder are higher amongst non-ruminants than ruminants. Commercial, maize-plant protein blends may provide insufficient Zn for pigs and turkeys because phytate lowers zinc availability. Growth improvements from multi-element supplementation of starter pig rations (Veum *et al.*, 2009) were probably due to lack of Zn in an unsupplemented diet that contained only 23mg Zn kg^{-1}DM. Animals given more calcium than they require (e.g. 1% Ca for broiler chicks) and laying or breeder hens on diets high in Ca (3–4%) that they do require are at particular risk. Addition of ZnO to a corn/SBM ration, improved egg weight and fertility in breeder hens, though not when phytase was also added (Sharideh *et al.*, 2016). Risk of deprivation is lower when cereals with higher phytase contents than maize (e.g. wheat, triticale) are used. Footpad dermatitis is a welfare problem in poultry but claims for protective effects of supplementary Zn (Viera *et al.*, 2013) were not supported by the data. Zinc status can also be pushed from the marginal to inadequate by excesses of copper. Mortality and lesions similar to those of parakeratosis and rectifiable by Zn supplements were observed in growing pigs given 250mg Cu kg^{-1}DM as a growth stimulant (O'Hara *et al.*, 1960; Suttle and Mills, 1966). The need to routinely supplement plant-based diets with Zn will diminish as use of phytase supplements increases, as both Ca and Cu supplementation decrease and should insect protein replace vegetable protein. Natural corn/SBM mixtures often provide sufficient zinc for growing pigs.

Biochemical Consequences of Lack of Dietary Zinc

Identification of a biochemical state of insufficiency requires a comprehensive description and understanding of the limits of normality and how they are preserved.

Zinc homeostasis

Absorbed Zn is transported to the liver via the portal bloodstream loosely bound to albumin, which accounts for about two thirds of plasma Zn: the remainder includes Zn bound to α-2 macroglobulin and MT. At the liver, Zn is removed by hepatic MT and directed to functional and excretory pathways (Bremner, 1993; Lee *et al.*, 1994). Stressors such as infections reduce plasma Zn through the action of glucocorticoids and cytokines, which increase hepatic Zn by inducing hepatic MT synthesis (Cousins, 1996). Beyond the liver, albumin-bound plasma Zn is taken up by endocytosis (Rowe and Bobilya, 2000) and sequestrated by a family of at least 14 Zip proteins and shuttled by transporter (ZnT) proteins: some are organ- and organelle-specific and variously stimulated by high and low Zn intakes (Fig 16.1).[iii] Excretion is primarily instigated by the exocrine pancreas, where ZnT2 pumps Zn into zymogen granules (Kondaiah *et al.*, 2019). Muscle, rich in Zn, provides the largest exchangeable body pool (Brugger and Windisch, 2019), followed by bone: the large amounts in the fleece of sheep are non-exchangeable. Most of the zinc in blood (80%) is present in erythrocytes as carbonic anhydrase, with far less in CuZnSOD.[iv] Plasma Zn is not tightly regulated and affected by many factors other than dietary Zn,[vi] some acting indirectly by lowering food intake. Endogenous losses occur principally via faeces, uninfluenced by Zn intakes that are well above requirements (e.g. Schryver *et al.*, 1980; Suttle *et al.*, 1982)[vii] but increase when intakes are excessive (Kondaiah *et al.*, 2019). Low apparent absorption of Zn (AA_{Zn}) in pigs on diets high in phytate (Brugger *et al.*, 2014) may indicate that phytate binds endogenous Zn, secreted by the pancreas, thus increasing faecal endogenous loss (FEL_{Zn}).

Depletion

The sequence of biochemical changes in livestock deprived of zinc is typical of a Type 2 nutrient (Golden, 1996) (Fig. 3.2), having a minimal depletion phase. Capacity to store and mobilize Zn is limited and there is no accumulation of a fetal hepatic reserve in late pregnancy, at least in sheep (Williams *et al.*, 1978). Egg yolk constitutes an important reserve for the chick embryo but a threefold increase in dietary Zn above need only increased yolk Zn from 67 to 72 ± 0.4mg kg^{-1}DM (Mayer *et al.*, 2018). Furthermore, exposure to

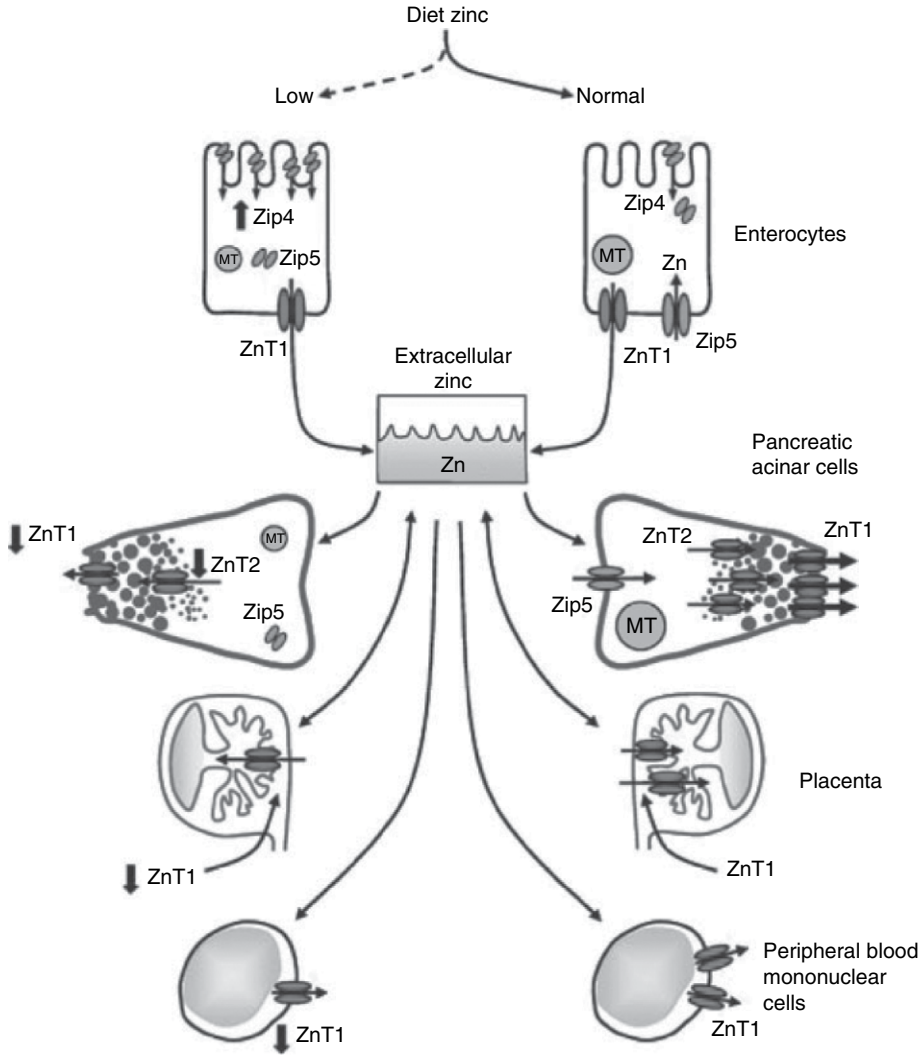

Fig. 16.1. Changes in murine zinc transporter (T), Zip protein and metallothionein (MT) expression in response to dietary Zn content. When dietary Zn is low, enterocytes increase Zip4 expression in the apical membrane and Zip5 is internalized to increase intestinal absorption, while decreases in ZnT1 and ZnT2 expression in pancreatic acinar cells reduce intestinal losses of zinc; elsewhere, MT and ZnT1 expression in placenta and peripheral blood mononuclear cells are reduced, conserving Zn in cells with a high turnover, including those of the immune system. With adequate dietary Zn, enterocytes express more MT and Zip5 is localized to the basolateral membrane (Lichten and Cousins, 2009) but lactation may change the normal rules of homeostasis (McCormick *et al*., 2015).

excess Zn in early development may decrease subsequent retention.[v] Feeding chicks diets with 300 mg rather than 10.6mg Zn kg^{-1}DM for eight days merely delayed growth retardation by three days (Emmert and Baker, 1995) but yolk Zn is decreased by deprivation (Mayer *et al*., 2018). Mobilization of bone Zn during chronic depletion of pregnant ewes may delay onset of deficiency and explain tolerance of newborn lambs to maternal Zn depletion (Masters and Moir, 1983). Studies with rats suggest that Zn can be rapidly mobilized from trabecular bone when the diet lacks Zn (Brugger *et al*., 2018). Femur Zn spontaneously decreased during lactation in mice given diets adequate in Zn but not

when dietary Zn was halved (McCormick *et al.*, 2015). Studies with human volunteers (Ryu *et al.*, 2001) indicated early upregulation of mucosal, Zip and ZnT genes and upregulation of mucosal and pancreatic genes was evident in Zn-deprived, weanling pigs (Fig. 16.2). Any anorexia may release Zn from the skeleton by stimulating bone resorption.

Deficiency

Plasma Zn soon decreases in all species. In lambs, plasma Zn decreased by about 20% within a week when Zn was reduced from the equivalent of about 12 to 4mg kg^{-1}DM: growth rate was maintained for a further week, by which time plasma Zn had fallen below 0.4 mg (6.2 µmol) l^{-1} (Mills *et al.*, 1967; White *et al.*, 1994), a threshold for growth-related dysfunction (White, 1993). Similar relationships were found in marginally deprived piglets (Brugger *et al.*, 2014; Brugger and Windisch, 2016, 2019). Rate of decline in plasma Zn and other parameters of Zn status depends on the intensity of deprivation, i.e. the margin between daily supply and demand. In young piglets, given a diet high in phytate and without phytase, stepped reductions from 88 to 28mg Zn kg^{-1}DM reduced plasma Zn by 80% after eight days but there were many accompanying changes, as illustrated in Fig. 16.2 (Brugger *et al.*, 2014). Some pre-clinical changes correlated with decreases in pancreatic Zn and all were proportional to severity of deprivation (Brugger and Windisch, 2016). Zinc was redistributed from skin, liver, kidney and spleen, to cardiac and skeletal muscle, thymus and immune cells, where concentrations increased despite short-term deprivation (Brugger and Windisch, 2019). The authors describe the situation as one of 'marginal deficiency' but describe responses to deprivation of moderate intensity, due to low availability of dietary Zn. Zinc enzyme activities were reduced in piglets, as in previous studies with various species (see Suttle, 2010 for review). In marginally deprived lambs, plasma alkaline phosphatase (AP), vitamin A and CuZnSOD all declined (Fadayifar *et al.*, 2012). However, enzymes and vitamins may be present in excess of need and reductions do not necessarily indicate dysfunction. Changes in non-enzymic zinc proteins may be more important.[viii] In particular, Zn-deprivation uncharacterstically lowers expression of DMT1 in enterocytes and changes the expression of Fe-transporting proteins, causing Fe to accumulate in the intestinal mucosa (Kondaiah *et al.*, 2019). Reduced expression of Zip4 in enterocytes plays a key role and appearance of extracellular vesicles (ESV), containing Zn,

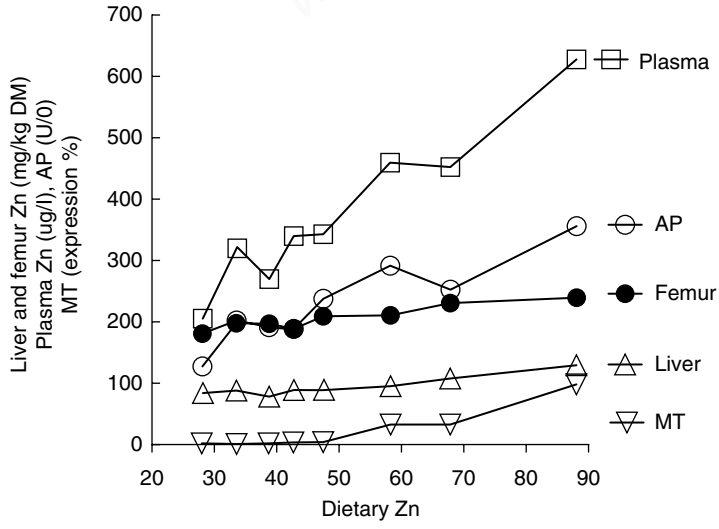

Fig. 16.2. A 'snapshot' of biochemical changes in young pigs, eight days after partially or wholly withdrawing supplementary inorganic zinc from what proved to be a marginally deficient diet: subliminal increases in liver and femur Zn are typical of Group 2 minerals; an index with a breakpoint in response (MT) indicates systemic recognition of a change in status, whereas plasma Zn and alkaline phosphatase (AP) largely reflect Zn intake. (From Brugger *et al.*, 2014; Brugger and Windisch, 2016, 2019)

indicates intracellular redistribution of Zn (King *et al.*, 2016). Activity of ACE 2 decreased at a slower rate than plasma Zn in pregnant ewes deprived of Zn (Fig. 16.3). Production of apoenzyme gave a further indication of inadequate dietary Zn and restoration of activity *in vitro* provided a simple confirmatory test (Apgar *et al.*, 1993), which should be followed up.

Dysfunction

In piglets, decreased digestibilities of dietary DM, protein and fat, the latter from 77% to 64% (Fig. 16.4) indicated pancreatic dysfunction as a possible rate-limiting factor. Low elastase activity may have compromised basal membrane integrity in pancreatic acinar cells, a vulnerable site in the Cu-deprived calf. Inflammatory effects of low Zn status may trigger a secondary dysfunction in Fe metabolism, redistributing Fe to the liver and down-regulating DMT1 (Kondaiah *et al.*, 2019). Monitoring changes in the differential display of mRNA (e.g. Huang *et al.*, 2007) identified further possible rate-limiting, Zn-dependent functions in chicks. Activities of CuZnSOD were reduced in testes, intestine and liver of Zn-deprived goats (Chhabra and Arora, 1993) but may only disturb cell functions at times and sites of peak activity (e.g. spermatozoa). A study of zinc depletion in human volunteers may have significance in this regard (Lukaski, 2005). After nine weeks depletion, erythrocyte Zn, serum Zn and CA activity had all fallen by about 20%, without evidence of dysfunction. During exercise, however, cardiorespiratory function was compromised. Areas requiring future study include:

- carbonic anhydrase activity in zinc-deprived livestock;
- the importance of metabolic activity on zinc-dependent pathways;
- Fe-metabolism in livestock deprived of Zn; and
- comparing threshold figures for genomic responses to dietary Zn at different sites, as indicators of requirement (Brugger and Windisch, 2019).

However, such thresholds do not necessarily rank performance and health in the same order.

- A solution to this dilemma may be to correlate new biochemical and molecular markers with performance *within* large groups on a marginal dietary Zn supply that has begun to retard growth or impair FCE in some chronically deprived individuals.

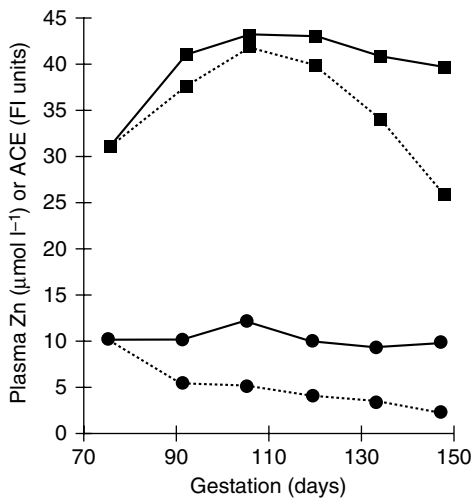

Fig. 16.3. Activity of angiotensin-converting enzyme (ACE) (■) declined at a later stage during zinc deprivation (broken line) than plasma Zn (●) in pregnant ewes. Zn-supplemented ewes are represented by solid line. (Data from Apgar *et al.*, 1993)

Disorder

Teratogenic effects of maternal zinc deprivation in rats provided unequivocal evidence of transition from dysfunction to disorder and were attributed to impaired DNA synthesis due to low fetal thymidine kinase activity in the central nervous system (Hurley, 1981). However, other zinc-responsive enzymes (e.g. DNA polymerases) and proteins were probably reduced in activity and abundance. The fundamental disorder may be in gene expression, through impairment in the G1 phase of the cell cycle (Lo *et al.*, 2020) but skeletal development may also be compromised.

- Deprivation *in utero* can cause disorder in later life (e.g. in immunocompetence).
- Lesions of the integument (p. 414) in older, Zn-deprived animals may arise because scarce supplies of zinc are centralized for life-sustaining internal purposes.
- As a Type 2 element, deprivation of Zn may cause secondary disorders, attributable to systemic lack of other nutrients, such as Fe and vitamin A.

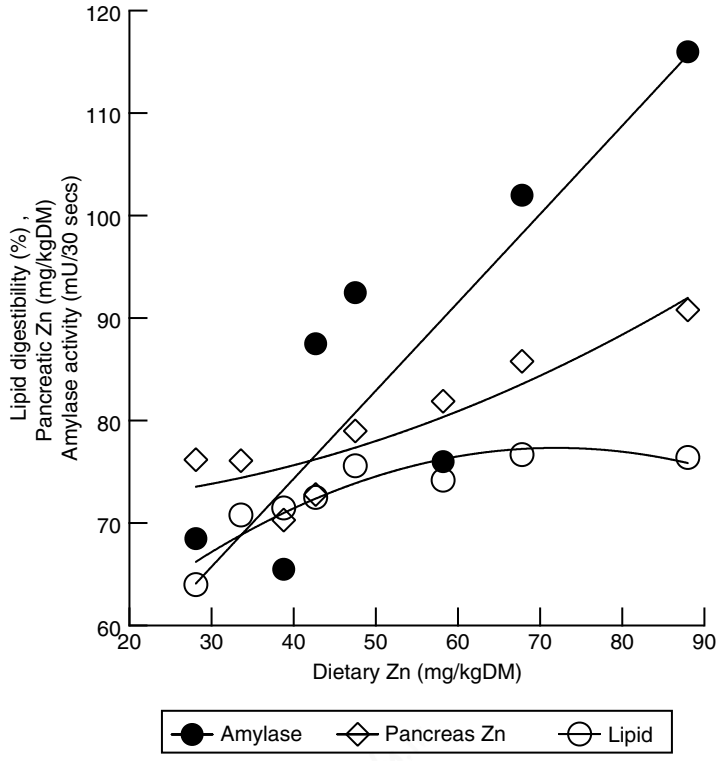

Fig. 16.4. Effects of dietary zinc (Zn) concentration on lipid digestibility, pancreatic Zn and pancreatic amylase activity in weaned piglets after eight days. (Data from Brugger and Windisch, 2016)

Diagnosis of Chronic Lack of Dietary Zinc

Prolonged insufficiency after birth can be diagnosed from combined evidence of skin, horn, hoof and feather abnormalities and low Zn in affected parts (Table 16.2) but diagnosis of early or marginal lack of dietary Zn presents difficulties. Low dietary Zn is more helpful in ruminants than in pigs and poultry but not definitive. Other biochemical abnormalities must be cross-referenced to contemporary impairment of health and productivity.

- Biochemical changes induced by acute and complete, primary deprivation of zinc (e.g. Ryu *et al.*, 2001) by diet or chelation are unlikely to represent those encountered under farm conditions and secondary problems may arise due to sudden Fe overload.

Plasma and erythrocyte zinc

Zinc deprivation was over-diagnosed by widespread use of a high normal range for mean serum and plasma Zn (0.8–1.2 mg l^{-1}), which frequently accorded 'subnormal' status to healthy stock, including sheep (Grace, 1972). Lower limits of normality and marginal bands from the previous edition (Table 16.2) are supported by new data for cows (Wang *et al.*, 2019), pigs (Brugger *et al.*, 2014) and poultry (Schlegel *et al.*, 2010, 2013). Little weight should be given to isolated low values because of the hypozincaemic effects of stressors. Since plasma Cu rises under the influence of most stressors, abnormal plasma Zn:Cu can distinguish false 'lows' in plasma Zn but Zn:Cu in serum will be unreliable in most species (p. 268).

- The view that plasma Zn is the most reliable biochemical measure of zinc status in humans (King *et al.*, 2016) is questionable (Choi *et al.*, 2018) and at odds with experience in livestock.
- Erythrocyte Zn is sensitive to depletion in humans (Lukaski, 2005), less affected by stressors and should arguably replace plasma Zn as the index of first resort.

Zinc-dependent proteins and enzymes

Erythrocyte (and plasma) MT concentrations are also reduced by lack of dietary Zn but unaffected by the stressors that affect plasma Zn (Bremner, 1991) and offer an alternative measure(s) of zinc status. Mononuclear cell MT mRNA also reflects zinc status (Sandoval *et al.*, 1997b; Cao and Cousins, 2000) and is considered to hold great promise (Choi *et al.*, 2018), as is mRNA for Zip4 (Kondaiah *et al.*, 2019). Changes in genomic markers in young pigs on diets varying in dietary Zn are shown in Fig. 16.4. However, it is unlikely that such sophisticated assays will be used to diagnose rare and easily avoided Zn-responsive disorders in livestock. Serum AP activity follows a similar time course to serum Zn during zinc deprivation in sheep (Apgar, 1979), pigs (Swinkels *et al.*, 1996; Schlegel *et al.*, 2010) and hens (Mayer *et al.*, 2018) but the enzyme can be released from gut and bone in various disease states. A return to the first metalloenzyme shown to be zinc-dependent, CA, is long overdue, whichever isoforms in blood (and serum) are measured (Lukaski, 2005).

Other indices

Bone, hair, wool and feathers are normally rich in Zn (100–200mg kg^{-1}DM) but not in animals lacking dietary Zn (Table 16.2). However, individual variability is high, samples being vulnerable to contamination before, during and after collection and affected by age, breed, sampling site, season (Miller *et al.*, 1965; Grace and Sumner, 1986; White *et al.*, 1991) and the period over which sampled appendage has grown.

- Zinc profiles along hoof, hair or wool fibre probably indicate fluctuations in previous zinc nutrition.

Testis Zn and seminal fluid Zn, normally high, are also reduced by lack of dietary Zn: values of 105 ± 4.4 and 74 ± 5.0mg Zn kg^{-1}DM were reported for testes of normal and infertile, zinc-deprived rams, respectively (Underwood and Somers, 1969): similar reductions were reported in goats (Miller *et al.*, 1964). *Post mortem* assays of bone Zn add to the diagnostic template, summarized in Table 16.2. Although less rich in Zn than bone, the toe is readily sampled in poultry and toe Zn reflects zinc intake (Linares *et al.*, 2007). Soft tissue Zn is generally unreliable. Liver Zn can increase greatly during infection and inflammation, giving false 'normal' or 'high' values. In mice, lactation *per* se changed the partition of zinc amongst soft tissues and response to dietary Zn, so that plasma Zn rose when Zn intake declined to a marginal level (McCormick *et al.*, 2015). An immediate improvement in appetite following Zn supplementation indicates previous lack of dietary Zn, but FCE rather than appetite can be affected first in Zn-deprived cattle (Engle *et al.*, 1997).

Natural Sources of Zinc

Forages

Information on Zn in forages and other feeds is summarized in Table 16.3. Worldwide values for

Table 16.2. Marginal bands[a] for assessing the risk of zinc deprivation in livestock from the most diagnostically useful biochemical indices of zinc status.

	Diet (mg kg^{-1}DM)	RBC	Serum (mg l^{-1})	'Coat' (mg kg^{-1}DM)	Pancreas (mg kg^{-1}WM)	Bone (ff) (mg kg^{-1}DM)
Cattle & goat	10–20	1.8–2.0	0.4–0.6	75–100	20–25	50–70 (r)
Pig	30–50[b]	?	0.4–0.7	140–150	30–35	80–90 (t)
Poultry growers	20–30[b]	<10	0.7–1.3	200–275	20–30	100–160 (t)
breeders, layers	50–70					45–60 (toe)
Sheep	10–20		0.4–0.6	80–100	<18	Unknown

For bone: ff, fat-free; r, rib; t, tibia

[a]Mean values for a population sample below the given ranges for more than one criterion indicate probable benefits from zinc supplementation in sufficient individuals to merit interventions. Values within bands indicate the possibility of future benefits if zinc status does not improve.

[b]For cereal–vegetable protein diets, need decreases with age: dietary phytate:zinc molar ratio should be <15.

For units in mmol, multiply values by 0.0153.

pasture Zn ranged from 7 to >100 but most lay between 25 and 50mg kg^{-1}DM, influenced by soil Zn and sward maturity (Minson, 1990).[ix] Towards the low extreme, herbage yields may be increased by application of zinc fertilizers (Masters and Somers, 1980). Pasture Zn can fall by almost 50% in successive cuts, irrespective of fertilizer usage (Underwood and Suttle, 1999) but the decrease can be only a trend when pasture Zn is low, as it was in cool-season Bahia grass in Florida (contained 22 ± 3mg kg^{-1}DM; Myer *et al*., 2009). A survey in New Zealand has revealed little variation about a median value of 43mg Zn kg^{-1}DM (Knowles and Grace, 2014). Hays tend to contain less Zn than silages, concentrations varying little between grass species, although Rhodes grass is often low in Zn (e.g. Jumba *et al*., 1995). Legume species can vary widely, irrespective of soil Zn (Minson, 1990).

Concentrates

Cereal (grain) Zn varies little among species, either side of the Atlantic Ocean (Table 16.3) and values are influenced more by soil Zn status. Wheat, oats, barley and millet in Europe contain 26–35mg Zn kg^{-1}DM on average, and maize (corn) slightly less (20; Sauvant *et al*., 2004) but cereals grown on low-Zn soils can contain <20mg Zn kg^{-1}DM: applications of Zn in fertilizers can triple values (Cakmak, 2008). Cereal Zn is concentrated in outer layers of the grain in association with phytate. In wheat from a low-Zn area, containing 16mg Zn kg^{-1}DM, bran, pollard and white flour contained 49, 41 and 5mg Zn kg^{-1}DM (Underwood and Suttle, 1999) but wheat flour in the USA, where wheat grain contained far more Zn (Table 16.3), contained 39 ± 10.0mg Zn kg^{-1}DM (Kerr *et al*., 2008). The degradability of phytate determines the nutritive value in phytate-rich feeds. Cereal straws usually contain only a third of the concentration found in the grain and frequently <12mg Zn kg^{-1}DM (White, 1993). Vegetable and animal protein sources are richer but more variable in Zn than cereals, with feather meal outstandingly so (Table 16.3), although European (mean-only) data (Sauvant *et al*., 2004) fails to show this. Zinc in vegetable protein sources is largely present as complexes in siderophores.

Milk and milk products

Normal cow's milk contains 3–5 mg Zn l^{-1} and colostrum far more (14 mg l^{-1}) mostly associated with α2 casein. The large amounts of Zn secreted after birth by cows may contribute to the temporary fall in plasma Zn around parturition (Goff and Stabel, 1990). Supplementation of the mother will not increase milk Zn unless the diet lacks Zn (see Miller, 1970; Schwarz and Kirchgessner, 1975). Both dried skimmed milk and buttermilk are therefore good sources, commonly containing between 30 and 40mg Zn kg^{-1}DM.

Table 16.3. Mean zinc concentrations (± standard deviation) in common livestock forages and feeds in UK (MAFF, 1990) and USA (values in *italics*; Kerr *et al*., 2008) (more data given in Table 16.4).

Roughages	Zn	Concentrates	UK / *USA*	By-products	UK/*USA*
Straw[a]	14	Barley	33 (8.5)	Wheat feed	104 (22.7)
		Rice	*30 (6.7)*		*115 (13.7)*
Grass		Maize	19 (2.6) *23 (3.5)*	Rice bran	77 (17.0)
Fresh[a]	36	Oats	26 (3.7) *42 (4.4)*	Brewers grains	73 (12.5)
Dried	33 (7.1)	Wheat	26 (7.0) *39 (5.7)*	Distillers grains	55 (4.7)
					114 (84.7)
Grass		Corn gluten	80 (10.3) *73 (3.2)*	Rapeseed meal	82 (9.1)
Hay	21 (2.7)	Cassava meal	12 (0.6)	Meat & bone meal	130 (43)
Silage	28 (6.6)	Linseed meal	66 (0.8)	Feather meal	152 (3)
			52 (7.5)	Beet pulp	*22 (2.4)*
Kale	27	Soybean meal	49 (9.9)	Soybean hulls	*49 (4.1)*
			61 (5.5)		
Maize silage	45 (13.9)	Sorghum	14 (1.0)		
Lucerne silage		Field beans	38 (2.7)		

[a]See text for sources and countries.

Availability of Zinc in Feeds

Availabilities as attributes of experiments

Zinc is absorbed according to need by an active saturable process in apical mucosal cells from the duodenum to the colon (Martin *et al*., 2013a) and involves a series of ZnT and Zip proteins (Fig. 16.1) (Suttle, 1983). Efficiency of absorption (A_{Zn}) declines exponentially as dietary Zn increases (Fig. 16.5) and potential absorbability ($\boldsymbol{A}_{Zn}$, p. 10) can only be measured with dietary Zn close to requirement. In pregnant women, A_{Zn} for a standard sandwich meal increased from 0.21 to 0.31 as pregnancy progressed: the increase was attributed to an increased fetal requirement for Zn (Harvey *et al*., 2007). Apparent absorption (AA_{Zn}) (p. 26) decreased from 0.47 to 0.22 as dietary Zn increased from 40 to 1000mg kg^{-1}DM in a milk replacer for calves (Jenkins and Hidirolglou, 1991). Relatively high $AA_{Zn,}$ at high Zn intakes (Jenkins and Hidiroglou, 1991; Neathery *et al*., 1980) may be attributable to a second, non-specific process, involving DMT1 (p. 30) and/or mucosal binding, resulting from induction of MT, which limits further transport (Cousins, 1996). Enhancement of A_{Zn} during depletion (Miller *et al*., 1967; Brugger *et al*., 2014) and inhibition during overload (Stake *et al*., 1975) both occur within a week of changes in Zn supply.

In monogastric species, antagonism of A_{Zn} by phytate is a major determinant of availability and inclusion rates for dietary components that influence that antagonism affect the recorded A_{Zn} or AA_{Zn}. Relative biological values (RBV_{Zn}) (p. 28) are also subject to dietary influences. Comparison of linear growth responses in broilers to inorganic Zn (Zn_I) and Zn in a soy protein concentrate, added to diets either low or high in phytate, gave soy protein an RBV_{Zn} of either 0.5 or 1.0 (Fig. 16.6). Using basal diets low in phytate underestimated RBV_{Zn} in phytate rich feeds (Wedekind *et al*., 1992; Edwards and Baker, 2000). Brans rich in phytase improve AA_{Zn} when included in the diet (Roberson *et al*., 2005) but efficacy will not be fully expressed if zinc is provided in excess of requirements. Choice of response index (e.g. growth rate, plasma, liver, pancreatic or tibia Zn) influenced RBV_{Zn}, as did high dietary Ca, which exacerbates antagonism from phytate. Use of newborn, newly-hatched or -weaned animals is popular but the models may be inherently unstable (e.g. p. 140) and fail to predict availability for growing, fattening and reproducing animals. Such factors affect assessment of the merits of chelates (p. 434).

- Indices of performance reflect availability for all purposes, whereas Zn in a tissue reflects partial retainability and may overestimate functionality (p. 28).

Availability of zinc in milk and by-products

Zinc is absorbed well from milk by suckled young but less well from milk 'replacers', based on bovine milk than from human milk itself (Lönnerdal, 2000): this observation may have implications for non-bovine livestock reared on bovine milk by-products. Is whey a superior source of Zn for pigs?

Fig. 16.5. Efficiency of zinc absorption in mature, non-pregnant female ewes on a maintenance ration of hay decreased sharply as zinc intake increased; plasma Zn was unaffected (Suttle *et al*., 1982).

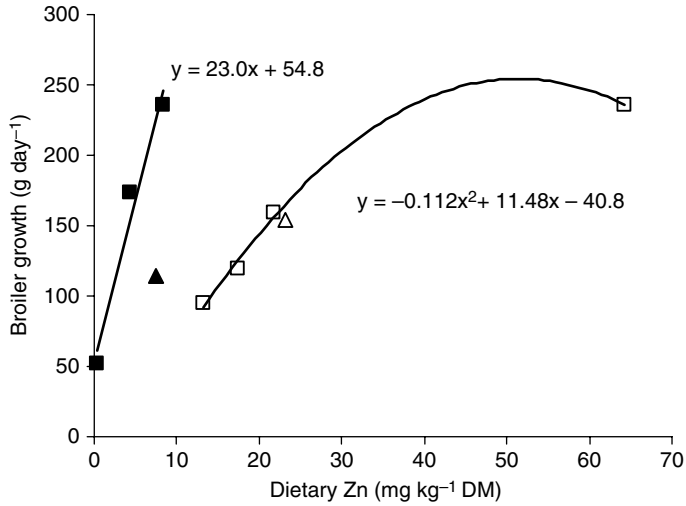

Fig. 16.6. Problems in assessing relative biological value (RBV) of zinc from comparisons of growth responses of broilers to inorganic (Zn_i) and feed zinc, added to an egg-albumen-based diet (EA, solid symbols) or a phytate-rich soy diet (open symbols). A conventional 'slopes ratio' comparison underestimates RBV of Zn_i added to the soy diet because the response curve is curvilinear. RBV for zinc provided by a soybean meal supplement (triangles) is only lower than that of Zn_i if added to the EA diet (▲). (From Edwards and Baker, 2000)

Could formation of ligands between α_2 casein and Zn lower A_{Zn} in the artificially reared calf or lamb on milk replacers based on soybean protein? Low plasma and liver Zn in calves given soy protein-based milk replacers suggests that A_{Zn} may be low (Xu *et al.*, 1997).

Availability of zinc in feeds for pigs and poultry

There are no published values for A_{Zn} in specific feedstuffs for either pigs or poultry but coefficients for AA_{Zn} in corn SBM blends are given in Table 16.4. On average, less than one fifth of ingested Zn was apparently absorbed, whether present in the feed or added as Zn_i or Zn_o, but addition of phytase increased that proportion to about one third. The AA_{Zn} in phytate-rich rations can be improved by factors that decrease antagonism from phytate:

- decreasing seed phytate by plant breeding (Fig. 16.7);
- manipulating animal genotypes to increase capacity to degrade phytate;
- exposure to plant phytases (e.g. by cereal choice and treatments such as soaking);
- adding microbial phytases (Lei *et al.*, 1993; Adeola *et al.*, 1995; Ao *et al.*, 2007; Zeng *et al.*, 2014);
- adding hydroxylated vitamin D_3 (OHD_3) to enhance efficacy of endogenous phytase[x]; and
- minimizing dietary calcium and phosphorus supplements.

Increasingly complex equations for predicting effects of Ca and phytate on A_{Zn} were proposed to predict zinc availability (Davies *et al.*, 1985; Wing *et al.*, 1997), based on molar ratios of Ca:phytate or phytate:Zn (see Fig. 16.8) and the latter are now routinely used to rank foods and estimate requirements for humans (Gibson *et al.*, 2018). A larger number of influential variables can be accommodated by meta-analysis and mathematical modelling. A model that included non-hydrolysable phytate, zinc source (feed, Zn_f, inorganic, Zn_i or organic supplement Zn_o) and phytase accounted for >89% and >96% of variation in markers of zinc status in pigs and broilers, respectively, leaving little apparent scope for effects of Ca, cereal type or protein source (Schlegel *et al.*, 2013) but widespread over-supplementation with Ca may have limited the scope for interaction. A partial model for AA_{Zn} in pigs was:

$$AA_{Zn}(\text{mg Zn kg}^{-1}\text{DM}) = 44.4 - 1.21Zn_f + 0.21Zn_i$$

Table 16.4. Apparent absorption coefficients for zinc in pigs given diets with or without added zinc and phytase.

	Phytase		Units	Reference
Dietary Zn	0	+	FU kg^{-1}DM	
30	–0.34	0.30	1350	Lei *et al*. (1993)
55	0.09	ND		
27	0.28	0.35	1500	Adeola *et al*. (1995)
32	0.17	0.43	1200	Revy *et al*. (2004)
55_i	0.21	0.35	1200	
55_o	0.22	0.34	1200	
15	0.03	0.10	700	Bikker *et al*. (2012)
15	0.31	0.37	250–300	Arredondo *et al*. (2019)

ND, not determined; Zn_i and Zn_o = inorganic and organic Zn, respectively

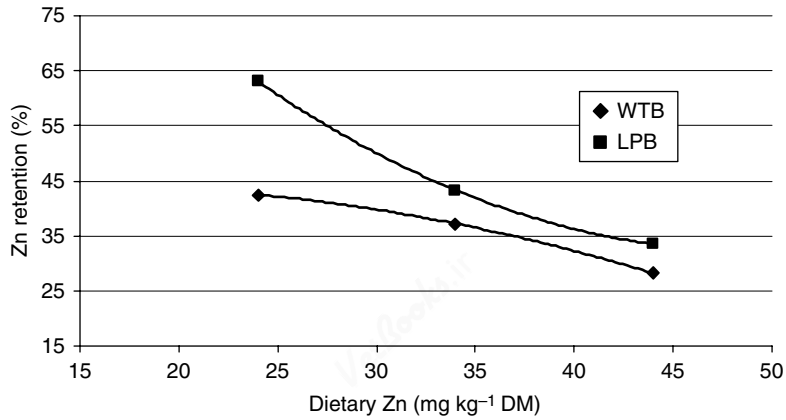

Fig. 16.7. Zinc retention in broiler chicks is determined by need, up to a limit set principally by dietary phytate as shown in a comparison of genetically modified low-phytate barley (LPB) and wild-type, high-phytate barley (WTB) diets (Linares *et al.*, 2007).

Within recorded ranges for Zn_f and Zn_i of 25–35 and 0–100mg kg^{-1}DM, respectively, AA_{Zn} coefficient for Zn_f decreased from 0.56 to 0.06 as Zn_F concentration increased, possibly reflecting a high contribution from poorly available, phytate-bound Zn in SBM in diets for starter pigs and low phytase activity, contrived by re-pelleting the ration (Brugger *et al*., 2014). It was concluded that piglets used phytate-bound Zn far less efficiently than broilers and were thus more responsive to phytase supplementation. There were insufficient data for meta-analysis of AA_{Zn} in broilers, for which contrasts were made in relative rather than absolute terms, using plasma AP, plasma Zn and bone Zn, but phytase supplementation improved zinc status far more in pigs than in poultry (Schlegel *et al*., 2013). The broiler chick is able to degrade most of the phytate ingested (Zeller *et al*., 2015). In turkey poults, AA_{Zn} declined from 0.32 to 0.18 between 7 and 21days of age but remained high in broilers: both received phytase-supplemented diets containing *c*.100mg Zn kg^{-1}DM but the poult diet contained 50% more soybean meal and Ca (13.4 g and 9.0 g) and twice as much available P (6.0 versus 3.1g kg^{-1}DM) as the broiler diet (Olukosi *et al*., 2020).

Zinc availability in feeds for herbivores

Hind-gut fermenters such as the horse are vulnerable to antagonism from phytate. Pellets containing 50% corn and 35mg Zn kg^{-1}DM had an AA_{Zn} of only 0.07 in gelding ponies, rising to 0.27 with 125mg Zn kg^{-1}DM added as ZnO (Schryver *et al*., 1980). With cereal replaced by alfalfa, AA_{Zn} for

orally administered ^{65}Zn was 0.16 for both treatments, suggesting that inherent Zn was poorly utilized from the phytate-rich diet. Horses may be capable of absorbing Zn from forage with the same high efficiency as sheep. An A_{Zn} of 0.75 was obtained for a low-Zn forage (12mg kg^{-1}DM) given to non-productive sheep (Suttle *et al.*, 1982) (Fig. 16.5). In unsupplemented, mixed diets based on sugarcane bagasse and corn silage, containing 18mg Zn kg^{-1}DM and given to growing steers, AA_{Zn} was 0.64 and 0.70 ± 0.031, respectively, but lower when soybean hulls were added to the former (0.46) and raised by adding 50 mg Zn_i (to 0.70) (Zanetti *et al.*, 2017). Phytate is extensively degraded by microbial phytases in the rumen but undegraded phytate may lower Zn availability for ruminants given diets rich in processed cereals.

Dietary Zinc Requirements

Dietary trials give requirements that reflect choices of dietary components, amounts of added Zn and target values for markers of zinc status (p.11) (Figs 16.2 and 16.8). Asymptotes on dose–response curves were around 65mg Zn kg^{-1}DM for maximal pancreatic Zn, tibia Zn and mRNA MT abundance in broilers compared to ≤28mg Zn kg^{-1}DM for optimal growth, liver CuZnSOD and plasma AP, for example (Liao *et al.*, 2013). Adding Zn as $ZnSO_4$ to the pelleted diet of Targhee rams improved wool growth but not body growth or semen characteristics (Page *et al.*, 2020).

- Cut-off points for biochemical and immunological parameters should be tied to actual or prospective performance or health outcomes.

Determination of needs by factorial modelling is still hampered by lack of data on model components for maintenance and absorbability (p. 9). Only the net requirement for growth was not in dispute and similar for all classes, at around 23 mg kg^{-1}DM, from pigs (Ruiz-Ascacibar *et al.*, 2019), to steers (Zanetti *et al.*, 2017). However, a value 50% higher was estimated when a questionable comparative slaughter technique (see p. 8) was applied in sheep (Zhang et al., 2018). In non-ruminants, reabsorption of secreted Zn may be restricted on diets with high phytate: Zn ratios, thus increasing FEL_{Zn} (Oberleas, 1996; Brugger and Windisch, 2017) and reducing AA_{Zn}. The pragmatic solution to the problem, as with calcium and all elements that are absorbed according to need (p. 77), is to use optimal AA_{Zn} coefficients; i.e. commercially attainable AA_{Zn}, measured at Zn, intakes close to requirement in phytase-supplemented rations. Whatever the species, factorially derived requirements in growing animals will decrease with approach to mature body weight, but increase with growth rate and FCE.

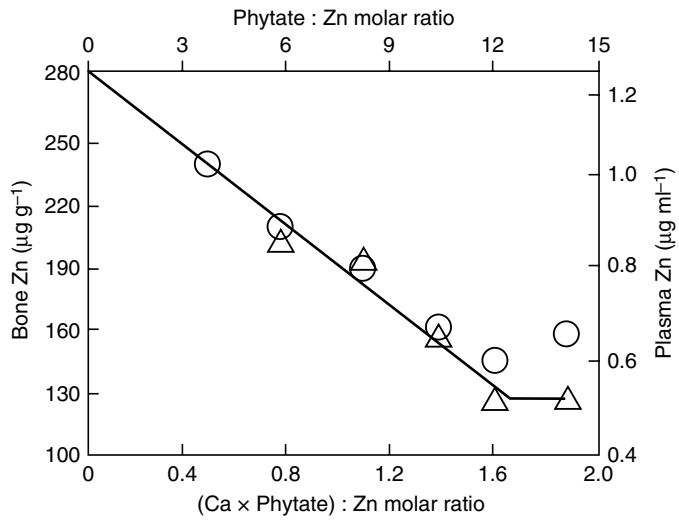

Fig. 16.8. Zinc requirements of pigs vary with the chosen criterion of adequacy, being lower for optimal plasma Zn (circle) than bone Zn (triangle) (data from Wedekind *et al.*, 1994b). Expression as dietary Zn:phytate and calcium ratios smoothed relationships but are avoidable by restricting dietary Ca and minimizing the impact of phytate (Table 16.5).

Pigs

Early dietary trials probably overestimated requirements.[xi] Performance was not improved by raising dietary Zn from 27 to 32mg kg^{-1}DM in low- (Dourmad *et al.*, 2002) or high-phytate diets (Hill *et al.*, 1986; Wedekind *et al.*, 1994b; Revy *et al.*, 2004) but a similar concentration was associated with phytase-responsive skin lesions in nursery pigs (Shelton *et al.*, 2005). Requirements cannot realistically be covered by a single figure because of variation in AA_{Zn} (Table 16.4). Results of a hypothetical, restricted factorial model for phytase-supplemented diets (Table 16.5) gives requirements that are roughly half those recommended by NRC (2012) for starters (20 kg LW) and decreasing with age. Replacing plant with animal protein sources (whey or meat, fish and insect meals) and using loose mixes with phytase-rich cereals would create needs similar to those catered for in Table 16.5. Requirements of >100mg Zn kg^{-1}DM for optimal immune responses in weanling pigs (Kloubert *et al.*, 2018) are unacceptable until shown to reduce susceptibility to infections. Breakpoint analysis of responses in pancreatic and mucosal mRNA, together with mucosal mRNA for MT (Fig. 16.2) and fat digestibility (Fig. 16.4) produced a concensus requirement of 50–55mg kg^{-1}DM for starter pigs on a diet with a very low AA_{Zn} of 0.06: needs for 3–3.3 mg apparently absorbed Zn would be well met with far less Zn in phytase-rich diets (Table 16.5). The suggestion that a corn/SBM ration containing 30–34mg Zn kg^{-1}DM and 16g Ca kg^{-1}DM was marginal for sows (Hoekstra *et al.*, 1967) probably overestimated Zn requirements for reproduction.

Poultry

Growth responses in starter chicks indicated a need to add 5–10 mg Zn to a corn/SBM blend containing 23 mg inherent Zn kg^{-1}DM (Ao *et al.*, 2006) (Fig. 16.9) but experiments conducted since 2010 indicate a need of ≤25mg Zn kg^{-1}DM.[xii] Requirement will be greatly influenced by availability of inherent Zn, which may have been low in the 2006 study, due to high dietary Ca (10 g) and available P (4.5g kg^{-1}DM). The lower need in chicks than in piglets was confirmed by feeding both species on the same diet and attributed to a low pH in gizzard and stomach, which promoted phytate degradation and zinc solubilization (Schlegel *et al.*, 2010). The species difference may have been exaggerated on a diet that contained 14g Ca kg^{-1}DM, high even for 1 d.o. chicks. Phytate degradation decreases as dietary Ca increases in both species (p. 147). As much as 84mg Zn kg^{-1}DM was needed for optimum serum 5'-nucleotidase activity and pancreas ZnT-2 mRNA expression (Huang *et al.*, 2007) but justification for these as 'preferred indices' of need was unconvincing. Given the high, constant net requirement for growth (24mg kg^{-1}LWG), gross requirements are likely to decline in terms of dietary concentration as birds grow (p. 11). Hatchlings from eggs laid by hens deprived of Zn required >50mg Zn kg^{-1}DM to prevent feather fraying (Underwood and Suttle, 1999) but hatchlings normally draw on reserves in the egg yolk. An earlier spurt in growth of feathers, rich in Zn, in females than in faster-growing males evens up needs in the first month of life. Feather replacement uniquely adds to the maintenance need for Zn in avian species.

Table 16.5. Hypothetical factorial net and gross zinc requirements of growing pigs, assuming that dietary Ca is minimal (Table 4.6), plant or microbial phytase activity is ≥500FU kg^{-1}DM and the apparent availability of zinc (coefficients in parentheses) rises as less phytate-rich protein is fed.[a]

Liveweight (kg)	Liveweight gain (kg day^{-1})	Food intake (kg day^{-1})	Zn requirement Net (mg day^{-1})	Gross[a] (mg kg^{-1}DM)
10	0.3	0.6	8.0	53 (0.25)
20	0.4	1.1	9.6	35 (0.25)
40	0.6	1.9	14.4	30 (0.25)
80	0.8	2.7	19.2	24 (0.30)
120	1.0	3.1	24.0	22 (0.35)

[a]In the absence of data for faecal endogenous loss of zinc in pigs, a simplified model where $R = G / AA$ rather than a complete model (p. 10) is used to generate gross needs. *AA* is assumed to be 0.25 for younger pigs but rises to 0.36 by final growth stage as dietary Ca and protein concentrations decrease and antagonism from phytate decreases.

Fig. 16.9. Effect of dietary zinc concentration and source on liveweight gain (LWG) in chicks: adding between 10 and 20 mg Zn to a basal diet (triangle) containing 23 mg Zn kg^{-1}DM optimized growth, whether the source was inorganic (white circle) Zn_i or organic (black circle) (Zn_o) (data from Ao *et al*., 2006) who concluded that Zn_o was 80% more available than Zn_i by comparing linear slopes over different ranges of dietary Zn.

- Since commercial diets for chicks contain >20mg Zn kg^{-1}DM, tables of requirements for growth are of largely academic interest.
- For starter chicks, 25mg Zn kg^{-1}DM should be sufficient in diets with phytase and without excess Ca: grower and fattening birds will need less Zn.

The 75% higher need for turkey poults than broilers, recommended by NRC (1994), may be largely justified: growth requirements were 20% higher in poults on a phytate-free diet (Dewar and Downie, 1984); phytate is less degradable by poults (p. 140) and AA_{Zn} lower by 21 d.o. (p. 11) in poults than broiler chicks. Turkey poults needed <64 and <50mg Zn kg^{-1}DM in the first and third months of life on well-formulated wheat/SBM diets (Jankowskia *et al*., 2019). Poults may benefit more than chicks from nutritional strategies for lessening exposure to phytate (p. 425):

- With optimal AA_{Zn}, 50mg Zn kg^{-1}DM is probably sufficient for all turkey poults.

Egg production and breeding

The old recommendation of 65mg Zn kg^{-1}DM for egg production (NRC, 1994) was confirmed but more (70–80 mg) recommended to optimize tibia Zn and plasma AP (Liu *et al*., 2015; Qin *et al*., 2017). However, 28 mg inherent Zn kg^{-1}DM was sufficient towards the end of lay in diets with added phytase (Zhang *et al*., 2017b). Changing the pattern of Zn supplementation to follow circadian rhythms affected expression of a Ca-binding protein and an antioxidant enzyme in the eggshell gland (Lin *et al*., 2020) but practical advantages are unclear. Breeder hens have similar needs for egg production to layers but higher needs for optimum fertility (Fig. 16.10). Average optimum concentration for six indices was 73mg Zn kg^{-1}DM: requirements for egg production and settability were some 20–25mg kg^{-1}DM higher in the first period after prior depletion than later periods and much higher for AP than other indices (Mayer *et al*., 2018). Requirements for a different breed on a diet containing more corn and SBM were slightly higher (Li *et al*., 2019a). Raising phytase activity by choice of cereal or supplementation can lower need by improving AA_{Zn} (Rojas *et al*., 2018; Kamberi *et al*., 2018) but the improvement can disappear with addition of Zn_i (Sharideh *et al*., 2016).

- The higher requirement for laying and breeding than growing birds is partly due to a high net requirement for each egg produced and partly to the strong antagonism from phytate on diets rich in Ca throughout lay (>30g kg^{-1}DM).
- In the transition to lay, zinc provision for pullets should increase in line with calcium provision from 25 to 70mg kg^{-1}DM.

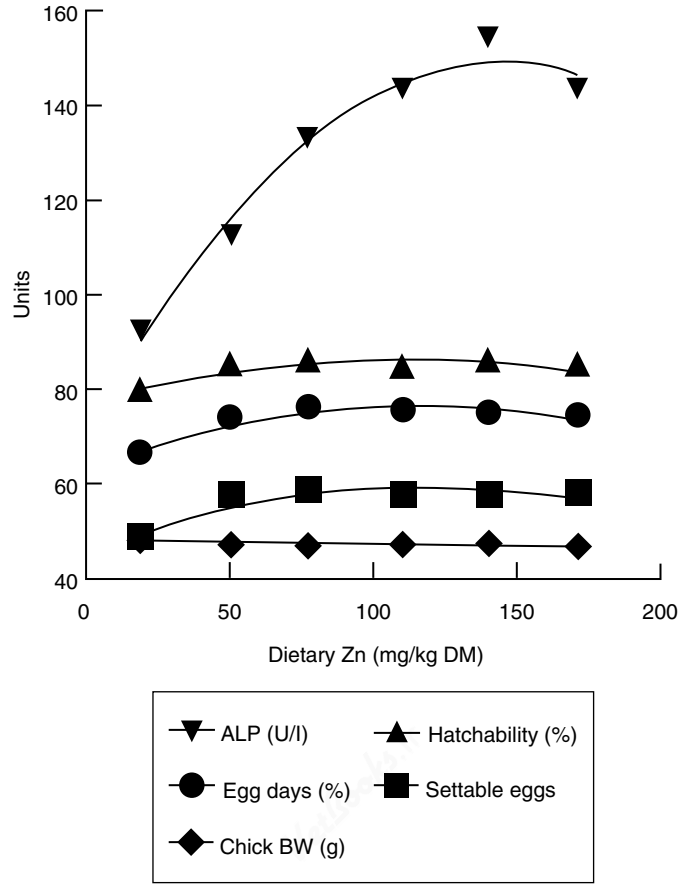

Fig. 16.10. Responses of layer hens to supplementation of a corn, SBM and wheat meal diet, containing 30g Ca kg^{-1}DM, with $ZnSO_4$: values are averaged over three periods from 33–44 weeks of lay, following seven weeks depletion in Cobb 500 hens. (Data from Mayer *et al*., 2018)

The proportionate increase in zinc requirement for breeding turkeys compared with hens may be less than that for growers because the phytate antagonism is already strongly expressed in poults. Zinc content of the egg increases curvilinearly with dietary Zn, and

- a case can be made for maximal supplementation to increase Zn intake in rural communities, where there is risk of zinc insufficiency (p. 532).

Zinc requirements for cattle

Beef and dairy cattle commonly grazed pastures with <20mg Zn kg^{-1}DM and remained healthy in Scotland (Price and Humphries, 1980) and New Zealand (N.D. Grace, personal communication, 1998) but there have been exceptions (e.g. Mayland *et al*., 1980). Housed cattle may have marginally higher needs than grazing cattle. Weaned calves grew faster when zinc was added to corn silage diets containing 24 and 33mg Zn kg^{-1}DM (Spears, 1989 and Spears and Kegley, 2002) and on a hay/ground-corn diet containing 17mg Zn kg^{-1}DM (Engle *et al*., 1997). In one study (Spears and Kegley, 2002), growth improvement was marginal, plasma Zn remained normal but food intake tended to be higher in Zn-supplemented groups, raising the possibility that palatability was improved. However, phytate in corn-based diets may partially escape degradation in the rumen (p. 150), reducing A_{Zn} and raising the requirement. Lactating cows on commercial rations probably need more zinc than those in

early experiments with semi-purified diets (<17mg Zn kg^{-1}DM; Neathery *et al.*, 1973a; Schwarz and Kirchgessner, 1975). Cows became marginally hypozincaemic on a corn/corn silage diet containing 37.6mg Zn kg^{-1}DM, mostly from phytate-rich rapeseed meal, SBM and wheat bran, and a Zn supplement increased milk yield (Wang *et al.*, 2013). Responses to zinc when plasma Zn is normal may indicate either pharmacological responses, as in veal calves given soybean protein in a milk replacer, containing 149mg Zn kg^{-1}DM (Xu *et al.*, 1997), or effects on rumen microflora, as in low-yielding cows on a predominantly concentrate diet of unspecified zinc content (Shakweer *et al.*, 2010).

- Zinc requirements may be at least twice as high for cattle on cereal-based rations than for those consuming long forage as grass, silage or hay.

Factorial estimates of requirement from previous editions are given in Table 16.6 but additional allowances are recommended for cattle on grain-based diets. The original estimate of FEL_{Zn} came from a sheep study (Suttle *et al.*, 1982) but is supported by a new estimate of 107µg Zn kg^{-1}LW for cattle (Zanetti *et al.*, 2017), which was four times greater than the only previous value for cattle (Hansard *et al.*, 1968): potential flaws in the regression techniques employed (p. 9) are less likely to affect zinc, a Group 2 mineral, than Group 1 minerals (p. 40).

- Theoretically, 18mg Zn kg^{-1}DM is sufficient from forages but 40mg Zn kg^{-1}DM may be needed in cereal-rich, total mixed rations.
- Improvements in somatic cell count (Cope *et al.*, 2009; Ianni *et al.*, 2019) and changes in cheese quality (Ianni *et al.*, 2019) in response to supplementary Zn that did not improve milk yield may bring a new dimension to the setting of zinc requirements.

Zinc requirements for sheep

As with cattle, early studies with semi-purified diets gave low estimates of requirement that varied with criterion of adequacy and between studies (Underwood and Suttle, 1999). Requirements for optimal wool growth, plasma Zn and male fertility (14mg Zn kg^{-1}DM) were approximately twice those for LWG (Fig. 16.11).[xiii] Exceeding the higher NRC (2007) requirement did not improve sperm numbers in rams (Page *et al.*, 2020). Sheep commonly graze pastures with <20mg Zn kg^{-1}DM and remain healthy and fertile (Grace, 1972; Pond,

Table 16.6. Factorial estimates of minimum zinc requirements for cattle on fresh or conserved grass or lucerne diets: housed cattle should be allowed twice as much zinc to counter lower availability.[a]

	Liveweight (kg)	Production (kg day^{-1})	Requirement (mg day^{-1})		DMI (kg day^{-1})	Zn requirement (mg kg^{-1}DM)[d]
			Net[b]	Gross[c]		
Growth	40	0.5	16.0	18.8	1.0	18.7
	100	0.5	22.0	31.4	4.0–2.2	7.9–14.3
		1.0	34.0	48.6	4.5–2.8	10.8–17.4
	200	0.5	32.0	45.7	6.0–3.5	7.6–13.1
		1.0	44.0	62.9	6.5–4.0	9.7–15.7
	300	0.5	43.0	61.4	8.0–4.5	7.7–13.6
		1.0	54.0	77.1	8.4–5.4	10.1–14.3
Adult	500	0	50.0	71.4	7.6–4.6	9.4–15.5
Pregnancy	500	90–100 days	51.1	73.0	9.0–6.6	8.1–11.1
		180–270 days	56.3	80.4	11.2–6.9	7.2–11.7
Lactation	500	10	90.0	128.6	11.7–8.3	11.0–15.5
		20	129.5	185.0	17.8–12.7	10.4–14.6
		30	170.0	242.9	19.2–15.5	12.7–15.7

[a]On corn or corn silage-based diets, A_{Zn} may be as low as 0.35 and requirements twice as high as those in the table.
[b]Net requirement consists of: maintenance, 0.1 mg Zn kg^{-1} liveweight; growth, 24 mg Zn kg liveweight gain; pregnancy, 1.1 and 6.3 mg Zn day^{-1} at mid and late stages; milk, 4 mg Zn kg^{-1}.
[c]The absorption coefficient (A_{Zn}) is 0.70 for all but the youngest (milk-fed) calves, for which the assumed value was 0.85.
[d]In ranges, higher values correspond to a low DMI on highly digestible diets.

1983; Masters and Fels, 1985; White *et al.*, 1991) but feedlot lambs may require more Zn. Performance, plasma Zn and bone-specific AP activity were improved by adding Zn to a barley-based diet containing 22.5mg Zn kg^{-1}DM of low availability (AA_{Zn} 0.19; Aliarabi *et al.*, 2015). Male lambs given a similar diet, providing 19.7mg Zn kg^{-1}DM, grew faster and had higher plasma Zn within the normal range (1.4 versus 0.9 mg l^{-1}) when given zinc from any one of four sources (p. 433) (Alimohamady *et al.*, 2019). The manner of supplementation may have enhanced antagonism from phytate. Zinc supplements were fed and consumed as a premix with phytate-rich constituents, wheat bran (5%) and SBM (3%) *before* the main ingredients were offered. Zinc supplementation did not improve lamb growth on concentrate diets containing 29.3mg Zn kg^{-1}DM (Nagalakshmi and Himabindu, 2013).

- Needs given in Table 16.7, like those for cattle, may be higher on fine, cereal-rich diets, due to antagonism from phytate that escapes rumen degradation (Humer and Zebeli, 2015).

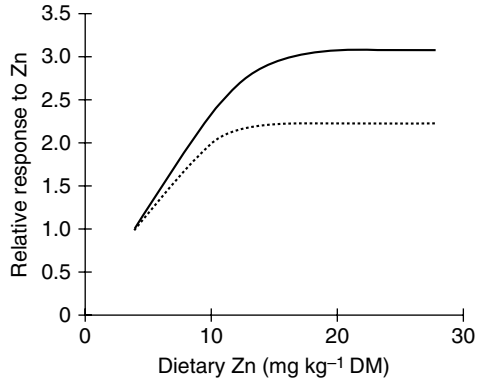

Fig. 16.11. Responses in wool growth (—) and live-weight gain (LWG) (····) to zinc added to a semi-purified diet indicate a higher zinc requirement for wool growth than LWG (White, 1993).

Zinc requirements for goats

Clinical abnormalities were produced in a quarter of goats given a diet consisting partly of wheat straw and containing 15mg Zn kg^{-1}DM for 171–200 days (Chhabra and Arora, 1993). Requirements for non-lactating goats are probably similar to those for roughage-fed sheep and cattle (i.e. approximately 20mg kg^{-1}DM). Growth rate and FCE were improved in cashmere goats by raising dietary Zn from 22mg Zn kg^{-1}DM during the cashmere growing period, and requirement lay between 37 and 52mg Zn kg^{-1}DM (Jia *et al.*, 2008). Intact male cashmere goats showed improvements in wool yield, fibre length, plasma Zn and plasma testosterone when 20 mg Zn was added to a diet containing 46mg kg^{-1}DM (Liu *et al.*, 2015).

Table 16.7. Factorial estimates of minimum zinc requirements for sheep from forage-based diets: feedlot lambs should be allowed twice as much zinc to counter lower availability.

	Liveweight (kg)	Production rate (kg day^{-1})	Requirement (mg day^{-1})		DMI (kg day^{-1})	Zn requirement (mg kg^{-1} DM)[c]
			Net[a]	Gross[b]		
Growth	5	0.15	4.6	5.4	0.2	27.0
	10	0.15	5.6	8.0	0.4	20.0
	20	0.15	6.6	9.4	0.64–0.57	14.7–16.5
	40	0.075	6.8	9.7	1.0–0.6	9.7–16.2
		0.150	8.6	12.3	1.4–0.8	8.8–15.4
		0.300	12.2	17.4	1.1	15.8
Adult	50	–	6.0	8.6	0.8–0.5	10.8–17.2
Pregnancy (late)	75	2 fetuses	11.9	17.0	1.7–1.4	10.0–12.1
Lactation	75	1	15.7	22.4	1.9–1.5	11.8–14.9
		2	22.9	32.7	2.8–2.2	11.7–14.9
		3	30.1	43.0	3.7–2.4	11.6–17.9

[a]Net requirement consists of: maintenance, 0.1 mg Zn kg^{-1} liveweight; growth, 24 mg Zn kg^{-1} liveweight gain; pregnancy, 0.28 and 1.5 mg Zn day^{-1} for mid and late stages; wool growth, 115 mg Zn kg^{-1} fresh weight; milk, 7.2 mg Zn kg^{-1}.
[b]The absorption coefficient is 0.70 for all but the youngest (milk-only diet) lambs.
[c]In ranges, higher values correspond to a low DMI on highly digestible diets.

- High rates of fibre growth in cashmere goats, coupled with high need for sperm development, may create high seasonal needs for zinc.

Zinc requirements for herbivores

Stabled horses require more zinc than pastured horses because cereal phytate lowers A_{Zn}, but the adverse effect may be partially offset if phytase-rich wheat bran is added to rations. Horses, like ruminants, thrive on pastures containing 15–20mg Zn kg^{-1}DM, but stabled horses given grains should be allowed 30–40mg Zn kg^{-1}DM. Camelids may resemble horses in having high requirements on grain-based diets and, like cashmere goats, have high needs for zinc during periods of rapid fibre growth.

Avoiding Zinc Insufficiency

Inorganic zinc supplements

Zinc insufficiency can be rectified by adding ZnO or $ZnSO_4$ to concentrate supplements and whole mixed rations, effectiveness varying as much within as between sources and affected by choice of diet (Fig. 16.6) and response index.[xv]

- Differences in availability within Zn_i sources can influence margins of superiority, if any, attributed to Zn_o.

The coefficients in the equation on p. 425 indicated availability of Zn_I was 0.15, below that of Zn_i (0.31) for pigs, presumably lowered by a molar excess of phytate. Phytase supplementation combats antagonism from phytate (Adeola *et al.*, 1995; Schlegel *et al.*, 2013) and improves utilization of supplementary as well as inherent feed Zn. Ultra-fine (nano) particles (NP) of Zn_i might have enhanced reactivity and availability but no differences in performance were found by giving ZnO_{NP} to pigs (Li *et al.*, 2016), heat-stressed broilers and unstressed turkeys (Jankowskia *et al.*, 2019): susceptibility to antagonism from phytate may increase with surface area of such particles. Increased immune responses (King *et al.*, 2016; Akhavan-Salamat and Ghasemi, 2019) may indicate adjuvant effects rather than primary improvements in resistance to infections.

Quality as well as formula affects the value of Zn_i sources for ruminants as well. Three different ZnO sources had RBVs of 106%, 87% and 79% in sheep (Sandoval *et al.*, 1997a, b) and similar results were obtained with horses (Wichert *et al.*, 2002). Differences may be related to particle size and solubility. Supplementation of cattle with relatively insoluble hydroxychloride (ZnOHCl) (together with CuOHCl and MnOHCl) rather than as sulfate(s) lowered soluble Zn (and Cu) in rumen liquor (Genther and Hansen, 2015; Caldera *et al.*, 2019), without affecting either zinc solubility in abomasal digesta (Genther and Hansen, 2015) or AA_{Zn} (Faulkner *et al.*, 2017). Advantages of NP_{Zn} have been investigated in goats but none were found (Zaboli *et al.*, 2013).

Organic zinc sources for ruminants

Comparisons between Zn_o and Zn_i sources, published since the last edition was written (Suttle, 2010) still show no consistent advantage for Zn_o sources, despite extravagant claims.[xiv] Three studies have agreed that Zn_o and $ZnSO_4$ are of similar value to lambs (Nagalakshmi and Himabindu, 2013; Aliarabi *et al.*, 2015; Alimohamady *et al.*, 2019). Lactation performance was improved when Zn-amino acid chelates replaced 75 mg Zn, added as $ZnSO_4$ in a diet already adequate in Zn (Nayeri *et al.*, 2014) but may have no practical advantage.

- Absence of an unsupplemented control group in the latter study, a common feature of many such comparisons.
- The design avoids the question 'Was adding ZnO beneficial?', one which powerful multinationals with chelates to sell are happy to avoid.

Adding Cu- and Zn-methionates (ZnMet) rather than sulfates lowered Cu and Zn excretion in faeces and raised plasma and liver Cu in ewes (Pal, 2010) but supplements were incorporated daily into a premix containing 22% wheat bran and limestone: these conditions may have favoured formation of insoluble Zn_i-phytate complexes, *ex* and *in vivo*. Adding 40mg Zn kg^{-1}DM as $ZnSO_4$ or ZnOHMB to a concentrate supplement improved digestibility of a Beersem hay/rice straw diet: milk yield was increased more by Zn_o but group size was small (Shakweer *et al.*, 2010). Milk yield was raised by reducing provision from 63 to 42mg Zn kg^{-1} DM with zinc proteinate but not by adding less ZnO (Cope *et al.*, 2009). Somatic cell counts in milk, an indicator of udder health, were lower with Zn_o but unaffected by dietary Zn concentration. Zn-chelation with either weak or strong bonds increased milk yield and secondary antibody

responses, whereas $ZnSO_4$ and a chelate with moderate binding properties did not (Wang *et al.*, 2013). In cultures, numbers of rumen microorganisms were reduced at the higher of two concentrations (10 versus 20 mg Zn l^{-1}), regardless of source, but chelation enhanced fermentation at the lower concentration (Wang *et al.*, 2013). Species differences emerged in the rumen microflora after prolonged feeding of pelleted diets to Targhee rams with 100mg Zn kg^{-1}DM added as either an amino-acid chelate or $ZnSO_4$, the former reducing microbial diversity (Ishaq *et al.*, 2018): Zn_o increased weight gain but Zn_i increased serum Zn and wool yield: neither source affected sperm quality (Page *et al.*, 2020).

- Complexation may have benefits in protecting feed ingredients and rumen microflora from free zinc and free zinc from antagonism from phytate; but
- adverse effects on feed constituents and rumen microbes are best avoided by not raising dietary Zn unless there is a proven need.

Effects of chelation in newborn calves

Unique effects of chelation have recently been reported in newborn calves, prone to diarrhoea (Chang *et al.*, 2020; Ma *et al.*, 2020a, b) but may be attributes of the experimental model rather than source (p. 424). Supplements of ZnO or ZnMet, providing 80mg Zn, were given in 200ml colostrum or milk for 14–21 days, a method that would stimulate the oesophageal groove and abruptly increase concentrations of Zn in digesta to macromineral levels (around 3g Zn kg^{-1}DM). As in piglets (p. 435), such high doses controlled diarrhoea but relative efficacy of ZnO and ZnMet changed with time and differed between studies. Both sources increased plasma Zn, MT, alkaline phosphatase, hepatic Zn and three different ZnT similarly. However, ZnMet induced Zip4 more strongly (Ma *et al.*, 2020a) and lowered permeability of the duodenum and plasma MDA more than ZnO (Ma *et al.*, 2020b). The authors linked all effects of source to enhanced availability of chelated Zn but other explanations are possible. Control of diarrhoea by Zn in calves is accompanied by changes in gut microbiota, including pathogenic species, (Chang *et al.*, 2020) and gut permeability, interrelated effects. In early-weaned pigs, control of diarrhoea and gut permeability were enhanced by providing ZnO at relatively low concentrations (500mg kg^{-1}DM), coated by lipid, protecting the upper intestine from inflammatory effects of excess Zn, while exposing pathogens in lower regions (Dong *et al.*, 2019).

- Lower accessibility of chelated Zn may affect diarrhoea incidence and gut permeability by protecting the gut mucosa from Zn cytotoxicity.
- ZnMet may be partially absorbed intact through a 'leaky' gut, enter protein pools, like selenomethionine (Fig. 15.6) and accumulate in non-functional forms.
- Results from animals prone to diarrhoea should not be used to make general assertions about availability of sources of Zn or any other mineral.

Organic zinc sources for pigs and poultry

Previous reviews found little or no evidence for consistent benefits in performance from providing Zn_o (Baker and Ammerman, 1995; Spears, 1996; Underwood and Suttle, 1999; Suttle, 2010), in pigs and poultry, as illustrated in Fig. 16.12. Subsequent meta-analysis of data for pigs and poultry found no difference in RBV_{Zn} between Zn_o and Zn_i (Schlegel *et al.*, 2013; Romero, 2013). Of three claimed production benefits from doubling dietary Zn to 200mg kg^{-1}DM for sows as Zn proteinate rather than $ZnSO_4$, only one (increased total weaned weight) was significant: differences in villus morphology may have indicated inflammatory responses to unnecessary additions of Zn_i (Payne *et al.*, 2006). In laying hens, Zn_o increased eggshell thickness without increasing breaking strength or egg production, compared to Zn_i (Zhang *et al.*, 2017b).

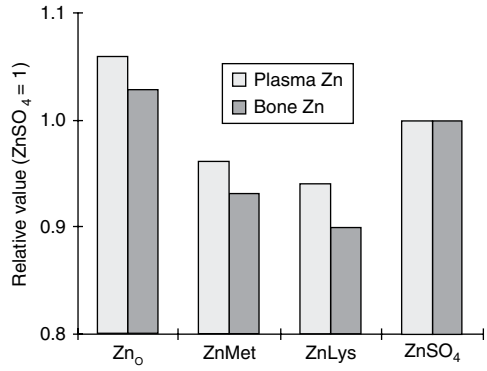

Fig. 16.12. Pigs given zinc complexed with either lysine (ZnLys) or methionine (ZnMet) had lower plasma and bone Zn than those given inorganic supplements to an inadequate diet. (Data from Wedekind *et al.*, 1994b)

How then, can a widely held view that chelated (or 'organic') zinc sources are more absorbable than inorganic sources be explained?

- Firstly, superiority of performance and/or zinc status has often been exaggerated.

In chicks, data presented in Fig. 16.9 led Ao *et al.* (2007) to conclude that a Zn-proteinate provided 83% more available Zn than $ZnSO_4$: the difference was obtained by questionable manipulation of the data as in other such comparisons[xvi], reliance on linear slopes to assess relative availability introduces errors when the response curve changes with age and differs between markers (e.g. Huang *et al.*, 2009). Occasional benefits in performance and differences in biochemical and genomic markers can vary with time and differ quantitatively with each other (e.g. Huang *et al.*, 2009). Many claimed advantages are artefacts of statistical procedures, experimental design or untruths.

- Secondly, effects of source on biochemical, immunological and genomic markers do not necessarily indicate merit for Zn_o.

Increased plasma Ig might indicate enhanced yet harmful inflammatory reactions.[xvii] Chelates of different strength may elicit different systemic responses (e.g. Huang *et al.*, 2009). Neither ZnMet nor $ZnSO_4$ enhanced performance in laying hens but the former increased expression of mRNA for jejunal MT and changed morphology of the jejunum (Li *et al.*, 2019b).

- The role of MT is generally protective and such responses may be to an unwelcome zinc complex that has gained entry by an unorthodox route.

Tissue accretion of an element that is not normally stored (Fig 3.4) may not be beneficial (Brugger and Windisch, 2019b). Zn-glycine, a chelate of moderate strength, increased bone Zn far more than ZnO and changed femur and tibial geometry without improving performance after six weeks in broilers (Kwiecień *et al.*, 2016) but source did not affect soft tissue Zn, CuZnSOD or peroxidation in another study (Ivanišinová *et al.*, 2016).[xviii] In a comparison of three Zn-chelates the effect of chelation strength was greater in broilers than that of doubling dietary Zn or tripling dietary phytate on expression of the pancreatic MT gene, assumed to indicate availability of Zn: performance was not measured (Fig. 16.13). Real differences in functional availability of that order should greatly affect faecal excretion, performance and requirement but remain unreported.

- Thirdly, improvement in availabilty may be dependent on the strength of antagonism from phytate and relatively small.

A small increase from 6.0 to 7.4g Ca kg^{-1}DM reduced RBVZn for Zn-Met in chicks by 3.8-fold (Wedekind *et al.*, 1992, 1994a): such a large effect may indicate differences in faecal endogenous losses (*FEL*Zn). Increases of around 20% have been reported in tissue Zn or pancreatic MT RNA when ZnMet and $ZnSO_4$ were compared but were either not significant (Azad *et al.*, 2017) or age-dependent (Sou *et al.*, 2015). Phytate degradation begins in the gizzard (p. 140), which is underdeveloped in the hatchling.

- Fourthly, chelates may be absorbed as intact molecules from the immature or leaky gut (p. 424), though not from a healthy gut (Spears, 2015). This possibility was described in newborn calves given ZnMet (p. 434) and may occur in newly hatched chicks, particularly those deliberately infected with gut pathogens, and early-weaned pigs.

Numerous interactions between zinc source and infection were reported when chicks were exposed to single or mixed infections with gut pathogens (He *et al.*, 2019). The normal reduction in serum Zn after microbial infection was absent in chicks fed Zn-proteinate rather than $ZnSO_4$. Expression of several agents of zinc trafficking was affected by source but might be an adjuvant effect: there was no evidence that outcome of infection changed. A new chelating agent, pectin-oligosaccharide, increased functional, non-functional and performance indices of Zn status in chicks in a dose-dependent manner (Wang *et al.*, 2019). Decreased foot-pad lesions in chicks given Zn_o rather than Zn_i without improved humoral immunity (Vieira *et al.*, 2013) may be pharmaceutical in origin.

Pharmacological Responses to Zinc in Piglets

Reliance on large Zn supplements, providing 2–4g kg^{-1}DM, to improve growth and health of early-weaned piglets (Hill *et al.*, 2001) has been banned in the European Community (EFSA, 2014), following evidence that transferable antibiotic resistance is induced in gut microbes (Martin *et al.*, 2013b; Vahjen *et al.*, 2015).[xix] Young pigs strongly discriminated against

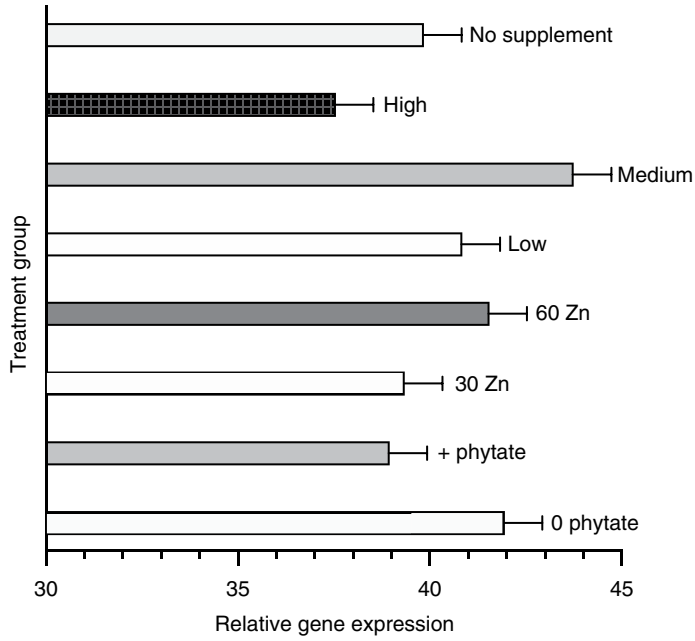

Fig. 16.13. Main treatment effects on expression of mRNA for the pancreatic metallothionein gene of adding phytate (10 g) and zinc (30 or 60mg kg^{-1}DM) as $ZnSO_4$ or one of three Zn chelates, with low, medium or high bonds, to the corn/SBM diet of broiler chicks six days old: no zinc supplement was given for the first two days of life and effects of source diminished with age in previous experiments. (Data from Huang *et al*., 2013)

a diet containing so much Zn, when given a choice (Reynolds *et al*., 2010). Such supplements are also potentially disruptive of Fe metabolism (Kondaiah *et al*., 2019) and reduce responses to phytase supplements in pigs and chicks (Augspurger *et al*., 2004; Blavi *et al*., 2017). Pharmacological supplementation had already been restricted to 0.5g Zn kg^{-1}DM on environmental protection grounds in the EC, prompting advocates of Zn_o to claim that hyperavailability would allow health benefits to be obtained with less dietary Zn. However, faecal Zn:dietary Zn ratios were unaffected by source in pigs (e.g. Case and Carlson, 2002) and poultry (e.g. Bao *et al*., 2007), indicating similar availabilities. Attention turned to sources with different structural properties, such as NP (Jankowskia *et al*., 2019) and porous forms of ZnO (Long *et al*., 2017), hoping that higher surface area would increase reactivity and efficacy at relatively low levels of supplementation in pigs (0.5g Zn kg^{-1}DM) (Long *et al*., 2017) and turkeys (Jankowskia *et al*., 2019). However, improved reactivity may increase capacity to induce antibiotic resistance and inhibit Fe metabolism.

Facial eczema

Facial eczema is a disease that can affect sheep, cattle, deer, goats and camelids grazing pastures infected with the fungus *Pithomyces chartarum*. The fungus produces sporidesmin, a hepatotoxic mycotoxin (Di Menna *et al*., 2009). Ryegrass pastures are particularly susceptible to infection during periods of hot humid weather. The disease is particularly prevalent in New Zealand, where warnings are issued when *P. charterum* spore counts on regional pastures exceed 30,000 g^{-1}. High zinc intakes treated and gave protection from the condition from developing in sheep (Towers *et al*., 1975; Smith *et al*., 1977) and zinc therapy was extended successfully to other grazing species of ruminant. The disease is characterized by liver damage, photosensitization, weight loss and death and monitored by measuring activity of gamma glutamyl transferase (GGT in serum), indicative of bile duct damage. Zinc affords protection by countering oxidant and other effects of metabolites normally cleared by a healthy liver. The degree of protection afforded by oral ZnO increased with

intakes providing up to 2 g Zn d^{-1} in sheep and 23mg Zn kg^{-1}LW in cattle. Dosage has to be maintained throughout the period of risk but is some 50 times normal zinc requirements and can induce signs of zinc toxicity and lower copper status (Towers *et al.*, 1975; Smith *et al.*, 1977; Di Menna *et al.*, 2009). An intraruminal bolus was developed that afforded safe and prolonged protection by releasing about 0.8 g Zn from ZnO per day for around six weeks (Munday *et al.*, 1997). Other essential trace elements can be provided to sheep in the same bolus (Se and Co; Grace *et al.*, 1997) and one has been produced for camelids (Cuttance *et al.*, 2016). Drinking water can be supplemented with zinc salts during periods of risk and zinc is also given by drench (Cuttance *et al.*, 2016).

Zinc Toxicity

Clinical toxicity

Viewed from the standpoint of specific clinical symptoms, simple-stomached livestock are extremely tolerant of high Zn intakes, tolerance depending partly on species but mainly on dietary factors that limit availability, like phytate and calcium. Weanling pigs can consume diets containing 2–3g Zn kg^{-1}DM for several weeks (p. 435) but growth and appetite are depressed at 4–8g kg^{-1}DM and mortality is high (NRC, 2005). Raising dietary Ca from 7 to 11g kg^{-1}DM increased tolerance (Underwood and Suttle, 1999). Broilers and layer hens tolerated 1–2g Zn kg^{-1}DM and showed only slight growth and appetite depression at 4g Zn kg^{-1}DM (Oh *et al.*, 1979). Laying hens were fed diets with 20g Zn kg^{-1}DM for 14 days to reduce food intake and induce moult, without adverse long-term consequences (Stevenson and Jackson, 1984): high Ca intakes of laying birds may have been protective. By contrast, growth rates of pre-ruminant calves were reduced on a milk replacer containing >500mg Zn kg^{-1}DM, added as ZnO to a diet low in antagonists (Jenkins and Hidiriglou, 1991): digesta Zn will reach extremely high levels on such highly digestible diets. Weaned ruminants are also relatively intolerant of Zn, probably due to lack of protection from phytate and vulnerability of rumen microflora: ≥1.7g Zn kg^{-1}DM induced pica and disrupted rumen fermentation in lambs (Ott *et al.*, 1966). For diagnosis, high Zn in feed, digesta and faeces (i.e. g >mg kg^{-1}DM concentrations) are indicative of toxicity. Soft tissue Zn shows little change in the short term (for pigs, see Miller *et al.*, 1968) and the largest responses occur in bone (for sheep, see Stake *et al.*, 1975; for poultry, see Wedekind *et al.*, 1992) and eggs. Fears that reserves of nutrients such as zinc become limiting in late embryonic life have prompted interest in supplementation *in ovo* but injected Zn can be harmful (Sun *et al.*, 2018).

Subclinical toxicity

Zinc shares agents of cellular uptake (e.g DMT1 and Zip4) with Fe, Mn, Cu and Cd, and 'enteric crosstalk' between messengers allows each element to affect uptake of others (Kondaiah *et al.*, 2019). Shared intracellular transport proteins (MRE, MTF1 and FPN1) allow similar crosstalk to affect intracellular distribution, even in the brain. Zinc tolerance is attributable to multiple changes in zinc transactions at the genomic level in host and symbiote (see Elgendy *et al.*, 2017) but secondary effects on co-transported elements have received little attention. Adding 300mg Zn kg^{-1}DM to the diet of broilers lowered plasma Cu and Fe (Kakhki *et al.*, 2016). It is often claimed that chelated trace elements are less toxic yet more available than inorganic sources. While this might be true for gut microflora, particularly in ruminants, there is the possibility that stronger chelates may be absorbed intact by DMT1-independent pathways and affect expression of intracellular transporters (p. 435). In a comparison of zinc methionine and ZnO, providing up to 0.5g Zn kg^{-1}DM in the diets of broilers, performance and accretion of zinc in pancreas and tibia did not differ between sources (FEEDAP, 2018). However:

- blanket assumptions that all chelates are equally non-toxic are unjustified.

Notes

[i] Spermatogenesis practically ceased within 20 weeks in lambs on a diet containing only 2.4mg Zn kg^{-1}DM, due possibly to anorexia and reduced secretion of gonadotrophin-releasing hormone, but recovered completely during repletion (Martin and White, 1992).

[ii] Parakeratosis-like lesions were reported in cattle in British Guiana (Legg and Sears, 1960) and Greece (Spais and Papasteriadis, 1974) on pastures containing 18–83mg Zn kg^{-1}DM.

[iii] A slightly smaller number of transporters (ZnT) move zinc within and from, cells. Movements of zinc are orchestrated by ZnT in zinc intracellular (ICV) and extracellular

vesicles (ECV) (Choi *et al*., 2018), a feature unique to zinc metabolism.

[iv] Erythrocytes contain about 1 mg Zn/10^6 cells: of this, over 85% is present as carbonic anhydrase and about 5% as CuZnSOD. Reticulocytes are particularly rich in zinc and MT (approximately 6 mg/10^6 cells) (Cousins, 1996). Mutant mice lacking the genes for MTI and II have increased susceptibility to both deficiency and toxicity of zinc (Kelly *et al.*, 1996). Although MT has a high affinity for zinc, other elements such as cadmium and copper also induce MT synthesis and have higher affinities for the metalloprotein. MT therefore lies at the heart of the many reported interactions between zinc, cadmium and copper (Fig. 2.6).

[v] In chicks, increased Zn intake in the first four days after hatching can lower subsequent Zn retention (Mwangi *et al*., 2017). The phenomenon was described as 'imprinting' but was probably a residual effect of treatment (25 or 45mg Zn kg^{-1}DM) rather than a memory effect.

[vi] Non-dietary factors that influence plasma Zn include: microbial infection (e.g. sheep; Corrigall *et al.*, 1976; Orr *et al.*, 1990); 24-h fasting (e.g. Japanese quail, Harland *et al.*, 1974; pigs, Wedekind *et al.*, 1994b), raising concerns that standard pre-prandial sampling underestimates zinc status; parturition (in cows, the effect was magnified by difficult calvings, with values of 0.38 ± 0.14 compared to 0.68 ± 0.23 mg l^{-1}, 18–24 h after normal parturition; Dufty *et al.*, 1977) and hyperthermic stress (e.g. cattle; Wegner *et al.*, 1973; Japanese quail; Sahin and Kucuk, 2003). In quail, serum Zn fell from 31.3 (much higher in normal avians than other species) to 24.9 µmol l^{-1} during heat stress and was not prevented by a threefold increase in dietary Zn from 36 to 108mg kg^{-1}DM.

[vii] In mature sheep given hay, direct estimates of FEL_{Zn} were approximately 100 µg Zn kg^{-1} liveweight (LW) and were unaffected by zinc intake above the marginal (Suttle *et al.*, 1982). In rats, FEL_{Zn} was reduced by zinc intakes below the marginal but made a smaller contribution to homeostasis than an increased efficiency of absorption, the primary regulator of retention (Underwood and Suttle, 1999).

[viii] Mild maternal zinc depletion lowers MTI in plasma, erythrocytes and soft tissues of offspring (Morrison and Bremner, 1987) and may compromise anti-oxidant defence. Angiotensin-converting apoenzyme levels increased in pregnant sheep during prolonged, severe zinc deprivation and were detectable by reconstitution *in vitro* (Apgar *et al.*, 1993). Such changes might enhance recovery during repletion.

[ix] Improved, mixed pastures in New Zealand contained more zinc in North than South Island (38 versus 22mg kg^{-1}DM) (Grace, 1972), with the range embracing values found in Scotland in uncontaminated pastures (Mills and Dalgarno, 1972).

[x] Phytase and hydroxylated vitamin D_3 (OHD_3) analogue supplements to phytate-rich diets released Zn and P in a proportionate (ratio of P to Zn) and additive manner in broiler chicks (Biehl *et al.*, 1995).

[xi] Requirement for growth in weanling pigs, given corn/SBM diets was around 46mg Zn kg^{-1}DM and slightly less to eliminate parakeratosis (Smith *et al.*, 1960; Miller *et al.*, 1970; Wedekind *et al.*, 1994b).

[xii] Adding zinc to rations containing 25 (Mwanga *et al*., 2017), 28 (Liao *et al*., 2013), 30 (Bun *et al*., 2011), 32 (Akhavan-Salamat and Ghasemi, 2019) and 38 (Schlegel *et al*., 2010) mg Zn kg^{-1}DM did not improve growth or FCE.

[xiii] Higher requirements between 17 and 32mg kg^{-1}DM, were suggested for normal testicular growth and spermatogenesis (Ott *et al.*, 1965; Underwood and Somers, 1969) but a subsequent study with smaller increments in dietary Zn found optimal male fertility and growth with 14mg Zn kg^{-1}DM (Martin and White, 1992).

[xiv] Effects of wholly or partially replacing a cluster of inorganic trace minerals with organic sources (e.g. Hackbart *et al*., 2010) cannot indicate a specific advantage for zinc chelation. Partial substitution of ZnMet for ZnOHCL to provide 145mg Zn kg^{-1}DM, tended to increase expression of one of eight markers of mammary epithelial cell integrity in heat-stressed dairy cows, insufficient to justify a conclusion that ZnMet 'improves integrity of the mammary epithelium' (Weng *et al*., 2018). Heat stress increased the expression of several genes and reduced milk yield by 22%, regardless of zinc source. Fattening steers were given either $ZnSO_4$ or ZnMet, respectively adding 28 and 66 mg Zn to an adequate 94mg Zn kg^{-1}DM, with or without anabolic implants: expression of some genes was increased by ZnMet, while others decreased, but there were no interactions with implant and no effects on feedlot performance (Hergenreder *et al*., 2016). The conclusion that ZnMet had 'positive biological effects on musculo-skeletal tissue' is again questionable.

[xv] Feed-grade sources of ZnO had RBV_{Znl} of 44% or 61% of analytical-grade (AR) $ZnSO_4$, depending on index of status (tibia Zn or growth) (Wedekind and Baker, 1990; Wedekind *et al.*, 1992). RBV of three feed-grade ZnO sources varied from 30% to 90%, regardless of response index, and two feed-grade sources of $ZnSO_4$ had RBV of 87–88% compared with AR $ZnSO_4$ (Edwards and Baker, 1999). Tetrabasic zinc chloride has a similar RBV to $ZnSO_4$ for chicks (Batal *et al.*, 2001).

[xvi] A claimed 1.64 advantage for Zn_O over Zn_I in terms of response in tibia Zn in broilers (Star *et al*., 2012) was due entirely to use of a slopes ratio test over different ranges of added dietary Zn (0-15 v 0-20mg kg^{-1}DM: tibia Zn was the same at the maximal level used for Zn_I. Addition of copper at a supra-nutritional concentration (250mg Cu kg^{-1}DM) to a Zn-deficient, semi-purified diet inhibited broiler growth less with chelated rather than Zn_i (30mg kg^{-1}DM) also added (Zhao *et al*., 2016). Superior availability for chelated Zn was claimed but tibia Zn did not differ between sources. Tibia zinc also

did not differ in a comparison of Zn-methionine and ZnO added to a conventional broiler diet (FEEDAP, 2018). Provision of 125mg Zn kg^{-1}DM as glycinate or proteinate (Ivanišinová *et al.*; 2016) rather than $ZnSO_4$ did not improve broiler performance or zinc status but the unsupplemented corn/SBM diets were adequate in Zn (84 and 65mg kg^{-1}DM for starter and grower chicks, respectively). Chelated sources raised MnSOD activity and may have presented an oxidative stress. Adding 80 rather than 20mg Zn kg^{-1}DM as Zn-methionine rather than ZnO to diets of heat-stressed broilers improved antibody responses, raised serum CuZnSOD and reduced muscle peroxidation (MDA) without improving performance, although improved growth was listed amongst benefits (Akhavan-Salamat and Ghasemi, 2019). No unsupplemented controls were used.

[xvii] Zinc glycinate supplementation increased performance in breeder hens more than $ZnSO_4$ in taking dietary Zn from 24 to 104mg kg^{-1} DM and gave higher values of markers for antioxidant status (Zhang *et al*., 2017a). However, similar breeder hens only needed 40 mg added Zn_i for optimum performance and antioxidant status (Li *et al*., 2019b) (also, see Fig. 16.10). Feeding more zinc than required may be a source of oxidant stress, as indicated by a rise in glutathione peroxidase activity in livers of breeder hens receiving >100mg Zn kg^{-1} DM (Li *et al*., 2019b).

[xviii] Chelation strength influenced zinc uptake from isolated loops of duodenum (Yu *et al*., 2008) but the model is a questionable indicator of functional availability (p. 27). Provision of Zn, Cu, Fe and Mn, together, as glycinates, did not improve broiler performance, carcase yield or reduce faecal excretion of those elements, when compared with the same concentrations (100mg kg^{-1}DM) of grouped inorganic sources (De Marco *et al*., 2017).

[xix] Large Zn supplements reduced incidence of diarrhoea in nursery piglets, partly by altering the host response to bacterial populations in the gut (Kreuzer-Redmer *et al*., 2018). Responses were additional to those obtained from concurrent antibiotic administration but more likely to induce antibiotic resistance when both were added, and not additional to those obtained with pharmacological Cu supplements (Smith *et al.*, 1997): Ca and P availabilities were reduced (Blavi *et al*., 2017). Changes in gut morphology, pancreatic enzyme activity and cellular immune responses were reported without evidence of related benefits (Suttle, 2010). In a collaborative study to define optimal supplementation (Hill *et al.*, 2001), growth responses tended to be larger and optimum dietary Zn higher in piglets weaned at 15 rather than 20 days of age.

References

Adeola, O., Lawrence, B.V., Sutton, A.C. and Cline, T.R. (1995) Phytase-induced changes in mineral utilisation in zinc-supplemented diets for pigs. *Journal of Animal Science* 73, 3384–3391.

Akhavan-Salamat, H. and Ghasemi, H.A. (2019) Effect of different sources and contents of zinc on growth performance, carcass characteristics, humoral immunity and antioxidant status of broiler chickens exposed to high environmental temperatures. *Livestock Science* 223, 76–83.

Alcantara, E.H., Lomeda, R.A., Feldmann, J., Nixon, G.F., Beattie, J.H. and Kwun, I.S. (2011) Zinc deprivation inhibits extracellular matrix calcification through decreased synthesis of matrix proteins in osteoblasts. *Molecular Nutrition and Food Research* 55, 1552–1560.

Aliarabi, H., Fadayifar, A., Tabatabaei, M.M., Zamani, P., Bahari, A., Farahaver, A. and Dezfulian, A.H. (2015) Effect of zinc source on hematological, metabolic parameters and mineral balance in lambs. *Biological Trace Element Research* 168, 82–90.

Alimohamady, R., Aliarabi, H., Bruckmaier, H.M. and Christensen, R.C. (2019) Effect of different sources of supplemental zinc on performance, nutrient digestibility, and antioxidant enzyme activities in lambs. *Biological Trace Element Research* 189, 75–84.

Alloway, B.J. (2004) *Zinc in Soils and Crop Nutrition*. IZA Publications, International Zinc Association, Brussels.

Ao, T. Pierce, J.L. Power, R., Dawson, K.A., Pescatore, A.J., Cantor, A.H. and Ford, M.J. (2006) Evaluation of Bioplex Zn® as an organic zinc source for chicks. *International Journal of Poultry Science* 5, 808–811.

Ao, T., Pierce, J.L., Pescatore, A.J., Cantor, A.H., Dawson, K.A., Ford, M.J. and Shafer, B.L. (2007) Effects of organic and phytase supplementation in a maize–soybean meal diet on the performance and tissue zinc content of broiler chicks. *British Poultry Science* 48, 690–695.

Apgar, J. (1979) Alkaline phosphatase activity and zinc level in plasma as indicators of zinc status in pregnant and lactating sheep. *Nutrition Reports International* 19, 371–376.

Apgar, J., Everett, G.A. and Fitzgerald, J.A. (1993) Dietary zinc deprivation effects parturition and outcome of pregnancy in the ewe. *Nutrition Research* 13, 319–330.

ARC (1980) *The Nutrient Requirements of Ruminants*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 184–185.

Arredondo, M.A., Casas, G.A. and Stein, H.H. (2019) Increasing levels of microbial phytase increases the digestibility of energy and minerals in diets fed to pigs. *Animal Feed Science and Technology* 248, 27–36.

Augspurger, N.R., Spencer, J.D., Webel, D.M. and Baker, D.H. (2004) Pharmacological zinc levels reduce the phosphorus-releasing efficacy of phytase in young pigs and chickens. *Journal of Animal Science* 82, 1732–1739.

Azad, K.S., Shariatmadari, F., Torshizi , K.M.A. and Hamed, A. (2017) Effect of zinc concentration and

source on performance, tissue mineral status, activity of superoxide dismutase enzyme and lipid peroxidation of meat in broiler chickens. *Animal Production Science* 58, 1837–1846.

Baker, D.H. and Ammerman, C.B. (1995) Zinc bioavailability. In: Ammerman, C.B., Baker, D.H. and Lewis, A.J. (eds) *Bioavailability of Nutrients for Animals*. Academic Press, New York, pp. 367–398.

Bao, Y.M., Choct, M., Iji, P.A. and Bruerton, K. (2007) Effect of organically complexed copper, iron, manganese and zinc on broiler performance, mineral excretion and accumulation in tissues. *Journal of Applied Poultry Research* 16, 448–455.

Batal, A.B., Parr, T.M. and Baker, D.H. (2001) Zinc bioavailability in tetrabasic zinc chloride and the zinc requirement of young chicks fed a soya concentrate diet. *Poultry Science* 80, 87–90.

Beach, R.S., Gershwin, M.E. and Hurley, L.S. (1981) Nutritional factors and autoimmunity. I. Immunopathology of zinc deprivation in New Zealand mice. *Journal of Immunology* 126, 1999–2006.

Beattie, J.H. and Kwun, I.-S. (2004) Is zinc deficiency a risk factor in atherosclerosis? *British Journal of Nutrition* 91, 177–181.

Beattie, J.H. and Trayhurn, P. (2002) Metallothioneins and oxidative stress. *Nutritional Sciences* 5, 228–233.

Beattie, J.H., Gordon, M.-J., Rucjlidge, G.J., Reid, M.D., Duncan, G.J. *et al*. (2008) Aorta protein networks in marginal and acute zinc deficiency. *Proteonomics* 8, 2126–2135.

Berg, J.M. (1990) Zinc fingers and other metal-binding domains: elements for interactions between molecules. *Journal of Biological Chemistry* 265, 6513–6516.

Bernstein, K.E., Khan, Z., Giani, J.F., Cao, D.Y., Bernstein, E.A. and Shen. X.Z. (2018) Angiotensin-converting enzyme in innate and adaptive immunity. *Nature Reviews in Nephrolology* 14, 325–336.

Biehl, R.R., Baker, D.H. and DeLuca, H.F. (1995) 1α-Hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese utilization in chicks fed soy-based diets. *Journal of Nutrition* 125, 2407–2416.

Bikker, P., Jongbloed, A.W. and Thissen, J.T.N.M. (2012) Meta-analysis of effects of microbial phytase on digestibility and bioavailability of copper and zinc in growing pigs. *Journal of Animal Science* 90(suppl 4), 134–136.

Blavi, L., Sola-Oriol, D., Perez, J.F. and Stein, H.H. (2017) Effects of zinc oxide and microbial phytase on digestibility of calcium and phosphorus in maize-based diets fed to growing pigs. *Journal of Animal Science* 95, 847–854.

Bremner, I. (1991) A molecular approach to the study of copper and zinc metabolism. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals*. IMI, Zagreb, pp. 1-1–1-3.

Bremner, I. (1993) Metallothionein in copper deficiency and copper toxicity. In: Anke, M., Meissner, D. and Mills, C.F. (eds) *Proceedings of the Eighth International Symposium on Trace Elements in Man and Animals*. Verlag Media Touristik, Gersdorf, Germany, pp. 507–515.

Brugger, D. and Windisch, W. (2016) Subclinical zinc deficiency impairs pancreatic digestive enzyme activity and digestive capacity of weaned piglets. *British Journal of Nutrition* 116, 425–433.

Brugger, D. and Windisch, W. (2017) Short-term subclincial zinc deficiency in weaned piglets affects cardiac redox metabolism and zinc concentration. *Journal of Nutrition* 147, 521–527.

Brugger, D. and Windisch, W. (2019a) Adaption of body zinc pools in weaned piglets challenged with subclinical zinc deficiency. *British Journal of Nutrition* 121, 849–858.

Brugger, D. and Windisch, W. (2019b) Zn metabolism of monogastric species and consequences for the definition of feeding requirements and the estimation of feed availability. *Journal of Zhejiang University of Science B* 20, 617–627.

Brugger, D., Buffler, M. and Windisch, W. (2014) Development of an experimental model to assess the bioavailability of zinc in practical piglet diets. *Archives of Animal Nutrition* 68, 73–92.

Brugger, D., Schlati, M. and Windisch, W. (2018) Short-term kinetics of tissue zinc exchange in 65Zn-labelled adult rats receiving a sufficient dietary zinc supply. *Proceedings of the Society of Nutrition Physiology* 27.

Bun, S.D., Guo, Y.M., Guo, F.C., Ji, F.J. and Cao, H. (2011) Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with Eimeria tenella. *Poultry Science* 90, 1220–1226.

Cakmak, I. (2008) Enrichment of cereal grains with zinc: agronomic or genetic biofortification. *Plant and Soil* 302, 1–17.

Caldera, E., Weigel, B., Kucharczyk, V.N., Sellins, K.S., Archibeque, S.L. *et al*. (2019) Trace mineral source influences ruminal distribution of copper and zinc and their binding strength to ruminal digesta. *Journal of Animal Science* 97, 1852–1864.

Cao, J. and Cousins, R.J. (2000) Metallothionein mRNA in monocytes and peripheral blood mononuclear cells and in cells from dried blood spots increases after zinc supplementation of men. *Journal of Nutrition* 130, 2180–2187.

Case, C.L. and Carlson, M.S. (2002) Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. *Journal of Animal Science* 80, 1917–1924.

Chang, M.N., Wei, J.Y., Hao, L.Y., Ma, F.T., Li, H.Y. *et al* (2020) Effects of different types of zinc supplement on the growth, incidence of diarrhea, immune function, and rectal microbiota of newborn dairy calves. *Journal of Dairy Science* 103, 6100–6113.

Chesters, J.K. (1992) Trace element–gene interactions. *Nutrition Reviews* 50, 217–223.

Chhabra, A. and Arora, S.P. (1993) Effect of vitamin A and zinc supplement on alcohol dehydrogenase and superoxide dismutase activities of goat tissues. *Indian Journal of Animal Sciences* 63, 334–338.

Choi, S., Liu, X. and Pan, Z. (2018) Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. *Acta Pharmacologica Sinica* 39, 1120–1132.

Coleman, J.E. (2002) Zinc proteins: enzymes, storage proteins, transcription factors and replication proteins. *Annual Reviews of Biochemistry* 61, 897–946.

Cope, C.M., Mackenzie, A.M., Wilde, D. and Sinclair, L.A. (2009) Effects of level and form of dietary zinc on dairy cow performance and health. *Journal of Dairy Science* 92, 2128–2135.

Corrigall, W., Dalgarno, A.C., Ewen, L.A. and Williams, R.B. (1976) Modulation of plasma copper and zinc concentrations by disease status in ruminants. *Veterinary Record* 99, 396–397.

Cousins, R.J. (1996) Zinc. In: Filer, L.J. and Ziegler, E.E. (eds) *Present Knowledge in Nutrition*, 7th edn. International Life Science Institute–Nutrition Foundation, Washington, DC.

Cousins, R.J., Blanchard, R.K., Moore, J.B., Cui, L., Green, C.L. *et al*. (2003) Regulation of zinc metabolism and genomic outcomes. *Journal of Nutrition* 133, 1521S–1526S.

Cousins, R.J., Liuzzi, J.P. and Lichten, L.A. (2006) Mammalian zinc transport, trafficking and signals. *Journal of Biological Chemistry* 281, 24085–24089.

Cuttance, E., Stevenson, M.A., Laven, R. and Mason, W. (2016) Facial eczema management protocols used on dairy farms in the North Island of New Zealand and associated concentrations of zinc in serum. *New Zealand Veterinary Journal* 64, 1–23.

Davies, N.T., Carswell, A.J.P. and Mills, C.F. (1985) The effect of variation in dietary calcium intake on the phytate–zinc interaction in rats. In: Mills, C.F., Bremner, I. and Chesters, J.K. (eds) *Proceedings of the Fifth International Symposium on Trace Elements in Man and Animals*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 456–457.

De Marco, M., Zoon, M.V., Margetyal, C., Picart, C. and Ionescu, C. (2017) Dietary administration of glycine complexed trace minerals can improve performance and slaughter yield in broilers and reduces mineral excretion. *Animal Feed Science and Technology* 232, 182–189.

Demertzis, P.N. (1973) Oral zinc therapy in the control of infectious pododermatitis in young bulls. *Veterinary Record* 93, 219–222.

Devishree, K., Pan, W., Beggs, M.R., Trepiccione, F., Chambrey, R., *et al*. (2018) Deficiency of carbonic anhydrase II results in a urinary concentrating defect. *Frontiers in Physiology* 8, 1108.

Dewar, W.A. and Downie, J.N. (1984) The zinc requirements of broiler chicks and turkey poults fed on purified diets. *British Journal of Nutrition* 51, 467–477.

Di Menna, M.E., Smith, B.L. and Miles, C.O. (2009) A history of facial eczema (pithomycotoxicosis) research. *New Zealand Journal of Agricultural Research* 52, 345–376.

Dourmad, J.Y., Guinotte, F. and Nys, Y. (2002) Bioavailability of two sources of zinc in weanling pigs**.** *Animal Research* 51 315–326.

Droke, E.A. and Spears, J.W. (1993) *In vitro* and *in vivo* immunological measurements in growing lambs fed diets deficient, *marginal or adequate in zinc. Journal of Nutrition* 123, 71–90.

Droke, E.A., Spears, J.W., Armstrong, J.D., Kegley, E.B. and Simpson, R. (1993a) Dietary zinc affects serum concentrations of insulin and insulin-like growth factor I in lambs. *Journal of Nutrition* 123, 13–19.

Droke, E.A., Spears, J.W., Brown, T.T. and Quereshi, M.A. (1993b) Influence of dietary zinc and dexamethasone on immune responses and resistance to *Pasteurella haemolytica* challenge in growing lambs. *Nutrition Research* 13, 1213–1216.

Dufty, J.H., Bingley, J.B. and Cove, L.Y. (1977) The plasma zinc concentration of nonpregnant, pregnant and parturient Hereford cattle. *Australian Veterinary Journal* 53, 519–522.

Edwards, H.M. III and Baker, D.H. (1999) Zinc bioavailability in soybean meal. *Journal of Animal Science* 78, 1017–1021.

Edwards, H.M. III and Baker, D.H. (2000) Bioavailability of zinc in several sources of zinc oxide, *zinc sulfate and zinc metal. Journal of Animal Science* 77, 2730–2735.

EFSA (2014) Scientific opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. *EFSA Journal* 12. DOI:10.2903/j.efsa.2014.3668

Egan, A.R. (1972) Reproductive responses to supplemental zinc and manganese in grazing Dorset Horn ewes. *Australian Journal of Experimental Agriculture and Animal Husbandry* 12, 131–135.

Elgendy, R., Palazzo, F., Castellani, F., Giantin, M., Grotta, L. *et al*. (2017) Transcriptome profiling and functional analysis of sheep fed with high zinc-supplemented diet: a nutrigenomic approach. *Animal Feed Science and Technology* 234, 195–204.

Emmert, J.L. and Baker, D.H. (1995) Zinc stores in chickens delay the onset of deficiency symptoms. *Poultry Science* 74, 1011–1021.

Engle, T.E., Nockels, C.F., Kimberling, C.V., Weaber, D.L. and Johnson, A.B. (1997) Zinc repletion with organic and inorganic forms of zinc and protein turnover in marginally zinc-deficient calves. *Journal of Animal Science* 75, 3074–3081.

Fadayifar, A., Aliarabi, H., Tabatabaei, M.M., Zamani, P., Bahari, A., Malecki, M. and Dezfoulian, A.H. (2012) Improvement in lamb performance on barley based

diet supplemented with zinc. *Livestock Science* 144, 285–289.

Faulkner, M.J., St-Pierre, N.R. and Weis, W.P. (2017) Effect off source of trace minerals in either forage- or by-product-based diets fed to dairy cows: 2. *Apparent availability of minerals*. *Journal of Dairy Science* 100, 5368–5377.

FEEDAP (2018) Scientific Opinion of EFSA panel: safety of zinc chelate of methionine sulfate for the target species. *European Food Safety Authority Journal* 16, 1–7.

Fischer Walker, C. and Black, R.E. (2004) Zinc and the risk for infectious disease. *Annual Review of Nutrition* 24, 255–275.

Flanagan, P.R. (1984) A model to produce pure zinc deficiency in rats and its use to demonstrate that dietary phytate increases the excretion of endogenous zinc. *Journal of Nutrition* 114, 493–502.

Gajula, S.S., Chelasani, V.K., Panda, A.K., Raju-Mantena, V.L.N. and Savaram, R.R. (2011) Effect of supplemental inorganic Zn and Mn and their interactions on the performance of broiler chicken, mineral bioavailability, and immune response. *Biological Trace Element Research* 139, 177–187.

Genther, O.N. and Hansen, S. (2015) The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. *Journal of Dairy Science* 98, 566–573.

Gibson, R.S., Raboy, V. and King, J.C. (2018) Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutrition Reviews* 76, 793–804.

Goff, J.P. and Stabel, J.R. (1990) Decreased plasma retinol α-tocopherol and zinc concentration during the periparturient period: effect of milk fever. *Journal of Dairy Science* 73, 3195–3199.

Golden, M.H.N. (1996) Specific deficiencies versus growth failure: type i and type ii nutrients. *Journal of Nutritional and Environmental Medicine* 6, 301–308.

Grace, N.D. (1972) Observations on plasma zinc levels in sheep grazing New Zealand pastures. *New Zealand Journal of Agricultural Research* 15, 284–288.

Grace, N.D. and Sumner, R.M.W. (1986) Effect of pasture allowance, season and breed on the mineral content and rate of mineral uptake by wool. *New Zealand Journal of Agricultural Research* 29, 223–230.

Grace, N.D., Munday, R., Thompson, A.M., Towers, N.R., O'Donnell *et al*. (1997) Evaluation of intraruminal devices for combined facial eczema control and trace element supplementation in sheep. *New Zealand Veterinary Journal* 45, 236–238.

Gunther, V., Lindert, U. and Schaffner, W. (2012) The taste of heavy metals: gene regulation by MTF-1. *Biochimica et Biophysica Acta-Molecular Cell Research* 1823, 1416–1425.

Haase, H. and Rink, L. (2014) Zinc signals and immune function. *BioFactors* 40, 27–40.

Hackbart, K.S., Ferreira, R.M., Dietsche, A.A., Socha, M.T., Shaver, R.D. *et al*. (2010) Effect of dietary organic zinc, manganese, copper, and cobalt supplementation on milk production, follicular growth, embryo quality, and tissue mineral concentrations in dairy cows. *Journal of Animal Science* 88, 3856–3870.

Hansard, S.L., Mohammed, A.S. and Turner, J.W. (1968) Gestation age effects upon maternal–foetal zinc utilization in the bovine. *Journal of Animal Science* 27, 1097–1102.

Harland, B.F., Fox, M.R.S. and Fry, B.E. Jr (1974) Changes in plasma zinc related to fasting and dietary protein intake of Japanese quail. *Proceedings of the Society for Experimental Biology and Medicine* 145, 316–322.

Harvey, L.J., Dainty, J.R., Hollands, W.J. and Bull, V.J. (2007) Effects of high-dose iron supplements of fractional zinc absorption and status in pregnant women. *American Journal of Clinical Nutrition* 85, 131–136.

He, B., Bortoluzzi, C., King, W.D., Graugnard, D., Dawson, K.A. and Applegate, T. (2019) Zinc source influences the gene expression of zinc transporters in jejunum and cecal tonsils during broiler challenge with *Eimeria maxima* and *Clostridium perfringens*. *Poultry Science* 98, 1146–1152.

Hergenreder, J.E., Legako, J.F., Dinh, T., Spivey, K.S., Baggerman, J.O. *et al*. (2016) Zinc methionine supplementation impacts gene and protein expression in calf-fed Holstein steers with minimal impact on feedlot performance. *Biological Trace Element Research* 171, 315–327.

Hill, D.A., Peo, E.R., Lewis, A.J. and Crenshaw, J.D. (1986) Zinc–amino acid complexes for swine. *Journal of Animal Science* 63, 121–130.

Hill, G.M., Mahan, D.C., Carter, S.D., Cromwell, G.L. and Ewan, R.C. (2001) Effect of pharmacological concentrations of zinc oxide with or without the inclusion of an antibacterial agent on nursery pig performance. *Journal of Animal Science* 79, 934–941.

Hoekstra, W.G., Faltin, E.C., Lin, C.W., Roberts, H.F. and Grummer, R.H. (1967) Zinc deficiency in reproducing gilts fed a diet high in calcium and its effect on tissue zinc and blood serum alkaline phosphatase. *Journal of Animal Science* 26, 1348–1357.

Huang, Y.L., Lu, L., Luo, X.J. and Liu, B. (2007) An optimal dietary zinc level of broiler chicks fed a corn–soybean meal diet. *Poultry Science* 86, 2582–2589.

Huang, Y.L., Lu, L., Xie, J.J., Li, S.F., Li, X.L. *et al*. (2013) Relative bioavailabilities of organic zinc sources with different chelation strengths for broilers fed diets with low or high phytate content. *Animal Feed Science and Technology* 179, 144–148.

Humer, E. and Zebeli, Q. (2015) Phytate in feed ingredients and potentials for improving the utilization of phosphorus in ruminant nutrition. *Animal Feed Science and Technology* 209, 1–15.

Humer, E., Schwarz, C. and Schedle, K. (2015) Phytate in pig and poultry nutrition. *Animal Physiology and Animal Nutrition* 99, 605–625.

Hurley, L.S. (1981) Teratogenic effects of manganese, zinc and copper in nutrition. *Physiological Reviews* 61, 249–295.

Ianni, A., Iannaccone, M., Martino, C., Innosa, D., Grotta, L., Bennato, F. and Martino, G. (2019) Zinc supplementation of dairy cows: effects on chemical composition, nutritional quality and volatile profile of Giuncata cheese. *International Dairy Journal* 94, 65–71.

Ishaq, S.L., Page, C.M., Yeoman, C.J., Murphy, T.W., Van Emon, M.L. and Stewart, W.C. (2018) Zinc AA supplementation alters yearling ram rumen bacterial communities but zinc sulfate supplementation does not. *Journal of Animal Science* 97, 687–697.

Ivanišinová, O., Grešáková, D., Ryzner, M., Oceľová, V. and Čobanová, K. (2016) Effects of feed supplementation with various zinc sources on mineral concentration and selected antioxidant indices in tissues and plasma of broiler chickens. *Acta Veterinaria Brno* 85, 285–291.

Jankowskia, J., Kozłowskia, K., Ognik, K., Otowskia, K., Juśkiewiczc, J. and Zduńczyk, Z. (2019) The effect of the dietary inclusion levels and sources of zinc on the performance, metabolism, redox and immune status of turkeys. *Animal Feed Science and Technology* 252, 103–114.

Jenkins, K.J. and Hidiriglou, M. (1991) Tolerance of the pre-ruminant calf to excess manganese and zinc in a milk replacer. *Journal of Dairy Science* 74, 1047–1053.

Jia, W., Jia, Z., Zhang, W., Wang, R., Zwhang, S. and Zhu, X. (2008) Effects of dietary zinc on performance, nutrient digestibility and plasma zinc status in Cashmere goats. *Small Ruminant Research* 80, 68–72.

Jumba, I.O., Suttle, N.F., Hunter, E.A. and Wandiga, S.O. (1995) Effects of soil origin and mineral composition and herbage species on the mineral composition of forages in the Mount Elgon region of Kenya. 2. Trace elements. *Tropical Grasslands* 29, 47–52.

Kakhki, R.A.M., Bakhshalinejad, R. and Shafiee, M. (2016) Effect of dietary zinc and tocopheryl acetate on broiler performance, immune responses, antioxidant enzyme activities, minerals and vitamin concentration in blood and tissues of broilers. *Animal Feed Science and Technology* 221, 12–26.

Kamberi, M.A., Muji, S., Kryeziu, A., Kasrati, R. and Mestani, N. (2018) The excretion of Ca, Mg, Zn and Cu via excreta of laying hens fed low phosphorus diets and phytase. *Agronomy Research* 16, 443–453.

Keilin, D. and Mann, T. (1940) Carbonic anhydrase: purification and nature of the enzyme. *Biological Journal* 34, 1163–1176.

Kelly, E.J., Quaife, C.J., Froelick, G.J. and Palmiter, R.D. (1996) Metallothionein I and II protect against zinc deficiency and toxicity in mice. *Journal of Nutrition* 126, 1782–1790.

Kennedy, K.J., Rains, T.M. and Shay, N.F. (1998) Zinc deficiency changes preferred macronutrient intake in subpopulations of Sprague-Dawley outbred rats and reduces hepatic pyruvate kinase gene expression. *Journal of Nutrition* 128, 43–49.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S.L., Bearson, B.L, Shurson, G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Kienholz, E.W., Turk, D.E., Sunde, M.L. and Hoekstra, W.G. (1961) Effects of zinc deficiency in the diets of hens. *Journal of Nutrition* 75, 211–221.

Kim, E.-S., Noh, S.K. and Koo, S.I. (1998) Marginal zinc efficiency lowers the lymphatic absorption of *a*-tocopherol in rats. *Journal of Nutrition* 128, 265–270.

King, J.C., Brown, K.H., Gibson, R.S., Krebs, N.N., Lowe, N.M., Stekmann, J.H. and Raiten, D.J. (2016) Biomarkers of nutrition for development (BOND)-Zinc review. *Journal of Nutrition* 146 (Suppl), 858S–85S.

Kloubert, V., Blaabjerg, K., Dalgaard, T.S., Poulsen, H.D., Rink, L. and Wessels, I. (2018) Influence of zinc supplementation on immune parameters in weaned pigs. *Journal of Trace Elements in Medicine and Biology* 49, 231–240.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–310

Kogan, S., Sond, A. and Granick, M.S. (2017) Zinc and wound healing: a review of zinc physiology and clinical applications. *Wounds* 29, 102–106.

Kondaiah, P., Yaduvanshi, P.S., Sharp, P.A. and Pullakhandam, R. (2019) Iron and zinc homeostasis and interactions: does enteric zinc excretion cross-talk with intestinal iron absorption? *Nutrients* 11, 1885–1899.

Kreuzer-Redmer, S., Arends, D., Schulte, J., Karweina, D., Korkuc, P. *et al.* (2018) High dosage of zinc modulates T-cells in a time-dependent manner within porcine gut-associated lymphatic tissue. *British Journal of Nutrition* 120, 1349–1358.

Kul'kova, J., Bremner, I., McGaw, B.A., Reid, M. and Beather, J.H. (1993) Mercury–zinc interactions in marginal zinc deficiency. In: Anke, M., Meissner, D. and Mills, C.F. (eds) *Proceedings of the Eighth International Symposium on Trace Elements in Man*

and Animals. Verslag Media Touristik, Gersdorf, Germany, pp. 635–637.

Kwiecień, M., Winiarska-Mieczan, A., Milczarek, A., Tomaszewska, E. and Matras, J. (2016) Effects of zinc glycine chelate on growth performance, carcass characteristics, bone quality, and mineral content in bone of broiler chicken. *Livestock Science* 191, 43–50.

Kwun, I.-S., Cho, Y.-E., Lomeda, R.-A.R., Kwon, S.-T., Kim, Y. and Beattie, J.H. (2007) Marginal zinc deficiency in rats decreases leptin expression independently of food intake and corticotrophin-releasing hormone in relation to food intake. *British Journal of Nutrition* 98, 485–489.

Lee, J., Treloar, B.P. and Harris, P.M. (1994) Metallothionein and trace element metabolism in sheep tissues in response to high and sustained zinc dosages. 1. Characterisation and turnover of metallothionein isoforms. *Australian Journal of Agricultural Research* 45, 303–320.

Legg, S.P. and Sears, L. (1960) Zinc sulfate treatment of parakeratosis in cattle. *Nature, UK* 186, 1061–1062.

Lei, X.G., Ku, P.K., Miller, E.R., Ullrey, D.E. and Yokoyama, M.T. (1993) Supplemental microbial phytase improved bioavailability of dietary zinc to weanling pigs. *Journal of Nutrition* 123, 1117–1123.

Li, L., Abouelezz, K.F.M., Gou, Z., Lin, X., Wang, Y. *et al*. (2019a) Optimization of dietary zinc requirement for broiler breeder hens of chinese yellow-feathered chicken. *Animals* 9, 472–486.

Li, L., Li, H., Zhou, W., Feng, J. and Zou, X. (2019b) Effects of zinc methionine supplementation on laying performance, zinc status, intestinal morphology, and expressions of zinc transporters' mRNA in laying hens. *Journal of Science in Food and Agriculture* 99, 6582–6588.

Li, M.Z., Huang, J.T., Tsai, Y.H., Mao, S.Y., Fu, C.M. and Lien, T.F. (2016) Nanosize of zinc oxide and the effects on zinc digestibility, growth performances, immune response and serum parameters of weanling piglets. *Animal Science Journal* 87, 1379–1385.

Liao, X., Li, A., Lu, L., Liu, S., Li, S. *et al.* (2013) Optimal dietary zinc levels of broiler chicks fed a corn–soybean meal diet from 22 to 42 days of age. *Animal Production Science* 53, 388–394.

Lichten, L.A. and Cousins, R.J. (2009) Mammalian zinc transporters: nutritional and physiologic regulation. *Annual Review of Nutrition* i29, 153–176.

Lin, X., Meng, T., Yang T., Xu, X., Zhao, Y. and Wu, X. (2020) Circadian zinc feeding regime in laying hens related to laying performance, oxidation status, and interaction of zinc and calcium. *Poultry Science* 99, 6783–6796.

Linares, L.B., Broomhead, J.N., Guaiume, E.A., Ledoux, D.R., Veum, T.L. and Raboy, V. (2007) Effects of a low phytate barley (*Hordeum vulgare* L.) on zinc utilisation in young broiler chicks. *Poultry Science* 86, 299–308.

Liu, Y.H., Sun, M.H.,Yang, J.Q., Jia, C.L., Zhang, M., Zhu, Y.J. and Zhang, Y. (2015) Influence of different dietary zinc levels on cashmere growth, plasma testosterone level and zinc status in male Liaoning Cashmere goats. *Journal of Animal Physiology and Animal Nutrition* 99.

Lo, M.N., Damon, L.J., Tay, J.W., Jia, S. and Palmer, A.E. (2020) Single cell analysis reveals multiple requirements for zinc in the mammalian cell cycle. *eLife*, 9, 51107.

Long, L., Chen, J., Zhang, Y., Liang, X., Ni, H., Zhang, B. and Yin, Y. (2017) Comparison of porous and nano zinc oxide for replacing high-dose dietary regular zinc oxide in weaning piglets. *PLOS ONE* 12, e0182550.

Lonnerdal, B. (2000) Dietary factors affecting zinc absorption. *Journal of Nutrition* 130, 1378–1383S.

Lukaski, H.C. (2005) Low dietary zinc decreases erythrocyte carbonic anhydrase activities and impairs cardiorespiratory function in men during exercise. *American Journal of Clinical Nutrition* 81, 1045–1051.

Ma, F., Wo, Y., Li, H., Chang, M., Wei ,J., Zhao, S. and Sun, P. (2020a) Effect of the source of zinc on the tissue accumulation of zinc and jejunal mucosal zinc transporter expression in Holstein dairy calves. *Animals* 10, 1246. DOI: 10.3390/ani10081246. PMID: 32707966.

Ma, F.T. Wo, Y.Q.L., Shan, Q., Wei, J.Y., Zhao, S.G. and Sun, P. (2020b) Zinc-methionine acts as an anti-diarrheal agent by protecting the intestinal epithelial barrier in postnatal Holstein dairy calves. *Animal Feed Science and Technology* 270, 114686.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Mahmoud, O.M., El Samani, F., Bakheit, R.O. and Hassan, M.A. (1983) Zinc deficiency in Sudanese desert sheep. *Journal of Comparative Pathology* 93, 591–595.

Martin, A.B., Aydemir, T.B., Guthrie, G.J., Samuelson, D.A., Chang, S.M. and Cousins, R.J. (2013a) Gastric and colonic zinc transporter ZIP11 (Slc39a11) in mice responds to dietary zinc and exhibits nuclear localization. *Journal of Nutrition* 143, 1882–1888.

Martin, G.B. and White, C.L. (1992) Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. *Journal of Reproduction and Fertility* 96, 497–507.

Martin, L., Pieper, R., Schunter, N., Vahjen, W. and Zentek, J. (2013b) Performance, organ zinc concentration, jejunal brush border membrane enzyme activities and mRNA expression in piglets fed with different levels of dietary zinc. *Archives of Animal Nutrition* 67, 248–261.

Masters, D.G. and Fels, H.E. (1980) Effect of zinc supplementation on the reproductive performance of grazing Merino ewes. *Biological Trace Element Research* 2, 281–290.

Masters, D.G. and Fels, H.E. (1985) Zinc supplements and reproduction in grazing ewes. *Biological Trace Element Research* 7, 89–93.

Masters, D.G. and Moir, R.J. (1983) Effect of zinc deficiency on the pregnant ewe and developing foetus. *British Journal of Nutrition* 49, 365–372.

Masters, D.G. and Somers, M. (1980) Zinc status of grazing sheep: seasonal changes in zinc concentrations in plasma, *wool and pasture. Australian Journal of Experimental Agriculture and Animal Husbandry* 26, 20–24.

Mayer, A.N., Vieira, S.L, Berwanger, E., Angel, C.R., Kindlein, L., Franca, I. and Noetzold, T.L. (2018) Zinc requirements of broiler breeder hens. *Poultry Science* 98, 1288–1301.

Mayland, H.F., Rosenau, R.C. and Florence, A.R. (1980) Grazing cow–calf responses to zinc supplementation. *Journal of Animal Science* 51, 966–974.

McCormick, N.H., King, J., Krebs, N., Soybel, D.I. and Kelleher, S.L. (2015) Redistribution of tissue zinc pools during lactation and dyshomeostasis during marginal zinc deficiency in mice. *Journal of Trace Elements in Medicine and Biology* 29, 170–175.

McDonald, R.S. (2000) The role of zinc in growth and cell proliferation. *Journal of Nutrition* 130, 1500–1508S.

Miller, W.J. (1970) Zinc nutrition of cattle: a review. *Journal of Nutrition* 53, 1123–1135.

Miller, J.K. and Miller, W.J. (1962) Experimental zinc deficiency and recovery of calves. *Journal of Nutrition* 76, 467–474.

Miller, W.J., Pitts, W.J., Clifton, C.M. and Schmittle, S.C. (1964) Experimentally produced zinc deficiency in the goat. *Journal of Dairy Science* 47, 556–559.

Miller, W.J., Morton, J.D., Pitts, W.J. and Clifton, C.M. (1965) The effect of zinc deficiency and restricted feeding on wound healing in the bovine. *Proceedings of the Society for Experimental Biology and Medicine* 118, 427–431.

Miller, W.J., Blackmon, D.M., Gentry, R.P., Pitts, W.J. and Powell, G.W. (1967) Absorption, excretion and retention of orally administered zinc-65 in various tissues of zinc deficient and normal goats and calves. *Journal of Nutrition* 92, 71–78.

Miller, E.R., Luecke, R.W., Ullrey, D.E., Baltzer, B.V., Bradley, B.L. and Hoefer, J.A. (1968) Biochemical, skeletal and allometric changes due to zinc deficiency in the baby pig. *Journal of Nutrition* 95, 278–286.

Miller, E.R., Liptrap, D.O. and Ullrey, D.E. (1970) Sex influence on zinc requirement of swine. In: Mills, C.F. (ed.) *Trace Element Metabolism in Animals – 1*. Livingstone, Edinburgh, pp. 377–379.

Mills, C.F. and Dalgarno, A.C. (1972) Copper and zinc status of ewes and lambs receiving increased dietary concentrations of cadmium. *Nature, UK* 239, 171–173.

Mills, C.F., Dalgarno, A.C., Williams, R.B. and Quarterman, J. (1967) Zinc deficiency and the zinc requirements of calves and lambs. *British Journal of Nutrition* 21, 751–768.

Minson, D.J. (1990) *Forages in Ruminant Nutrition*. Academic Press, San Diego, California, pp. 208–229.

Mohanna, C. and Nys, Y. (1999) Effect of zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. *British Poultry Science* 40, 108–114.

Morrison, J.N. and Bremner, I. (1987) Effect of maternal zinc supply on blood and tissue metallothionein I concentrations in suckling rats. *Journal of Nutrition* 117, 1588–1594.

Munday, R., Thompson, A.M., Fowke, E.A., Wesselink, C., Smith, B.L. *et al*. (1997) A zinc-containing intraruminal device for faecal eczema control in lambs. *New Zealand Veterinary Journal* 45, 93–98.

Mwangi, S., Timmons, J., Ao, T., Paul, M., Macalintal, L. *et al.* (2017) Effect of zinc imprinting and replacing inorganic zinc with organic zinc on early performance of broiler chicks. *Poultry Science* 96, 861–868.

Myer, B., Chelliah, G., McDowell, L., Blount, A. and Mackowiak, C. (2009) Mineral concentrations in cool-season annual grass pastures in north Florida. UF-IFAS, EDIS, AN224.

Nagalakshmi, D. and Himabindu, D. (2013) Effect of zinc supplementation from organic and inorganic sources on performance, nutrient utilization and carcass characteristics in lambs. *Indian Journal of Animal Sciences* 83, 411–418.

Nayeri, A., Upah, N.C., Sucu, E., Sanz-Fernandez, M.V., DeFrain, J.M., Gorden, P.J. and Baumgard, L.H. (2014) Effect of the ratio of zinc amino acid complex to zinc sulfate on the performance of Holstein cows. *Journal of Dairy Science* 97, 4392–4404.

Neathery, M.W., Miller, W.J., Blackmon, D.M. and Gentry, R.P. (1973a) Performance and milk zinc from low zinc intake in dairy cows. *Journal of Dairy Science* 56, 212–217.

Neathery, M.W., Miller, W.J., Blackmon, D.M., Pate, F.M. and Gentry, R.P. (1973b) Effects of long-term zinc deficiency on feed utilisation, reproductive characteristics and hair growth in the sexually mature male goat. *Journal of Dairy Science* 56, 98–105.

Neathery, M.W., Moos, W.H., Wyatt, R.D., Miller, W.J., Gentry, R.P. and George, L.W. (1980) Effects of dietary aflatoxin on performance and zinc metabolism in dairy calves. *Journal of Dairy Science* 63, 789–799.

Noh, S. and Koo, S.I. (2001) Intraduodenal infusion of lysophosphatidylcholine restores the intestinal absorption of vitamins A and E in rats fed a low-zinc diet. *Experimental Biology and Medicine* 226, 342–348.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2005) *Mineral Tolerances of Animals*, 2nd edn. National Academy of Sciences, Washington, DC.

NRC (2007) *Nutrient Requirements for Small Ruminants and New World Camelids*. National Academy of Sciences, Washington, DC.

NRC (2012) *Nutrient Requirements of Dairy Cows*, 8th edn. National Academy of Sciences, Washington, DC.

Oberleas, D. (1996) Mechanisms of zinc homeostasis. *Journal of Inorganic Biochemistry* 62, 231–241.

O'Connor, J.P., Kanjilal, D., Teitelbaum, M. and Lin, S.S. (2020) Zinc as a therapeutic agent in bone regeneration. *Materials* 13, 2211.

O'Dell, B.L. and Reeves, P.G. (1989) Zinc status and food intake. In: *Zinc in Human Biology. ILSI Press*, Washington, DC, pp. 173–181.

O'Dell, B.L. and Savage. J.E. (1957) Symptoms of zinc deficiency in the chick. *Federation Proceedings* 16, 394.

O'Dell, B.L., Newberne, P.M. and Savage J.E. (1958) Significance of dietary zinc for the growing chicken. *Journal of Nutrition* 65, 503–508.

Oh, S.H., Nakane, H., Deagan, J.T., Whanger, P.D. and Arscott, G.H. (1979) Accumulation and depletion of zinc in chick tissue metallothioneins. *Journal of Nutrition* 109, 1720–1729.

O'Hara, P.J., Newman, A.P. and Jackson, R. (1960) Parakeratosis and copper poisoning in pigs fed a copper supplement. *Australian Veterinary Journal* 36, 225–229.

Olukosi, O.A., Cowieson, A.J. and Adeola, O. (2007) Age-related influence of a cocktail of xylanase, amylase and protease or phytase individually or in combination in broilers. *Poultry Science* 86, 77–86.

Olukosi, O.A., González-Ortiz, G., Whitfield, H. and Bedford, M.R. (2020) Comparative aspects of phytase and xylanase effects on performance, mineral digestibility, and ileal phytate degradation in broilers and turkeys. *Poultry Science* 99, 1528–1539.

Orr, C.L., Hutcheson, D.P., Grainger, R.B., Cummins, J.M. and Mock, R.E. (1990) Serum copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. *Journal of Animal Science* 68, 2893–2900.

Ott, E.A., Smith, W.H., Stob, M. and Beeson, W.M. (1964) Zinc deficiency syndrome in young lamb. *Journal of Nutrition* 82, 41–50.

Ott, E.A., Smith, W.H., Stob, M., Parker, H.E., Harrington, R.B. and Beeson, W.M. (1965) Zinc requirement of the growing lamb fed a purified diet. *Journal of Nutrition* 87, 459–463.

Ott, E.A., Smith, W.H., Harrington, R.B. and Beeson W.M. (1966) Zinc toxicity in ruminants. 1. Effect of high levels of dietary zinc on gains, feed consumption and feed efficiency of lambs. *Journal of Animal Science* 25, 414–418.

Page, C.M., Van Emon, M., Murphy, T., Larson, C., Berardinelli, J. *et al.* (2020). Effects of zinc source and dietary concentration on serum zinc concentrations, growth performance, wool and reproductive characteristics in developing rams. *Animal* 14, 520–528.

Pal, D.L. (2010) Effect of copper- and zinc-methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. *Journal of Trace Elements in Medicine and Biology* 24, 89–94.

Payne, R.L., Beidner, T.D., Fakler, T.M. and Southern, L.L. (2006) Growth and intestinal morphology of pigs from sows fed two zinc sources during gestation and lactation. *Journal of Animal Science* 84, 2141–2149.

Pitts, W.J., Miller, W.J., Fosgate, O.T., Morton, J.D. and Clifton, C.M. (1966) Effect of zinc deficiency and restricted feeding from two to five months of age on reproduction in Holstein bulls. *Journal of Dairy Science* 49, 995–1000.

Pond, W.G. (1983) The effect of dietary calcium and zinc levels on weight gain and blood and tissue mineral concentrations in growing Columbia- and Suffolk-sired lambs. *Journal of Animal Science* 56, 952–959.

Prasad, A.S. (2007) Zinc: mechanisms of host defence. *Journal of Nutrition* 137, 1345–1349.

Price, J. and Humphries, W.R. (1980) Investigation of the effect of supplementary zinc on growth rate of cattle in farms in N. Scotland. *Journal of Agricultural Science, Cambridge* 95, 135–139.

Purkerson, J.M. and Schwartz, G.J. (2007) The role of carbonic anhydrases in renal physiology. *Kidney International* 71, 103–115.

Qin, S., Lu, L., Zhang, X., Liao, X., Zhang, L., Guo, Y. and Luo, X. (2017) An optimal dietary zinc level of brown-egg laying hens fed a corn-soybean meal diet. *Biological Trace Element Research* 177, 376–383.

Read, S.A., Obeid, S., Ahlenstiel, C. and Ahlenstiel, G. (2019) The role of zinc in antiviral immunity. *Advances in Nutrition* 10, 696–710.

Revy, P.S., Jondreville, C., Dourmad, J.Y. and Nys, Y. (2004) Effect of zinc supplemented as either an organic or an inorganic source and of microbial phytase on zinc and other minerals utilisation by weanling pigs. *Animal Feed Science and Technology* 116, 93–112.

Reynolds, F.H., Forbes, J.M. and Miller, H.M. (2010) Does the newly weaned piglet select a zinc oxide supplemented feed, when given the choice? *Animal* 4, 1359–1367.

Roberson, K.D., Kalbfleish, J.L., Pan, W., Applegate, T.J. and Rosenstein, D.S. (2005) Comparison of wheat bran phytase and a commercially available phytase on turkey tom performance and litter phosphorus content. *International Journal of Poultry Science* 4, 244–249.

Rojas, I.Y.M., González, E.A., Menocal, J.A., Santos, T.T.D., Arguello, J.R. and Coello, L.C. (2018)

Assessment of a phytase included with lactic acid on productive parameters and on deposition of phosphorus, calcium, and zinc in laying hens fed with sorghum–soybean-meal-based diets. *Journal of Applied Animal Research* 46, 341–321.

Romero, A. (2013) The bioavalability of zinc: what does the literature say? *International Poultry Production* 23, 11–13.

Rowe, D.J. and Bobilya, D.J. (2000) Albumin facilitates zinc acquisition by endothelial cells. *Proceedings of the Society for Experimental Biology and Medicine* 224, 178–186.

Ruiz-Ascacibar, I., Stoll, P., Bee, G. and Schlegel, P. (2019) Dynamics of the mineral composition and deposition rates in the empty body of entire males, castrates and female pigs. *Animal* 13, 50–58.

Ryu, M.-S., Langkamp-Henken, B., Chang, S.-M., Shankar, M.N. and Cousins, R.J. (2001) Genomic analysis, cytokines expression and micrRNA profiling reveal bio-markers of human dietary zinc depletion and homeostasis. *Proceedings of the National Academy of Sciences* 108, 20970–20975.

Sahin, K. and Kucuk, O. (2003) Zinc supplementation alleviates heat stress in laying Japanese quail. *Journal of Nutrition* 133, 2808–2811.

Sandoval, M., Henry, P.R., Ammerman, C.B., Miles, R.D. and Littell, R.C. (1997a) Estimation of the relative bioavailability of zinc from inorganic zinc sources for sheep. *Animal Feed Science and Technology* 66, 223–235.

Sandoval, M., Henry, P.R., Littell, R.C., Cousins, R.J. and Ammerman, C.B. (1997b) Relative bioavailability of supplemental inorganic zinc sources for chicks. *Journal of Animal Science* 75, 3195–3205.

Sauvant, D., Perez, J.M. and Tran, G. (2004) Tables INRA-AFZ de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage: 2nd édition. INRA Editions, Versailles, France.

Schlattl, M., Reindl, S., Buffler, M., Brugger, D. and Windisch, W. (2019) Effect of clay minerals on the bioavailability of dietary zinc in rumen fluid and duodenal chyme in vitro. *Advances in Animal Bioscience* 10, 514.

Schlegel, P., Nys, Y. and Jondreville, C. (2010) Zinc availability and digestive zinc solubility in piglets and broilers fed diets varying in their phytate contents, phytase activity and supplemented zinc source. *Animal* 4, 200–209.

Schlegel, P., Sauvant, D. and Jondreville, C. (2013) Bioavailability of zinc sources and their interaction with phytates in broilers and piglets. *Animal* 7, 47–59.

Schryver, H.F., Hintz, H.F. and Lowe, J.E. (1980) Absorption excretion and tissue distribution of stable zinc and ^{65}zinc in ponies. *Journal of Animal Science* 51, 896–902.

Schwarz, W.A. and Kirchgessner, M. (1975) Experimental zinc deficiency in lactating dairy cows. *Veterinary Medical Review* 1/2, 19–41.

Shakweer, M.E., EL-Mekass, A.A.M. and EL-Nahas, H.M. (2010) Effect of two different sources of zinc supplementation on productive performance of Friesian dairy cows. *Egyptian Journal of Animal Production* 47, 11–22.

Sharideh, H., Zhandi, M., Zaghari, M. and Akhlaghi, A. (2016) Dietary zinc oxide and 6-Phytase effects on fertility rate in old broiler breeder hens. *Journal of Agricultural Science and Technology* 18, 327–336.

Shelton, J.L., LeMieux, F.M., Southern, L.L. and Bidner, T.D. (2005) Effect of microbial phytase addition with or without the trace mineral premix in nursery, growing, *and finishing pig diets. Journal of Animal Science* 83, 376–385.

Smith, I.D., Grummer, R.H., Hoekstra, W.G. and Phillips P.H. (1960) Effects of feeding an autoclaved diet on the development of parakeratosis in swine. *Journal of Animal Science* 19, 568–579.

Smith, B.L., Embling, P.P., Towers, N.R., Wright, D.E. and Payne, E. (1977) The protective effect of zinc sulfate in experimental sporidesmin poisoning of sheep. *New Zealand Veterinary Journal* 25, 121–127.

Smith, J.C. Jr, McDaniel, E.G. and Chan, W. (1976) Alterations in vitamin A metabolism during zinc deficiency and food and growth restriction. *Journal of Nutrition* 106, 569–574.

Smith, J.W., Tokach, M.D., Goodband, R.D., Nelsson, J.L. and Richert, B.T. (1997) Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early weaned pigs. *Journal of Animal Science* 75, 1861–1866.

Spais, A.G. and Papasteriadis, A.A. (1974) Zinc deficiency in cattle under Greek conditions. In: Hoekstra, W.G., Suttie, J.W., Ganther, T.T.E. and Mertz, W. (eds) *Trace Element Metabolism in Animals – 2*. University Park Press, Baltimore, Maryland, pp. 628–631.

Spears, J.W. (1989) Zinc methionine for ruminants: relative bioavailability of zinc in lambs and effects on growth and performance of growing heifers. *Journal of Animal Science* 67, 835–843.

Spears, J.W. (1996) Organic trace minerals in ruminant nutrition. *Animal Feed Science and Technology* 58, 151–163.

Spears, J.W. (2015) Advances in trace mineral absorption, homeostasis. *Feedstuffs* 87, May 4.

Spears, J.W. and Kegley, E.B. (2002) Effect of source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics and immune response of growing and finishing steers. *Journal of Animal Science* 80, 2747–2752.

Stake, P.E., Miller, W.J., Gentry, R.P. and Neathery, N.W. (1975) Zinc metabolic adaptations in calves fed a high but non-toxic zinc level for varying time periods. *Journal of Animal Science* 40, 132–137.

Star, L., van der Klis, J.D., Rapp, C. and War, T. (2012) Bioavailability of organic and inorganic zinc sources in male broilers. *Poultry Science* 91, 3115–3120.

Stevenson, M.H. and Jackson, N. (1984) Comparison of dietary hydrated copper sulfate, dietary zinc oxide and a direct method for inducing moult in laying hens. *British Poultry Science* 25, 505–517.

Stevenson, J.W. and Earle, I.P. (1956) Studies on parakeratosis in swine. *Journal of Animal Science* 15, 1036–1045.

Sun, G., Chen, D., Zhang, K. and Yu, B. (2009) Effects of dietary zinc level and an inflammatory challenge on performance and immune response of weaning pigs. *Asian-Australian Journal of Animal Science* 22, 1303–1310.

Sun, X., Liao, X., Lu, L., Zhang, L., Ma, Q., Lin Xi, L. and Luo, X. (2018) Effect of *in ovo* zinc injection on the embryonic development, tissue zinc contents, antioxidation, and related gene expressions of broiler breeder eggs. *Journal of Integrative Agriculture* 17, 648–656.

Suo, H., Lu, L., Zhang, L., Zhange, X., Li, H., Lu, Y. and Luo, X. (2015) Relative bioavailability of zinc-methionine chelate for broilers fed a conventional corn–soybean meal Diet. *Biological Trace Element Reserarch* 165, 206–213.

Suttle, N.F. (1983) Assessment of the mineral and trace element status of feeds. In: Roberds, G.E. and Packham, R.G. (eds) *Proceeding of the Second Symposium of the International Network of Feed Information Centres*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 211–237.

Suttle, N.F. (2010) Zinc. In: *Mineral Nutrition of Livestock*, 4th edn. CAB International, Wallingford, UK, pp.389–392.

Suttle, N.F. and Mills, C.F. (1966) Studies on the toxicity of copper to pigs. 1. The effects of oral supplements of zinc and iron salts on the development of copper toxicosis. *British Journal of Nutrition* 20, 135–148.

Suttle, N.F., Lloyd-Davies, H. and Field, A.C. (1982) A model for zinc metabolism in sheep given a diet of hay. *British Journal of Nutrition* 47, 105–112.

Suzuki, H., Asakawa, A., Li, J.B., Tsai, M., Amitani, H. *et al*. (2011) Zinc as an appetite stimulator – the possible role of zinc in the progression of diseases such as cachexia and sarcopenia. *Recent Patents in Food Nutrition and Agriculture* 3, 226–231.

Swinkels, J.W.G.M., Kornegay, E.T., Zhou, W., Lindemann, M.D., Webb, K.E. and Verstegen, M.W.A. (1996) Effectiveness of a zinc amino acid chelate and zinc sulphate in restoring serum and soft tissue zinc concentrations when fed to zinc-depleted pigs. *Journal of Animal Science* 74, 2420–2430.

Todd, W.R., Elvehjem. C.A. and Hart, E.B. (1934) Zinc in the nutrition of the rat. *American Journal of Physiology* 107, 146–156.

Towers, N.R., Smith, B.L., Wright, D.E. and Sinclair, D.P. (1975) Preventing facial eczema by using zinc. *Proceedings of the Ruakura Farmers' Conference*, pp. 57–61.

Tucker, H.F. and Salmon, W.D. (1955) Parakeratosis or zinc deficiency disease in the pig. *Proceedings of the Society for Experimental Biology and Medicine* 88, 613–616.

Tunçdemir, M., Ertürküner, S. and Özçelik, D. (2017) Investigation of lipid peroxidation and antiapoptotic effects of zinc aganist liver damage in diabetic rats. *Human and Experimental Toxicology* 36, 813–822.

Underwood. E.J. and Somers, M. (1969) Studies of zinc nutrition in sheep. 1. The relation of zinc to growth, testicular development and spermatogenesis in young rams. *Australian Journal of Agricultural Research* 20, 889–897.

Underwood, E.J. and Suttle, F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Vahjen, W., Pietruszynska, D.I., Starke, C. and Zentek, J. (2015) High dietary zinc supplementation increases the occurence of tetracycline and sulfonamide resistance genes in the intestine of weaned pigs. *Gut Pathology* 7, 23.

Vallee, B.L. and Falchuk, K.H. (1993) The biochemical basis of zinc physiology. *Physiological Reviews* 73, 79–118

Veum, T.L., Ledoux, D.R., Shannon, M.C. and Raboy, V. (2009) Effect of graded levels of iron, zinc, and copper supplementation in diets with low-phytate or normal barley on growth performance, bone characteristics, hematocrit volume, and zinc and copper balance of young swine. *Journal of Animal Science* 87, 2625–2634.

Vieira, M.M., Ribeiro, A.M.L., Kessler, A.M., Moraes, M.L., Kunrath, M.A. and Ledur, V.S. (2013) Different sources of dietary zinc for broilers submitted to immunological, nutritional, and environmental challenge. *Journal of Applied Poultry Research* 22, 855–861.

Wang, R.L., Liang, J.G., Lu, L., Zhang, L.Y., Li, S.F. and Luo, X.G. (2013) Effect of zinc source on performance, zinc status, immune response, and rumen fermentation of lactating cows. *Biological Trace Element Research* 152, 16–24.

Wang, Z.C., Yu, H.M., Xie, J.J., Cui, H., Nie, H., Zhang, T. and Gao, X.H. (2019) Effect of dietary zinc pectin oligosaccharides chelate on growth performance, enzyme activities, Zn accumulation, metallothionein concentration, and gene expression of Zn transporters in broiler chickens. *Journal of Animal Science* 97, 2114–2124.

Wedekind, K.J. and Baker, D.H. (1990) Zinc bioavailability of feed grade sources of zinc. *Journal of Animal Science* 68, 684–689.

Wedekind, K.J., Hortin, A.E. and Baker, D.H. (1992) Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate and zinc oxide. *Journal of Animal Science* 70, 178–187.

Wedekind, K.J., Collings, G., Hancock, J. and Titgemeyer, E. (1994a) The bioavailability of zinc-methionine relative to zinc sulfate is affected by calcium level. *Poultry Science* 73 (Suppl. 1), 114.

Wedekind, K.J., Lewis, A.J., Giesemann, M.A. and Miller, P.S. (1994b) Bioavailability of zinc from inorganic and organic sources for pigs fed corn–soybean meal diets. *Journal of Animal Science* 72, 2681–2689.

Wegner, T.N., Ray, D.E., Lox, C.D. and Stott, G.H. (1973) Effect of stress on serum zinc and plasma corticoids in dairy cattle. *Journal of Dairy Science* 56, 748–752.

Weng, X., Monteiro, A.P.A., Guo, J., Li, C., Orellana, R.M. *et al.* (2018) Effects of heat stress and dietary zinc source on performance and mammary epithelial integrity of lactating dairy cows. *Journal of Dairy Science* 101, 2617–2630.

White, C.L. (1993) The zinc requirements of grazing ruminants. In: Robson, A.D. (ed.) *Zinc in Soils and Plants: Developments in Plant and Soil Sciences*, Vol. 55. Kluwer Academic Publishers, London, pp. 197–206.

White, C.L., Chandler, B.S. and Peter, D.W. (1991) Zinc supplementation of lactating ewes and weaned lambs grazing improved Mediterranean pastures. *Australian Journal of Experimental Agriculture* 31, 183–189.

White, C.L., Martin, G.B., Hynd, P.T. and Chapman, R.E. (1994) The effect of zinc deficiency on wool growth and skin and wool histology of male Merino lambs. *British Journal of Nutrition* 71, 425–435.

Wichert, B., Kreyenberg, K. and Kienze, E (2002) Serum response after oral supplementation of different zinc compounds in horses. *Journal of Nutrition* 132, 1769S–1770S.

Williams, R.B., McDonald, I. and Bremner, I. (1978) The accretion of copper and of zinc by the foetuses of prolific ewes. *British Journal of Nutrition* 40, 377–386.

Wing, K., Wing, A., Sjostrom, R. and Lonnerdal, B. (1997) Efficacy of a Michaelis-Menton model for the availabilities of zinc, iron and cadmium from an infant formula diet containing phytate. In: Fischer, P.W.F., L'Abbe, M.R., Cockell, K.A. and Gibson, R.S. (eds) *Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals (TEMA 9)*. NRC Research Press, Ottawa, pp. 31–32.

Xu, C., Wensing, T. and Beynen, A.C. (1997) The effect of dietary soybean versus skim milk protein on plasma and hepatic concentrations of zinc in veal calves. *Journal of Dairy Science* 80, 2156–2161.

Yu, Y., Lu, L., Luo, X.G. and Liu, B. (2008) Kinetics of zinc absorption by in situ ligated intestinal loops of broilers involved in zinc transporters 1. *Poultry Science* 87, 1146–1155.

Zaboli, K., Aliarabi, H., Bahari, A.A. and Abbasalipourkabir, R. (2013) Role of dietary nano-zinc oxide on growth and blood levels of mineral: a study in Iranian Angora (Markhoz) goat kids. *Journal of Pharmaceutical and Health Sciences* 2, 19–26.

Zago, M. and Oteiza, P.I. (2001) The antioxidant properties of zinc: interactions with iron and antioxidants. *Free Radicals in Biology and Medicine* 31, 266–274.

Zanetti, D., Godoi, L.A., Estrada, M.M., Engle, T.E., Silva, B.C. *et al.* (2017) Estimating mineral requirements of Nellore beef bulls fed with or without inorganic mineral supplementation and the influence on mineral balance. *Journal of Animal Science* 95, 1696–1706.

Zeller, E., Schollenberger, M., Kühn, I. and Rodehutscord, M. (2015) Hydrolysis of phytate and formation of inositol phosphate isomers without or with supplemented phytases in different segments of the digestive tract of broilers. *Journal of Nutritional Science* 4(e1), 1–12.

Zeng, Z.K., Wang, D., Piao, X.S., Li, P.F., Zhang, H.Y., Shi, C.X. and Yu, S.K. (2014) Effects of adding super dose phytase to the phosphorus-deficient diets of young pigs on growth performance, bone quality, minerals and amino acids digestibilities. *Asian-Australasian Journal of Animal Sciences* 27, 237–246.

Zhang, L., Wang, Y.X, Xiao, X., Wang, J.S., Wang, Q. *et al*. (2017a) Effects of zinc glycinate on productive and reproductive performance, zinc concentration and antioxidant status in broiler breeders. *Biological Trace Element Research* 178, 320–326.

Zhang, Y.N., Zhang, H.J., Wang, J., Yue, H.Y., Qi, X.L., Wu, S.G. and Qi, G.H. (2017b) Effect of dietary supplementation of organic or inorganic zinc on carbonic anhydrase activity in eggshell formation and quality of aged laying hens. *Poultry Science* 96, 2176–2183.

Zhang, H., Nie, H., Wang, Z. and Wang, F. (2018) The net iron, manganese, copper and zinc requirements for maintenance and growth of Dorper × Hu ewe lambs. *Italian Journal of Animal Science* 17, 941–949.

Zhao, J., Shirley, R.B., Dibner, J.J., Wedekind, K.J., Yan, F., Fisher, P. and Vazquez-Añon, M. (2016) Superior growth performance in broiler chicks fed chelated compared to inorganic zinc in presence of elevated dietary copper. *Journal of Animal Science and Biotechnology* 7, 13. DOI:10.1186/s40104-016-0072-1.

Ziegler, T.R., Leach, R.M., Norris, L.C. and Scott, M.L. (1961) Zinc requirement of the chick: factors affecting requirement. *Poultry Science* 40, 1584–1593.

17 Occasionally Beneficial Elements

The 12 minerals covered so far were deemed essential to health and well-being because deprived animals showed three criteria of essentiality:

- failure to thrive and remain healthy;
- physiological responses to small supplements when repleted; and
- homeostatic mechanisms for modulating retention of the element.

Eight more elements: boron, chromium, lithium, molybdenum, nickel, rubidium, silicon and vanadium are grouped together here as occasionally beneficial elements (OBE) because they meet the above criteria of essentiality, albeit in 'ultratrace' concentrations (<1 mg kg^{-1}) (Nielsen, 1996). A similar situation applies to a group of five potentially toxic elements (PTE), grouped in Chapter 18. Other ubiquitous and reactive elements with specific functions in lower organisms may eventually have their essentiality to humans and livestock confirmed (Nielsen, 1996, 2002) but the list of minerals claimed to be essential shortened in previous updates (Underwood and Suttle, 1999; Suttle, 2010). Dietary provision of some OBE is still receiving attention in poultry nutrition (Baloš *et al*., 2017). A number of important questions remain to be answered before essentiality is unequivocally demonstrated for all OBE and PTE:

- Was the deficient diet adequate with respect to all other essential elements?
- Were the physiological responses life-enhancing?
- Were the homeostatic mechanisms any more than homeorhetic mechanisms providing defence against excess (p. 1)?
- Where does a nutritional response end and a pharmacological response begin?

Doubts on the first point led to tin being considered non-essential and essentiality of others, such as rubidium, called into question (NRC, 2005). Clinical consequences of deprivation of OBE and PTE are rarely specific (e.g. poor growth, low viability of newborn) and the same abnormalities were progressively attributed to a series of 'newer' ultratrace elements (see Chapter 18): each may have been unknowingly lacking from the diet in trials to establish essentiality of its predecessor in the series. Many of the biochemical consequences of deprivation and responses to supplementation are also shared with 'essential' elements; for example, chromium and zinc both reduce plasma insulin in cold-stressed birds; lack of either lithium or nickel impairs glycolysis.

- The above questions are important to farmers who may be tempted to purchase novel supplements that contain rare elements from the far reaches of the Periodic Table; but
- Do livestock need them on their 'table'?

For the most part, OBE are so abundant in farm environments that natural deficiencies are unlikely to arise. The last decade has again produced new information on OBE (and PTE) but none to justify a change of classification to 'essential element' status.

Boron

Essentiality

Boron is an essential nutrient for plants but evidence for essentiality to animals remains unconvincing to some (Spears, 2019) though convincing to others (Abdelnour *et al*., 2018). Boron binds strongly to *cis*-hydroxyl groups and may thus form complexes with riboflavin, oestradiol and vitamin D metabolites. For example, by binding with 24,25-dihyroxyvitamin D, produced by microsomal 24-hydroxylase, boron may upregulate the enzyme (Miljkovic *et al*., 2004). Dosing obese rabbits with 10–50mg B kg^{-1}LW by gavage increased tibia Ca, P and Mg (Hakki *et al*., 2013): the diet contained 3.9mg B kg^{-1}DM but responses may

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have been pharmacological rather than indicative of insufficiency. Supplementing the diet of quail with 5mg Cd kg^{-1}DM produced correlated decreases in tibia B and shear strength (p. 401). Boron supplementation of equine feeds is commonly practised in the hope that it will decrease the risk of leg injuries and osteochondrosis. The presence of 43mg B kg^{-1}DM in a marine supplement may have affected bone metabolism in yearlings (Nielsen *et al.*, 2010). Boron deprivation is most likely to occur in livestock fed grain-based diets.

Poultry

Benefits from raising dietary B in animals were first noted in vitamin D-deficient chicks in 1981 and subsequently confirmed and extended (Hunt *et al.*, 1994). However, the basal diet was exceedingly low in B (<0.18mg kg^{-1}DM) and supplement so small (1.2mg kg^{-1}DM) that it would only simulate B-deficient crops. Raising dietary B in commercial rations for broilers by 5 or 60mg B kg^{-1}DM marginally increased growth rate, more so in males than females, but significantly improved tibia weight and strength (Rossi *et al.*, 1993). The latter findings were confirmed (Wilson and Ruszler, 1997) and extended to hens, in which improvement in bone strength occurred sooner in laying than non-laying birds (Wilson and Ruszler, 1998): similar supplements have not always improved egg production (Eren *et al.*, 2004). Addition of 5 or 25mg B kg^{-1}DM to a corn/soy starter diet improved growth rate and feed conversion efficiency (FCE) of broiler chicks, whether the diet was low or adequate in vitamin D_3 (Kurtoglu *et al.*, 2001), but benefit disappeared when 15% wheat was included in the diet. Plasma Ca and alkaline phosphatase activity were lowered but their relationship to performance was unclear. Epiphyseal growth plate depth was reduced in B-supplemented groups at 45 days of age (Kurtoglu *et al.*, 2005). Addition of 30 but not 60 mg B to a corn/SBM diet containing 2mg kg^{-1}DM improved food intake, FCE and tibia Ca and P but not growth and mortality in broiler chicks (Bozkurt *et al.*, 2012). Addition of 100 mg B l^{-1} to drinking water improved absolute and relative thymus weight in broiler chicks (Jin *et al.*, 2014). In laying hens, 75–150 mg supplementary B kg^{-1}DM had a sparing effect on Ca and P requirements (Küçükyilmaz *et al.*, 2014), while a smaller supplement (60 mg) increased egg yolk lipids, without improving production (Olgun *et al.*, 2013)

Pigs

Young pigs ate more food and gained proportionately more weight when 5 or 15 mg B was added (as sodium borate) to a diet based on ground corn and containing 2.2mg B kg^{-1}DM. The higher level increased the shear strength and bone P, without affecting plasma concentrations or retentions of other minerals (Armstrong and Spears, 2001): continued supplementation through a first pregnancy did not affect fertility, fecundity, birth weight and bone mineralization in newborn piglets (Armstrong *et al.*, 2002). In offspring reared on a similar diet, inflammatory responses to intradermally injected mitogen (lipopolysaccharide) were muted but cytokine responses to intramuscular challenge were increased by boron supplementation (Armstrong and Spears, 2003).

Ruminants

An association between poor conception and 'low' serum B (9.2–13.6 μmol l^{-1}) was reported within a beef cow herd (Small *et al.*, 1997) but association is not proof of essentiality. A supplement of 40mg B kg^{-1}DM had an apparent sparing effect in lambs on calcium when dietary Ca was reduced from 5.3 to 3.6g kg^{-1}DM, countering reductions in growth rate, gene expression of SOD1 (p. 256) and total antioxidant capacity of plasma; both cellular and humoral immune responses were improved during a six-month experiment (Bhasker *et al.*, 2017).

- Pseudo-endocrine activity would be required for a trace element to 'spare' a macro-element.

Disruption of lobule architecture in liver and abnormalities in the kidney glomerulus were also reversed by raising dietary B from 8.4mg kg^{-1}DM. However, lambs gained only 60g d^{-1}, on a poorly digestible basal diet. Supplements of 5 and 25mg B kg^{-1}DM did not affect inflammatory or clinical responses to bovine herpes virus infection in growing steers (Fry *et al.*, 2011).

Sources

Soils in England and Wales are consistently rich in boron, with 50% of values falling between 20 and 40mg B kg^{-1}DM (Table 17.1), but only 2.5–5.0% was extractable in hot water, the common test of availability. An Indian survey revealed an average concentration of 17.6 ± 0.96mg B kg^{-1}DM in

foodstuffs, with forages 2.4 times richer than concentrates (Bhasker *et al*., 2015). Seasonality (or maturity) may be important, values in wheat plants falling from 7 to 2mg B kg^{-1}DM between May and August (Silanpaa, 1982). Grasses tend to contain less B than legumes (Table 17.2) but marked exceptions have been reported.[i] Root crops are rich sources; turnips, swedes and sugarbeet contained roughly ten times more boron (13.5–20.8mg kg^{-1}DM) than four cereal grains (1.4–1.7mg kg^{-1}DM) grown at the same site in Finland (Fig. 17.1; Silanpaa, 1982). Mixed rations for sheep contained 32mg kg^{-1}DM (Brown *et al*., 1989) and those for poultry contained less (9–16mg B kg^{-1}DM) (Rossi *et al*.,

Table 17.1. Median and ranges of concentrations (mg kg^{-1}DM) for occasionally beneficial trace elements found in soils.

Element	B	Cr	Mo	Li	Ni	Rb	V
	34 (20–40)	20 (10–121)	5 (0.1–20.0)	28[a]	4 (7.3–70.0)	4[a]	40[a]

[a]Mean values from Eastern Europe: Kabata-Pendias and Pendias (1992); all other data are for England and Wales: Archer and Hodgson (1987).

Table 17.2. Concentrations (mg kg^{-1}DM) of occasionally beneficial trace elements commonly found in feeds: ranges of means from surveys in different eastern European countries (E) are from Kabata-Pendias and Pendias (1992) and from the USA from Kerr *et al*. (2008).

Element	Grasses (E)	Legume[c] (E)	Cereals (E)	Cereals (US)	Oilseeds[d] (US) `	Fibrous[a] (US)
B	4.9–7.4	14–78	0.7–7.3	45–61[b]	51–66	35–102
Cr	0.1–0.35	0.2–4.2	0.01–0.55	2–4.6	1.6–2.5	2.3–23.0
Li	0.07–1.5	0.01–3.1	0.05	–	–	–
Mo	0.33–1.4	0.5–2.5	0.16–0.92	–	–	–
Ni	0.13–1.1	1.2–2.7	0.22–0.34	0.5–5.0	1.6–2.5	0.8–9.0
Rb	130	44–98	3–4	–	–	–
V	0.1–0.23	0.18–0.24	0.007–0.060	–	–	–

[a]Fibrous foods were soybean hulls, wheat middlings and sugarbeet pulp, the latter giving the exceptionally high values, probably due to soil contamination.
[b]Uniformly high values may indicate analytical error in European or USA data.
[c]Forage legumes.
[d]Linseed and soybean meals.

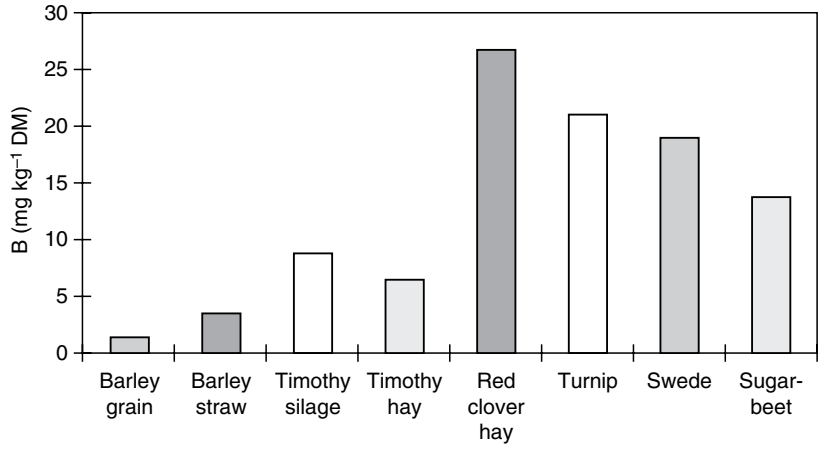

Fig. 17.1. Crops and forage species grown at the same location in Finland show considerable variation in boron concentrations. (Data from Silanpaa, 1982)

1993) but a corn/SBM broiler diet only 2.7mg B kg^{-1}DM (Jin *et al*., 2014). Bovine milk normally contains 0.09–0.25 mg B l^{-1} but concentrations greatly increase as B intake increases (Kirchgessner *et al*., 1967).

Metabolism

There is little evidence of homeostatic control at high B intakes. In cattle, plasma and urine B were almost linearly related to intake over the range 1–45mg B kg^{-1}LW (Weeth *et al*., 1981) and up to 69% is excreted in bovine urine (Green and Weeth, 1977). Plasma contained 2.4 mg B l^{-1} in sheep given a boron-rich hay (40mg B kg^{-1}DM). In sheep, faeces was the major route of excretion but urine became the more important when borate (45 and 175mg^{-1} $head^{-1}$ day^{-1}) was added (Brown *et al*., 1989). In broiler chicks, liver and muscle increased linearly from <2 to >16mg B kg^{-1}DM as dietary B increased to 330mg kg^{-1}DM (Rossi *et al*., 1993). In mature horses, given a diet consisting predominantly of hay, containing 24g B kg^{-1}DM, apparent absorption (AA_B) was 46% but plasma contained only 0.2 mg B l^{-1} after 13 days (O'Connor *et al*., 2007). Daily drenching of periparturient cows with 30 g sodium borate attenuated customary reductions in plasma Ca and Mg, associated with calving (p. 58) (Kabu *et al*., 2013) but may have lowered pH in rumen and bloodstream (p. 173).

Toxicity

Boron is less well tolerated by poultry than was once thought (Underwood and Suttle, 1999). Eggshells became much weaker when dietary B was increased from 100 to 200mg kg^{-1}DM and egg yield decreased in laying hens (Fig. 17.2) (Eren *et al*., 2004). Consumption of drinking water containing 200–400 mg B l^{-1} decreased absolute and relative weights of immune organs (thymus, bursa and spleen), induced histological abnormalities and reduced organ lymphocyte counts (Jin *et al*., 2014). Heifers given 150–300 mg B l^{-1} in drinking water (multiply by 3.33 and 2.5 for approximate B kg^{-1} diet DM in cattle and sheep, respectively) lost appetite and body weight (Green and Weeth, 1977), but drinking water from natural sources is unlikely to contain so much B. Plasma B >4mg l^{-1} indicates abnormally high intakes. Ingestion of borate fertilizer caused toxicity in cattle and raised tissue

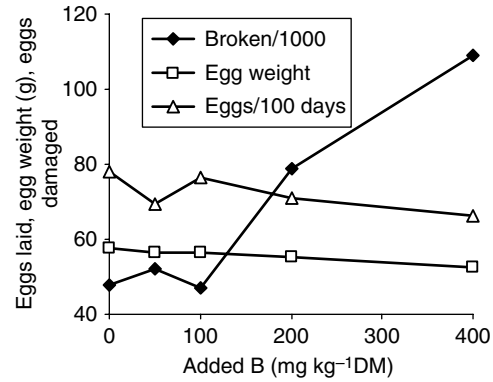

Fig. 17.2. Supplementation of laying hens with >100mg B kg^{-1}DM decreased egg production rate and increased the proportion of damaged eggs: no benefits from smaller supplements were recorded. (Data from Eren *et al*., 2004)

B from <10 to >100mg kg^{-1}DM (Puls, 1994). Riboflavin protects animals from toxicity and enrichment of edible products in livestock exposed to excess B does not present a hazard to consumers (NRC, 2005).

Chromium

Essentiality

Essentiality for mammals was first indicated when supplements of trivalent chromium (Cr III) salts improved glucose tolerance in rats. This property was shared by organic chromium (Cr_o) in brewers yeast and termed 'glucose tolerance factor' (GTF). A low-molecular-weight, Cr-binding protein (chromodulin) that facilitated uptake of glucose by insulin-sensitive cells was eventually isolated (Vincent and Stallings, 2007). Chromodulin is probably synthesized *in vivo* (Schwarz and Mertz, 1959) because Cr is found in GTF-like forms in livers of mammals given inorganic chromium (Yamamoto *et al*., 1988). Supplementation of livestock diets with Cr III changed protein, nucleic acid and lipid metabolism (Anderson, 1987). The nutritional biochemistry of Cr III was reviewed by Vincent and Stallings (2007). Ruminants use acetate rather than glucose to build carbon chains and this may lower insulin sensitivity. However, a supplement of 1mg Cr kg^{-1}DM (as amino acid chelate) increased ATP-citrate lyase activity, thereby increasing

potential use for fat synthesis in adult sheep by 30% (Gardner *et al*., 1998). Responsiveness may be heightened in twin-bearing ewes, which have a uniquely high requirement for glucose: this possibilty has not been specifically investigated. Feeding 3 mg chromium methionine (CrMet) kg^{-1}DM for five weeks before and after lambing lowered serum cholesterol and decreased both loss in body weight and serum MDA in ewes but cholesterol was also lowered by SeMet (Mousaie *et al*., 2017).

Natural sources

Chromium is far more abundant in soils than in crops. The average English soil contained 20mg Cr kg^{-1}DM (Table 17.1) but in Canada, less impacted by industrialization, soils contained 1–25mg Cr kg^{-1}DM (Puls, 1994). Livestock feeds have generally contained <1mg Cr kg^{-1}DM (Table 17.2), despite being prone to contamination.

- Grinding feed samples through a conventional laboratory mill, with stainless steel screen, can double chromium concentrations and feed processes such as grinding and pelleting may add Cr (Spears and Lloyd, 2017).

In the USA, whole cereals were generally poor (0.02–0.06 mg), soybeans moderate (0.069 ± 0.15 s.d.) and legume forage rich sources (alfalfa hay, 0.52 ± 0.22, white clover 0.36 ± 0.05mg Cr kg^{-1}DM; Spears and Lloyd, 2017); corn contained only 0.026 ± 0.015mg, far less than wheat, and corn silage (0.22 ± 0.087mg Cr kg^{-1}DM). Mixed, corn-based diets for dairy cows contained 1–2mg Cr kg^{-1}DM in one study (Bryan *et al*., 2004) but only 0.38–0.48mg Cr kg^{-1}DM in another (Lloyd *et al*., 2010). Significant amounts of Cr can be ingested as soil (p.20) and contaminants of phosphate supplements (135 ± 20mg; Spears and Lloyd, 2017). High concentrations found in sugarbeet pulp (1.2 ± 0.39mg Cr kg^{-1}DM) may be due in part to soil contamination (Spears and Lloyd, 2017). Bovine milk contained, on average, only 1.7 µg Cr l^{-1} and was unaffected by raising dietary Cr but values fell by 50% between the 1st and 90th days of lactation (Lloyd *et al*., 2010); far higher concentrations, found previously in milk (55 µg Cr l^{-1}; Hayirli *et al*., 2001) were probably due to contamination. Some chromium is present in feeds as organic complexes. Brewers yeast contained chromium in an ethanol-extractable, small-molecular-weight complex (400 Da), while alfalfa contained a much larger complex (2600 Da) that was insoluble in non-polar solvents (Starich and Blincoe, 1983). Similar chromium complexes were found in wheat (Toepfer *et al*., 1973) but emphasis has been on Cr_i.

Metabolism

Little is known about the absorption (A_{Cr}) and metabolism of Cr in any species. The success of oxides, salts and chelates of Cr as inert markers in digestibility studies with weaned animals implies that inorganic sources (Cr_i) are poorly absorbed but the milk-fed animal may absorb Cr more efficiently. Estimates of A_{Cr} are generally low, ranging from 0.5% to 2.8% for Cr III in rats and humans (NRC, 2005). Absorbed Cr is assumed to bind to plasma transferrin, like injected Cr (Feng, 2007). Insulin activates transferrin receptors in cell membranes and induces a flux of chromium that leads to formation of holochromodulin from stores of the apoprotein. Holochromodulin in turn facilitates insulin uptake by adipocytes via tyrosine kinase and thus serves as an autoamplifier in insulin signalling (Feng, 2007). Contaminant Cr may be partially available, contrary to widespread belief (Spears and Lloyd, 2017). Ingestion of two chromium-rich, sludge-amended soils, adding 105 and 408mg Cr kg^{-1}DM to the diet of sheep for six weeks, raised liver Cr from 0.25 to 0.64 and 0.90mg kg^{-1}DM, respectively, although <1% was apparently absorbed (Suttle *et al*., 1991). Greater urinary and tissue Cr may not indicate superior functional availability of Cr_o than Cr_i (see NRC, 2005). The synthetic chelate with picolinic acid (CrPic) is highly stable at acid pH and may be absorbed intact: Cr must then be released and reduced before it can participate in insulin signalling (Vincent, 2000). The CrMet complex escapes rumen degradation (Kim *et al*., 2005) but may be drawn into the host's methionine pool in the same manner as Se given as SeMet (Fig. 15.6), if the molecule is absorbed intact.

- Scarcity of direct comparisons between Cr_o and Cr_i suggests deliberate avoidance of anticipated similar potencies.

Responses to Chromium Supplements

Evidence for benefits of increasing dietary Cr was (NRC, 1995) and remains (EFSA, 2009) equivocal, despite widespread use of corn, corn silage and SBM (i.e. all low in Cr) in experiments. Commercial

supplementation with Cr_o sources is permitted in the USA and Europe and benefits have been widely sought (Lindemann *et al*., 2008). Superiority of Cr_o over Cr_i has been assumed, relying heavily on higher tissue Cr, but never proved. A wide spectrum of metabolic, hormonal and carcase-quality indices have been changed by adding Cr_o to diets, usually without associated health and production benefits, and despite being sought in stressful, insulin-sensitive circumstances.

Responses in transported, vaccinated ruminants

Recently transported feedlot calves have poor appetites, are susceptible to respiratory infections and are usually vaccinated against them. Appetite, early weight gain and humoral immune function were often improved by raising dietary Cr (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Mowat *et al*., 1993). In a 2 × 2 factorial study involving an amino acid chelate (raising dietary Cr from 0.32 to 0.42mg kg^{-1}DM) and multiple vaccination, improvements were mostly confined to vaccinated calves (Wright *et al*., 1994). Addition of 0.5mg Cr_o kg^{-1}DM improved antibody titres after vaccination against infectious rhinotracheitis in one study (Burton *et al*., 1994) but not others (Burton *et al*., 1993; Kegley *et al*., 1997b). Multiple vaccines, particularly those directed against Gram-negative bacteria (e.g. *Pasteurella haemolytica*) are themselves 'stressful' and raise plasma cortisol, while lowering blood glucose in recently transported lambs (Suttle and Wadsworth, 1991). Thus, vaccination may add to the stress of transportation, enhancing responses to cortisol-limiting, insulin-facilitating Cr supplements. Incidence of placental retention was reduced from an exceptional 56% to 16% in a Mexican dairy herd by supplementation with CrPic (Villalobos *et al*., 1997). Transport stress was implicated but persistence of retained placenta in settled unsupplemented controls suggests other contributory factors, such as vaccination, were involved.

- Animals should be vaccinated well in advance of transportation.

Responses in heat-stressed ruminants

Heat stress increases peripheral glucose metabolism and may increase responsiveness to chromium. Supplementing heat-stressed cows with 3.5 mg Cr d^{-1}, as CrPic, in mid-lactation did not attenuate a drop in plasma glucose and biochemical evidence of peroxidation but may have enhanced the inflammatory response: production of a heat shock protein (Hsp72) and interleukin 10 increased (Zhang *et al*., 2014). Dietary supplementation of heat-stressed cows with 0.08 mg Cr as CrPic kg^{-1} $LW^{0.75}$ lowered plasma glucose and vaginal temperature without affecting milk yield (Ribeiro *et al*., 2019): pair-fed groups were used to discern specific effects of Cr on insulin function and lipid metabolism during the anorexia induced by heat stress. Raising dietary Cr from 0.11mg kg^{-1}DM by adding 0.18–0.54 mg Cr as chromium yeast (CrY) improved DMI and reduced rectal temperature and respiration rate during a hot summer: some plasma cytokines were decreased, others were unaffected and antioxidant status was improved but neither milk production nor composition were affected (Shan *et al*., 2020). However, supplementation of a lucerne–maize diet for heatstressed sheep with CrY (1mg Cr kg^{-1}DM) did not alter responses in blood glucose or insulin (Sano *et al*., 2000).

Responses in the unstressed bovine

In unstressed calves, adding 0.37mg Cr kg^{-1}DM as CrPic lowered blood cholesterol and improved glucose tolerance without improving growth, food consumption or FCE (Bunting *et al*., 1994). Similar responses were reported in growing heifers given chromium propionate (CrPr, 5–15mg d^{-1}; Sumner *et al*., 2007; 3–9 mg d^{-1}; Spears *et al*., 2012), without improving performance. In steers, 0.2mg Cr kg^{-1}DM as CrPic had no effects on either blood metabolites or performance but reduced serum and liver triglycerides (Besong *et al*., 2001). Adding 0.4mg Cr kg^{-1}DM to a milk replacer containing 0.31mg Cr kg^{-1}DM 'intensified' responses to insulin in calves but biochemical responses to $CrCl_3$ and chromium–nicotinic acid complex (CrNA) were similar and neither source improved growth (Fig. 17.3) (Kegley *et al*., 1997a). Addition of 0.4mg Cr kg^{-1}DM as CrNA tended to improve growth in steers (Kegley *et al*., 1997b). Carbohydrate source may be important. Providing the equivalent of 0.4mg Cr kg^{-1}DM as CrMet by bottle-feeding in milk, thus bypassing the rumen, improved insulin sensitivity in newly weaned calves given barley as the major dietary constituent but not in those given corn (Habibi *et al*., 2019). Duration of pneumonic episodes was reduced in this experiment but not their frequency.

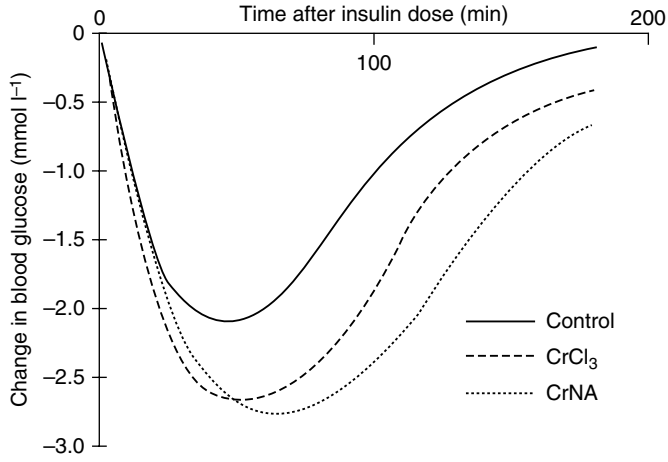

Fig. 17.3. Continuous dietary supplements of chromium chloride ($CrCl_3$) and chromium–nicotinic acid complex (CrNA) both accentuate the hypoglycaemic response of pre-ruminant calves to intravenous insulin (Kegley *et al*., 1997a).

Early lactation milk yield was improved by 7% and 13% by adding 5mg Cr kg^{-1}DM (as amino acid chelate) to the rations of primiparous cows in two experiments but was of no benefit to multiparous cows (Yang *et al*., 1996). In further studies, either gluconeogenesis or glycogenolysis may have been enhanced by Cr_o (Subiyatno *et al*., 1996). Provision of 40 mg Cr kg^{-1} as CrPic from 75 days pre-partum decreased body-weight loss in two- to three-year-old, but not older, beef cows; pregnancy rates also tended to improve (Stahlhut *et al*., 2005). Daily doses of encapsulated CrMet (0.03–0.12 mg kg^{-1} $LW^{0.75}$) increased food intake pre- and post-partum in dairy cows, decreasing weight loss and improving glucose tolerance (Hayirli *et al*., 2001), but daily dosing for seven weeks was arguably stressful. Yields of milk, milk fat and lactose showed a negative quadratic response, being reduced by the highest Cr dose (Fig. 17.4) and were poorly correlated with plasma insulin response. Effects of Cr_o on hepatic gluconeogenesis *in vitro* depended on carbohydrate source (Smith *et al*., 2008). Providing 8 mg Cr d^{-1} as CrMet improved milk yield and composition in multiparous cows during the first two months after parturition (Targhibi *et al*., 2012).

Under grazing conditions, CrMet (6.25 mg d^{-1}) did not improve milk yield, although serum non-esterified fatty acids (NEFA) were decreased peri-partum and supplemented cows tended to conceive earlier (Bryan *et al*., 2004). Adding 10 mg d^{-1} CrPic to pre- and immediate post-partum diets of dairy

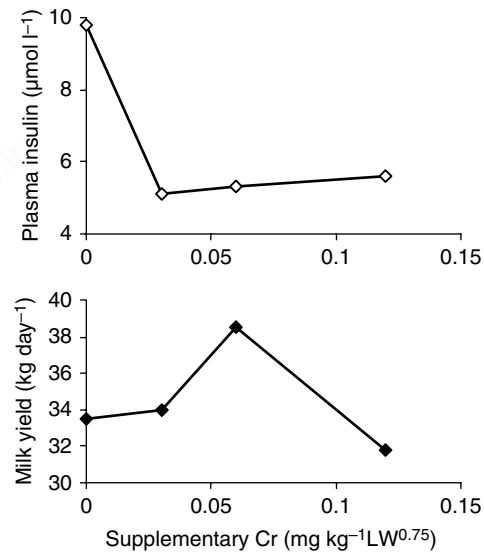

Fig. 17.4. Effects of supplementary chromium concentration, given as Cr methionine, on plasma insulin and milk yield in dairy cows (Hayirli *et al*., 2001). Benefit from supplementation may be affected by chromium concentration in the basal diet.

cows increased food intake without significantly increasing milk yield (McNamara and Valdez, 2005). Providing an estimated 0.5–1.0 mg Cr as CrPic kg^{-1}DM to grazing steers in Brazil had little effect on overall performance (Moreira *et al*., 2020). Effects of chromium on rumen metabolism

must be considered since CrPic altered volatile fatty acid (VFA) production by rumen microbes during *in vitro* culture (Besong *et al*., 2001).

Responses in lambs

More consistent responses have been reported in lambs. Growth, FCE, blood metabolites and isolated carcase traits were improved on corn-based diets, following supplementation with chromium yeast (CrY) (e.g Domínguez-Vara *et al*., 2009). Transportation stress was not necessary to invoke such a response (Kraidees *et al*., 2009). An interaction with dietary protein was reported in one study, in which basal diets contained 0.7–0.9mg Cr kg^{-1}DM: raising dietary Cr was thought to have improved energy metabolism by promoting glycolysis and lipolysis, through modulation of the insulin signal transduction system (Yan *et al*., 2010). The specific contribution of Cr in studies with CrY, particularly live yeast, cannot be ascertained.

Responses in pigs

Supplementation with Cr_o (Ward *et al*., 1997; Matthews *et al*., 2001, 2005; Shelton *et al*., 2003) did not consistently support the positive results of an initial study (Page *et al*., 1993). With CrPic commonly used to provide 0.2mg Cr kg^{-1}DM, occasional metabolic and re-partitioning effects were found but direction could depend on dietary energy density (Shelton *et al*., 2003) and evidence of commercial benefit was generally unconvincing.[ii] Effects of stressors have received little attention. Blood glucose, lactate and cortisol concentrations, measured after shipping to and exsanguination at the abattoir, were unaffected by CrPr supplementation (Matthews *et al*., 2005). Adding 0.2 mg Cr kg^{-1}DM as $CrCl_3$, CrPic or CrNA did not improve immune responses (Van Heugten and Spears, 1997) but the basal diet contained 5.2mg Cr kg^{-1}DM. Heat stress revealed only a tendency for CrPr to improve performance in fattening pigs and markers of glycolysis and lipolysis were unaffected; of immune markers, only blood neutrophil counts were increased (Mayorga *et al*., 2019).

- In a rare comparison between Cr_i and Cr_o sources ($Cr(SO_4)_3$ and CrMet, adding 0.2mg Cr kg^{-1}DM), both lowered plasma glucose by similar margins (Peres *et al*., 2014).
- Consistent commercial benefits of Cr_i and Cr_o supplementation have yet to be demonstrated.

Responses in unstressed avians

Benefits of adding Cr_o to diets (Balos *et al*., 2017), first claimed over 40 years ago, are still exaggerated.[iii] Supplements of CrPic (0.8–3.2mg kg^{-1}DM) improved growth and food intake of broilers while lowering blood glucose and NEFA concentrations and changing the distribution of body fat (increased in liver, decreased in abdomen; Lien *et al*., 1999). Similar responses in broiler performance and blood metabolites were reported on a diet high in animal fat (approximately 5.0%) (Sahin *et al*., 2002). In Japanese quail, adding 0.4–1.2 mg Cr as CrPic to a diet containing 1.3 mg inherent Cr kg^{-1}DM improved food intake, egg production and egg quality, while raising plasma insulin and lowering cortisol (Sahin *et al*., 2001).

- In another rare comparison between sources ($CrCl_3$, CrMet, CrY and CrPic) in broiler chicks, adding Cr again lowered plasma glucose, irrespective of source: organic sources differed in their effects on plasma lipids and $CrCl_3$ altered lipid distribution in plasma and total cholesterol but lowered triglyceride concentrations (Kani, 2015).
- In a third comparison between Cr_i and Cr_o (as Cr_2Cl_3 and CrY) at similar concentrations (2.7mg Cr kg^{-1}DM), CrY produced two- to threefold higher tissue Cr but 30% lower serum Cr than Cr_i (Król *et al*., 2017) but neither supplement affected performance.

Responses in stressed avians

Commercial layers, kept under conditions of cold (Fig. 17.5; Sahin *et al*., 2001) and heat stress (Fig. 17.6; Sahin *et al*., 2002), exhibited expected changes in blood glucose, insulin, cortisol, thyroxine and triiodothyronine, which were attenuated by CrPic supplementation (0.2–0.4 mg kg^{-}DM). However. in a 2 × 2 factorial study that compared responses to CrPic and zinc, both elements improved egg production and blood biochemistry similarly but not additively: CrPic was only advantageous to cold-stressed birds if the diet contained no added Zn (Sahin *et al*., 2001).

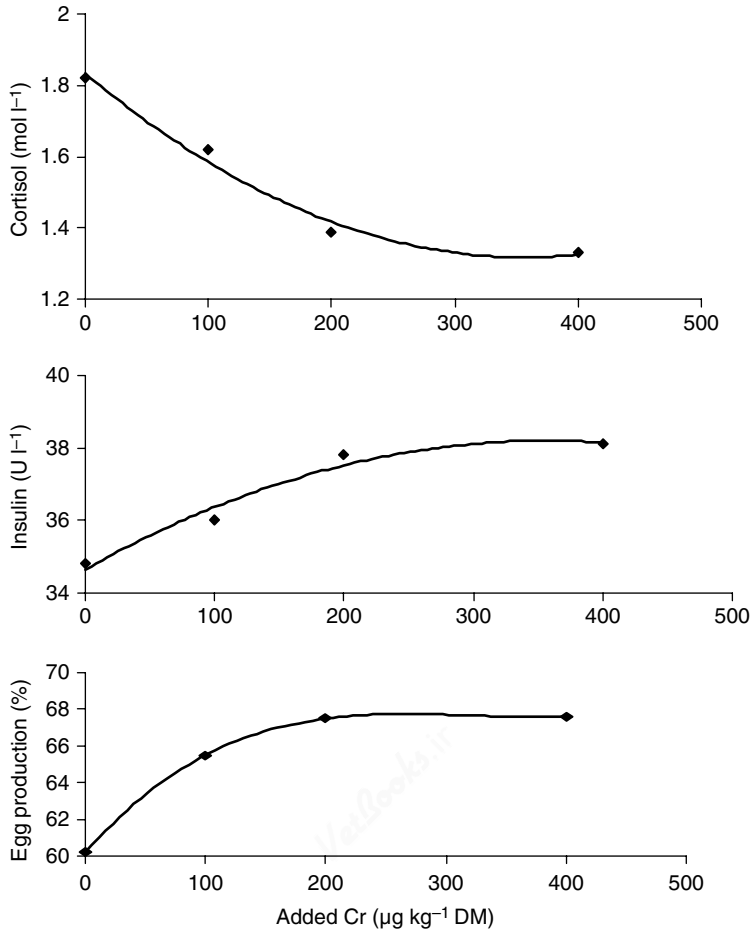

Fig. 17.5. Addition of chromium picolinate to the diet of cold-stressed laying hens lowers plasma cortisol and raises both plasma insulin and egg production (data from Sahin *et al*., 2001).

In a similar experiment, lipid peroxidation during cold stress was reduced by CrPic and zinc, as was the digestibility of protein and fat: responses to both elements were additive and obtained without increases in food intake (Onderci *et al*., 2002). Qualitatively similar responses in blood insulin, glucose, cortisol and cholesterol were found with vitamin C (250mg kg^{-1}DM) and CrPic in cold-stressed laying hens (Sahin and Onderci, 2002): a common biochemical lesion, possibly peroxidative damage to the pancreas, healed by Cr, Zn and/or vitamin C is suggested. Addition of CrY (0.15 mg kg^{-1}) to a basal diet high in Cr (4mg kg^{-1}DM) had little effect on performance in laying hens, other than to decrease food consumption by 3%, and did not raise yolk Cr (Eseceli *et al*., 2010).

- In summary, benefits from raising dietary Cr were often confined to groups with special needs in extreme environments and of limited duration.
- Investigations of responses to Cr_i on diets other than corn and corn silage are needed.
- There is no evidence that Cr_o sources are functionally hyperavailable.
- Measuring extractable Cr in feed samples and commercial feedstuffs may, by discounting contamination by elemental Cr, improve assessments of bioavailability.

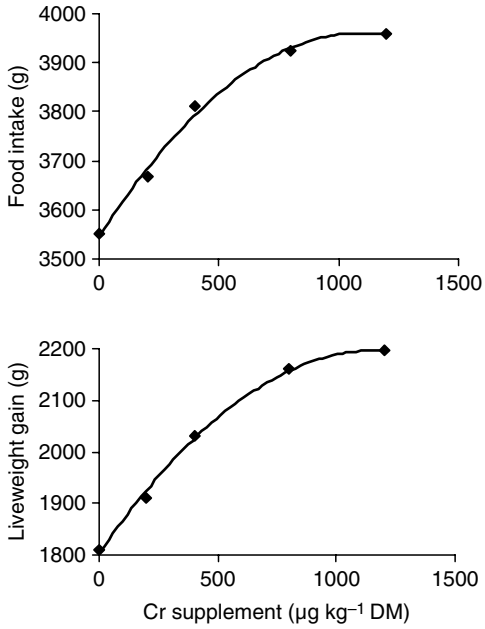

Fig. 17.6. Beneficial effects of supplementary chromium on liveweight gain and food consumption in broiler chicks after 42 days exposure to heat stress (32.8°C) (Sahin *et al*., 2002).

Requirements

Requirements are ill-defined (NRC, 1995) and will remain so while potencies of feed sources remain untested. Strenuous exercise, transportation and infection increase urinary Cr and may increase requirements (Anderson, 1987). Social, behavioural and metabolic stress in first calvers may also raise needs. Exercise in sheep, sufficient to increase energy demands by 9% over a ten-week period, halved liver Cr (Gardner *et al*., 1998). In unstressed heifers, the smallest of three supplements, adding 0.47mg Cr kg^{-1}DM, was sufficient to optimize glucose tolerance but not necessarily performance (Spears *et al*., 2012). Bovine milk contains only 0.014mg Cr kg^{-1}DM (from Lloyd *et al*., 2010) but appears to be sufficient for suckled calves.

- Pastures and feeds for housed livestock, which normally contain 1.0–2.0mg Cr kg^{-1}DM, have rarely limited performance in unstressed livestock other than poultry, which may need <1mg added Cr kg^{-1}DM.

Requirements of broilers were relatively high during heat stress (Fig. 17.6), but the diet was high in fat (6–7%), also 'heat-stressed' and therefore prone to oxidation.

Chromium status

Assessment of status of both diet and livestock would present difficulties if Cr_i and Cr_o differed in functionality and metabolism. Organic sources varied in the extent to which they increased tissue Cr in pigs given 5mg Cr kg^{-1}DM: CrPic and CrMet caused chromium to accumulate in bone, liver, kidney and ovary, whereas CrPr and CrY only increased levels in bone and kidney (Lindemann *et al*., 2008). CrPic raised tissue Cr more than CrMet and it was concluded that CrPic had by far the highest 'bioavailability'. However, differences in tissue Cr have rarely been associated with improved performance and overestimate functional availability (see p. 28).

Total blood and tissue Cr have been questioned as measures of sufficiency (Subiyatno *et al*., 1996) and values rarely reported. Serum Cr was increased from 0.12 to 0.15–0.24 µg l^{-1} following supplementation with CrPic (Onderci *et al*., 2002). Milk Cr was not increased by oversupplementation with CrPr (Lloyd *et al*., 2010). Baseline values in porcine bone, kidney, liver, muscle and ovary were 34, 62, 53, 49 and 55µg Cr kg^{-1}DM, respectively, in Lindemann *et al*. (2008): similar values were found in the liver and kidney of unsupplemented lactating cows but increased two- and threefold, respectively, by quadrupling intake as CrPr (Lloyd *et al*., 2010).

Inorganic chromium as indigestible marker

Compounds such as Cr_2O_3 have a long history of use as inert markers in digestibility studies. Ingested chromium is almost totally excreted in faeces and faecal Cr can provide information on food intake, digestibility of organic matter and total faecal excretion of other minerals and nutrients, when given orally in regular known doses (e.g. in sows, Saha and Galbraith, 1993). Chromium has an affinity for fibrous particles and this has been

exploited by impregnating fibrous feeds (a process known as 'mordanting') to study fibre digestion of fibre by ruminants (Uden *et al*., 1980; Coleman *et al*., 1984). Information on sites of absorption and rates of passage of digesta can be generated by administering chromium to suitably cannulated or fistulated animals, including grazing animals, given Cr_2O_3 continuously from an intraruminal capsule (Parker *et al*., 1990). Radioisotopes of chromium in a soluble chelated form (^{75}CrEDTA) have also been used as inert markers (Downes and McDonald, 1964), showing that chelation does not necessarily enhance absorbability. In the milk-fed animal, significant absorption and urinary excretion of CrEDTA occurs, indicating temporary enhancement of gut permeability to the molecule (Suttle, unpublished data). Controlled release devices can deliver Cr_2O_3 steadily to grazing animals (Judson *et al*., 2018).

Toxicity

Bioreduction of Cr^{6+} to the less-toxic Cr^{3+} state is effected by many organisms (Starich and Blincoe, 1983) but may generate free radicals and constitute a potential hazard. Reduction of CrPic is essential for biological potency and risk of causing peroxidative tissue damage has been flagged (Vincent, 2000), though unlikely to occur at the low levels of supplementation employed commercially. The predominant form in feeds is Cr^{3+}, but it is important to distinguish the two forms (Hamilton *et al*., 2018). Chromium toxicity is rare and even soluble Cr^{3+} sources, such as the chloride and chromate, are tolerated at 1000mg Cr kg^{-1}DM by livestock (NRC, 2005) but lower limits are advised (Table 17.3). Tissue Cr is normally low (<0.1mg kg^{-1}FM) (Lindemann *et al*., 2008) and exposure to excess Cr is readily distinguished by raised tissue Cr (>10mg Cr kg^{-1}FW; Puls, 1994). Disturbances of metabolism, in the form of inhibition of cytochrome P450-linked monooxygenases and hydroxylation of testosterone, were reported with as little as 50mg Cr kg^{-1}DM in laying hens, particularly when added as aminoacinate rather than $CrCl_3$ (Guerra *et al*., 2002). CrMet was considered to be a safe but not necessarily efficacious source for livestock (EFSA, 2009).

Table 17.3. Tolerances of livestock towards occasionally beneficial elements, given as soluble salts (mg kg^{-1}DM; NRC, 2005).

Element	Pigs	Poultry	Ruminants
B	Unknown	200^{L}	800
Cr (III)^{Cr}	Unknown	500 (50^{L})	Unknown
Li	25	25	25
Mo	Unknown	100	2–20^{Cu}
Ni	250	250	100
V	<200	25 (<5^{L})	>200, <400

^{Cr}Tolerance of hexavalent (VI) Cr is much lower.
^{Cu}Tolerance can be only 2mg Mo kg^{-1}DM on diets high in S and low in Cu but >20mg Mo kg^{-1}DM with S low and Cu high.
^{L}Tolerance of layers < growers.

Lithium

Essentiality

Lithium was proposed to be an essential dietary constituent in 1981, when research led by Anke (reviewed by Mertz, 1986) reported growth retardation, impaired fertility, low birth weights and reduced longevity in goats on a diet containing 3.3 µg Li kg^{-1}DM; supplemented cohorts given 24 µg Li kg^{-1}DM remained healthy. Diets with < 1.7mg Li kg^{-1}DM lowered monooxygenase activity in liver and raised creatine kinase (Mueller *et al*., 2010). Similar clinical abnormalities were obtained with goats deprived of PTE by the same group and may reflect hormesis rather than essentiality (p. 505). Reduced fertility was also reported in second- and third-generation rats, given a diet low in Li (5–15 µg kg^{-1}DM), consisting principally of ground yellow corn (67%). Conservation of relatively high tissue concentrations in endocrine organs in the face of depletion (57 µg Li kg^{-1}FW in adrenal, 140 µg Li kg^{-1}FW in pituitary) may indicate an endocrine function for lithium (Patt *et al*., 1978). Essentiality of lithium in the human diet has been claimed (Schrauzer, 2002).

Sources

Lithium ranks 27th in abundance amongst elements in the earth's crust. Soils in eastern Europe contained an average of 28mg Li kg^{-1}DM (Table 17.1). Crops, particularly cereal grains, are low in Li but leguminous forages and field beans are richer sources (Table 17.2). Within food types, values can be two- to fourfold richer in those grown on high- than on low-Li soils, while concentrations in different cereals rank in the order rye > wheat > barley > oats (NRC, 2005).

Metabolism and status

The metabolism of lithium resembles that of the more abundant alkali metal, sodium. High solubility and low molecular weight allow almost complete absorption by a paracellular route. Lithium turnover is rapid, urinary excretion extensive and there is minimal storage (NRC, 2005). Plasma normally contains <0.02 mg Li l^{-1}, which is not protein bound: values increase linearly as Li intake increases, allowing Li salts to be used as a marker for intake of supplementary foods at pasture (Vipond *et al.*, 1996). When dietary Li increased to 162mg kg^{-1}DM, plasma Li rose to 3–6 mg l^{-1}. Any Li-dependent pathway would have to be protected from competition from Na and studies of ruminants reared under saline conditions (p. 197) would be of interest. In rats, feed sources supported lower tissue Li than $LiCO_3$ and may be of lower availability (Patt *et al.*, 1978). Indicators of normal status have yet to be described for livestock. Human population distributions of whole blood and hair Li in Croatia suggested that values < 3.4 μg and 14 ηg g^{-1}, respectively, were subnormal (Mimica *et al.*, 2019).

Toxicity

Farm livestock are rarely exposed to excess Li but 50–200mg Li kg^{-1}DM can suppress appetite, with chicks the most sensitive and cattle the least sensitive species (Table 17.3) (NRC, 2005). Poisoning following exposure to industrial products (e.g. lithium greases) has occasionally been reported. The symptoms in mature beef cattle were severe depression, diarrhoea, ataxia and death, and were reproduced by acute oral exposure to 500–700mg Li kg^{-1}LW (as LiCl) (Johnson *et al.*, 1980). Serum Li rose to 40–60 mg l^{-1}, while soft-tissue Li rose from 5–20 to 50–120mg kg^{-1}FW with little difference between muscle, brain, heart, liver and kidney. Lower doses (250mg Li kg^{-1}LW) caused mild diarrhoea and are sufficiently unpleasant to illicit aversive behaviour in cattle (Ralphs, 1997) and sheep (Thorhallsdottir *et al.*, 1990). Aversion to Li salts has been exploited by training livestock to associate aversion with concurrent intake of toxic plants (e.g. tall larkspur), which are subsequently avoided when given a choice under range conditions (Lane *et al.*, 1989). Livestock clearly recover well after brief exposure to sub-lethal lithium doses, but aversive behaviour is quickly overridden by social influences, such as the presence of untrained cohorts. In sheep, continuous exposure to lithium was necessary to maintain aversion to vermeerbos in South Africa (Snyman *et al.*, 2002). In humans, toxicity has arisen from the use of lithium as an antidepressant by subjects on salt-restricted diets (see Johnson *et al.*, 1980 for citations).

- Toxic and deficient concentrations of dietary Li may decrease as dietary Na increases.

Molybdenum

Essentiality

Early nutritional interest in molybdenum (Mo) centred on antagonism of copper (Cu) metabolism in ruminants (p. 255). However, Mo is an essential trace element by virtue of its presence in and activation of the flavoprotein enzyme, xanthine oxidoreductase (XO/XDH) (de Renzo *et al.*, 1953), aldehyde oxidase and sulfite oxidase (Johnson, 1997). Absorbed molybdate (MoO_4) is normally transported in plasma in the free ionic state. On reaching tissues, Mo is stored as molybdopterin or incorporated into one of three enzymes, listed above, in mitochondrial membranes (Johnson, 1997). High activities of XDH are found in liver, endothelial cells lining blood vessels, macrophages and mast cells (Helsten-Westing *et al.*, (1991) and in the mammary gland, in close association with sulfite oxidase (Blakistone *et al.*, 1986). Interconversion of XO and XDH, catalysed by sulfite oxidase, generates free superoxide radicals and may cause muscle damage during intense exercise in humans (Helsten-Westing *et al.*, 1991). Reactivity hinges upon an ability to change valency and is both life-threatening and life-sustaining (Johnson, 1997). Addition of tungstate (WO_4), a Mo antagonist, to a diet low in Mo retarded growth and induced anaemia in chicks: tissue Mo fell and capacity to convert xanthine to uric acid was reduced (Higgins *et al.*, 1956; Johnson *et al.*, 1974). Anaemia may be attributable to impaired Fe metabolism since XO/XDH interconversion promotes ferroxidase activity in intestinal mucosa (Topham *et al.*, 1982b) and liver (Topham *et al.*, 1982a), releasing Fe bound to ferritin (p. 329). Adding Mo to a diet for goats, containing only 24 μg Mo kg^{-1}DM for several generations averted what was to become a litany of abnormalities, alleviated by other OBE and PTE (p. 460), including abortions and neonatal deaths (Anke *et al.*, 1978).

Natural occurrence of molybdenum insufficiency

Lack of Mo can limit yields of crops and animal performance (Anderson, 1956). Chicks tolerate diets containing only 0.2mg Mo kg^{-1}DM (NRC, 2005). Growth and cellulose digestibility were improved in lambs when Mo was added to a semi-purified diet containing 0.36mg Mo kg^{-1}DM. Cattle can tolerate pastures containing such low concentrations but sheep may succumb to chronic copper poisoning (CCP) because Cu is so well absorbed when dietary Mo is so low (p. 255). Induction of XO may be required to trigger inflammatory responses to trauma (Friedl *et al.*, 1989). Variations in dietary Mo within the range encountered by grazing sheep can inhibit (at <5 mg) (Suttle *et al.*, 1992) and enhance (at >5mg Mo kg^{-1}DM) (McClure *et al.*, 1999) invasion of the gut mucosa by parasitic nematodes in lambs. Nematodiasis is a perennial problem for most grazing livestock in temperate climates.

Sources for ruminants

Cereals are relatively low in Mo, rarely containing >1mg Mo kg^{-1}DM (Table 17.2) For practical nutritional purposes, only pasture Mo is important and then only as an antagonist of Cu. Pasture Mo is determined by available soil Mo, which varies widely. Sandy soils are low and those of marine origin high in Mo (Table 17.1), of which approximately 10% is normally extractable. Extractability rises with soil pH and, with it, crop and pasture Mo (Fig. 2.1). In British Columbia, mean forage Mo ranged from 0.9 to 2.6mg Mo kg^{-1}DM, with corn silage having least, sedge the most and legumes and grasses, intermediate values, respectively (Miltimore and Mason, 1971). However, red clover accumulated ten times more Mo than grasses on a Mo-rich soil (Kincaid *et al.*, 1986). A median value of only 1.1mg Mo kg^{-1}DM was reported for 20 limed and improved hill pastures in Scotland, expected to be relatively high in Mo: the maximum value was 60mg kg^{-1}DM (Suttle and Small, 1993), one of a cluster of raised values found in association with outcrops of marine black shale in Caithness (Suttle, 2008a). A recent survey of >800 samples from New Zealand found 95% of values were ≤0.7 mg and the maximum 3.8mg Mo kg^{-1}DM (Knowles and Grace, 2014). Differences between plant species are not generally large (Table 17.2). Animal feed sources are mostly low in Mo, with the exception of marine products and milk from animals grazing Mo-rich pastures.

Molybdenum metabolism in ruminants

In milk-fed ruminants, Mo is absorbed from the stomach (Miller *et al.*, 1972). The three-way interaction between Cu, Mo and S in the rumen that determines susceptibility to hypocuprosis, involving formation of unabsorbable thiomolybdate (TM) complexes (p. 272), will determine tolerance of Mo insufficiency and excess. Molybdenum can be well absorbed from water-soluble sources, like MoO_4 and Mo-rich herbage, by cattle (Ferguson *et al.*, 1943) and sheep (Grace and Suttle, 1979) but is largely excreted in faeces on diets high in Cu, S and fibre (Fig. 17.7), partly as Cu-TM complexes. Molybdate absorption can be inhibited by sulfate (SO_4) (Mason and Cardin, 1977) but little sulfur leaves the rumen in that form (Fig. 9.2). Above dietary Mo thresholds determined partly by dietary Cu:Mo ratio, TM leaves the rumen in absorbable forms and perturbs Cu metabolism (Suttle and Field, 1983; Price *et al.*, 1987). Absorption of TM is indicated by accumulation in plasma of albumin-bound, trichloroacetic acid-insoluble (I) copper complexes (TCAI-Cu; Dick *et al.*, 1975; Smith and Wright, 1975a, b). The rise in TCAI-Cu decreases as dietary Cu:Mo ratio increases (Fig. 17.8). Lower dietary Cu:Mo ratios are needed to induce TCAI-Cu in sheep than in deer (Freudenberger *et al.*, 1987) and cattle (Wang *et al.*, 1988).

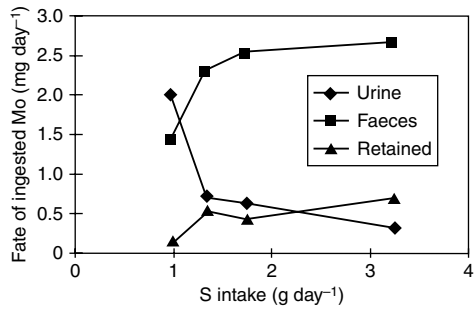

Fig. 17.7. Increases in daily sulfur intake can shift the route of excretion of a dietary molybdenum supplement (5mg kg^{-1}DM) from urine to faeces while increasing Mo retention in sheep (Grace and Suttle, 1979).

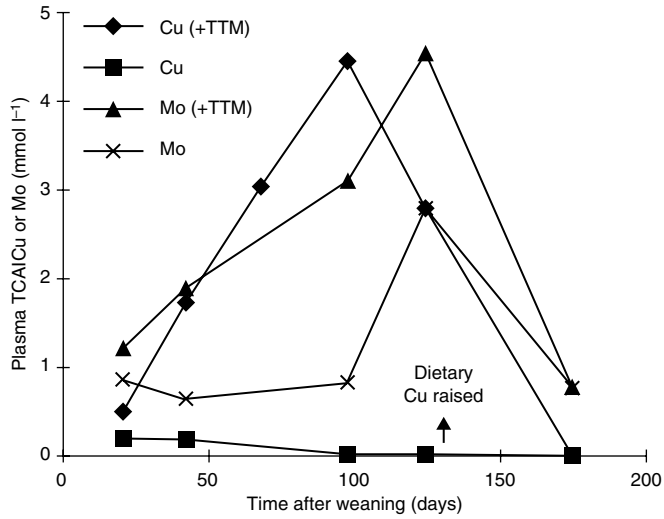

Fig. 17.8. Biochemical consequences of exposing lambs to tetrathiomolybdate (TTM, 5.4mg Mo kg^{-1}DM) via the diet depend on the Cu:Mo created. Initially, when dietary Cu was low (3.5mg kg^{-1}DM, Cu:Mo 0.65), excess TTM was absorbed, causing trichloroacetic acid-insoluble Cu (TCAI-Cu) to accumulate in plasma. When dietary Cu was raised after 130 days (to 15.5mg kg^{-1}DM, Cu:Mo 2.9), TCAI-Cu disappeared and plasma Mo was markedly reduced, presumably due to insoluble Cu–TTM complex formation in the gut (from Suttle and Field, 1983).

It has been inferred that absorbed TM may impair Cu trafficking in ruminants exposed to dietary Mo (Clarkson *et al*., 2020) but addition of 5 mg Mo and 1.5 g S to a corn silage diet containing 6mg Cu kg^{-1}DM failed to affect any of the seven transporters and chaperones, measured in cattle after 112 days treatment (Fry *et al*., 2013). Since TCAI-Cu has yet to be reported in grazing cattle and is not seen in moderately exposed sheep (Smith and Wright, 1975a, b; Suttle and Small, 1993),

- impaired Cu trafficking will rarely, if ever, be encountered on Scottish and NZ pastures, which rarely contain >1mg Mo kg^{-1}DM.

Appearance of TM metabolites in the bloodstream of rats on high MoO_4 intakes, given via drinking water, raised the possibility that TM can also be synthesized in and absorbed from the caecum (Suttle, 2012b).

- Caecal synthesis of TM should be investigated further in all species.

Excess Mo is normally excreted via the kidneys. Competitive inhibition of MoO_4 reabsorption by SO_4 in the renal tubule can increase urinary Mo excretion in sheep (Bishara and Bray, 1978). Ruminants and non-ruminants will express the same antagonism because sulfur absorbed from the rumen or lungs as sulfide and from the intestine as microbial sulfur amino acids is metabolized to SO_4. Marked reduction of Mo retention in sheep given supplementary SO_4 (Dick, 1956) was an artefact of the experiment.[iv]

Natural occurrence of molybdenum overload in cattle

Within a few days of grazing pastures containing 20–100mg Mo kg^{-1}DM in Somerset, UK, cattle developed severe diarrhoea ('teart') and rough hair coats. The condition was controlled with large doses of $CuSO_4$ (1–2 g day^{-1}) or intravenous injections of 200–300 mg Cu day^{-1}, although blood and liver Cu were normal (Ferguson *et al*., 1938). Diarrhoea was prevented by much smaller amounts of Cu, given via the drinking water (Farmer *et al*., 1982). The Somerset pastures were underlain by Mo-rich Lower Lias clay. A similar diarrhoea in New Zealand was associated with organic soils and termed 'peat scours' but liver and blood Cu were generally low in affected stock (Cunningham, 1950). Without treatment, cows on teart pastures conceive with difficulty. Male infertility was not studied but young bulls lacked libido, showed testicular

damage and little evidence of spermatogenesis when exposed to excess Mo under experimental conditions (Thomas and Moss, 1952). Skeletal abnormalities may also develop and appear as 'beading' of the ribs, widening of metatarsal epiphyses and lameness but are indistinguishable from those caused by lack of dietary Cu. Teart scours may be caused by acute lack of Cu in the caecal mucosa, induced by secondary generation of TM (Suttle, 2012b) and peat scours by chronic copper insufficiency, i.e. they may be different points in a spectrum of copper-responsive disorders (CuRD), illustrated in Fig. 17.9 (Suttle, 1991), clinically and therapeutically indistinguishable (Suttle, 2012a; Raisbeck *et al.* 2006).

'The thiomolybdate toxicity hypothesis'

Infertility in normocupraemic cattle on pastures or rations with normal dietary Cu:Mo ratios was claimed to be widespread in the UK and caused by thiomolybdate (TM) toxicity (McKenzie *et al.*, 1997; Kendall *et al.*, 2001; Gould and Kendall, 2011). Induction of infertility in heifers with severe hypocupraemia, induced by excess Mo but not excess Fe, was claimed to support the hypothesis (Fig. 11.8),

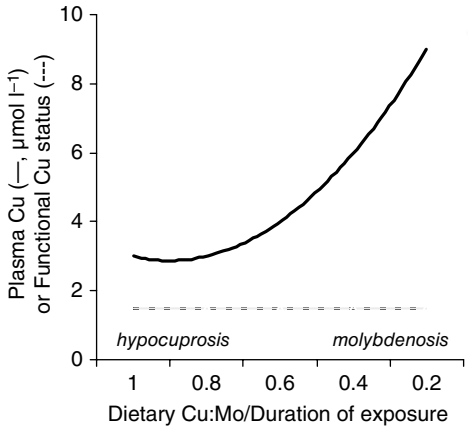

Fig. 17.9. 'Molydenosis' and 'hypocuprosis' should be used to describe clinical disorders that lie at opposite extremes on a continuous scale. Underlying both conditions is low functional copper status at critical loci (hair follicle in hypocuprosis; intestinal mucosa in molybdenosis); as Cu:Mo ratio declines, time taken for symptoms to develop shortens and an abnormal, trichloroacetic acid insoluble constituent accumulates in plasma, causing total plasma Cu to paradoxically increase.

despite the subtle difference in Cu status. Systemic influences of TM were held to explain unexpectedly wide variation in the relationship between plasma Cu and caeruloplasmin oxidase activity (Cp) but were attributable to a procedural anomaly.[v] Natural exposure of cattle to high-Mo pastures did not affect the Cp:plasma Cu relationship (Suttle, 2008b). The TM hypothesis has been more fully criticized (Suttle, 2012b) and has yet to pass peer scrutiny but has recently been linked to possible inhibition of intracellular Cu trafficking (Clarkson *et al.*, 2020). The hypothesis continues to run counter to facts: dietary Mo >5mg kg^{-1}DM and dietary Cu:Mo around 1.0, abnormal Cu distribution in plasma, hallmark for TM absorption, was not evident (e.g. Fry *et al.*, 2013). The thiomolybdate toxicity hypothesis continues to be promulgated worldwide and provision of 'sacrificial Cu', from a slow-release, soluble glass bolus, retained in the rumen, advocated as the perfect antidote, preventing TM absorption. However,

- supplementing cattle of normal blood Cu status without knowing liver Cu status is a potentially dangerous practice.

It may be no coincidence that Cu is now commonly overfed and bovine copper toxicity an emerging problem from the UK to the Antipodes (Knowles and Grace, 2014; Suttle, 2016). The TM hypothesis fitted properties of the bolus rather than biochemical and therapeutic evidence from the field. Providing Cu as CuO particles, which slowly release Cu in the abomasum, protects cattle and sheep from Mo-induced hypocuprosis (p. 279).

Molybdenum overload in other grazing species

Although sheep exposed to high dietary Mo in artificial diets (25mg kg^{-1}DM) can develop diarrhoea (Suttle and Field, 1968), species other than cattle grazing Mo-rich pastures rarely do so. Joint abnormalities, lameness, osteoporosis and spontaneous bone fractures were observed in some lambs grazing pastures heavily fertilized with Mo (Pitt, 1976). Goats and yak (Xiao-Yun *et al.*, 2006) can succumb to Mo-induced CuRD but deer (Grace *et al.*, 2005) and horses (Pearce *et al.*, 1999) are particularly tolerant. Tolerance in sheep may be attributable to differences in physiology of digestion in the caecum, where DM content is higher and pH lower than in cattle. In horses, Mo is probably extensively absorbed before the site of potential

TM synthesis, the caecum, is reached. If this is the case, tolerance of Mo may be shared by other hindgut fermenters such as camelids.

Therapeutic properties of thiomolybdates

Tetrathiomolybdate (TTM) is a powerful chelator of Cu *in vitro* and used to control haemolytic copper poisoning (CCP) in sheep (p. 281) and mammary tumour growth in women.

- Biochemical responses during TTM treatment give fresh insights into the metabolism of TTM and suggest that it is less harmful than assumed by proponents of the thiomolybdate hypothesis.

A course of parenteral TTM injections immediately reduces leakage of GDH from hepatocytes in sheep with pre-haemolytic copper poisoning (PCP) and abruptly inhibits erythrocyte superoxide dismutase activity (ESOD) but both effects are short-lived (Fig. 17.10). TTM inactivates ESOD by binding to Cu *in situ* and presumably does the same to labile Cu that is damaging hepatocyte membranes. TTM does not bind to less-accessible Cu in the Cu molecule (Suttle, 2012b; Clarkson *et al.*, 2020). Spontaneous recovery of ESOD activity indicated breakdown of TTM/SOD complexes in erythrocytes, and subsequent increases in plasma GDH suggest copper released elsewhere can cause further damage to hepatocyte membranes (Fig. 17.10). The whole process was clinically silent, suggesting that TTM had been unable to inhibit life-sustaining, Cu-dependent functions.

In using parenteral TTM to deplete initial liver Cu in calves, liver Cu was initially depleted but in the longer term, depletion was slowed (Fig. 17.11). Plasma Cu remained 3.1 ± 0.41 μmol l^{-1} higher in calves given ATTM but was always within normal limits and unaffected by injecting Cu. Taken together, responses in liver and plasma Cu suggested that injecting ATTM created a slow-release pool of Cu that augmented the hypercupraemic effect of parenteral Cu being translocated from an opposing injection site. Lambs, dosed with CuO particles or unsupplemented while grazing pastures with 10–50mg Mo kg^{-1}DM, showed delayed responses in ESOD, after earlier increases in TCAI Cu (Fig. 17.12): these responses resemble those seen after parenteral TTM treatment of lambs (Fig. 17.10) and are consistent with responses to parenteral ATTM in cattle (Fig. 17.11), indicating build-up of a systemic pool of slowly released Cu (Suttle 2013).

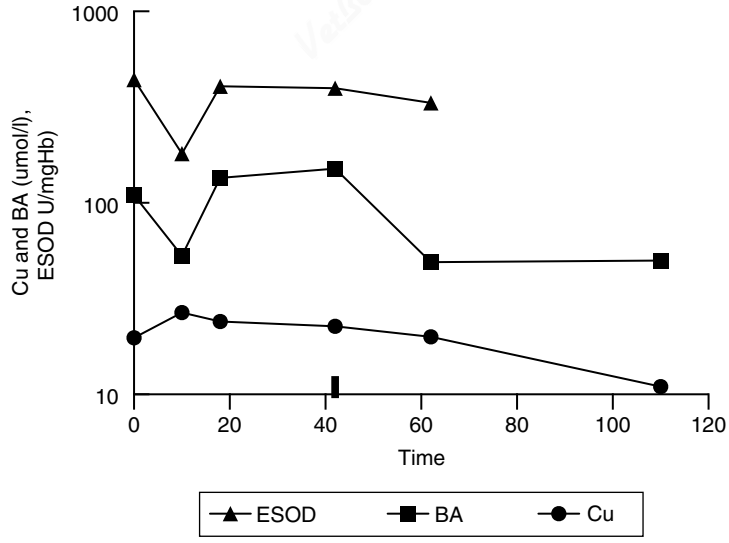

Fig. 17.10. Responses in plasma copper (Cu), bile acids (BA) and erythrocyte superoxide dismutase activity in a cohort of lambs with pre-haemolytic copper poisoning after conservative treatment with parenteral ammonium tetrathiomolybdate (ATTM) on days 0 and 42. Initial treatment inhibits ESOD but also reduces liver damage, seen as a fall in BA: recovery of activity and resurgence of liver damage suggest that insufficient Cu had been removed and release of temporarily deactivated Cu in the liver was causing further dysfunction; repeated ATTM treatment provided complete protection. (Data from the uninfected control group in Suttle, 2012a)

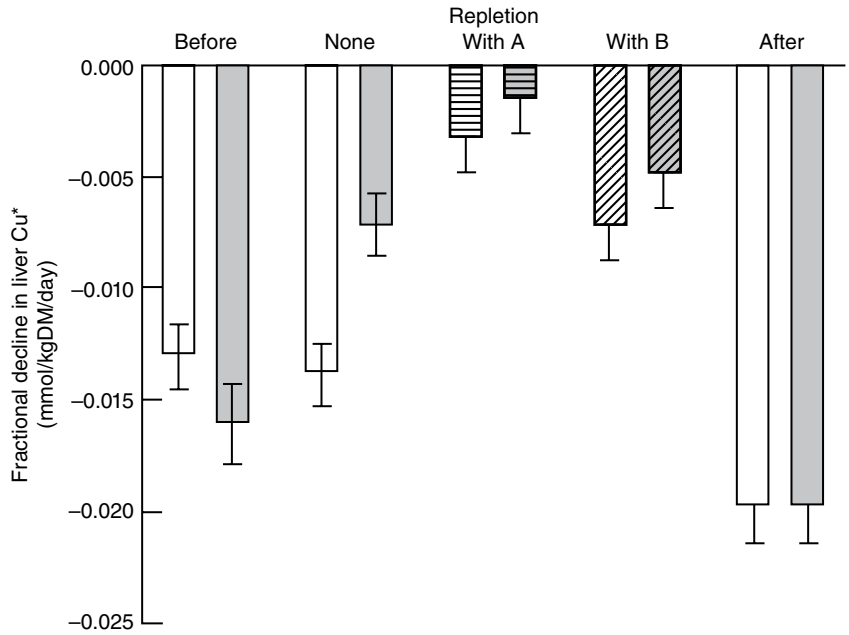

Fig. 17.11. Fractional decline in liver copper (Cu) in calves given a diet low in available Cu, with (shaded) or without (clear columns) initial subcutaneous injections of ammonium tetrathimolybdate (TTM) to reduce liver Cu in those with high values and before or after comparing responses to two injectable Cu products (A and B). TTM tended to decrease liver Cu over 50 days prior to injecting Cu but slowed depletion over the next 80 days, whether calves were injected with Cu or not: no residual effect of TTM was evident during a final 30-day period.* Products A and B differed in capacity to halt the decline in liver Cu and $\log_e$ transformed values are used for the middle repletion period to linearize changes in liver Cu with time. (Data from Suttle, 2013)

In women with breast cancer, TTM is used orally and continuously to produce a managed low Cu status. Tumour angiogenesis has a relatively high need for Cu and is inhibited when TTM reduces Cu absorption from the gut. The only harmful side-effects are anaemia and neutropenia. Repeated parenteral TTM doses can cause histopathological changes in the pituitary of sheep (Haywood *et al*., 2004). Endocrine changes in adult ewes given 24 mg TTM d^{-1} by continuous intravenous infusion and responses of bovine follicular cells to TTM *in vitro* indicate possible mechanisms for TM-induced infertility (Kendall *et al*., 2003) but the degree to which dietary exposure was simulated is unknown. Natural exposure to Mo can be sufficient to raise serum $TCAI_{Cu}$ and liver Mo without harming sheep or cattle (Raisbeck *et al*., 2006).

Episodic thiomolybdate exposure

In isolated locations, symptoms of hypocuprosis (diarrhoea and browning of black coats) can briefly appear in cattle grazing pasture high in Mo (>10mg kg^{-1}DM; McAloon *et al*., 2017). A similar syndrome has been seen on the teart pastures in Somerset, by the author, and on Duthie Farm in Aberdeenshire (M. Phillippo, personal communication). The syndrome is repeatable and associated with particular fields, even parts of fields. From the responses to parenteral TTM and pasture high in Mo, described above, it is conceivable that a rapid increase in herbage Mo causes TM to be absorbed and flush Cu from the liver but inhibits cuproenzyme activity in the large intestine and skin, causing immediate diarrhoea and loss of hair colour after a time lag. Release of Cu from a concurrently formed, slow-release pool of copper could lead to a spontaneous recovery. Whether such episodes are called hypocuprosis or thiomolybdate toxicity is a semantic argument. In animals about to conceive, fertility may be adversely affected. However, such incidents will be rare and unlikely to occur on the vast majority of farms in the UK and New Zealand, where herbage Mo is far too low (MAFF, 1990; Knowles and Grace, 2014).

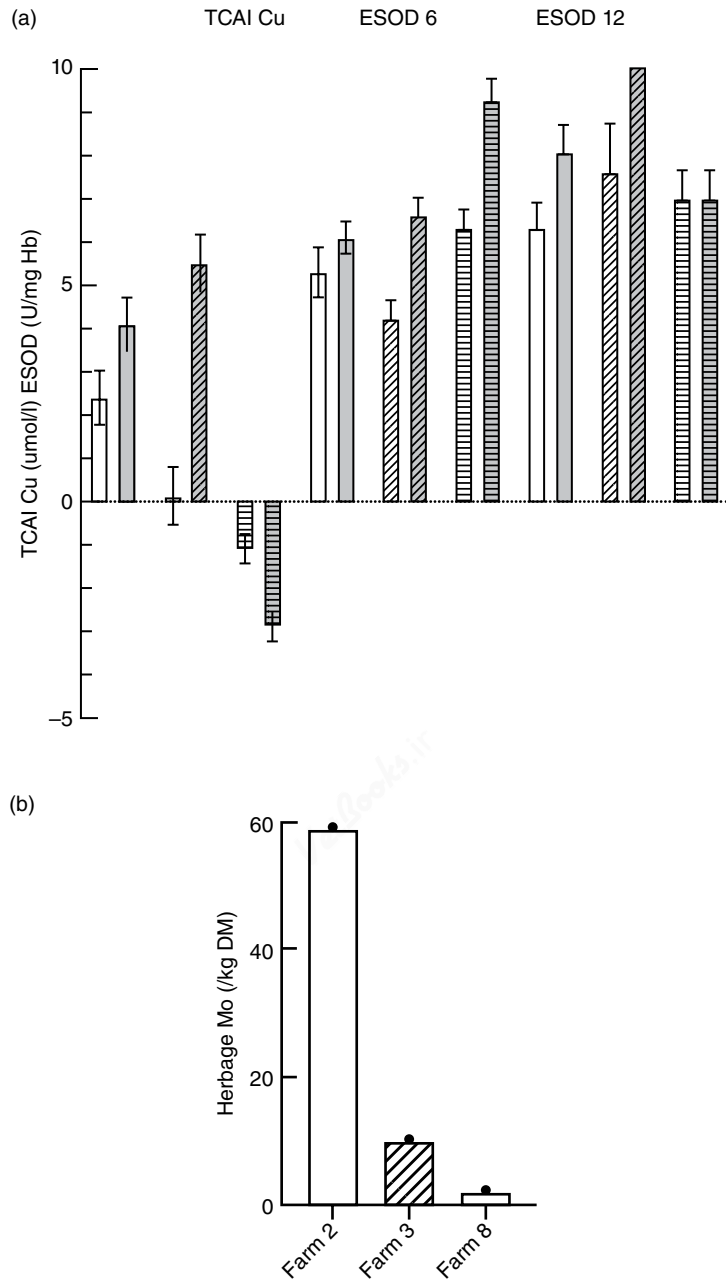

Fig. 17.12. (a) Responses in trichloracetic acid-insoluble (TCAI) Cu in plasma after six weeks and erythrocyte superoxide dismutase (ESOD) after 6 and 12 weeks in suckling lambs, treated (shaded) or untreated (open columns) with cupric oxide needles on three hill farms, indicated by different column patterns. (b) Herbage molybdenum (Mo) concentrations at 6 weeks: negative values for TCAI Cu on the farm with lowest herbage Mo may have been partly attributable to a lag of several months between determination of total and TCA-soluble Cu, but exposure to ≥10mg Mo kg^{-1}DM raised TCAI Cu, especially in treated lambs; ESOD was improved by treatment on all farms but later on the higher-Mo farms. (Data from Suttle and Small, 1993)

- Episodes of systemic TM exposure would be best treated and prevented by short periods of oral or parenteral Cu supplementation.

Molybdenum tolerance in non-grazing species

Pigs are unaffected by diets containing 1g Mo kg^{-1}DM but growth in chicks and turkey poults is inhibited at 200 mg (NRC, 2005) and 300mg Mo kg^{-1}DM, respectively (Kratzer, 1952); higher levels can cause anorexia and weight loss. The least-tolerant monogastric species (avian) normally have much lower plasma Cu values than other classes (Table 11.8), due to lack of caeruloplasmin. High tolerance is explained by the absence of a rumen to generate TM. Differences between non-ruminant species are harder to explain but may also involve antagonisms of Cu metabolism. Exposure of rats to Mo via drinking water induces TCAI-Cu in plasma and causes Cu and Mo to accumulate in tissues (Suttle, 2012b). Molybdenum can be more toxic when given to non-ruminants of low than of high Cu status but sulfur supplements afforded protection, whether given as SO_4, high-protein diets, thiosulfate, cysteine or methionine (Underwood and Suttle, 1999): effects on TM uptake from the caecum and renal tubular reabsorption of Mo may be involved.

Nickel

Essentiality

Non-ruminants

Nickel was first shown to be essential for chicks fed on a highly purified diet under strict environmental control (Nielsen and Sauberlich, 1970). Depigmentation of the shank skin, thickened legs, swollen hocks, growth retardation, ultrastructural liver changes and anaemia developed in chicks given a diet with only 2–15 μg Ni kg^{-1}; an additional 50 μg Ni kg^{-1} alleviated all evidence of deprivation (Nielsen *et al*., 1975). The symptoms of deficiency were confirmed in first-generation rat offspring as growth retardation, impaired reproduction and anaemia, accompanied by lowered liver activities of many enzymes involved in the tricarboxylic acid cycle and biochemical evidence of liver damage (Stangl and Kirchgessner, 1996). The essentiality of nickel has been reviewed by Kumar and Trivedi (2016). A specific functional role for nickel has still not been identified but high concentrations are present in nucleic acids, especially RNA, and nickel may stabilize their structure. Nickel may also be involved in haematopoiesis. More nickel was needed to reverse some effects of vitamin B_{12} deprivation in pigs; a supplement of 6mg Ni kg^{-1}DM lowered liver Fe and serum homocysteine, while increasing serum vitamin B_{12} and folate (Stangl *et al*., 2000). Mechanisms for the vitamin B_{12}-sparing effect of nickel have yet to be elucidated but may involve stimulation of the gut microflora. Lower organisms undoubtedly require nickel to activate a hydrogenase and a urease. The pathogenic *Helibacterum pylori* is rich in Ni and colonization by the pathogen is restricted in mice given diets low in Ni (Benoit *et al*., 2013). The finding opens up a new possibility for the commonality of abnormalities seen in animals given diets low in OBE and PTE:

- Is health improved by dietary OBE and PTE that either favour colonization by commensal species or inhibit pathogenic bacteria?

Anaerobic bacteria

Nickel is essential to some anaerobic microbes. The presence of Ni^{1+} in the tetrapyrrole coenzyme, Cofactor F430, assists methyl coenzyme M reductase and thus powers methanogenesis in the rumen. Oxidation of Ni^{1+} by nitrate in 3-nitroxy propopanol is a promising tool to decrease enteric methane emission from ruminants without apparent negative side-effects and may help to mitigate anthropogenic climate change (Evert *et al*., 2016). Paddy fields are also an important source of methane emissions and vulnerable to pollution with Ni. *In vitro* studies suggest that with 50 mg Ni kg^{-1} dry soil, methane production from paddies is enhanced but a tenfold increase inhibited methane production: effects are partially due to changes in the numbers of methane-producing species present (Wang *et al*., 2019). Similar quadratic responses in methane production to added Ni were found during biogas generation from dairy excreta slurry (Hassan Dar and Tandon, 1987). Methane-producing soil anaerobes cannot utilize Ni bound to EDTA (Pramanik and Kim, 2013).

Ruminants

Supplementation of diets containing 0.26–0.85mg Ni kg^{-1}DM with 5 mg Ni (as $NiCl_3$) increased ruminal urease activity, growth rate and FCE of

lambs and steers, given diets high in energy and low in protein (Spears, 1984), but urease activity can be increased without affecting growth (Spears *et al.*, 1986), methanogenesis (Oscar *et al.*, 1987) or nitrogen metabolism (Milne *et al.*, 1990). Nickel supplementation had inconsistent effects on VFA production in the rumen (Milne *et al.*, 1990).

- Relationships between available nickel supply and methane synthesis in the rumen merit further study.

Sulfate reduction and vitamin B_{12} synthesis are potentially limiting, Ni-dependent processes in rumen microbes (Nielsen, 1996). Similar biochemical abnormalities to those found in Ni-deprived rats and pigs have been reported in goats reared on diets with <100 µg Ni kg^{-1}DM (Anke *et al.*, 1991a), accompanied by non-specific clinical abnormalities, also seen with diets low in lithium, molybdenum and some PTE (see Chapter 18).

Sources

Nickel is not abundant in soils and crops (Tables 17.1 and 17.2) and only 5% of that present in soils is easily extractable (Archer and Hodgson, 1987). Concentrations of Ni in pasture grasses are lower than in soils, but legumes such as alfalfa contain more (Table 17.2). Wheat from 12 locations in North America contained from 0.08 to 0.35 about a mean of 0.18mg Ni kg^{-1}DM (Welch and Cary, 1975). Oilseeds are richer sources (0.5–1.0; EFSA, 2015) but only sunflower meal reached the level quoted by NRC (2005) of 5–8mg Ni kg^{-1}DM. Bovine milk normally contains about 0.02–0.05 mg Ni l^{-1}, but colostrum is four times richer (Kirchgessner *et al.*, 1967), a similar increase to that found for essential minerals (Table 2.5). Proprietary feeds for fattening and milking livestock contained 1.2–3.2mg Ni kg^{-1}DM but rations for laying hens much more (5.3; EFSA, 2015).

Metabolism

Nickel is poorly absorbed (1–5% by calves; O'Dell *et al.*, 1971) but absorption increases during pregnancy, lactation and iron deprivation, suggesting involvement of active transport mechanisms shared with iron (NRC, 2005). Plasma contains <0.017 µmol Ni l^{-1}, bound chiefly to albumin, but it is histidine-bound Ni that may facilitate uptake by cells. Tissue Ni varies from organ to organ, being much higher in kidney and lung than elsewhere in calves (O'Dell *et al.*, 1971). Kidney and liver from rat pups, born of dams given adequate Ni, contained 2.78 and 0.77 µmol Ni kg^{-1}FW, respectively; depletion reduced these concentrations by 39% and 44%, (Stangl and Kirchgessner, 1996). Supplementing cows with 365 or 1835 mg Ni day^{-1} as $NiCO_3$ did not increase milk Ni (O'Dell *et al.*, 1970a). Excess Ni is excreted via urine rather than faeces (NRC, 2005) but small supplements (5mg Ni kg^{-1}DM) increased bovine kidney Ni sixfold to 0.3mg Ni kg^{-1}DM, suggesting a gross dietary excess (Spears *et al.*, 1986).

Requirements

Milk containing 0.2–0.5mg Ni kg^{-1}DM presumably meets or exceeds the needs of suckled calves. There have been no reports of grazing livestock being adversely affected by lack of Ni, as might be expected from low requirements in other species and relatively high pasture Ni. In view of the low nickel content of maize (0.2 mg kg^{-1}DM) (NRC, 2005), requirements are least likely to be met on maize-based diets. Prospects of nickel requirements of poultry not being met are remote (Balos *et al.*, 2017). However, contamination of feeds with non-available elemental Ni from stainless steel, as with Cr (p. 454), may overestimate the adequacy of dietary Ni provision.

Toxicity

There is a wide margin between beneficial and toxic doses of Ni for all species, particularly non-ruminants. Susceptibility of the ruminant may reflect susceptibility of rumen microflora to Ni toxicity from soluble sources. Diets with >50mg Ni kg^{-1}DM, as $NiCl_2$, disturbed rumen function and caused inappetence but no specific toxicity signs or pathology, other than nephritis (O'Dell *et al.*, 1970b). With less-soluble forms such as the carbonate, 250mg kg^{-1}DM is tolerated by ruminants (O'Dell *et al.*, 1971). Poultry and pigs tolerate up to 400mg Ni kg^{-1}DM, as sulfate or acetate (NRC, 2005). The kidney is particularly vulnerable and

kidney Ni in exposed animals can reach 30–50mg kg^{-1}DM. In broiler chicks, >300mg Ni kg^{-1}DM unfolded proteins in the endoplasmic reticulum lumen of kidney tubule cells (Guo *et al.*, 2016). At such concentrations, antioxidant enzyme activities are decreased and peroxidative damage occurs in the intestine (Wu *et al.*, 2013). Nickel in edible livestock products poses no threat to consumers (EFSA, 2015).

Rubidium

Essentiality

Evidence that rubidium might be essential to sustain animal life came from application of the 'Anke goat model', which produced the familiar, non-specific signs of disorder on a diet containing <0.28mg Rb kg^{-1}DM (p. obe). Abortion rate was 80% in goats deprived of rubidium (Anke and Angelov, 2004), The syndrome was not repeatable elsewhere (NRC, 2005).

Sources

The earth's crust is rich in Rb (310 mg kg^{-1}). Rubidium enters groundwaters, those draining through gneisses and alluvial soils containing 18 and 3 µg l^{-1}, respectively (Anke and Angelov, 2004). Soil origin has a major effect on plant uptake, which can vary fivefold between species. Crops grown on gneisses are particularly rich in Rb (Anke and Angelov, 2004). Pig feeds contained 2.6–26mg Rb kg^{-1}DM (NRC, 2005) but components of the human diet less, values ranging from 0.3 mg in carbohydrates-rich to 13mg Rb kg^{-1}DM in protein-rich foods (Anke and Angelov, 2004). The contrasts are similar for livestock feeds, with cereals relatively poor, forage grasses and legumes relatively rich in Rb (Table 17.2) but high values may reflect contamination with soil.

Metabolism

There is a striking similarity between the metabolism of rubidium and that of potassium and evidence of interactions between the two elements. Like potassium, rubidium is extensively absorbed, may share the same active absorption pathway and is primarily excreted in urine (NRC, 2005; Anke and Angelov, 2004). Rats given diets containing 0.5 or 8.1mg Rb kg^{-1}DM showed five- to tenfold increases in blood and tissue Rb but reductions in blood and tissue K (Yokoi *et al.*, 1994).

Requirements and tolerance

If there is a requirement for rubidium, it is likely to be met by diets containing >0.4 µg kg^{-1}DM, i.e. all natural diets (Anke and Angelov, 2004). Risk of toxicity is also remote, diets with 250–1000mg Rb kg^{-1}DM being tolerated (NRC, 2005). Requirements and tolerances might be affected by interactions with dietary K, which is normally present in far higher 'macro' concentrations, particularly in the diets of grazing livestock (p. 174): risks of insufficiency would be greatest on sandy soils, on which plant Rb is low (Anke and Angelov, 2004).

Silicon

Essentiality

Chicks reared on a diet containing only 1mg Si kg^{-1}DM, in a plastic isolator with filtered air supply,

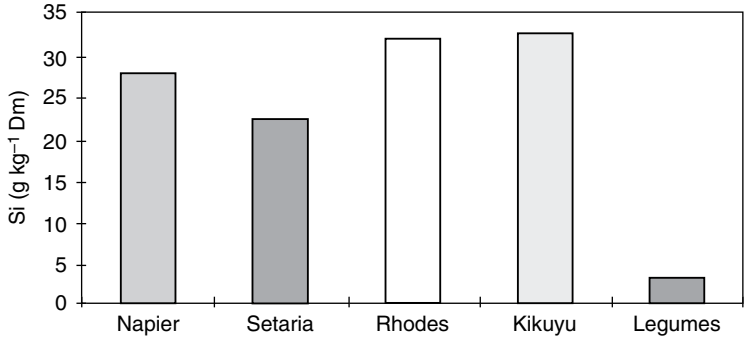

Fig. 17.13. Silicon concentrations are high in tropical grasses, but much lower in legumes (Jumba, 1989).

became decidedly less healthy than those supplemented with 250mg Si kg^{-1}DM (Carlisle, 1986). Growth rate was reduced by 30–50%, wattles did not develop, combs were stunted and development of skull and long bones was impaired. Silicon is involved in glycosaminoglycan formation in cartilage and connective tissue. In bone, lack of silicon reduces calcification. Weakening of connective tissues, including cartilage, caused defects at articular surfaces. and wound healing may be impaired in animals deprived of silicon (Seaborn and Nielsen, 2002). Connective tissues, such as tendons, and organs rich in connective tissue (e.g. aorta and trachea) are high in Si compared to others (11–17 versus 2–4mg kg^{-1}FW) and here the role of silicon may be structural rather than functional (p. 2). It is highly unlikely that silicon deprivation will occur naturally on crop- or forage-based diets but human studies have shown a correlation between silicon intake and bone mineral density (Jugdaohsingh, 2007).

Sources

As the second most abundant element in the earth's crust, Si is a ubiquitous contaminant of the environment and presents problems of nutritional excess rather than deficiency. Grasses commonly contain silicon in macro-element proportions (20–40g kg^{-1}DM) and coarser fodder or range species can contain twice as much (Smith and Urquhart, 1975). Leaves are richer in Si than stems in rice plants. Cereal grains are also rich in Si, particularly the more fibrous types such as oats (Carlisle, 1986). Leguminous forages contain relatively little silicon (Fig. 17.13), sometimes as little as 1.8g kg^{-1}DM. Silicon occurs in crops and forages in inorganic (silica, SiO_2, and silicic acid) and in organic forms (e.g. pectin and mucopolysaccharides). Most animal products are low in Si: eggs contain 3mg Si kg^{-1}DM) and milk only 1 mg Si l^{-1}. Risks of insufficiency are theoretically possible in the suckled or milk-fed animal, but probably avoided in practice by environmental contamination. Sodium zeolite A, an aluminosilicate, became a fashionable food additive in livestock nutrition and remedy for arthritic problems in horses (O'Connor *et al*., 2007).

Metabolism

Analysis of feed Si is complicated by the difficulty of 'digesting' the element even with strong acids, so it is hardly surprising that Si is often poorly absorbed. Sheep given dry gramineous or leguminous diets excreted 99% of ingested Si in faeces; absorbed silicon was mostly recovered in urine (Jones and Handreck, 1965). However, in grazing situations, Si is excreted predominantly via urine and may be far more absorbable from green than from dry feeds (Nottle and Armstrong, 1966). Humans can apparently absorb 40–50% of Si in natural foods (NRC, 2005; Jugdaohsingh, 2007) and horses slightly more (O'Connor *et al*., 2007). When aluminium silicate and orthosilicic acid were compared in horses, the latter was better absorbed and retained (apparent absorption, 41%) and additionally improved boron absorption, AA_B increasing from 46% to 66% (O'Connor *et al*., 2007). Silicon is partially converted to monosilicic acid in the gut and probably absorbed and transported as such, readily entering tissues and crossing membranes in this free form (Carlisle, 1986). Silicon is uniformly distributed within the cells of soft tissues such as liver. Plants contain small grains of amorphous silica (opal) or phytoliths, some fine enough to be absorbed and excreted in urine unchanged, while some are large enough to become trapped in lymph nodes (Carlisle, 1986). Absorption does not guarantee a supply of utilizable Si. Absorbed Si is rapidly excreted and blood Si does not reflect the absorbed load but urinary excretion does (Jugdaohsingh, 2007).

Anti-nutritional effects

Silicon toxicity is not a recognized condition but high Si intakes can adversely affect health and nutrition. In grazing livestock, silicate-rich soil particles may abrade the teeth and cause excessive tooth wear (Healy and Ludwig, 1965). Complexation of elements such as Ca, Mg, Fe and Mn as unavailable silicates has been described in non-ruminants (Carlisle, 1986) and implied in ruminants (Smith and Nelson, 1975; Smith and Urquhart, 1975). Lowering of availability is inevitable on dry diets and coarse forages containing 6–8% of largely unabsorbable Si. High urinary Si (some present as phytoliths) in grazing animals may trigger urolithiasis. Risk is accentuated by low water intakes, which lower urine volume, and physicochemical properties, such as pH, which influence initiation and growth of calculi. Urolithiasis in sheep in Western Australia was associated with inland rather than coastal regions and with dry seasons, when low water intakes gave rise to maximal urine Si (Nottle and Armstrong, 1966). Siliceous calculi consist of polysilicic acid–protein

complexes (Keeler, 1963) and are less likely to form when urine is high in phosphate and of low pH (Emerick, 1987). Reciprocal antagonism between Si and Mo has been reported in chicks, whereby supplements of one element reduce blood and tissue concentrations of the other (Carlisle and Curran, 1979). Sodium zeolite supplements lower availability of dietary P, although this may be the result of antagonism from Al rather than Si. When dietary P is adequate, sodium zeolite A supplementation of laying hens can improve eggshell strength (Roland, 1990).

Vanadium

Essentiality

Female goats given a diet containing <10 μg V kg^{-1}DM had a higher abortion rate, produced less milk and reared fewer offspring than control goats given 2mg V kg^{-1}DM (Anke *et al.*, 1991b). In the rat, vanadium deprivation tended to decrease growth while increasing thyroid weight. Diets low in iodine may amplify responses to dietary vanadium (Nielsen, 1996) and those high in goitrogens may have similar effects. However, prospects of requirements of poultry for V not being met are remote (Balos *et al.*, 2017).

Sources

Vanadium is abundant in soils, particularly in the finer clay fractions (65–200mg V kg^{-1}DM). Only a small fraction (<10%) is extractable (Berrow *et al.*, 1978) and levels in pasture and crops are usually low (<0.1mg kg^{-1}DM) (Table 17.2). Natural foodstuffs contain V mostly in the form of vanadyl (VO^{2+}) (NRC, 2005). Since grazing animals cannot avoid consuming soil when they graze and contamination of herbage varies from approximately 2% in summer to 10% or more of DMI in winter, soil ingestion is a potential source of V for pastured animals. Free-range hens may also ingest significant amounts of soil V. Contamination of the environment may be increasing due to combustion of V-rich oils (Davison *et al.*, 1997) and use of V-rich dietary phosphate supplements, which contain on average 135mg V kg^{-1}DM and as much as 185mg V kg^{-1}DM (Sell *et al.*, 1982; Sullivan *et al.*, 1994). However, prolonged use of phosphatic fertilizers did not increase V in soil or maize (or As, Cr or Pb) (Goodroad and Campbell, 1979).

Metabolism

Absorption is affected by source, being higher from ammonium metavanadate (AMV) and a chelate than from vanadyl sulphate, in which availability is low (Thompson *et al.*, 2002): most dietary studies have used AMV. Sheep absorbed only 1.6% of ingested V, whether the diet contained 2.6 or 202.6mg V kg^{-1}DM (Patterson *et al.*, 1986). Intravenously administered dioxovanadium is metabolized differently to orally dosed elements, raising questions as to the physiological significance of *in vitro* studies with V sources. Reactivity stems from the wide range of oxidation states for V sources; movements between the tetravalent and pentavalent state have reduction–oxidation potential in mammalian tissues (Nielsen, 1996; Davison *et al.*, 1997). Vanadium is readily transported across membranes by non-specific anion channels and binds to proteins such as transferrin and lactoferrin. Vanadate can substitute for phosphate, mimicking the effects of cyclic AMP. As peroxo-vanadate, V influences free-radical generation and mimics insulin (Nielsen, 1996). Tissue V is normally low (<10 μg kg^{-1}FW) but increases substantially in liver and bone when large dietary supplements of AMV are given (Fig. 17.14). In simulated soil-ingestion studies with sheep, ingestion of as little as 4.9 and 8.3mg soil V kg^{-1}DM for 41 days raised kidney V from undetectable levels (<0.2) to means of 0.65 and 1.85mg kg^{-1}DM despite very low apparent availability (Suttle *et al.*, 1991).

Toxicity

Vanadium has been studied mostly for its toxicological and pharmacological properties. Toxicity increases with valency state of the source and is characterized by diuresis, natriuresis, diarrhoea and hypertension, with irreversible damage to both liver and kidney at high doses (Nechay, 1984). Peroxidative effects were apparent as reductions in CuZnSOD, catalase and glutathione reductase activities in erythrocytes of rats given 125 μg l^{-1}, as $NaVO_3$, in drinking water (Ascibior and Zaporowska, 2010). Species vary widely in tolerance, with poultry the most and sheep the least susceptible of farmed species (Table 17.3). As little as 5mg V kg^{-1}DM, added as AMV, retarded growth of broiler chicks, given a corn/SBM diet, but previous work had indicated tolerance of 13–20mg V

kg^{-1}DM from various sources, including superphosphates (Cervantes and Jensen, 1986). Egg quality is particularly affected as little as 6–7mg V kg^{-1}DM, added as dicalcium phosphate, lowering egg albumin content (Sell *et al*., 1982).[iii] Hen body weight and egg production were both reduced by 50 mg V, added as calcium orthovanadate for140 days, while 100 mg added V kg^{-1}DM caused up to 56% mortality, without specific pathology (Kubena and Phillips, 1983). Higher sensitivity to AMV was reported in laying hens. As little as 50 mg V kg^{-1}DM, fed for 56 days, reduced hatchability and increased moisture content of faeces (Bressman *et al*., 2002). Brown eggs were 'bleached' by 15mg V kg^{-1}DM but not when accompanied by 100 mg vitamin C (Odabasi *et al*., 2006).

Growth suppression, mortality, enteritis and cystitis were recorded in weanling pigs given 200mg V kg^{-1}DM for ten weeks (Van Vleet *et al*., 1981) but lambs tolerated 200 mg added V for at least 84 days – higher levels (400 and 800mg V kg^{-1}DM) caused immediate diarrhoea and anorexia (Hansard *et al*., 1978). Dietary factors such as high iron, sulfur, amino acid and vitamin C concentrations reduce the toxicity of vanadium (Nielsen, 1996) and ruminants may be protected by reduction of vanadium in the rumen. Tissue concentrations are normally low (<0.25mg V kg^{-1}FW) (Puls, 1994) but increased in lambs given diets with 202mg V kg^{-1}DM to 3.3, 2.8 and 11.1 mg kg^{-1}FW in bone, liver and kidney, respectively, without signs of toxicity (Fig. 17.14). The increase in muscle V was exceedingly small by comparison (from 0.04 to 0.41mg kg^{-1}DM).

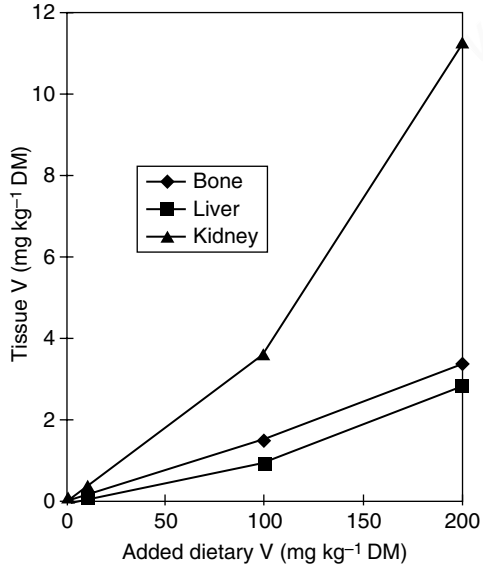

Fig. 17.14. Exposure of lambs to vanadate via the diet caused proportional increases in vanadium in liver and bone. Large increases in kidney V suggest that the urinary route of excretion is important for dealing with dietary excesses. (Data from Hansard *et al*., 1978)

Notes

[i] Consistently high levels of 38–40mg kg^{-1}DM were found in grass hays from Nevada (Green and Weeth, 1977; Weeth *et al*., 1981).

[ii] Glucose tolerance was not always affected (Matthews *et al*., 2001). Plasma cholesterol was consistently lowered but there was only a tendency for pigs to grow more *latissimus dorsi* muscle and deposit less rib fat: other measures of muscling and fatness were not improved (Boleman *et al*., 1995). In another study, only three out of >20 diverse carcase attributes were changed and none related to fat or lean content (Matthews *et al*., 2005). In the sow, CrPic supplementation during pregnancy changed isolated metabolic and hormonal markers in plasma, including insulin-like growth factor, without affecting the course of pregnancy or viability of offspring (Woodworth *et al*., 2007). Addition of 0.2mg Cr kg^{-1}DM as chromium propionate to corn/SBM diets of growing/fattening pigs did not improve LWG but improved FCE, lowered backfat thickness and saturation of belly fat (Jackson *et al*., 2009).

[iii] Growth of newly hatched turkey poults on a corn/SBM diet, was improved by 10% by adding 20mg Cr kg-^{1}DM (as CrCl3) and hepatic lipogenesis increased by 60% (Steele and Roseburgh, 1981). Fermentation by-products, supplying 5mg Cr kg^{-1}DM, improved egg quality and protected the egg interior from harmful effects of vanadium (Jensen *et al*., 1978).

[iv] Sheep were switched from a low- to a high-sulfur diet, having built up high blood and tissue Mo concentrations while receiving excess molybdenum. When SO_4 and MoO_4 are added simultaneously to the diet, Mo retention increases when compared with MoO_4 alone (Grace and Suttle, 1979).

[v] The poor relationships between oxidase activity, measured in serum, and plasma copper were largely attributable to loss of caeruloplasmin during clot formation (p. cu).

References

Abdelnour, S.A., El-Hack, M.E.A., Swelum, A.A., Perillo, A. and Losacco, C. (2018) The vital roles of boron in animal health and production: a comprehensive review. *Journal of Trace Elements in Medicine and Biology* 50, 296–304.

Anderson, R.A. (1956) Molybdenum as a fertiliser. *Advances in Agronomy* 8,163–202.

Anderson, R.A. (1987) Chromium. In: Mertz, W. (ed.) *Trace Elements in Human and Animal Nutrition*. Academic Press, New York, pp. 225–244.

Anke, M.K. and Angelov, L. (2004) Rubidium. In: Merian, E., Anke, M., Ihnat, M. and Stoeppler, M. (eds) *Elements and Their Compounds in the Environment*. Wiley online library.

Anke, M., Grun, M., Partschefeld, M. and Groppel, B. (1978) Molybdenum deficiency in ruminants. In: Kirchgessner, M. (ed.) *Proceedings of the 3rd International Symposium on Trace Element Metabolism in Man and Animals.* Arbeitskries fur Tierernahrungsforchung, Weihensstephan, Germany, pp. 230–235.

Anke, M., Groppel, B. and Krause, V. (1991a) The essentiality of the toxic elements cadmium, arsenic and nickel. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals (TEMA 7), Dubrovnik*. IMI, Zagreb, pp. 11-6–11–8.

Anke, M., Groppel, B. and Krause, V. (1991b) The essentiality of the toxic elements aluminium and vanadium. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals (TEMA 7), Dubrovnik*. IMI, Zagreb, pp. 11-9–11-10.

Archer, F.C. and Hodgson, J.H. (1987) Total and extractable trace element content of soils in England and Wales. *Journal of Soil Science* 38, 421–431.

Armstrong, T.A. and Spears, J.W. (2001) Effect of dietary boron on growth performance, calcium and phosphorus metabolism and bone mechanical properties in growing barrows. *Journal of Animal Science* 79, 3120–3127.

Armstrong, T.A. and Spears, J.W. (2003) Effects of boron on supplementation of pig diets on the production of tumour necrosis factor-*a* and interferon-γ. *Journal of Animal Science* 81, 2552–2561.

Armstrong, T.A., Flowers, W.L., Spears, J.W. and Nielsen, F.H. (2002) Long-term effects of boron supplementation on reproductive characteristics and bone mechanical properties in gilts. *Journal of Animal Science* 80, 154–161.

Ascibior, A. and Zaporowska, H. (2010) Effects of combined vanadate and magnesium treatment on erythrocyte antioxidant defence system in rats. *Environmental Toxicology and Pharmacology* 30, 153–161.

Baloš, M.Z., Ljubojevic, D. and Jakšic, S. (2017) The role and importance of vanadium, chromium and nickel in poultry diet. *World's Poultry Science Journal* 73, 5–16.

Benoit, S.L., Miller, E.F. and Maier, R.J. (2013) *Helicobacter pylori* stores nickel to aid its host colonization. *Infection and Immunity* 81, 580–584.

Berrow, M.L., Wilson, M.J. and Reaves, G.A. (1978) Origin of extractable titanium and vanadium in the horizons of Scottish podzols. *Geoderm* 21, 89–103.

Besong, S., Jackson, J.A., Trammell, D.S. and Akay, V. (2001) Influence of supplemental chromium on concentrations of liver triglycerides, blood metabolites and rumen VFA profile in steers on a moderately high fat diet. *Journal of Dairy Science* 84, 1679–1685.

Bhasker, T.V., Gowda, N.K.S., Pal, D.T., Bhat, S.K. and Pattanaik, A.K. (2015) Boron profile in common feedstuffs used in tropical livestock systems. *Animal Feed Science and Technology* 209, 280–285.

Bhasker, T.V., Gowda, N.K.S., Pal, D.T., Bhat, S.K., Krishnamoorthy, P., Mondal, S. *et al.* (2017) Influence of boron supplementation on performance, immunity and antioxidant status of lambs fed diets with or without adequate level of calcium. *PLOS ONE* 12(11), e0187203.

Bishara, H.N. and Bray, A.C. (1978) Competition between molybdate and sulfate for renal tubular reabsorption in sheep. *Proceedings of the Australian Society of Animal Production* 12, 123.

Blakistone, B.A., Aurand, L.W. and Swaisgood, H.E. (1986) Association between sulfhydryl oxidase and xanthine oxidase in bovine mammary tissue. *Journal of Dairy Science* 69, 2803–2809.

Boleman, S.L., Boleman, S.J., Bidner, T.D., Southern, L.L., Ward, T.L., Pontif, J.E. and Pike, M.M. (1995) Effects of chromium picolinate on growth, body condition and tissue accretion in pigs. *Journal of Animal Science* 73, 2033–2042.

Bozkurt, M., Küçükyılmaz, K., Catlı, A.U., Cınar, M., Cabuk, M. and Bintas, E. (2012) Effects of boron supplementation to diets deficient in calcium and phosphorus on performance with some serum, bone and fecal characteristics of broiler chickens. *Asian-Australasian Journal of Animal Science* 25, 248–255.

Bressman, R.B., Miles, R.D., Comer, C.W., Wilson, H.R. and Butcher, G.D. (2002) Effect of vanadium supplementation on commercial egg-laying type hens. *Journal of Applied Poultry Research* 11, 46–53.

Brown, T.F., McCormick, M.E., Morris, D.R. and Zerinque, L.K. (1989) Effects of boron on mineral balance in sheep. *Nutrition Research* 9, 503–512.

Bryan, M.A., Socha, M.T. and Tomlinson, D.J. (2004) Supplementing intensively grazed late-gestation and early lactating dairy cattle with chromium. *Journal of Dairy Science* 87, 4269–4277.

Bunting, L.D., Fernandez, J.M., Thompson, D.L. and Southern, L.L. (1994) Influence of chromium picolinate on glucose usage and metabolic criteria in growing Holstein calves. *Journal of Animal Science* 72, 1591–1599.

Burton, J.L., Mallard, B.A. and Mowat, D.N. (1993) Effects of supplemental chromium on immune responses of peri-parturient and early lactation dairy cows. *Journal of Animal Science* 71, 1532–1539.

Burton, J.L., Mallard, B.A. and Mowat, D.N. (1994) Effects of supplemental chromium on antibody responses of newly weaned feedlot calves to immunisation with infectious bovine rhinotracheitis and parainfluenza 3 virus. *Canadian Journal of Veterinary Research* 58, 148–151.

Carlisle, E.M. (1986) Silicon. In: Mertz, W. (ed.) *Trace Elements in Human and Animal Nutrition*, Vol. 2, 5th edn. Academic Press, New York, pp. 373–390.

Carlisle, E.M. and Curran, M.J. (1979) A silicon–molybdenum interaction *in vivo. Federation Proceedings* 38, 553.

Cervantes, H.M. and Jensen, L.S. (1986) Interaction of monensin with dietary vanadium, potassium and protein and its effect on hepatic rubidium and potassium in chicks. *Poultry Science* 65, 1591–1597.

Chang, X. and Mowat, D.N. (1992) Supplemental chromium for stressed growing feeder calves. *Journal of Animal Science* 70, 559–565.

Clarkson, A., Paine, S., Martín-Tereso, J. and Kendall, N. (2020) Copper physiology in ruminants: trafficking of systemic copper, adaptations to variation in nutritional supply and thiomolybdate challenge. *Nutrition Research Review* 33, 43–49.

Coleman, S.W., Evans, B.C. and Horn, G.W. (1984) Some factors influencing estimates of digesta turnover rates using markers. *Journal of Animal Science* 58, 979–986.

Cunningham, I.J. (1950) Copper and molybdenum in relation to diseases of cattle and sheep in New Zealand. In: McElroy, W.D. and Glass, B. (eds) *Copper Metabolism: A Symposium on Animal, Plant and Soil Relationships, Baltimore, USA*. Johns Hopkins Press, Baltimore, Maryland, pp. 246–273.

Davison, A., Kowalski, L., Xuefeng, Y. and Siu-Sing, T. (1997) Vanadium as a modulator of cellular regulation: the role of redox reactivity. In: Fischer, P.W.F., L'Abbé, M.R. and Cockell, K.A. (eds) *Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals, Banff*. NRC Research Press, Ottawa, pp. 229–233.

de Renzo, E.C., Kaleita, E., Heytler, P., Oleson, J.J., Hutchings, B.L. and Williams, J.H. (1953) The nature of the xanthine oxidase factor. *Journal of the American Chemical Society* 75, 753.

Dick, H.T. (1956) Molybdenum in animal nutrition. *Soil Science* 81, 229–258.

Dick, A.T., Dewey, D.W. and Gawthorne, J.M. (1975) Thiomolybdates and the copper–molybdenum–sulfur interaction in ruminant nutrition. *Journal of Agricultural Science, Cambridge* 85, 567–568.

Domínguez-Vara, I.A., González-Muñoz, S.S., Pinos-Rodríguez, J.M., Bórquez-Gastelum, J.L., Bárcena-Gama, R. *et al.* (2009) Effects of feeding selenium-yeast and chromium-yeast to finishing lambs on growth, carcass characteristics, and blood hormones and metabolites. *Animal Feed Science and Technology* 152, 42–49.

Downes, A.M. and McDonald, I.W. (1964) The chromium–Si complex of ethylene-diamine tetra-acetic acid as a soluble rumen marker. *British Journal of Nutrition* 18, 153–163.

EFSA (2009) Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of chromium methionine (Availa®Cr) as feed additive for all species. *The EFSA Journal* 1043, 1–69.

EFSA (2015) Scientific Opinion on the risks to animal and public health and the environment related to the presence of nickel in feed. *European Food Safety Authority Journal* 13, 4074.

Emerick, R.J. (1987) Phosphate inhibition of protein–polysilicic acid complex formation *in vitro*, a factor in preventing silica urolithiasis. *Journal of Nutrition* 117, 1924–1928.

Eren, M., Uyanik, F. and Kucukersan, S. (2004) The influence of dietary boron on egg quality and serum calcium, inorganic phosphorus, magnesium levels and alkaline phosphatase activity in laying hens. *Research in Veterinary Science* 76, 203–210.

Eseceli, H., Degerincioglu, N. and Bilgic, N. (2010) The effect of inclusion of chromium yeast (Co-Factor III, Alltech Inc.) and folic acid to the rations of laying hens on performance, egg quality, egg yolk cholesterol, folic acid and chromium levels. *Journal of Animal and Veterinary Advances* 92, 384–391.

Evert, C.D., Wagner, T., Shima, S., Prakash, D., Cronin, B. *et al.* (2016) Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proceedings of the National Academy of Sciences, USA.* doi:10.1073/pnas.1600298113

Farmer, P.E., Adams. T.E. and Humphries, W.R. (1982) Copper supplementation of drinking water for cattle grazing molybdenum-rich pastures. *Veterinary Record* 111, 193–195.

Feng, W. (2007) The transport of Cr (III): implications for function. In: Vincent, J.B. (ed.) *The Nutritional Biochemistry of Chromium (III).* Elsevier, Amsterdam, pp. 121–138.

Ferguson, W.S., Lewis, A.H. and Watson, S.J. (1938) Action of molybdenum in milking cattle. *Nature, UK* 141, 553.

Ferguson, W.S., Lewis, A.H. and Watson, S.J. (1943) The teart pasture of Somerset: the cause and cure of teartness. *Journal of Agricultural Science, Cambridge* 33, 44–51.

Freudenberger, D.O., Familton, A.S. and Sykes, A.R. (1987) Comparative aspects of copper metabolism in silage-fed sheep and deer (*Cervus elaphus*). *Journal of Agricultural Science, Cambridge* 108, 1–7.

Friedl, H.P., Till, G.O., Trentz, O. and Ward, P.A. (1989) Roles of histamine, complement and xanthine

oxidase in thermal injury of skin. *American Journal of Pathology* 135, 203–217.

Fry, R.S., Brown, T.T., Lloyd, K.E., Hansen, S.L., Legleiter, L.R., Robarge, W.P. and Spears, J.W. (2011) Effect of dietary boron on physiological responses in growing steers inoculated with bovine herpesvirus type-1. *Research in Veterinary Science* 90, 78–83.

Fry, R.S., Spears, J.W., Lloyd, K.E., O'Nan, A.T. and Ashwell, M.S. (2013) Effect of dietary copper and breed on gene products involved in copper acquisition, distribution, and use in Angus and Simmental cows and fetuses. *Journal of Animal Science* 91, 861–871.

Gardner, G.E., Pethick, D.W. and Smith, C. (1998) Effect of chromium chelavite supplementation on the metabolism of glycogen and lipid in adult Merino sheep. *Australian Journal of Agricultural Research* 49, 137–145.

Goodroad, L.L. and Campbell, A.C. (1979) Effects of phosphorus fertilizer and lime on the As, Cr, Pb and V content of soils and plants. *Journal of Environmental Quality* 8, 493–496.

Gould, L. and Kendall, N.R. (2011) Role of the rumen in copper and thiomolybdate absorption. *Nutrition Research Reviews* 24, 176–182.

Grace, N.D. and Suttle, N.F. (1979) Some effects of sulfur intake on molybdenum metabolism in sheep. *British Journal of Nutrition* 41, 125–136.

Grace, N.D., Wilson, P.R. and Quinn, A.K. (2005) Impact of molybdenum on the copper status of red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 53, 31–38.

Green, G.H. and Weeth, H.J. (1977) Responses of heifers to boron in water. *Journal of Animal Science* 46, 812–818.

Guerra, M.C., Renzulli, C., Antelli, A., Pozzetti, L., Paolinin, M. and Speroni, E. (2002) Effects of trivalent chromium on hepatic CYP-linked monooxygenases in laying hens. *Journal of Applied Toxicology* 22, 161–165.

Guo, H., Cui, H., Peng, X., Fang, J., Zuo, Z. *et al.* (2016) Nickel chloride ($NiCl_2$) induces endoplasmic reticulum (ER) stress by activating UPR pathways in the kidney of broiler chickens. *Oncotarget* 7, 17508–17519.

Habibi, Z., Karimi-Dehkordi, S., Kargar, S. and Sadeghi. M. (2019) Grain source and chromium supplementation: effects on health, metabolic status, and glucose-insulin kinetics in Holstein heifer calves. *Journal of Dairy Science* 102, 8941–8951.

Hakki, S.S., Dundar, N., Kayis, S.A., Hakki, E.E., Hamurcu, M. *et al.* (2013) Boron enhances strength and alters mineral composition of bone in rabbits fed a high energy diet. *Journal of Trace Elements in Medicine and Biology* 27, 148–153.

Hamilton, E.M., Young, S.D., Bailey, E.H. and Watts, M.J. (2018) Chromium speciation in foodstuffs: A review. *Food Chemistry* 250, 105–112.

Hansard, S.L.I., Ammerman, C.B., Fick, K.R. and Miller, S.M. (1978) Performance and vanadium content of tissues in sheep as influenced by dietary vanadium. *Journal of Animal Science* 46, 1091–1095.

Hassan Dar, Gh. and Tandon, S.M. (1987) Response of a cattle dung methane fermentation to nickel. *Biological Wastes* 22, 261–268.

Hayirli, A., Bremmer, D.R., Bertics, S.J., Socha, M.T. and Grummer, R.R. (2001) Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. *Journal of Dairy Science* 84, 1218–1230.

Haywood, S., Dincer, Z., Jasani, B. and Loughram, M.J. (2004) Molybdenum-associated pituitary endocrinopathy in sheep treated with ammonium tetrathiomolybdate. *Journal of Comparative Pathology* 130, 21–31.

Healy, W.B. and Ludwig, T.G. (1965) Wear of sheep's teeth. 1. The role of ingested soil. *New Zealand Journal of Agricultural Research* 8, 737–752.

Helsten-Westing, Y., Sollevi, A. and Sjodin, B. (1991) Plasma accumulation of hypoxanthine, uric acid and creatine kinase following exhaustive rune of differing duration in man. *European Journal of Applied Physiology* 62, 380–384.

Higgins, E.S., Richert, D.A. and Westerfeld, W.W. (1956) Molybdenum deficiency and tungstate inhibition studies. *Journal of Nutrition* 59, 539–559.

Hunt, C.D., Herbel, J.L. and Idso, J.P. (1994) Dietary boron modifies the effects of vitamin D on indices of energy substrate utilisation and mineral metabolism in the chick. *Journal of Bone and Mineral Research* 9, 171–181.

Jackson, A.R., Powell, S., Johnston, S.L., Matthews, J.O., Bidner, T.D., Valdez, F.R. and Southern, L.L. (2009) The effect of chromium as chromium propionate on growth performance, carcass traits, meat quality, and the fatty acid profile of fat from pigs fed no supplemented dietary fat, choice white grease, or tallow. *Journal of Animal Science* 87, 4032–41.

Jensen, L.S., Maurice, D.V. and Murray, M.W. (1978) Evidence for a new biological role of chromium. *Federation Proceedings* 37, 404.

Jin, E., Gu, Y., Wang, J., Jin, G. and Li, S. (2014) Effect of supplementation of drinking water with different levels of boron on performance and immune organ parameters of broilers. *Italian Journal of Animal Science* 13, 205–214.

Johnson, J.H., Crookshank, H.R. and Smalley, H.E. (1980) Lithium toxicity in cattle. *Veterinary and Human Toxicology* 22, 248–251.

Johnson, J.L. (1997) Molybdenum. In: O'Dell, B.L. and Sunde, R.A. (eds) *Handbook of Nutritionally Essential Mineral Elements*. Marcel Dekker, New York, pp. 413–438.

Johnson, J.L., Rajagopalan, K.V. and Cohen, H.J. (1974) Effect of tungsten on xanthine oxidase and sulfite oxidase in the rat. *Journal of Biological Chemistry* 249, 859–866.

Jones, L.H.P. and Handreck, K.A. (1965) The relation between the silica content of the diet and the excretion of silica by sheep. *Journal of Agricultural Science, Cambridge* 65, 129–134.

Judson, G.J., McGregor, B.A., Ellis, K.J. and Howse, A.M. (2018) Evaluation of controlled-release devices for providing chromium sesquioxide and zinc in Huacaya alpacas at pasture. *Australian Veterinary Journal* 96, 458–463.

Jugdaohsingh, R. (2007) Silicon and bone health. *Journal of Nutrition, Health and Ageing* 11, 99–110.

Jumba, I.O. (1989) *Tropical soil–plant interactions in relation to mineral imbalances in grazing livestock*. PhD Thesis, University of Nairobi.

Kabata-Pendias, A. and Pendias, H. (1992) *Trace Elements in Soils and Plants*, 2nd edn. CRC Press, Boca Raton, Florida.

Kabu, M., Birdane, F.M., Civelek, T. and Uyarlar, C. (2013) Effects of boron administration on serum Ca, Mg and P of peripartum cows. *Archiv fur Tierzucht* 56, 733–741.

Kani, M.M. (2015) The effects of different sources of organic and inorganic chromium on blood parameters of broiler chickens. *Indian Journal of Science and Technology* 8, 1–7.

Keeler, R.F. (1963) Silicon metabolism and silicon–protein matrix interrelationship in bovine urolithiasis. *Annals of the New York Academy of Sciences* 104, 592–611.

Kegley, E.B., Spears, J.W. and Brown, T.T. (1997a) Effect of shipping and chromium supplementation on performance, immune response and disease resistance of steers. *Journal of Animal Science* 75, 1956–1964.

Kegley, E.B., Spears, J.W. and Eisemann, J.H. (1997b) Performance and glucose metabolism in calves fed a chromium–nicotinic acid complex or chromium chloride. *Journal of Dairy Science* 80, 1744–1750.

Kendall, N.R., Illingworth, D.V. and Telfer, S.B. (2001) Copper responsive infertility in British cattle: the use of a blood caeruloplasmin to copper ratio in determining a requirement for copper supplementation. In: Diskin, M.G. (ed.) *Fertility in the High-Producing Dairy Cow*. Occasional Publication No. 26, British Society of Animal Science, pp. 429–432.

Kendall, N.R., Marsters, P., Scaramuzzi, R.J. and Campbell, B.K. (2003) Expression of lysyl oxidase and effect of copper chloride and ammonium tetrathiomolybdate on bovine ovarian follicle granulosa cells cultured in serum-free media. *Reproduction* 125, 657–665.

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S.L., Bearson, B.L., Shurson G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Kim, C.H., Park, B.K., Park, J.G., Sung, K.I., Shin, J.S. and Ohh, S.J. (2005) Estimation of rumen by-pass rate of chromium methionine chelates by ruminal bacteria analysis. *Journal of Animal Science and Technology* 47, 759–768.

Kincaid, R.L., Gay, C.C. and Krieger, R.I. (1986) Relationship of serum and plasma copper and caeruloplasmin concentrations of cattle and the effects of whole sample storage. *American Journal of Veterinary Research* 47, 1157–1159.

Kirchgessner, M., Friesecke, H. and Koch, G. (1967) *Nutrition and the Composition of Milk*. Crosby Lockwood, London.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–10.

Kraidees, M., Al-Haidary, I., Mufarrej, S., Al-Saiady, M., Metwally, H. and Hussein, M. (2009) Effect of supplemental chromium levels on performance, digestibility and carcass characteristics of transport-stressed lambs. *Asian-Australasian Journal of Animal Science* 22, 1124–1132.

Kratzer, F.H. (1952) Effect of dietary molybdenum upon chicks and poults. *Proceedings of the Society for Experimental Biology and Medicine* 80, 483–486.

Król, B., Słupczynska, M., Kinal, S., Bodarski, R., Tronina, W. and Monka, M. (2017) Bioavailability of organic and inorganic sources of chromium in broiler chicken feeds. *Journal of Elementology* 22, 283–294.

Kubena, L.F. and Phillips, T.D. (1983) Toxicity of vanadium in female leghorn chickens. *Poultry Science* 62, 47–50.

Küçükyilmaz, K., Erkek, R. and Bozkurt, M. (2014) The effects of boron supplementation of layer diets varying in calcium and phosphorus concentrations on performance, egg quality, bone strength and mineral constituents of serum, bone and faeces. *British Poultry Science* 55, 804–816.

Kumar, S. and Trivedi, A.V. (2016) A review on role of nickel in the biological system. *International Journal of Current Microbiology and Applied Sciences* 5, 719–727.

Kurtoglou, F., Kurtoglou, V. and Coskun, B. (2001) Effects of boron supplementation of adequate and inadequate vitamin D_3-containing diet on performance and serum biochemical parameters of broiler chickens. *Research in Veterinary Science* 71, 183–187.

Kurtoglou, F., Kurtoglou, V., Celik, I., Kececi, T. and Nizamloglu, M. (2005) Effects of boron concentration on some biochemical parameters, peripheral blood lymphocytes and plasma cells and bone characteristics of broiler chicks given diets with adequate or inadequate cholecalciferol (vitamin D3) content. *British Poultry Science* 46, 87–92.

Lane, M.A., Ralphs, M.H., Olsen, J.D., Provenuza, F.D. and Pfister, J.A. (1989) Conditioned taste aversion: potential for reducing cattle loss to larkspur. *Journal of Range Management* 43, 127–131.

Lien, T.-F., Horng, Y.-M. and Yang, K.-H. (1999) Performance, carcase characteristics and lipid metabolism of broilers affected by chromium picolinate. *British Poultry Science* 40, 357–363.

Lindemann, M.D., Cromwell, G.L., Monegue, H.J. and Purser, K.W. (2008) Effect of chromium source on tissue concentration of chromium in pigs. *Journal of Animal Science* 86, 2971–2978.

Lloyd, K.E., Fellner, V., McLeod, S.J., Krafka, K., Lamptey, A. and Spears J.W. (2010) Effects of supplementing dairy cows with chromium propionate on milk and tissue chromium concentrations. *Journal of Dairy Science* 93, 4774–4780.

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Mason, J. and Cardin, C.J. (1977) The competition of molybdate and sulfate ions for a transport system in the ovine small intestine. *Research in Veterinary Science* 22, 313–315.

Matthews, J.O., Southern, L.L., Fernandez, J.M., Pontif, J.E., Bidner, T.D. and Odgaard, R.L. (2001) Effect of chromium picolinate and chromium propionate on glucose and insulin kinetics of growing barrows and on growth and carcass traits of growing-finishing barrows. *Journal of Animal Science* 79, 2172–2178.

Matthews, J.O., Guzik, A.C., Le Mieux, F.M., Southern, L.L., Fernandez, J.M. and Bidner, T.D. (2005) Effect of chromium propionate on growth, carcass traits and pork quality of growing-finishing pigs. *Journal of Animal Science* 83, 858–862.

Mayorga, E.J., Kvidera, S.K., Seibert, J.T., Horst, E.A., Abuajamieh, M. *et al.* (2019) Effects of dietary chromium propionate on growth performance, metabolism and immune biomarkers in heat-stressed finishing pigs. *Journal of Animal Science* 97, 1185–1197.

McAloon, C.G., Carty, C. and Mulligan, F. (2017) Molybdemosis in calves. *Veterinary Ireland Journal* 7, 678–680.

McClure, S.J., McClure, T.J. and Emery, D.L. (1999) Effects of molybdenum intake on primary infection and subsequent challenge by the nematode parasite *Trichostrongylus colubriformis* in weaned Merino lambs. *Research in Veterinary Science* 67, 17–22.

McKenzie, A.M., Illingworth, D.V., Jackson, D.W. and Telfer, S.B. (1997) The use of caeruloplasmin activities and plasma copper concentrations as an indicator of copper status in ruminants. In: Fischer, P.W.F., L'Abbé, M.R., Cockell, K.A. and Gibson, R.S. (eds) *Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* NRC Press, Ottawa, pp. 137–138.

McNamara, J.P. and Valdez, F. (2005) Adipose tissue metabolism and production responses to calcium propionate and chromium propionate. *Journal of Dairy Science* 88, 2498–2507.

Mertz, W. (1986) Lithium. In: Mertz, W. (ed.) *Trace Elements in Human and Animal Nutrition*, Vol. 2, 5th edn. Academic Press, New York, pp. 391–398.

Miljkovic, D., Miljkovic, N. and McCarty, M.F. (2004) Up-regulatory impact of boron on vitamin D function – does it reflect inhibition of 24-hyroxylase? *Medical Hypotheses* 63, 1054–1056.

Miller, J.K., Moss, B.R., Bell, M.C. and Sneed, N. (1972) Comparison of ^{99}Mo metabolism in cattle and swine. *Journal of Animal Science* 34, 846–850.

Milne, J.S., Whitelaw, F.G., Price, J. and Shand, W.J. (1990) The effect of supplementary nickel on urea metabolism in sheep given a low protein diet. *Animal Production* 50, 507–512.

Miltimore, J.E. and Mason, J.L. (1971) Copper to molybdenum ratio and molybdenum and copper concentrations in ruminant feeds. *Canadian Journal of Animal Science* 51, 193–200.

Mimica, N., Prejac, J., Skalny, A.A., Grabeklis, A.R. and Momcilovic, B. (2019) Assessing lithium nutritional status by analysing its cumulative frequency distribution in the hair and whole blood. *Trace Elements and Electrolytes* 36, 113–119.

Moonsie-Shageer, S. and Mowat, D.N. (1993) Effects of level of supplemental chromium on performance, serum constituents and immune status of stressed feeder calves. *Journal of Animal Science* 71, 232–238.

Moreira, P.S.A., Palhari, C. and Berber, R.C.A. (2020) Dietary chromium and growth performance animals: a review. *Scientific Electronic Archives* 13, 59–66.

Mousaie, A., Valizadeh, R. and Chamsaz, M. (2017) Selenium-methionine and chromium-methionine supplementation of sheep around parturition: impacts on dam and offspring performance. *Archives of Animal Nutrition* 71, 134–149.

Mowat, D.N., Chang, X. and Yang, W.Z. (1993) Chelated chromium for stressed feeder calves. *Canadian Journal of Animal Science* 73, 49–55.

Mueller, R., Betz, L. and Anke, M.R. (2010) Essentiality of the ultra-trace element lithium to the nutrition of animals and man. *Proceedings of the 30th Scientific Symposium of Industrial Toxicology*, pp. 134–143.

Nechay, B.R. (1984) Mechanisms of action of vanadium. *Annual Reviews of Pharmacology and Toxicology* 24, 501–524.

Nielsen, F.H. (1996) Other trace elements. In: Filer, L.J. and Ziegler, E.E. (eds) *Present Knowledge in Nutrition*, 7th edn. International Life Sciences Institute, Nutrition Foundation, Washington, DC.

Nielsen, F.H. (2002) The nutritional properties and pharmacological potential of boron for higher animals and humans. In: Goldbach, H.E., Rerkasem, B., Wimmer, M.A., Brown, P.H., Thellier, M. and Bell, R.W. (eds) *Boron in Plant and Animal Nutrition*. Kluwer Academic/Plenum, New York, pp. 37–49.

Nielsen, F.H. and Sauberlich, H.E. (1970) Evidence of a possible requirement for nickel by the chick. *Proceedings of the Society for Experimental Biology and Medicine* 134, 845–849.

Nielsen, F.H., Myron, D.R., Givand, S.H. and Ollerich, D.A. (1975) Nickel deficiency and nickel–rhodium interaction in chicks. *Journal of Nutrition* 105, 1607–1619.

Nielsen, B.D., Cate, R.E. and O'Connor-Robison, C.I. (2010) A marine mineral supplement alters markers of bone metabolism in yearling Arabians. *Journal of Equine Veterinary Science* 30, 419–424.

Nottle, M.C. and Armstrong, J.M. (1966) Urinary excretion of silica by grazing sheep. *Australian Journal of Agricultural Research* 17, 165–173.

NRC (1995) *The Role of Chromium in Animal Nutrition*. National Academy of Sciences, Washington, DC.

NRC (2005) *Mineral Tolerances of Animals*, 2nd edn. National Academy of Sciences, Washington, DC.

O'Connor, C.I., Nielsen, B.D., Woodward, A.D., Spooner, H.S., Ventura, B.A. and Turner, K.K. (2007) Mineral balance in horses fed two supplemental silicon sources. *Journal of Animal Physiology and Animal Nutrition* 92, 173–181.

Odabasi, A.Z., Miles, R.D., Balaban, M.O., Portier, K.M. and Sampath, V. (2006) Vitamin C overcomes the detrimental effect of vanadium on egg pigmentation. *Journal of Applied Poultry Research* 15, 425–432.

O'Dell, G.D., Miller, W.J., King, W.A., Ellers, J.C. and Jurecek, H. (1970a) Effect of nickel supplementation on production and composition of milk. *Journal of Dairy Science* 53, 1545–1548.

O'Dell, G.D., Miller, W.J., King, W.A., Moore, S.L. and Blackmon, D.M. (1970b) Nickel toxicity in the young bovine. *Journal of Nutrition* 100, 1447–1454.

O'Dell, G.D., Miller, W.J., Moore, S.L., King, W.A., Ellers, J.C. and Jurecek, H. (1971) Effect of dietary nickel level on excretion and nickel content of tissue in male calves. *Journal of Animal Science* 32, 769–773.

Olgun, O., Yazgan, O. and Cufadar, Y. (2013) Effect of supplementation of different boron and copper levels to layer diets on performance, egg yolk and plasma cholesterol. *Journal of Trace Elements in Medicine and Biology* 27, 132–136.

Onderci, M., Sahin, N., Sahin, K. and Kilic, N. (2002) Antioxidant properties of chromium and zinc: *in vivo* effects on digestibility, lipid peroxidation, antioxidant vitamins, and some minerals under low ambient temperature. *Biological Trace Element Research* 92, 139–150.

Oscar, T.P., Spears, J.W. and Shih, J.C.H. (1987) Performance, methanogenesis and nitrogen metabolism of finishing steers fed monensin and nickel. *Journal of Animal Science* 64, 887–896.

Page, T.G., Southern, L.L., Ward, T.L. and Thompson, D.L. (1993) Effect of chromium picolinate on growth and serum and carcass traits of growing-finishing pigs. *Journal of Animal Science* 71, 656–662.

Parker, W.J., Morris, S.T., Garrick, D.J., Vincent, G.L. and McCutcheon, S.N. (1990) Intraruminal chromium controlled-release capsules for measuring intake in ruminants: a review. *Proceedings of the New Zealand Society of Animal Production* 50, 437–442.

Patt, E.L., Pickett, E.E. and O'Dell, B.L. (1978) The effect of dietary lithium levels on tissue lithium concentrations, growth rate and reproduction in the rat. *Bioinorganic Chemistry* 9, 299–310.

Patterson, B.W., Hansard, S.L., Ammerman, C.B., Henry, P.R., Zech, L.A. and Fisher, W.R. (1986) Kinetic model for whole-body vanadium metabolism: studies in sheep. *American Journal of Psychology* 251, R325–R332.

Pearce, S.G., Firth, E.C., Grace, N.D. and Fennessy, P.F. (1999) The effect of high pasture molybdenum concentrations on the copper status of grazing horses in New Zealand. *New Zealand Journal of Agricultural Research* 42, 93–99.

Peres, L.M., Manha, B., Ana, M., Silva, C.A., Andreo, N. *et al*. (2014) Effect of supplementing finishing pigs with different sources of chromium on performance and meat quality. *Revista Brasileira de Zootecnia* 43(7), 369–375.

Pitt, M.A. (1976) Molybdenum toxicity: interactions between copper, molybdenum and sulfate. *Agents and Actions* 6, 758–769.

Pramanik, P. and Kim, P.J. (2013) Effect of limited nickel availability on methane emission from EDTA treated soils: Coenzyme M an alternative biomarker for methanogens. *Chemosphere* 90, 873–876.

Price, J., Will, A.M., Paschaleris, G. and Chesters, J.K. (1987) Identification of thiomolybdates in digesta and plasma from sheep after administration of 99Mo-labelled compounds into the rumen. *British Journal of Nutrition* 58, 127–138.

Puls, R. (1994) *Mineral Levels in Animal Health*, 2nd edn. Sherpa International, Clearbrook, Canada.

Raisbeck, M., Siemion, R.S. and Smith, M.A. (2006) Modest copper supplementation blocks molybdenosis in cattle. *Journal of Veterinary Diagnostic Investigation* 18, 566–572.

Ralphs, M.H. (1997) Persistence of aversions to larkspur in naive and native cattle. *Journal of Range Management* 50, 367–370.

Ribeiro, L.S., Brandão, F.Z., Carvalheira, L.Z., Goes, T.J.F., Filho, R.A.T. *et al.* (2020) Chromium supplementation improves glucose metabolism and vaginal temperature regulation in Girolando cows under heat stress conditions in a climatic chamber. *Tropical Animal Health and Production.* 52, 1661–1668.

Roland, D.A. (1990) The relationship between dietary phosphorus and sodium aluminosilicate to the performance of commercial leghorns. *Poultry Science* 69, 105–112.

Rossi, A.F., Miles R.D., Damron, B.L. and Flunker, L.K. (1993) Effect of dietary boron supplementation on broilers. *Poultry Science* 72, 2124–2130.

Saha, D.C. and Galbraith, R.L. (1993) A modified chromic oxide indicator ratio technique for accurate determination of nutrient digestibility. *Canadian Journal of Animal Science* 73, 1001–1004.

Sahin, K. and Onderci, M. (2002) Optimal dietary concentrations of vitamin C and chromium for alleviating the effect of low ambient temperature on serum insulin, corticosterone and some blood metabolites in lay-

ing hens. *Journal of Trace Elements in Experimental Medicine* 15, 153–161.

Sahin, N., Sahin, K. and Onderci, M. (2001) Effects of dietary chromium and zinc on egg production, egg quality and some blood metabolites of laying hens reared under low temperature conditions. *Biological Trace Element Research* 85, 47–58.

Sahin, K., Sahin, N., Onderci, M., Gursu, F. and Cikim, G. (2002) Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities and some serum metabolites of broiler chickens. *Biological Trace Element Research* 89, 1–12.

Sano, H., Konno, S. and Shiga, A. (2000) Effects of supplemental chromium and heat exposure on glucose metabolism and insulin action in sheep. *Journal of Agricultural Science, Cambridge* 134, 319–325.

Schrauzer, G.N. (2002) Lithium: occurrence, dietary intakes, nutritional essentiality. *Journal of American College of Nutrition* 21, 14–21.

Schwarz, K. and Mertz, W. (1959) Chromium (III) and the glucose tolerance factor. *Archives of Biochemistry and Biophysics* 85, 292–295.

Seaborn, C.D. and Nielsen, F.H. (2002) Silicon deprivation decreases collagen formation in wounds and bone and ornithine transaminase enzyme activity in liver. *Biological Trace Element Research* 89, 239–250.

Sell, J.L., Arthur, J.A. and Williams, I.L. (1982) Adverse effects of dietary vanadium, contributed by dicalcium phosphate, on albumen quality. *Poultry Science* 61, 2112–2116.

Shan, Q., Ma, F.T., Jin, Y.H., Gao, D., Li, H.Y. and Sun, P. (2020) Chromium yeast alleviates heat stress by improving antioxidant and immune function in Holstein mid-lactation dairy cows. *Animal Feed Science and Technology* 269, 114635.

Shelton, J.L., Payne, R.L., Johnston, S.L., Bidner, T.D., Southern, L.L., Odgaard, R.L. and Page, T.G. (2003) Effect of chromium propionate on growth, carcass traits, pork quality and plasma metabolites in growing-finishing pigs. *Journal of Animal Science* 81, 2515–2524.

Silanpaa, M. (1982) *Micronutrient and the Nutrient Status of Soils: A Global Study*. Food and Agriculture Organization, Rome.

Small, J.A., Charmley, E., Rodd, A.V. and Freeden, A.H. (1997) Serum mineral concentrations in relation to estrus and conception in beef heifers and cows fed conserved forage. *Canadian Journal of Animal Science* 77, 55–62.

Smith, G.S. and Nelson, A.B. (1975) Effects of sodium silicate added to rumen cultures on forage digestion, with interactions of glucose, urea and minerals. *Journal of Animal Science* 41, 891–899.

Smith, G.S. and Urquhart, N.S. (1975) Effect of sodium silicate added to rumen cultures on digestion of siliceous forages. *Journal of Animal Science* 41, 882–890.

Smith, B.S.W. and Wright, H. (1975a) Effect of dietary molybdenum on copper metabolism: evidence for the involvement of molybdenum in abnormal binding of copper to plasma proteins. *Clinica Chimica Acta* 62, 55–62.

Smith, B.S.W. and Wright, H. (1975b) Copper:molybdenum interactions: effects of dietary molybdenum on the binding of copper to plasma proteins in sheep. *Journal of Comparative Pathology* 85, 299–305.

Smith, K.L., Waldron, M.R., Ruzzi, L.C., Drackley, J.K., Socha, M.T. and Overton, T. (2008) Metabolism of dairy cows as affected by prepartum dietary carbohydrate source and supplementation with chromium throughout the periparturient period. *Journal of Dairy Science* 91, 2011–2020.

Snyman, L.D., Schulz, R.A., Kellerman, T.S. and Labuschagne, L. (2002) Continuous exposure to an aversive mixture as a means of maintaining aversion to vermeerbos (*Gergeiria ornativa* O. Hoffm.) in the presence of non-averted sheep. *Onderstepoort Journal of Veterinary Research* 69, 321–325.

Spears, J.W. (1984) Nickel as a 'newer trace element' in the nutrition of domestic animals. *Journal of Animal Science* 59, 823–835.

Spears, J.W. (2019) Boron, chromium, manganese, and nickel in agricultural animal production. *Biological Trace Element Research* 188, 35–44.

Spears, J.W. and Lloyd, K.E. (2017) Chromium concentrations in ruminant feed ingredients. *Journal of Dairy Science* 100, 3584–3590.

Spears, J.W., Harvey, R.W. and Samsell, L.J. (1986) Effects of dietary nickel and protein on growth, nitrogen metabolism and tissue concentrations of nickel, iron, zinc, manganese and copper in calves. *Journal of Nutrition* 116, 1873–1882.

Spears, J.W. Whisnant, C.S. Huntington, G.B., Lloyd, K.E., Fry, R.S., *et al.* (2012) Chromium propionate enhances insulin sensitivity in growing cattle. *Journal of Dairy Science* 95, 2037–2045.

Stahlhut, H.S., Whishant, C.S. and Spears, J.W. (2005) Effect of chromium supplementation and copper status on performance and reproduction of beef cows. *Animal Feed Science and Technology* 128, 266–275.

Stangl, G.I. and Kirchgessner, M. (1996) Effect of nickel deficiency on various metabolic parameters of rats. *Animal Physiology and Animal Nutrition* 75, 164–174.

Stangl, G.I., Roth-Maier, D.A. and Kirchgessner, M. (2000) Vitamin B_{12} deficiency and hyperhomocysteinaemia are partly ameliorated by cobalt and nickel supplementation in pigs. *Journal of Nutrition* 130, 3038–3044.

Starich, G.H. and Blincoe, C. (1983) Dietary chromium – forms and availabilities. *The Science of the Total Environment* 28, 443–454.

Steele, N.C. and Roseburgh, W. (1981) Effect of trivalent chromium on hepatic lipogenesis by the turkey poult. *Poultry Science* 60, 617–622.

Subiyatno, A., Mowat, D.N. and Yang, W.Z. (1996) Metabolite and hormonal responses to glucose or propionate infusions in periparturient dairy cows supplemented with chromium. *Journal of Dairy Science* 79, 1436–1445.

Sullivan, T.W., Douglas, J.H. and Gonzalez, N.J. (1994) Levels of various elements of concern in feed phosphates of domestic and foreign origin. *Poultry Science* 73, 520–528.

Sumner, J.M., Valdez, F. and McNamara, J.P. (2007) Effects of chromium propionate on response to an intravenous glucose tolerance test in growing heifers. *Journal of Dairy Science* 90, 3467–3474.

Suttle, N.F. (1991) The interactions between copper, molybdenum and sulfur in ruminant nutrition. *Annual Review of Nutrition* 11, 121–140.

Suttle, N.F. (2008a) Relationships between the concentrations of trichloroacetic acid-soluble copper and caeruloplasmin in the serum of cattle from areas with different soil concentrations of molybdenum. *Veterinary Record* 162, 237–240.

Suttle, N.F. (2008b) Lack of effect of parenteral thiomolybdate on ovine caeruloplasmin activity: diagnostic implications. *Veterinary Record* 162, 593–594.

Suttle, N.F. (2010) Occasionally beneficial elements. In: *Mineral Nutrition of Livestock*, 4th edn. CAB Ineternational, Wallingford, UK.

Suttle, N.F. (2012a) Residual effects of Mycobacterium avium infection on susceptibility of sheep to copper toxicity and efficacy of treatment with tetrathiomolybdate. *Veterinary Record* 17, 18. DOI:10.1136/vr.100716.

Suttle, N.F. (2012b) Copper imbalances in ruminants and humans: unexpected common ground. *Advances in Nutrition* 3, 666–674.

Suttle, N.F. (2013) Rate of change in liver copper concentration in cattle given a copper-deficient diet, with or without pre-treatment with tetrathiomolybdate, for evaluation of two parenteral copper supplements. *New Zealand Veterinary Journal* 61, 154–158.

Suttle, N.F. (2016) Reducing risk of copper toxicity in dairy cattle. *Veterinary Record.* DOI:10.1136/vr.i793

Suttle, N.F. and Field, A.C. (1968) The effect of intake of copper, molybdenum and sulfate on copper metabolism in sheep. I. Clinical condition and distribution of copper in the blood of the pregnant ewe. *Journal of Comparative Pathology* 78, 351–362.

Suttle, N.F. and Field, A.C. (1983) Effects of dietary supplements of thiomolybdates on copper and molybdenum metabolism in sheep. *Journal of Comparative Pathology* 93, 379–389.

Suttle, N.F. and Small, J.N.W. (1993) Evidence of delayed availability of copper in supplementation trials with lambs on molybdenum-rich pasture. In: Anke, M., Meissner, D. and Mills, C.F. (eds) *Proceedings of the Eighth International Symposium on Trace Elements in Man and Animals, Dresden*. Verlag Media Touristik, Gersdorf, Germany, pp. 651–655.

Suttle, N.F. and Wadsworth, I. (1991) Physiological responses to vaccination in sheep. *Proceedings of the Sheep Veterinary Society* 11, 113–116.

Suttle, N.F., Brebner, J. and Hall, J. (1991) Faecal excretion and retention of heavy metals in sheep ingesting topsoil from fields treated with metal-rich sewage sludge. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals*. IMI, Zagreb, pp. 32-7–32-8.

Suttle, N.F., Knox, D.P., Angus, K.W., Jackson, F. and Coop, R.L. (1992) Effects of dietary molybdenum on nematode and host during *Haemonchus contortus* infection in lambs. *Research in Veterinary Science* 52, 230–235.

Targhibi, M.R., Shabankareh, H.K. and Kafilzadeh, F. (2012) Effects of supplemental chromium on lactation and some blood parameters of dairy cows in late gestation and early lactation. *Asian Journal of Animal and Veterinary Advances* 7, 1295–1311.

Thomas, J.W. and Moss, S. (1952) The effect of orally administered molybdenum on growth, spermatogenesis and testis histology of young dairy bulls. *Journal of Dairy Science* 94, 929–934.

Thompson, K.H., Tsukada, Y., Xu, Z., Battell, M., McNeill, J.H. and Orvig, C. (2002) Influence of chelation and oxidation state on vanadium bioavailability, and their effects on tissue concentrations of zinc, copper, and iron. *Biological Trace Element Research* 86.

Thorhallsdottir, A.G., Proveuza, F.D. and Ralph, D.F. (1990) Social influences on conditioned food aversions in sheep. *Applied Animal Behaviour Science* 25, 45–50.

Toepfer, E.W., Mertz, W., Royinski, E.E. and Polansky, M.M. (1973) Chromium in foods in relation to biological activity. *Journal of Agricultural and Food Chemistry* 21, 69–73.

Topham, R.W., Walker, M.C. and Calisch, M.P. (1982a) Liver xanthine dehydrogenase and iron mobilisation. *Biochemical and Biophysical Research Communications* 109, 1240–1246.

Topham, R.W., Walker, M.C., Calisch, M.P. and Williams, R.W. (1982b) Evidence for the participation of intestinal xanthine oxidase in the mucosal processing of iron. *Biochemistry* 21, 4529–4535.

Uden, P., Colucci, P.E. and van Soest, P.J. (1980) Investigation of chromium, cerium and cobalt as markers in digesta: rate of passage studies. *Journal of Science in Food and Agriculture* 31, 625–632.

Underwood, E.J. and Suttle, N.F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Van Heugten, E. and Spears, J.W. (1997) Immune response and growth of stressed weanling pigs fed diets supplemented with organic and inorganic forms of chromium. *Journal of Animal Science* 75, 409–416.

Van Vleet, J.F., Boon, G.D. and Ferrans, V.J. (1981) Induction of lesions of selenium–vitamin E deficiency in weanling swine fed silver, cobalt, tellurium, zinc, cadmium and vanadium. *American Journal of Veterinary Research* 42, 789–799.

Villalobos, F., Romero, R.C., Farrago, C.M.R. and Rosado, A.C. (1997) Supplementation with chromium picolinate reduces the incidence of placental retention in dairy cows. *Canadian Journal of Animal Science* 77, 329–330.

Vincent, J.B. (2000) The biochemistry of chromium. *Journal of Nutrition* 130, 715–718.

Vincent, J.B. and Stallings, D. (2007) Introduction: a history of chromium studies, 1955–1995. In: Vincent, J.B. (ed.) *The Nutritional Biochemistry of Chromium (III).* Elsevier, Amsterdam, pp. 1–43.

Vipond, J.E., Horgan, G. and Anderson, D. (1996) Estimation of food intake in sheep by blood assay for lithium content following ingestion of lithium-labelled food. *Animal Science* 60, 513.

Wang, T., Li, Z., Chen, X., Long, X.-E. (2019) Effects of nickel and cobalt on methane production and methanogen abundance and diversity in paddy soil. *PeerJ* 7, e6274. DOI:10.7717/peerj.6274 ().

Wang, Z.Y., Poole, D.B.R. and Mason, J. (1988) The effects of supplementation of the diet of young steers with Mo and S on the intracellular distribution of copper in liver and on copper fractions in blood. *British Veterinary Journal* 114, 543–551.

Ward, T.L., Southern, T.L. and Bidner, T.D. (1997) Interactive effects of dietary chromium tripicolinate and crude protein level in growing-finishing pigs provided with inadequate pen space. *Journal of Animal Science* 75, 1001–1008.

Weeth, H.J., Speth, C.F. and Hanks, D.R. (1981) Boron content of plasma and urine as indicators of boron intake in cattle. *American Journal of Veterinary Research* 42, 474–477.

Welch, R.M. and Cary, E.E. (1975) Concentration of chromium, nickel and vanadium in plant materials. *Journal of Agricultural and Food Chemistry* 23, 479–482.

Wilson, J.H. and Ruszler, P.L. (1997) Effects of boron on growing pullets. *Biological Trace Element Research* 56, 287–294.

Wilson, J.H. and Ruszler, P.L. (1998) Long term effects of boron on layer bone strength and production parameters. *British Poultry Science* 39, 11–15.

Woodworth, J.C., Tokach, M.D., Nelssen, J.L., Goodband, R.D., Dritz, S.S., *et al.* (2007) Influence of dietary L-carnitine and chromium picolinate on blood hormones and metabolites of gestating sows fed one meal per day. *Journal of Animal Science* 85, 2524–2537.

Wright, A.J., Mowat, D.N. and Mallard, B.A. (1994) Supplemental chromium and bovine respiratory disease vaccines for stressed feeder calves. *Canadian Journal of Animal Science* 74, 287–295.

Wu, B., Cui, H., Peng, X., Fang, J., Zuo, Z., Deng, J. and Huang, J. (2013) Dietary nickel chloride induces oxidative intestinal damage in broilers. *International Journal of Environmental Research and Public Health* 10, 2109–2119.

Xiao-Yun, S., Guo-Zhen, D. and Hong, L. (2006) Studies of a naturally occurring molybdenum-induced copper deficiency in the yak. *The Veterinary Journal* 171, 352–357.

Yamamoto, A., Wada, O. and Suzuki, H. (1988) Purification and properties of biologically active chromium complex from bovine colostrum. *Journal of Nutrition* 118, 39–45.

Yan, X., Zhang, Z., Dong, L., Zhu, X. and Jia, Z. (2010) Effects of chromium on energy metabolism in lambs fed with different dietary protein levels. *Asian-Australian Journal of Animal Science* 23, 205–212.

Yang, W.Z., Mowat, D.N., Subiyatno, A. and Liptrap, R.M. (1996) Effects of chromium supplementation on early lactation performance of Holstein cows. *Canadian Journal of Animal Science* 76, 221–230.

Yokoi, K.M., Kimura, M. and Itokawa, Y. (1994) Effects of a low-rubidium diet on macro-mineral levels in rat tissues. *Journal of the Japanese Society for Nutrition and Food Science* 47, 295–299.

Zhang, F.J., Weng, X.G., Wang, J.F., Zhou, D., Zhang, W. *et al.* (2014) Effects of temperature-humidity index and chromium supplementation on antioxidant capacity, heat shock protein 72, and cytokine responses of lactating cows. *Journal of Animal Science* 92, 3026–3034.

18 Potentially Toxic Elements

Elements that are potentially toxic to animals (PTE) also have important industrial uses and past industrial removal of PTE from the earth's crust contaminates some farm environments (e.g. Geeson *et al.*, 1998). Environmental contamination from anthropogenic activity typically has subclinical effects, with no overt, adverse effects in livestock but PTE can accumulate in animal tissues and produce to present a threat to consumers. The insidious nature of PTE exposure in animals and man has caused concern since the 1980s (Chowdhury and Chandra, 1987; Fox, 1987; Scheuhammer, 1987) and continued in the new millennium (World Health Organization, 2000, 2001; NRC, 2005; Rahman and Singh, 2019). Perceived risks to human health set in motion a 'hazard-reduction juggernaut' that had repercussions for animal production, including the imposition of maximum permissible (MPL) and tolerable (MTL) levels for PTE in livestock feeds and maximum acceptable concentrations (MAC) in edible products (EFSA, 2006): MTL and MPL often bore no relationship to each other and can appear illogical (Table 18.1). In Europe, consignments that infringed MAC were recorded on a central database and checked at border controls, providing a useful dataset for research purposes (Mastilovic *et al.*, 2010). Abbatoir surveys of PTE in bovine liver between 2007 and 2018 give no cause for concern (Counotte *et al.*, 2019). Outside Europe, countries set their own limits, based on pragmatism as well as principle, with heavily populated and industrialized countries like Japan briefly accepting MTL for Cd and Hg that were two to four times higher than other countries.

Such regulations do little to protect livestock but occasionally threaten the sustainability of livestock production. Identification, regulation and amelioration of contaminated environments is a major challenge. Risk of PTE toxicity depends on:

- uptake of the PTE from soil by plants (translocation);
- inadvertent ingestion of PTE-rich soils by animals;
- absorbabilities of inherent and contaminant PTE from the diet;
- dietary constituents that enhance or inhibit PTE uptake and retention;
- predilection sites that act as repositories for PTE in the body; and
- duration and pattern of PTE exposure.

The above factors can make nonsense of regulatory limits by disrupting relationships between apparent PTE exposure and tissue accretion that vary between and within species (Table 18.1). With the exception of Hg, all PTE can, paradoxically, fulfil the criteria of essential elements for livestock (p. 451) but those criteria are reconsidered at the end this chapter.

Soils act as major sources and reservoirs of PTE (Table 18.2) but plant uptake is generally poor and bioconcentration factors between soil and plant are hugely negative. Soil ingestion by foraging livestock is often the major determinant of risk to both livestock and consumer. Soil ingestion is highly variable and determined by seasonal, husbandry and weather-related factors (p. 20), usually contributing 2–5% of dry-matter intake (DMI) and reaching maxima in outwintered animals. Contamination of pastures with fluorine and lead in an old mining area reached a maximum in winter, peak values in unwashed samples being two or more times those found in washed samples (Geeson *et al.*, 1998). Studies that measure PTE concentrations only in herbage cut from contaminated pastures in summer (e.g. Gaskin *et al.*, 2003) may greatly underestimate risk both to grazing livestock and humans. Animals that are exposed to PTE go through phases of

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Table 18.1. Maximum permitted level (MPL), maximum tolerable level (MTL) and ranges of tolerance for potentially toxic trace elements in complete feeds for ruminants in USA (NRC, 2005); standards that differ for pigs and poultry appear in parentheses.

Element	MPL (mg kg^{-1}DM)	MTL	Range of tolerance (mg kg^{-1}DM)	Factors that influence tolerance
As	2	30	Uncertain	Inorganic > organic
Cd[a]	1	10	3–30 (0.5–1.0)	Pb ↓: Cu, Zn →
Fl[b]	150 (100)	40 (150)	40–60[c] (225)	Inorganic < contaminant
Hg (organic)	0.1	2	Uncertain	Inorganic > organic
Pb[d]	5	100 (10)	150–800 (5–10)	Cd ↓

[a]The common low MPL for cadmium is fixed to protect the consumer but non-ruminants are both more vulnerable to Cd exposure and a greater threat to the consumer than ruminants.
[b]More fluorine is allowed for cattle than is tolerable: the more tolerant pig receives better protection.
[c]Cattle and sheep are ten times more tolerant of lead than pigs and the lesser threat to consumers because they accumulate less lead from a given level of exposure, yet a common MPL is decreed.
[d]Sheep are more tolerant than cattle.

Table 18.2. Median (maximal) ranges of concentrations of potentially toxic elements found in soils (mg kg^{-1}DM at sampling depth 0–7.5 cm).

Element	England and Wales[a]	New Zealand[b]
As	10.4 (140)	4.7 (58)
Cd	0.5 (10.5)	0.4 (1.5)
F	200–400[c]	217–454[d]
Pb	36.8 (2900)	10 (83)
Hg	0.09 (2.12)	–

[a]Archer and Hodgson (1987); [b]Longhurst *et al*. (2004); [c]Fuge and Andrews (1988); [d]Loganathan *et al*. (2001)

accretion and dysfunction before clinical toxicity occurs (see Fig. 3.7).

Aluminium

Sources

Aluminium is the most abundant mineral in most soils at 3–6% but little enters the soil solution and groundwater, which contain <2.25mg Al l^{-1} because the element is largely present in insoluble siliceous complexes. Most foodstuffs contained <5mg Al kg^{-1}DM (Muller *et al*., 1998). However, trees, ferns and tropical plants are 'aluminium accumulators' and may contain 3–4g Al kg^{-1}DM (Underwood and Suttle, 1999). The principal source of aluminium exposure for grazing livestock is from soil-contaminated pastures; aluminium may constitute 0.3–1.2% of DMI when soil constitutes 10–20% of DMI. The act of grazing itself can increase the degree of contamination of pasture with soil and aluminium ('poaching') (Robinson *et al*., 1984). High concentrations in the finest soil particles (Brebner *et al*., 1985) ensure that aluminium, like silicon, is a ubiquitous contaminant of farm environments. Addition of soil to continuous cultures of rumen microbes raised soluble Al in effluents (Brebner, 1987). Aluminium may also enter the diet through the use of contaminated mineral supplements (e.g. soft phosphate, 70g Al kg^{-1}DM), feed-pelleting agents, such as bentonite (110g Al kg^{--1}DM) or 'aids to digestion' such as zeolite (60g Al kg^{-1}DM). Aluminium may enter the environment following the treatment of public water supplies with alum (NRC, 2005). It is hardly surprising that aluminium presents problems of excess and not deficiency to livestock under farming conditions.

Metabolism

Less than 1% of ingested Al is normally absorbed (A_{Al}) and retained by weaned animals – even from simple, relatively soluble salts, such as $AlCl_3$: urinary excretion, which takes precedence over biliary excretion, only becomes important when intakes are excessive (Alfrey, 1986; NRC, 2005). Absorption can take place by passive paracellular processes or active processes involving non-specific, mucosal metal-binding proteins (NRC, 2005). Organic acids such as citric acid enhanced A_{Al} in non-ruminants (Cunat *et al*., 2000) but the effect

of weaker acids, produced during ensilage and rumen fermentation, is unknown. Aluminium ingested in coarse soil particles is probably trapped in the rumino-reticulum but Al in fine particles is rapidly excreted in faeces (Brebner *et al.*, 1985). Exposure of lambs to 2g Al kg^{-1}DM for two months raised soft tissues Al by no more than 2mg kg^{-1}DM above a similar norm, with liver the most- and brain the least-affected tissue (Valdivia *et al.*, 1982). Much more Al (10–80mg Al kg^{-1}DM) is found in silicon-rich tissues such as the lung and trachea, prompting suggestions of related functions for the two elements (Carlisle and Curran, 1993): common contaminants (aluminium silicates) and routes (inhalation, ingestion) of entry are more likely explanations. Adding Si to the diet reduces AA_{Al} (Domingo *et al.*, 2011). The anaemia of iron deficiency may increase tissue Al by reducing the rate of Al turnover rather than increasing A_{Al} (NRC, 2005).

- Aluminium deficiency has never been reported.

Toxicity

Various insoluble Al compounds are used as adjuvants in human and animal vaccines and vary in efficacy, though are assumed to be metabolically inert (Caufield *et al.*, 2007). Controversy has raged over the putative role of Al in Alzheimer's Disease (AD) but has recently taken a sharper focus (Exley, 2016). The presence of characteristic, Al-rich plaques in the brains of AD cadavers is beyond doubt, but is this cause or effect? Epidemiological studies were hampered by the difficulty of measuring lifetime exposure to such a ubiquitous contaminant of the human environment. The induction of similar plaques and an associated biochemical anomaly in the brains of mice by adding 4 g Al, as $Al_2(SO_4)_3$ to their drinking water for two months or more, with no outward signs of harm (Rodella *et al.*, 2008), has changed attitudes. Stronger advice on maximum Al intakes and how to minimize actual intakes has now been issued: edible animal products are not a significant source of ingested Al for humans and Al was not considered to be a risk factor for AD (EFSA, 2008). Poor absorption, ready urinary excretion and short lifespans mean that farm livestock is unlikely to be at risk from chronic exposure to excess Al and animal products contribute little Al to the human diet: a causative link with AD is still disputed (Food Safety Committee of Japan, 2019).

Non-ruminants

Adding 1g Al (as $Al_2(SO_4)_3$) kg^{-1}DM to the diet of laying hens for four months did not reduce egg production and served as an antidote to fluoride toxicity in mink (Aulerich *et al.*, 1987). However, lower exposure (0.6 g) inhibited calcium absorption in chicks via the regulation of calbindin (p. 72) at the post-transcriptional level and inhibited the calbindin response to calcium depletion (Cox and Dunn, 2001). A note of caution is sounded over the use of citrate to improve phosphorus utilization in pigs and poultry rations (see p. 148) because A_{Al} and tissue Al (and Cd, p. 487) may be increased.

Ruminants

An antagonism of phosphorus metabolism from ingested Al-rich soil (Krueger *et al.*, 1985) was implicated in phosphorus deficiency in cattle grazing tropical pastures (McDowell, 2003) but remains contentious.[i] A_{Al} may be higher during late gestation or lactation and also for the suckling but there are no data for livestock. High intakes of aluminium have been implicated in the aetiology of hypomagnesaemic tetany in cattle and intra-ruminal doses of $Al_2(SO_4)_3$, equivalent to 4g Al kg^{-1}DM, rapidly reduced in serum Mg and, subsequently, appetite (Allen *et al.*, 1984). Furthermore, additions of 2.9mg Al kg^{-1}DM as the citrate reduced plasma Mg while increasing urinary Ca (Allen *et al.*, 1986). Similar challenges, given as soil, have no effect on plasma Mg. Grazing livestock can clearly withstand considerable and sustained intakes of soil-borne Al (Robinson *et al.*, 1984; Allen *et al.*, 1986; Sherlock, 1989).

- The absorbability and antagonism of aluminium from tropical shrubs, used as forages, merits investigation.
- Accumulation of Al in edible farm products poses no threat to consumers.

Arsenic

Arsenic was once surpassed only by lead as a toxicological hazard to farm livestock (Selby *et al.*, 1977). Most incidents arose from exploiting toxic effects of As to control pests (rodenticides), common pathogens (including coccidia and cestodes) and crops (insecticides and fungicides). Incidents in Europe fell markedly following withdrawal of most arsenicals from the marketplace. In view of its long and colourful history as a poison, claims that

arsenic was an essential dietary constituent (Nielsen 1996) were hard to swallow and have been disputed (NRC, 2005). Recognition of arsenic as a carcinogen enhanced restrictions on routes of entry, via livestock, into the human food chain.

Sources

The natural abundance of As in agricultural soils is generally low in England and Wales, with a scatter of high values associated with mining activity (Table 18.2).[ii] A survey in New Zealand found far less soil As (mean 5.4 mg kg^{-1}DM) and no evidence of enrichment associated with farming (Longhurst *et al.*, 2004). Plants usually contained <0.2mg As kg^{-1}DM, with the highest values found on peat soils, relatively rich in As (10mg As kg^{-1}DM). Feeds of plant and animal origin vary in As content, with rice and rice by-products particularly high in As (Upadhyay *et al.*, 2019). Marine sources are much richer, fish meals containing 2–9 mg (NRC, 2005) and seaweeds (or their derivatives) 35mg As kg^{-1}DM. Marine algae contain up to 100mg As kg^{-1}DM, mostly as arsenosugars (Jackson and Punshon, 2014). Inorganic As is no longer added to pig and poultry rations to stimulate growth. The MAC for drinking water in the European Community is 50 µg As l^{-1}, but 'cancer scares' led authorities in the USA to reduce that level to 10 µg As l^{-1}. In arseniferous areas, groundwater can contain >1 mg l^{-1} (NRC, 2005). The European Community (2002) reduced permissible levels in air-dry animal feeds to 2 mg As kg^{-1}, making exceptions for some forage by-products (<4 mg As kg^{-1}). However, low arsenic intakes may predispose to colonic cancer by causing hypomethylation (NRC, 2005). Balancing the needs of animal production and human health is an issue returned to in Chapter 20.

Metabolism

Like most elements that exist primarily in the anionic state, arsenic is well absorbed (AA_{As} 0.9) from inorganic sources by non-ruminants (NRC, 2005). However, AA_{As} was only 0.45 for $^{75}AsCl_3$ in lambs: rates of tissue As accretion were ranked in the order liver > muscle > kidney (Beresford *et al.*, 2001). Bioavailability of contaminated soil As is relatively low (Casteel *et al.*, 2001). Some organic forms of arsenic, such as the arsenobetaines in seafoods, are highly absorbable but harmless and readily excreted in urine (Jackson and Punshon, 2014). Arsenic from most sources is well excreted in urine and tissue accretion is therefore slow but detectable in skin and its appendages (Selby *et al.*, 1977). Relatively high tissue As in poultry was attributed to the widespread use of arsenicals in diets (Doyle and Spaulding, 1978), now widely banned (Jackson and Punshon, 2014).

Toxicity to animals

Cases of arsenic toxicity are now extremely rare. Symptoms vary with the rate and duration of exposure and the source of As (Bahri and Romdane, 1991). Water-soluble salts such as arsenites and arsenates are the most toxic, particularly the trivalent forms, while insoluble elemental As is non-toxic (Arnold, 1988; NRC, 2005). Arsenic disturbs metabolism in three ways: impaired methylation through inhibition of sulfhydryl enzyme systems; oxidative stress though inhibition of keto acid oxidation and antagonism of other elements (NRC, 2005). Adding only 1.6mg As kg^{-1}DM to a rat diet as contaminated fish retarded growth and decreased sulfite and monoamine oxidase activities: the effects were larger than those of Cd and additive in testes (Ezedom and Asagba, 2016).

Acute toxicity

Accidental exposure to large oral doses of inorganic As causes gastroenteritis, a rapid drop in blood pressure and collapse, accompanied by muscular twitching, convulsions and gastrointestinal haemorrhage in exposed sheep (McCaughey, 2007). Confirmation of arsenic poisoning is provided by high arsenic concentrations in tissues, body fluids and gut contents, if oral exposure has been recent. In poisoned horses, concentrations were higher in liver than kidney (11–14 and 4.2–6.5mg As kg^{-1}FW, respectively; Pace *et al.*, 1997), some thrityfold greater than normal (Puls, 1994). Where the route of exposure has primarily involved the lungs or skin, the focus of pathology shifts to the liver and kidneys, which both show severe necrosis.

Chronic toxicity

Chronic toxicity was commonly caused by overexposure to organic As sources of medium

(e.g. phenylarsonic compounds) to low (e.g. arsanilic acid) toxicity in acaricides, rodenticides and feed additives. Early signs are those of neurological disorder, such as incoordination, swaying and ataxia ('drunken hog syndrome'), but affected animals remain alert and continue to eat and drink. Later, pigs show extreme agitation, screaming and pressing noses to the ground when disturbed. Demyelination of some peripheral nerves may be evident histologically (Kennedy *et al.*, 1986). Biochemical confirmation depends on the species and type of arsenic exposure, notably pattern and source (Puls, 1994). Arsenical sheep dips became trapped in the fleece and As was absorbed through the skin (McCaughey, 2007). In a few areas, such as south-west England (Thornton, 1996), disturbance of As-rich deposits during mining polluted soil, air and groundwater, increasing the risk of toxicity in grazing livestock. Disturbance of natural drainage systems by pipe-laying operations can further redistribute soil As. Only China still permits the use of organo-arsenicals in pigs and poultry: some sources are degradable to inorganic and more toxic forms (Jackson and Punshon, 2014).

Arsenic in edible animal products

Abattoir surveys indicated that liver and kidney As did not increase with age (Waegeneers *et al.*, 2009a) and have not increased in recent years. Meat, offal and egg As were invariably below MAC for consumers in Europe (Mastilovic *et al.*, 2010) and China but caution has been advised (Upadhyay *et al.*, 2019).

Cadmium

Cadmium is a highly reactive and toxic element but sparsely distributed in most agricultural ecosystems (Pinot *et al.*, 2002). Once absorbed by animals or humans, however, Cd is poorly excreted and sources of entry for Cd into the human food chain are being strictly regulated.

Sources

Soils, pasture and crops

Median soil Cd was low in England and Wales (Table 18.2) and similar to that found in New Zealand (Bramley, 1990; Longhurst *et al.*, 2004). In both countries, some soils have become enriched threefold by applications of Cd-rich inorganic (e.g. Loganathan *et al.*, 2008), organic fertilizers (e.g. Brebner *et al.* 1993) and dispersal of wastes from mining and smelting. Uptake of Cd by plants is generally poor, particularly from clay soils, and forages and crops grown on normal soils usually contain <1mg Cd kg^{-1}DM. If contamination continues, soil Cd slowly increases in the topsoil. Plant Cd also rises but the increase depends on competition from soil zinc, soil pH, plant species and tissue sampled (Van Bruwaene *et al.*, 1984; Hooser, 2018). Rice is exceptional in the latter respect and accumulates Cd in the grain, particularly the outer layers, when grown aerobically (Jackson and Punshon, 2014). Rice bran can be a significant contributor of available Cd in livestock diets and 94% can be extracted with 0.15M citric acid (Zou *et al.*, 2019). Because uptake by plants is poor, the major source of ingested Cd for grazing amials is soil that contaminates pasture. Uptake of Cd by sheep grazing pastures on acidic, sandy soils was higher than that on alkaline, clay soils (Morcombe *et al.*, 1994b).

Superphosphates and sewage sludges

The most important exogenous sources are superphosphate fertilizers, which contain from <5 to 134 mg Cd kg^{-1}, depending on country or region of origin (Bramley, 1990; Sullivan *et al.*, 1994). Regular use of Cd-rich Pacific sources of superphosphate in Australia and New Zealand increased soil and pasture Cd sufficiently to raise kidney Cd in grazing lambs above the MAC (>1 mg kg^{-1}FW) (Bramley, 1990; Morcombe *et al.*, 1994a). In New Zealand, soil Cd is correlated with soil P, confirming phosphorus fertilizers as a major source of Cd enrichment (Longhurst *et al.*, 2004). Pasture Cd can be contained within acceptable limits by avoiding Cd-rich sources of superphosphate and probably by liming, which reduces Cd uptake by cereals (Oliver *et al.*, 1996). Ingestion of fertilizer in topsoil adds to body burdens of cadmium in grazing animals (Stark *et al.*, 1998). Sewage sludges can contain up to 20mg Cd kg^{-1}DM and bans on dispersal to sea in some countries increased dispersal on agricultural land (Hill *et al.*, 1998a). In Europe, guidelines aimed to restrict accumulation in sludge-amended soils to <3mg Cd kg^{-1}DM (sampling depth 20 cm) were introduced (European Community, 2002). Under flooded rice culture in the Po valley, Italy, soil Cd increased by up to 6% per annum (Van Bruwaene *et al.*, 1984).

Absorption and metabolism

Apparent absorption of cadmium (AA_{Cd}; p. 26) by weaned animals is low in *all* species. In ruminants, AA_{Cd} is <1% for inorganic Cd salts (Neathery *et al.*, 1974; van Bruwaene *et al.*, 1982, 1984; Houpert *et al.*, 1995). With sludge-amended soil (Suttle *et al.*, 1991) or sewage sludge (Hill *et al.*, 1998a) providing Cd, AA_{Cd} is not significantly different from zero, although increases in tissue Cd indicates that a tiny fraction is absorbed. Inorganic Cd added to milk was relatively well absorbed (Bremner, 1978) but AA_{Cd} was still only 4% in neonatal pigs (Sasser and Jarboe, 1980) and Cd is not transferred naturally via the dam's milk (Suttle *et al.*, 1997).

- The high general A_{Cd} of 15% for cattle, cited by NRC (2005), is unsubstantiable.

Cadmium in superphosphates was of lower availability to pigs than that in $CdCl_2$ (King *et al.*, 1992). Phytase supplements (Rimbach *et al.*, 1996) and deficiencies of minerals, including Ca, Fe and Zn (Kollmer and Berg, 1989), increase A_{Cd}, while excesses (including Mn) reduce A_{Cd} (NRC, 2005).

Retention and maternal transfer

The vast literature on biochemical responses of animals to Cd has little nutritional relevance, because of either excessive dietary[iii] or unphysiological, parenteral exposure. The latter bypasses the protective gut mucosa and targets the liver, while excessive oral exposure targets the kidney. The small proportion of dietary Cd absorbed after oral (pulse) doses of ^{109}Cd was avidly retained: of the 0.75% absorbed by cows after 14 days, 17% was still present in tissues after 131 days; the 0.15–0.5% absorbed by sheep had a half-life in the body of 100–150 days (Houpert *et al.*, 1995). The principal sites of retention are the gastrointestinal tract, liver and kidneys (Neathery *et al.*, 1974; Houpert *et al.*, 1995). Half-life in the kidneys is measured in years. The slight reductions in liver and kidney Cd that followed cessation of oral supplementation in growing pigs were largely due to growth in organ size. Control of Cd retention is achieved largely by metallothionein (MT), a cysteine-rich and major Cd-binding protein in the body that transports Cd from liver to kidney. Exposure to Cd induces MT synthesis at many sites, including the gut mucosa and liver, in what is believed to be a protective role, but Cd–MT is nephrotoxic (Bremner, 1978). Cadmium is not readily transferred across the ovine (Mills and Dalgarno, 1972; Suttle *et al.*, 1997) or bovine (Neathery *et al.*, 1974; Smith *et al.*, 1991a) placenta, mammary gland and avian oviduct (Sell, 1975; Stoewsand *et al.*, 2005).

Interactions

Cadmium shares important biochemical properties with other metals, such as binding to MT (shared with copper and zinc; p. Cu), divalent metal transporter 1 (with iron, manganese and lead; see p. 30) and calbindin (with calcium and aluminium; p. 485). Shared elements are often mutually antagonistic, exposure to Cd lowering Cu status and exposure to Zn lowering Cd status (Fig. 2.6; Bremner, 1978). Addition of phytate (5g kg^{-1}DM) to a rat diet, supplemented with 5mg Cd kg^{-1}DM, increased liver Cd threefold, possibly by lowering A_{Ca} and inducing expression of calbindin in gut mucosa (Rimbach *et al.*, 1996). Concurrent exposure of pigs to Cd (1.0 or 2.5mg kg^{-1}DM) and Pb (5, 10 or 25mg kg^{-1}DM) raised tissue Cd more than exposure to Cd alone (Phillips *et al.*, 2003). Cadmium also interacts with other minerals through other mechanisms. Administration of Se lowered Cd toxicity by redistributing Cd from MT towards high-molecular-weight proteins (Bremner, 1978). Raising dietary S from a moderate (1.9g kg^{-1}DM) to a very high level (5.9g kg^{-1}DM) reduced liver and kidney Cd by 60% in sheep (Smith and White, 1997): Mo was less effective and the two antagonists did not act additively.

Chronic, primary toxicity in livestock

The listed mechanisms of primary toxicity are many and varied and include disruption of the cell reduction–oxidation cycle and calcium signalling (Pinot *et al.*, 2002; NRC, 2005) but were mostly ascertained under conditions of acute exposure to inorganic Cd, sometimes in cell cultures. Dietary exposure of rats to 3.3mg Cd kg^{-1}DM in contaminated fish, for three months, retarded growth and decreased both sulfite and monoamine oxidase activities in liver, kidney and testes: similar effects of arsenic exposure were additive in testes (Ezedom and Asagba, 2016). Normal, high and 'toxic' dietary Cd for species of agricultural interest are summarized in Table 18.3. Blood Cd is only marginally increased above normal (>0.01 μg l^{-1}), analytically problematic and diagnostically unhelpful, but

Table 18.3. Cadmium toxicity in farm livestock can be confirmed from cadmium concentrations in the diet, liver or kidneys. Normal (N), high (H) and toxic (T) ranges are given. (After Puls, 1994)

Species	Cd level	Diet (mg kg^{-1} DM)[a]	Liver (mg kg^{-1}FW)[a]	Kidney (mg kg^{-1}FW)[a]
Cattle and sheep	N	0.1–0.2	0.02–0.05	0.03–0.10
	H[b]	0.5–5.0	0.1–1.5	1.0–5.0
	T	>50	50–160	100–250
Pigs	N	0.1–0.8	0.1–0.5	0.1–1.0
	H[b]	1.0–5.0	1.0–5.0	2.0–5.0
	T	>80	>13	<270
Poultry	N	0.1–0.8	0.05–0.5	0.1–1.5
	H[b]	1.0–5.0	1.0–50	2.0–10.0
	T	>40	>15	>70

[a]Multiply by 8.9 to obtain units in mmol kg^{-1}DM.
[b]High dietary cadmium levels may lead to unacceptably high concentrations in offal. Maximum acceptable concentrations vary from country to country but are often 1mg Cd kg^{-1} FW and likely to fall. Cadmium may also induce copper or zinc deficiencies if the respective dietary concentrations are marginal.

excretion of MT in urine is indicative of Cd exposure. In avian species, dietary exposure to 75mg Cd kg^{-1}DM commonly caused kidney damage and delayed maturation of the testes (Scheuhammer, 1987). In laying hens, exposure to 48 (Leach *et al*., 1979) and 60mg Cd kg^{-1}DM (Sell, 1975) reduced egg production. With diets or animals of normal or high Cu and Zn status, relatively high dietary Cd is required to cause toxicity, the major abnormality being nephrotoxicity. Diagnosis is confirmed by elevated kidney Cd and histological evidence of kidney damage (e.g. Scheuhammer, 1987).

Secondary cadmium toxicity

Symptoms of secondary toxicity, involving interactions with Cu and Zn, are indistinguishable from those caused by deficiencies of each element. Less dietary Cd was required to induce secondary than primary Cd toxicity but the precise concentration was highly variable (p. 29).[iv] Exposure of pregnant ewes and/or lambs to 3.0–3.4mg Cd kg^{-1}DM, in a diet adequate in zinc, induced anaemia, impaired bone mineralization and wool crimp: some lambs were aborted, others stillborn. All abnormalities were associated with copper deprivation (p. 257) but prevented by adding 750mg Zn kg^{-1}DM (Mills and Dalgarno, 1972; Dalgarno, 1980): secondary copper deprivation had been induced. Housed lambs tolerated 15mg Cd kg^{-1}DM when given a diet that maintained high liver Cu (Doyle *et al.* 1974). In cattle, a supplement of 5mg Cd kg^{-1}DM, fed throughout pregnancy, reduced liver Cu in the newborn calf by 29%, while 1mg Cd kg^{-1}DM was sufficient to reduce liver Cu in the dam by 40% (Smith *et al.*, 1991a). High susceptibility of grazing ruminants to copper insufficiency makes them more vulnerable than other species to antagonism from Cd. Similar interactions between Cd, Zn and Cu occur in non-ruminants[v]. Hock and feather abnormalities were induced in turkey poults with 2mg Cd kg^{-1}DM in a low-zinc diet (10 versus 60mg Zn kg^{-1}DM: the higher zinc level did not fully protect against 20mg Cd kg^{-1}DM; Supplee, 1961).

- Tolerance will be determined by ratios (Cd:Zn and/or Cd:Cu) rather than dietary Cd alone.

Tolerance by livestock

Non-ruminants

NRC (2005) suggested the same high tolerance for all species, 10mg Cd kg^{-1}DM, but tolerance of cumulative poisons is a multiple of rate and duration of exposure. Non-ruminants are generally less tolerant of Cd exposure than ruminants but are less likely to be exposed for long periods in agricultural practice. Limits of tolerance are hard to define because they are determined by dietary factors, notably dietary Cu and Zn, and criteria of harmfulness. Studies with pigs illustrated the difficulties of setting limits on tolerance. With experimental exposure to $Cd(NO_3)_2$ alone and growth as probably the most sensitive index of harm, pigs tolerated between 0.5 to 1.0mg Cd kg^{-1}DM but with concurrent exposure to lead

acetate, <0.5mg Cd kg^{-1}DM was tolerated (Fig. 18.1a) (Phillips *et al.*, 2003). Combined exposure of sows harmed offspring (Fig. 18.1b) (Phillips *et al.*, 2003). High sensitivity may be partly attributable to inclusion of 50% wheat and barley in the diet and wheat bran as carrier for Cd, giving a higher phytase and lower phytate content than widely used corn/soybean meal diets. Free-range sows foraging on sludge-amended soils will be concurrently exposed to Cd and Pb. However, absorbabilities of Cd and Pb from sludge-amended soils would be lower than those from inorganic salts (Casteel *et al.*, 2001) and the experiments of Phillips *et al.* (2003) probably exaggerate risks on farms.

From outward appearances, broilers can tolerate up to 10mg Cd kg^{-1}DM (NRC, 2005) and Japanese quail, in lay, tolerated twice as much Cd for ten weeks in terms of egg production, shell strength (Fig. 18.2) and bone mineralization (not shown).

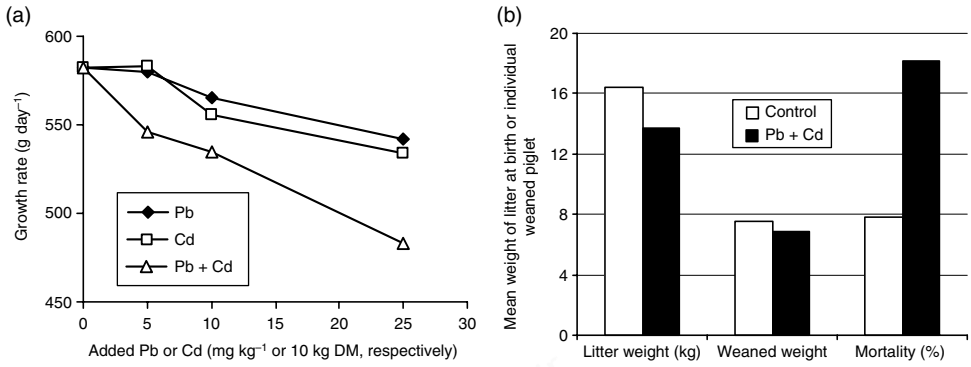

Fig. 18.1. Tolerance of added cadmium for 20 weeks in growing pigs (a) and six months in sows (b) was greatly reduced when combined with exposure to lead (1.0 mg Cd and 10mg Pb kg^{-1}DM). (Data from Phillips *et al.*, 2003)

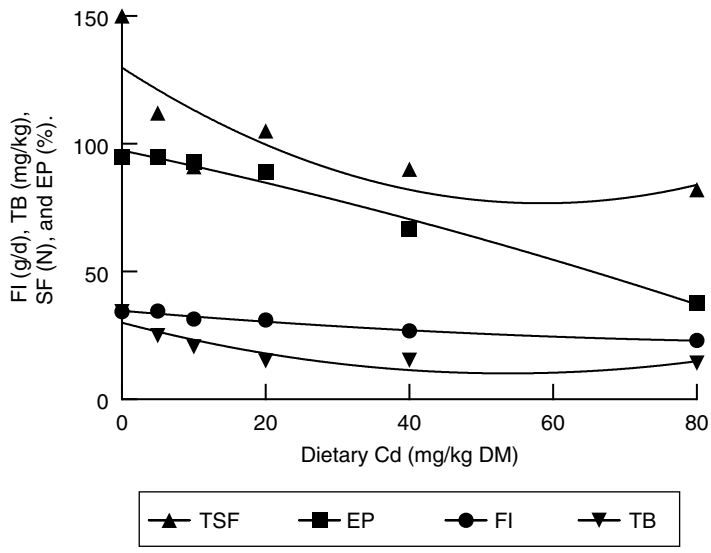

Fig. 18.2. Adding up to 20mg Cd kg^{-1}DM to a corn/soybean meal blend as $CdSO_4$ for ten weeks had relatively small effects on food intake (FI) and egg production (EP) in Japanese quail but tibia shear force (TSF) and tibia boron (TB) were reduced by only 5mg Cd kg^{-1}DM (From Olgun, 2015)

However, estimates of tolerance depend on the criteria of normality used and if bone strength is used as the yardstick, tolerance fell to <5mg Cd kg^{-1}DM. Tibia strength was strongly and negatively correlated with tibia B, which were reduced by 30% and 50%, respectively, by adding 20mg Cd kg^{-1}DM. Tolerance of laying birds is probably raised by high dietary Ca and studies on phytase-rich diets would be of interest.

Grazing livestock

Because plant uptake of Cd is poor, the major notional risk of toxicity in grazing or foraging livestock is presented by ingestion of soils enriched with Cd from inorganic (superphosphate) or 'biosolids' (sewage-sludge) fertilizers. Experimental PTE exposure has not caused ill-health or loss of production in either sheep or cattle and tolerance towards Cd from sewage sludge can be reckoned in mg kg^{-1} $days^{-1}$ from the following studies. In simulated soil-ingestion studies with lambs given grass pellets containing 10% soil for 84 days, kidney Cd increased linearly and liver Cd increased curvilinearly as soil Cd increased (Fig. 18.3), with higher accretion in liver from one soil (shown) than another, higher in iron and lower in calcium (not shown). These were arguably 'worst-case' simulations. On grazing sites where sewage sludge had been applied for several years, liver and kidney Cd showed little (Wilkinson *et al.*, 2001) or no increase (Rhind *et al.*, 2005) in lambs: soil Cd was not increased by sludge application in the latter study but soil ingestion reached a maximum of only 5% DMI over winter. Long-term exposure to sludge–amended soils involving reproducing sheep suggested that adaptive mechanisms

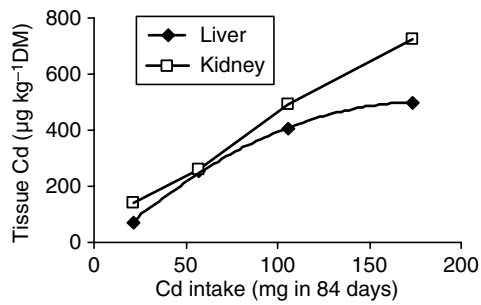

Fig. 18.3. Effects of cumulative cadmium (Cd) intake from a sludge-amended soil on liver and kidney Cd in lambs. (Data for Cassington soil from Hill *et al.*, 1998b)

limited Cd retention in liver. Ewes were exposed for three years to diets containing up to 0.4mg Cd kg^{-1}DM and liver Cd reached a plateau after about six months, the soil with most Cd having a relatively small cumulative effect (Fig. 18.4). Curvilinear increases in liver Cd over time (28 months) were also reported in lambs grazing pastures containing 0.5mg Cd kg^{-1}DM (Lee *et al.*, 1996). Higher liver and kidney Cd in ewes than in their lambs was partly attributed to greater duration of exposure (Rhind *et al.*, 2005). Kidney Cd increased linearly with exposure time in lambs grazing pastures on soils contaminated with fertilizer Cd and may exceed MAC (Langlands *et al.*, 1988; Petersen *et al.*, 1991) but accretion was less in liver and negligible in muscle (Petersen *et al.*, 1991; Fig. 18.5).

Liver Cd in heifers showed no further increase when exposure to 5mg Cd kg^{-1}DM (added as $CdCl_2$) continued from 394 days (parturition) and throughout lactation for a further 160 days (Smith *et al.*, 1991b), but this may reflect the negative effect of lactation rather than adaption to exposure. Five- to eightfold increases in kidney and liver Cd were reported in cattle exposed to pastures treated with sewage sludge by irrigation and soil incorporation for eight years (Fitzgerald *et al.*, 1985). Cadmium showed larger proportionate increases than any other element but no clinical or histopathological abnormalities were recorded and the maximal kidney Cd recorded (32mg kg^{-1}FW) remained well below that associated with kidney damage in humans (200mg kg^{-1}FW). In a contaminated region of Italy, abnormal but not pathognomic changes were found in association with kidney Cd of 1.1–1.5mg kg^{-1}FW (Barrasso *et al.*, 2018). Exposure to Cd from contaminated phosphates was blamed for 24%, 33% and 55% higher kidney, liver and mammary tissue Cd in dairy cows reared conventionally rather than 'organically' on the same farm (Olsson *et al.*, 2001). However, removal of older cows that entered the 'organic' herd as milkers removed the difference: the primary effect was of animal longevity and kidney Cd correlated with both age and milk yield.

Livestock as sources of cadmium exposure in humans

Regulations to limit flows of Cd into the human food chain mean that exposure of livestock to Cd

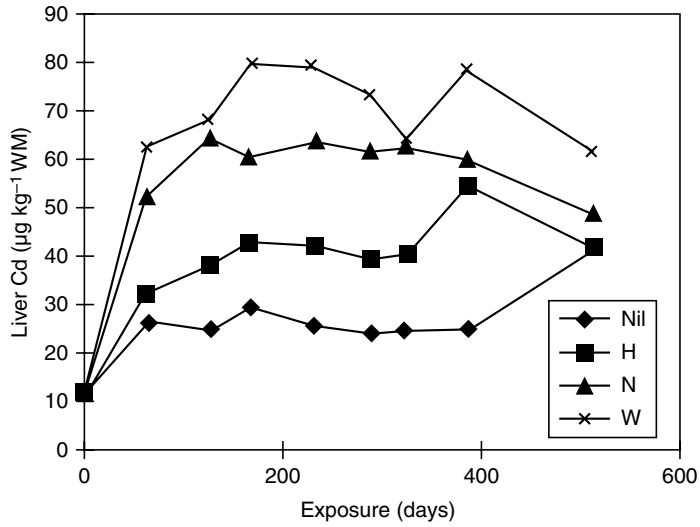

Fig. 18.4. Early high rates of cadmium (Cd) accumulation in the liver of lambs were not sustained during long-term exposure via three ingested soils (H, N and W), enriched with sewage sludges and containing 1.7, 3.9 and 7.3 mg Cd kg^{-1} DM, respectively: homeostatic adjustment to Cd exposure is implied (Stark *et al.*, 1998).

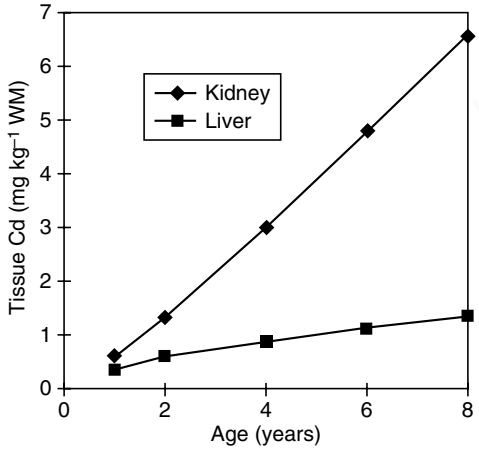

Fig. 18.5. Continuous exposure of sheep to pastures enriched with cadmium (Cd) from superphosphates in Western Australia caused linear increases in kidney and liver Cd over time, kidney Cd often exceeding maximum acceptable concentrations for human consumption (Peterson *et al.*, 1991).

assumed economic importance, through possible condemnation of carcases or offal (Loganathan *et al.*, 2008). Limits were set at 0.5 and 1.0mg Cd kg^{-1}FW for liver and kidney, regardless of species (EFSA, 2009). Since Cd is avidly retained, episodes of exposure have a cumulative effect. An abattoir survey in Belgium revealed a linear relationship between age and kidney Cd, such that bovines >2 y.o. were likely to exceed the EFSA limit (Waegeneers *et al.*, 2009a). Kidney Cd was higher in industrial than in rural areas (Waegeneers *et al.*, 2009b). A survey in the UK found far less accumulation: (Fig. 18.6). Livers from the oldest group generally contained <1mg Cd kg^{-1}DM and levels in muscle were often below the limit of detection. Kidney Cd in buffalo exceeded MAC in one region of Italy but not another (Barrasso *et al.*, 2018). Porcine kidney Cd showed considerable variation in animals of similar age from the same farm (Linden *et al.*, 2003). Liver and kidney Cd (and Pb) were about 50% lower in samples from goats than from sheep in Egypt (Darwish *et al.*, 2017). 17% of ovine kidney samples from a Spanish abattoir were unacceptably high in Cd (Pereira *et al.*, 2021). Kidney constitutes such a small fraction of food and Cd intakes as not to present a threat to human health (EFSA, 2009) but is largely present as MT, sufficiently heat-stable to survive cooking and of similar availability to cadmium in $CdCl_2$.

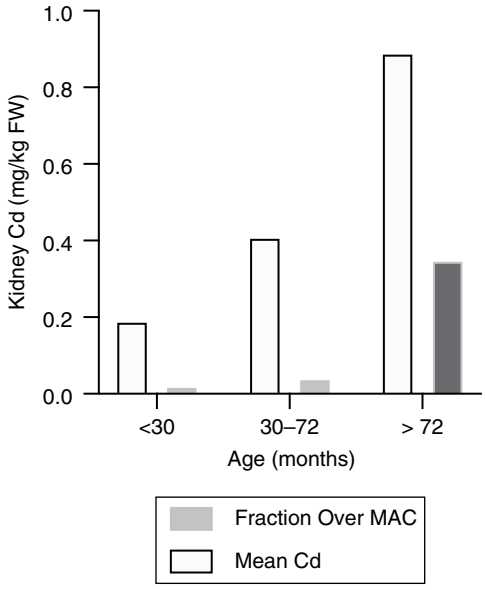

Fig. 18.6. An abattoir survey of PTE accumulation in cattle carcases confirmed that kidney cadmium (Cd) increases with age but only one third of samples from cattle over three years old exceeded the maximum acceptable concentration (MAC) of 1mg kg^{-1}FW. (Data from Brereton *et al*., 2014)

- Farm suppliers, butchers and customers are in a better position than farmers and regulators to limit entry of cadmium into the food chain.

Fluorine

Chronic endemic fluorosis in humans and farm animals was identified early in the last century in several countries (e.g. Jacob and Reynolds, 1928). Severe fluorosis in cattle during and after volcanic eruptions in Iceland has an even longer history (Roholm, 1937). Industrial contamination of herbage and agricultural use of fluorine-containing phosphates to supplement crops and animals increased the incidence of chronic fluorosis in livestock. The distribution of F in soils, plants and animal tissues, susceptibility of different animal species to chronic fluorosis and prevention of the disease became widely studied (Ranjan and Ranjan, 2015). Once the principal route of entry of F into farm environments, phosphates, became recognized and controlled, emphasis of research shifted briefly to verification of F as an essential nutrient for bodily, not just dental, health.

Sources

Pastures and crops

Most plant species have a limited capacity to absorb F from the soil, in which most is present as CaF_2. Cereals and other grains and their by-products contained only 1–3mg F kg^{-1}DM (McClure, 1949) and uncontaminated feeds are safe for farm livestock. Pastures and forages are naturally low in F unless contaminated by deposition of fumes and dusts of industrial origin or by irrigation with F-rich (often geothermal) waters (Shupe, 1980). Since the soil is usually far richer in F (>200mg kg^{-1}DM; Table 18.2) than the plant, ingestion of F-rich soil on overgrazed pastures can add significantly to F intakes (Cronin *et al*., 2000).[vi] As with all PTE, median and extreme rather than mean values should be reported to better characterize exposure.

Phosphate supplements

The principal sources of fluorine for livestock were commercial feeds that contained F-rich phosphate supplements. Fluoro-apatite rock phosphate (RP) sources varied widely[vii] until they became routinely, thermally 'defluorinated'. A variety of chemically processed P sources entered use but varied widely in P:F ratio and a minimum acceptable P:F ratio of 100:1 was set in the USA to minimize risk of fluorosis. The bones of mature animals, even without exposure, are far richer in F than soft tissues. Meat meal could contain as much as 200mg F kg^{-1}DM when it contained a high proportion of bone. Bone meals constituted a significant source, typically containing 0.7g F kg^{-1}DM (Thompson, 1980) but variable and occasionally low P:F ratios, high costs and possible transmission of zoonotic diseases lowered their use. Other components of animal origin are invariably low in F because soft tissues and body fluids rarely contain >2–4mg F kg^{-1}DM. Bovine milk and milk products contain only 1–2 mg kg^{-1}DM, regardless of fluorine intake.

Drinking water

Consumption of waters abnormally high in F, usually from deep wells or bores, has caused enzootic chronic fluorosis in sheep, cattle, goats and horses in parts of Turkey, India, Australia, Argentina, Africa and the USA. Surface waters from affected areas commonly contained <1 mg F l^{-1}, whereas bore waters often contained 5–15 mg F l^{-1} and as

much as 40 mg l^{-1} when evaporation has occurred in troughs or bore drains (Harvey, 1952). Elsewhere, drinking water is not normally a significant source, containing just 0.1–0.6 mg F l^{-1}.

Industrial and agricultural contamination

In parts of north Africa a chronic fluorosis, known as 'darmous', was caused by contamination of herbage and water with F-rich, phosphatic dusts, blown from rock phosphate (RP) deposits and quarries. Other sources of such pollution are fertilizer production, aluminium reduction, brick and tile production (e.g. Oelschlager *et al.*, 1970) and steel manufacturing. Stringent controls on industrial emissions greatly reduced (e.g. Lloyd, 1983) but did not eliminate problems (e.g. Patra *et al.*, 2000). Past mining activity raised soil F to >1000mg kg^{-1}DM (Geeson *et al.*, 1998). Prolonged use of phosphatic fertilizers containing 15–40mg F kg^{-1}DM in Australia and New Zealand raised soil fluorine to 217–454mg F kg^{-1}DM and ingestion of such soils as herbage contaminants by grazing sheep and cattle represents a potential risk of toxicity (Loganathan *et al.*, 2008).

Metabolism

The hazard presented by a particular source is determined partly by the extent to which F is absorbed and retained. Fluorine is well absorbed from natural feeds and inorganic halides. Roughages, such as hay (see Table 18.4) and lucerne pellets (Table 18.5) can contain F that is as available as that in soluble NaF: the apparent availability (p. 26; AA_F) for lucerne pellets, given to sheep, was 0.76 (Grace *et al.*, 2003a, b). Fluorine in relatively insoluble compounds, such as bone meal, CaF_2, cryolite (Na_3AlF_6), RP and defluorinated RP (DFP), is less well absorbed. Fluorine in RP, DFP and dibasic calcium phosphate (DCP) was 70%, 50% and 20% less available than F in NaF, as judged by skeletal F accretion in lambs given 60mg F kg l^{-1}DM as each source for ten weeks (Suttie, 1980), confirming the results of an earlier comparison between DFP and DCP (Hemingway, 1977). The AA_F in two F-enriched soils from New Zealand was roughly half the value found in lucerne pellets in sheep and cows (Grace *et al.*, 2003a, b) (Table 18.5), i.e. similar to that of the source of contamination, fertilizer phosphates, but less than that of NaF. Flume and brick dusts also contain F of relatively low availability (Oelschlager *et al.*, 1970).

Some 20–30% AA_F is excreted via the urine (Grace *et al.*, 2003a). Kidneys of exposed animals are richer in F than any other soft tissue but still contain <3mg F kg^{-1}DM in cattle (Shupe, 1980) and sheep (Grace *et al.*, 2003a). Fluorine is largely retained in the skeleton but there is a negative exponential relationship

Table 18.4. Mean alkaline phosphatase activity and bone fluoride content in heifers given different amounts and forms of fluoride (F) for 588 days. (Shupe *et al.*, 1962)

	F content (mg kg^{-1}DM)			Phosphatase activity[a]	
Treatment	Diet	Metatarsal	Ribs[b]	Metatarsal	Ribs[b]
Low-F hay	10	344	423	73	85
Low-F hay + CaF_2	69	931	1287	104	116
Low-F hay + NaF	68	1880	2753	146	248
High-F hay	66	2130	2861	163	191

[a]mg P hydrolysed in 15 min g^{-1} bone. [b]The 11th, 12th and 13th ribs.

Table 18.5. Contrasting apparent absorption (AA_F, %) of fluoride (F) from lucerne pellets, with or without added sodium fluoride (NaF), and soils contaminated by fertilizer F in young sheep and cows (from Grace *et al.*, 2003a, b). Tolerance of ingested soil F is underestimated by standards based on the higher AA_F of NaF (see Table 18.1).

	Source of F			
Species	Lucerne	+ NaF	+ Low-F soil	+ High-F soil
Sheep	76 (9)	69 (69)	32 (69)[a]	41 (86)
Cows	61 (110)	–	44 (450)	43 (1560)

[a]Daily fluorine intakes (mg) given in parentheses.

between F intake and rib F at a given exposure time in cattle (Fig 18.7) and sheep (Masters *et al.*, 1992). The relationship with serum F in sheep (Grace *et al.*, 2003a) and cows (Grace *et al.*, 2003b) also follows the law of diminishing returns (Fig. 18.7). Exposure of pregnant and lactating animals raises blood F in the neonate and milk F in the dam but increases are far greater in the dam's blood.[viii]

Toxicity

Fluorine is largely a cumulative poison and clinical signs of toxicity may not appear for many weeks or months in exposed animals. The mechanisms of chronic toxicity are unclear but may involve enzyme inhibition and substitution of fluoride ions (F^-) for hydroxide (OH^-) ions in soft tissues (NRC, 2005). Animals are protected by two physiological mechanisms, excretion in urine and deposition in bones or eggs. During a clinically 'silent' (latent) period in dairy cows, neither milk production (Suttie and Kolstad, 1977) nor digestibility and utilization of nutrients was affected (Shupe *et al.*, 1962). In other species, there may be transient subclinical changes in sensitive organs, such as the testes, and sperm development may be impaired in poultry (Mehdi *et al.*, 1983) and rabbits (Kumar and Susheela, 1995). Deposition of F in the skeleton proceeds more slowly as saturation is reached. Saturation is indicated by values of 15–20 g F kg^{-1} dry bone in cattle (30–40 times greater than normal) and 2.3 g F kg^{-1} in hens (five times normal). Once saturation is reached, fluorine floods into susceptible soft tissues, causing metabolic breakdown and death. Chronic skeletal abnormalities are usually apparent before this terminal stage is reached.

Tolerance to dietary fluorine

Direct signs of fluorosis are rare in newborn or suckling animals because placental and mammary transfer of F is limited (Forsyth *et al.*, 1972; Wheeler *et al.*, 1985). In weaned and adult animals, general undernutrition accentuates the toxic effects of fluorine (Suttie and Faltin, 1973). Whatever the measure of harm, be it clinical or subclinical, tolerance of dietary F depends largely on:

- species and age of the animal;
- chemical form of ingested fluorine;
- severity of contamination;
- duration and continuity of exposure; and
- whether the skeleton is in a state of net calcium accretion or resorption.

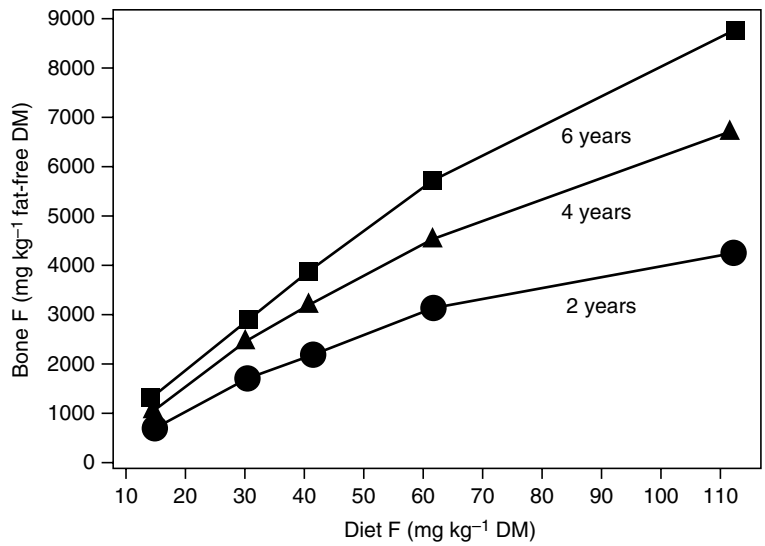

Fig. 18.7. Fluorine concentrations in the skeleton of dairy cows increase curvilinearly as dietary F concentration and duration of exposure increase. (Data from Shupe, 1980)

Pigs and Poultry

Pigs and poultry tolerate exposure well (Table 18.6).[ix] Exposure of hens to 100 mg F, as NaF, kg^{-1}DM, caused large increases in tibia F but little accumulation elsewhere and no fall in egg production; 1300mg F kg^{-1}DM raised eggshell, kidney, liver, egg yolk, plasma and muscle F, in decreasing order (Hahn and Guenther, 1986). Early moulting of feathers occurred, slight diarrhoea was induced and appetite and egg production decreased by 50% but mortality did not increase. Tolerance of laying hens may be attributable to the safe deposition of F in each egg laid but hatchability may be impaired.

Sheep and cattle

Ruminant species are relatively intolerant of exposure but tolerance is more variable than indicated by broad generalizations, such as that given in Table 18.6.[xi] Sheep consuming artesian bore water, containing 5 mg F l^{-1}, developed severe dental abnormalities and other signs of fluorosis in the hot climate of Queensland (Harvey, 1952) but in cooler conditions of South Australia, where little water is drunk during winter, no ill effects were seen in mature sheep given 20 mg F l^{-1} as NaF (Peirce, 1954). A seasonal peak in F ingestion in winter by pregnant ewes, grazing pastures contaminated by past mining, can be followed by outbreaks of lameness and stiffness in lambs (Geeson *et al.*, 1998).

- During early lactation, skeletal F probably released as bone calcium is resorbed (p. 58): secretion of fluorine in milk then exposes the suckling at risk.
- Tolerances of the the adult will be increased by physiological (lactation) and nutritional (lush pasture growth in spring) interruptions to exposure.
- Sheep and cattle will tolerate twice as much F ingested as phosphate-contaminated soil and DFP than in herbage, judging from respective availabilities (p. 494).

Table 18.6. Tolerance of fluoride in feed and water for different species[x] (Ranjan and Ranjan, 2015).

Species	Fluoride in feed (mg kg^{-1}DM)	Fluoride in water (mg l^{-1})
Beef and dairy heifers	30	2.5–4
Mature dairy cattle	30	3–6
Mature beef cattle	40	4–8
Sheep	50	12–15
Horse	60	4–8
Pig	70–100	5–8
Poultry	100	10–13

When compared on the basis of total yearly fluorine intake, skeletal F in cattle can be similar for continuous and intermittent exposure (Suttie, 1980). However, intermittent exposure can be the more harmful in terms of dental health and lameness: in tissue F increases rapidly when intakes are high (Suttie *et al.*, 1972).

Clinical signs of chronic fluorosis in ruminants

The need to combine clinico-pathological information and biochemical data in a flexible framework when diagnosing mineral imbalances is nowhere more apparent than in the diagnosis of fluorosis in dairy cattle. Table 18.7 summarizes the exhaustive studies of Shupe (1980) in cattle and illustrates the distinctive contribution that choice of diagnostic criterion can make: the criteria are broadly applicable to sheep:

- Incisor score indicates severity of past daily exposure.
- Fluorine in milk or blood indicates current intensity exposure.
- Fluorine in bone and urine, molar and hyperostosis score indicate the duration as well as intensity of exposure.

Each criterion can vary widely between individuals, with clinico-pathological features (i.e. the most important in welfare and economic terms) the most variable. Dental lesions are only seen in young animals exposed to excess F *before* the eruption of permanent teeth, which varies from 12 to 30 months of age from first incisors to last molars in sheep. Teeth become modified in size, shape, colour, orientation and structure because fluorine impairs the function of ameloblasts (enamel-forming cells) and abnormal enamel matrix fails to calcify (Shearer *et al.*, 1978a, b; Suttie, 1980). Incisors become pitted and blackened with erosion of the hypoplastic enamel. Molars become abraded and pulp cavities may become exposed due to fracture or wear. The uneven molar 'table' impairs mastication of food and is particularly debilitating (Suttie, 1980). There is little new information since reviews by Shupe (1980) and Suttie (1980). Exposure of immature stock to excess F decreases endochondral bone growth but the predominant lesion in adult stock is excessive periosteal bone formation (hyperostosis).[xii] Exostoses of the jaw and long bones become visible in severely exposed animals and joints may become

Table 18.7. A guide to the diagnosis of fluorosis in dairy cattle based on chronic experimental exposure studies and field cases (after Shupe, 1980).

		Fluorosis state				
	Age (years)	Normal	Marginal	Mild	Moderate	Severe
F in diet (mg kg^{-1}DM)[a]	–	>15	15–30	30–40	40–60	60–109
Incisor score	–	0–1	0–2	2–3	3–4	4–5
F in milk (mg l^{-1})[a]	–	<0.12	<0.12	0.12–0.15	0.15–0.25	>0.25
F in blood (mg l^{-1})[a]	–	<0.30	<0.30	0.3–0.4	0.4–0.5	0.5–0.6
F in rib bone[b] (g kg^{-1} fat-free DM)	2	0.4–0.7	0.71–1.60	1.6–2.1	2.1–3.0	3.0–4.2
	4	0.7–1.1	1.14–2.40	2.4–3.1	3.1–4.5	4.5–6.6
	6	0.65–1.22	1.22–2.80	2.8–3.8	3.8–5.6	5.6–8.7
F in urine (mg l^{-1})[a]	2	2.3–3.8	3.8–8.0	8.0–10.5	10.5–14.7	14.7–19.9
	4	3.5–5.3	5.3–10.3	10.3–13.3	13.3–18.5	18.5–25.6
	6	3.5–6.0	6.0–11.3	11.3–14.8	14.8–21.0	21.0–30.1
Molar score	2	0–1	0–1	0–1	0–1	0–3
	4	0–1	0–1	0–1	1–2	1–4
	6	0–1	0–1	0–1	1–3	1–5
Periosteal hyperostosis score	2	0	0–1	0–1	0–2	0–3
	4	0	0–1	0–1	0–3	0–4
	6	0	0–1	0–2	0–4	0–5

[a]Multiply by 52.6 to obtain values in μmol l^{-1} or kg^{-1}DM.
[b]To obtain approximate values in rib ash, multiply by 1.67 (assuming 60% ash in sample); since rib and tail vertebrae values are similar on an ash basis, the same factor is required for generating values from fat-free DM to vertebral ash; metacarpal and metatarsal bones contain 25% less F than rib on a fat-free DM basis.

thickened and ankylosed, due to calcification of tendons at their points of attachment. Stiffness and lameness make movement difficult and painful, which restricts foraging, slows growth in young cattle and reduces milk yield and fertility (Harvey, 1952; Phillips and Suttie, 1960). Symptoms are less prominent in young and pen-fed animals.

Biochemical evidence of fluorine exposure

Bone and tooth composition

Whole bones commonly contain 0.2–0.5g F kg^{-1} and values rarely exceed 1.2g kg^{-1}fat-free (ff) DM but normal teeth contain much less (<0.2 g). Dentin contains more F than the less metabolically active enamel and distribution varies within the fluorotic tooth (Shearer *et al.*, 1978b). Fluorine is incorporated more rapidly into: active areas of bone growth than static regions (epiphyses > shaft); cancellous bones (ribs, vertebrae and sternum) than compact cortical bones (metacarpals) (see Table 18.7) and surface areas (periosteal and endosteal) than inner regions of the shaft. Differences in bone F within and among bones (and teeth) and between results expressed on different bases influence assessment of exposure. For example, it was concluded from the lack of effect of short-term exposure to soil F on metacarpal F (625 to 1625mg F kg^{-1}DM), that fertilizer-enriched soils of New Zealand presented no fluorosis hazard to seven-year-old dairy cows (Grace *et al.*, 2003b). However, removal of fat and organic matter raises tail bone F, by approximately 50% (Suttie, 1967). Furthermore, rib F could have contained two- to threefold more F than metacarpals on a ffDM basis, changing the assessment of risk to mild/marginal (Table 18.7). In dairy cattle, experimental exposure raised compact bone F to >5.5 g kg^{-1} and cancellous bone F to >7.0g kg^{-1}ffDM: values of 4.5–5.5g F kg^{-1}DM indicate marginal status (Suttie *et al.*, 1958). Bovine tail vertebrae and rib F values are similar on an ash basis and values of 4–6 g F kg^{-1} ash were associated with lameness in the UK (Burns and Allcroft, 1962); tail bones can easily be biopsied. Toxic thresholds for cancellous bones of sheep may be 7g F kg^{-1}ffDM or 11g F kg^{-1}ash (Puls, 1994). The Ca:P ratio of fluorotic bone is normal but Mg is high and there may be a precipitation as CaF_2.

Urine

In unexposed sheep and cattle, urine rarely contains >10 mg F l^{-1}, whereas long-term exposure in dairy cows raised urine F to 20–30 mg l^{-1} in borderline toxicity and > 35 mg F l^{-1} in overt toxicity

(Suttie *et al.*, 1961; Shupe *et al.*, 1963a). Fluorine from the skeleton can be excreted in urine after exposure, particularly during periods of bone resorption (e.g. during early lactation). High urinary F therefore reflects either current or previous exposure and is easily measured with an ion-specific electrode. Urine samples are best taken in the morning on more than one occasion and results should be expressed on a specific gravity or creatinine basis (Shupe, 1980).

Blood constituents

Plasma F is higher than whole blood F and reflects current rather than cumulative F intake in sheep (Milhaud *et al.*, 1984; Grace *et al.*, 2003a) and cattle (Grace *et al.*, 2003b). During formation, tooth F is sensitive to small changes in plasma F: when the latter exceeds 0.5 mg l^{-1}, severe dental lesions appear in young cattle; with 0.2–0.5 mg l^{-1}, damage is less severe (Suttie *et al.*, 1972). In a long-term study of continuously or periodically exposed dairy cows, plasma F increased from 0.1 to 1.0mg l^{-1} (Suttie *et al.*, 1972). Good correlations between F intake and serum F in short-term studies do not indicate skeletal F status because: serum F reaches a plateau while bone F continues to increase (Grace *et al.*, 2003a, b). Furthermore, plasma F can show marked diurnal variations. In lambs given up to 2.5mg F kg^{-1}LW in a milk diet, plasma F reached 0.6 mg l^{-1} without signs of ill-health (Milhaud *et al.*, 1984), while in pregnant ewes a similar value was associated with marginal toxicity (Wheeler *et al.*, 1985). However, both studies were of short duration (4–8 months). Exposure of 14-month-old lambs for 55 days to 60mg F kg^{-1}DM caused no dental or skeletal abnormalities, although serum F was maintained at about 0.3 mg l^{-1} for the last 30 days (Grace *et al.*, 2003a). Serum alkaline phosphatase activity is increased in fluorotic chicks (Motzok and Branion, 1958), cows (Shupe *et al.*, 1962) and heifers but activities were raised in exposed buffalo without clinical abnormalities (Madan *et al.*, 2009) and are affected by many mineral imbalances and bone diseases.

- Serum F reflects current fluorine intake rather than cumulative exposure.

Treatment and prevention of fluorosis

Both the dental and skeletal lesions of chronic fluorosis are essentially irreversible, the latter being beyond the scope of remodelling. If livestock cannot be removed from the source of exposure, steps can be taken to delay progression of the disease. Aluminium salts protect against high fluorine intakes in sheep (Becker *et al.*, 1950) and cattle (1% as $AlSO_4$) (Allcroft *et al.*, 1965), apparently through reducing fluorine absorption from the intestinal tract. Similar additions of $AlSO_4$ greatly reduced fluorosis in hens given 1300 mg F kg^{-1}DM and reduced fluorine accretion, particularly in eggshell, liver and kidney (Hahn and Guenther, 1986). Borate and silicate supplements limited the effects of excess fluorine on the skeleton of pigs (Seffner and Tuebener, 1983).

Essentiality

Incidence of dental caries in the human populations is significantly higher where water supplies were virtually free from fluoride than in areas where water contains 1.0–1.5 mg F l^{-1}. Lifetime exposure to water with >1.5 mg F l^{-1} increased trabecular bone volume and osteoid when compared with exposure to low F waters (<0.3 mg l^{-1}) in Finland (Arnala *et al.*, 1985). Increases in bone mass induced by moderate F exposure are not accompanied by increased bone strength (Carter and Beaupré, 1990) but may lessen the impact of bone resorption during inactivity and proneness to bone fractures (Li *et al.*, 2001): beneficial effects rather than evidence of essentiality. Neither dental caries nor bone density appear to be affected in shorter-lived farm animals and there is as yet no evidence that fluorine performs any specific essential function.[xiii] Some health benefits may have been pharmacological in nature, fluoride improving iron utilization from a diet, marginal in Fe (Tao and Suttie, 1976; Ruliffson *et al.*, 1963) or changing the gut microbiome. All natural diets are likely to contain sufficient F to meet any dietary requirement of livestock.

Lead

Lead exposure was one of the most common causes of poisoning in farm livestock, with cattle most affected (Blakley, 1984) and some 50 incidents reported annually in the UK (VIDA, 2007). Young calves are particularly vulnerable: 50.9% of lead-poisoning cases in bovines in western Canada were in animals <6 months of age and

from dairy rather than in beef breeds (Blakley, 1984). These apparent effects of species, breed and age reflect three risk factors: ease of access to point sources of lead (e.g. old batteries, tins of paint), the trapping of heavy objects in the reticulum and lack of a functional and protective rumen microflora in sucklings. Most cases of lead poisoning are acute. Chronic lead exposure in farm livestock is rare but effects on tissue Pb are still receiving attention, due to concerns over subclinical effects of raised blood Pb in young children. Lead is commonly regarded as a cumulative poison with risk primarily determined by source of lead and duration of exposure (NRC, 2005) but this is far from true, particularly for ruminants.

Sources of chronic exposure

Soil Pb varies widely and concentrations are much higher in the UK than in New Zealand (Table 18.2). Mining activity (Moffat, 1993; Geeson *et al.*, 1998; Pareja-Carrera *et al.*, 2014) and the dispersal of sewage sludge (Suttle *et al.*, 1991; Hill *et al.*, 1998a, b; Gaskin *et al.*, 2003; Tiffany *et al.*, 2002) can raise soil Pb to ≥500mg kg^{-1}DM. Although soils contain some lead in EDTA-extractable forms (Archer and Hodgson, 1987), lead is poorly taken up by plants and pastures and crops generally contain <0.3mg kg^{-1}DM (Longhurst *et al.*, 2004). The principal threat to livestock and consumers comes from ingestion of contaminated soil by grazing livestock (Abrahams and Steismajer, 2003). Until the addition of lead to petrol was banned in 1985, automotive exhaust fumes caused local accumulations beside major roads and contributed to widespread atmospheric deposition, which still affects vast areas. These include upland peat soils of Scotland, in which levels of >50mg Pb kg^{-1}DM were frequently recorded (Bacon *et al.*, 1992). Dispersal of municipal sewage sludge on agricultural land is another, an ongoing source of anthropogenic contamination (Hill *et al.*, 1998a ,b). When Pb-contaminated pasture is ensiled, downward migration of Pb in the silo can lead to fourfold increases in silage Pb in the lowermost layers: 119 mg Pb kg^{-1}DM was reported in a case of clinical lead toxicity in a dairy herd (Coppock *et al.*, 1988). Calcium supplements can be rich in Pb (Ross, 2000).

Dietary effects on lead availability

Sheep and rabbits apparently absorbed (AA_{Pb}) only 0.01 of ingested Pb (Blaxter, 1950) and a negative AA_{Pb} was reported for lambs given Pb-contaminated grass nuts (Hill *et al.*, 1998a). However, an AA_{Pb} was 0.15 for a large supplement, providing 1g Pb kg^{-1}DM, as lead acetate (PbAc) (Pearl *et al.*, 1983) in sheep: the basal diet consisted of 56% corn, no long roughage, was low in Ca and probably low in S. Raising dietary Ca from 2.5 to 5.0 g kg^{-1}DM reduced AA_{Pb} from 0.11 to 0.06 in lambs (Morrison *et al.*, 1977). There is also an inverse relationship between dietary S and A_{Pb} that is only manifested in ruminants. In lambs, raising dietary S from 0.15 to 2.3 or 3.8g kg^{-1}DM increased mean survival time from 6 to 15 or 30 weeks, respectively, when 200mg Pb kg^{-1}DM was added, as lead acetate (PbAc), to the diet (Quarterman *et al.*, 1977). Precipitation of unabsorbable lead sulfide (PbS) in the sulfide-rich rumen at higher S intakes probably reduced A_{Pb}.[xiv]

Turning to non-ruminants, some lead is absorbed inadvertently through an affinity for calbindin (Fullmer *et al.*, 1985), which is downregulated when dietary Ca is increased (p. 72). Lead absorption can also be decreased by raising dietary Ca or P in avian species (Scheuhammer, 1987), probably through the formation of insoluble and unavailable Pb complexes with phytate. Lead also binds to divalent metal transporter 1, which is induced by iron deprivation (p. 329) and cadmium exposure: AA_{Pb} increases in iron deficiency (Morrison and Quarterman, 1987) and tissue Pb is raised in pigs by simultaneous exposure to Pb and Cd (Phillips *et al.*, 2003).

The Pb sources encountered by livestock vary greatly in availability. A soil enriched by lead-mining activity had a relative availability of 58–78% when compared with PbAc in young pigs (Casteel *et al.*, 1997). In other contaminated soils, relative availability varied from 3% to 86% for the pregnant pig (Casteel *et al.*, 2001). Lead in sewage and harbour sludges was about half as available as that in PbAc to lambs (Van der Veen and Vreman, 1986; cited by NRC, 2005). Lead in sewage sludge (Stark *et al.*, 1998), like that in roadside soils (Suttle, unpublished data), is extensively extracted by EDTA but AA_{Pb} was only 0.1–0.3, when added to grass nuts and fed to lambs (Hill *et al.*, 1998a). However, AA_{Pb} will have been a characteristic of the diet, which, uncontaminated (p. 511), had an AA_{Pb} of zero!

- Lead is thus poorly but variably absorbed by non-ruminant and ruminant species.
- Variations in dietary Ca, Cd and Fe influence lead availability and, hence, toxicity.
- Dietary S supplements strongly inhibit A_{Pb} and thus reduce toxicity in ruminants.

Metabolism

Transport and tissue distribution

Some 90% of absorbed Pb is taken up by red blood cells, while the remainder is largely bound to albumin (NRC, 2005). Eventually, lead is transferred to and firmly bound by cytosolic-binding proteins and MT, distribution varying widely between tissues. Blood and liver Pb show early rapid increases in lambs at a constant daily rate of exposure before reaching a plateau (Pearl *et al.*, 1983; Brebner *et al.*, 1993). After seven weeks intense exposure in lambs to 1g Pb kg^{-1}DM, kidney Pb and bone Pb increased markedly (Fig 18.8a), but liver, brain and bone Pb showed negative curvilinearity (Fig. 18.8b).

- Animals adapt to lead exposure and blood and liver Pb become poor indices of body lead burden and long-term risk of toxicity.

With lower, environmentally significant exposure, kidney Pb increased linearly with duration of exposure from a given sludge-amended, soil source (Stark *et al.*, 1998). Lead does not accumulate in soft tissues at low levels of exposure but does accumulate in bones of growing animals, particularly at growth point, and at times of rapid bone turnover in poultry. Four- to fivefold greater increases in bone Pb were reported in laying than in non-laying birds exposed to Pb (Finley and Dieter, 1978). Calcium and phosphorus supplements delayed release of Pb from the skeleton when exposure ceased in young rats (Quarterman *et al.*, 1978).

Maternal transfer

When cows (Pinault and Milhaud, 1985) and ewes (Carson *et al.*, 1974; Suttle *et al.*, 1997) were exposed to lead during gestation, raised liver and/or blood Pb in the newborn confirmed placental transfer. Tissue Pb was higher in gestating than in barren pigs but higher in the mother than in her unborn litter (Casteel *et al.*, 2001).

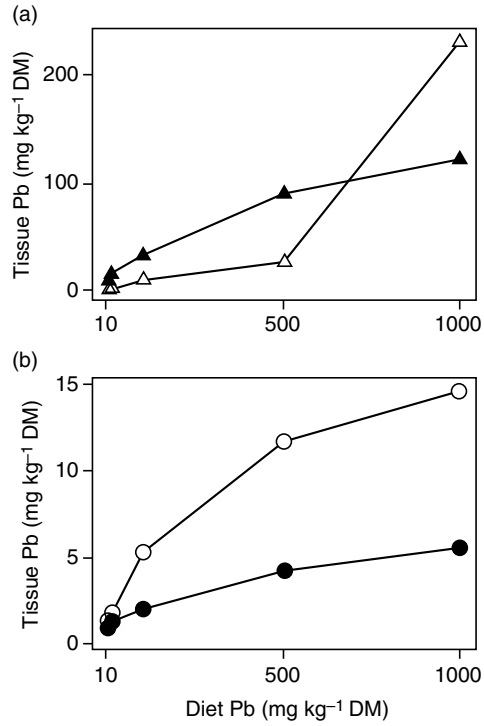

Fig. 18.8. Tissue lead reaches a plateau in soft tissue other than kidney as dietary Pb and duration of exposure increases (see text for the latter effect). Limits to liver accumulation occur soonest and partition shifts towards the kidney at very high exposures. (Data from Fick *et al.*, 1976). (Δ, kidney; ▲, bone; ○, liver; ●, brain)

Raised milk Pb indicated that maternal transfer continues during lactation and, while increases are small, Pb is well absorbed from milk (Morrison and Quarterman, 1987) and risk to the suckling or other consumer will be enhanced. Lead that has accumulated in the skeleton during previous exposure may, like F (p. 496), be released and secreted in milk during lactation (Fig. 18.9).

Toxicity

Acute toxicity is characterized by gastrointestinal haemorrhage, anaemia, liver necrosis and kidney damage; digesta and faeces Pb may be high. Diagnosis is confirmed by high kidney Pb (Table 18.8) and histological evidence of kidney damage, *post mortem*. Surviving cohorts are likely to have raised blood Pb (Table 18.8) and early dysfunction is indicated by a fall in erythrocyte aminolaevulinic acid dehydratase (ALAD) (Flora *et al.*, 2019; Bratton

Table 18.8. Marginal bands for assessing lead (Pb) exposure and likelihood of chronic toxicity from Pb concentrations in diet, blood and tissues; normal (N), high (H) and toxic (T) levels are given. Proximity to bold tissue figures indicates high risk of toxicity: with dietary Pb, risk is indicated by the product of concentration and duration of exposure

		Diet	Bone	Liver	Kidney	
Group	Status	(mg kg^{-1}DM)[b]		(mg kg^{-1}FM)[b]		Blood (mmol l^{-1})[a]
Cattle and sheep	N	1–6	1–7	0.1–0.5	0.1–0.5	0.005–0.25
	H[c]	20–1000	30–**75**	0.8–**2.0**	0.7–**4.0**	0.5–**1.5**
	T[d]	>2000	>75	>8.0	>20.0	>2.0
Pigs	N	2–8	1?	0.2–0.5	0.2–1.2	0.2–1.5
	H[c]	20–750	100–**500**	5.0–**35.0**	5.0–**25.0**	1.5–**4.8**
	T[d]	>750	>500	>35.0	>25.0	>4.8
Poultry	N	1–10	<50	0.1–5.0	0.1–5.0	0.2–1.0
	H[c]	20–200	150–**400**	5.0–**18.0**	5.0–**20.0**	1.0–**2.0**
	T[d]	>200	>400	>18.0	>20.0	>2.0

[a]Multiply by 207.2 to obtain values in µg l^{-1}.
[b]Multiply by 4.826 to obtain values in µmol kg^{-1}DM or FM.
[c]Values within the 'high' category indicate unusually high levels, but not high enough to harm animal health.
[d]Values in 'toxic' category will cause harm but not necessarily death.

et al., 1986) and rise in zinc protoporphyrin (ZPP) concentrations. However, ALAD activity is slightly reduced by heavy exposure to other metals such as cadmium and selenium.

In chronic lead exposure, neither gastrointestinal abnormalities nor anaemia are seen. However, osteoporosis may accompany hydronephrosis, particularly in young lambs (Butler *et al.*, 1957; Quarterman *et al.*, 1977). The first sign of malaise is loss of appetite. 'Lead lines' (probably porphyrin pigments arising from erythrocyte breakdown) may eventually be seen in the gingiva adjoining the teeth. Suckling lambs in a former lead-mining area were particularly vulnerable to lead-induced osteodystrophy between six and ten weeks of age (Butler *et al.*, 1957) and subsequent work in the same area found that blood Pb peaked at that time (Fig. 18.9) (Moffat, 1993). A similar peak in blood Pb occurred in offspring of housed ewes on constant lead intakes for over one year and coincided with a peak in milk Pb in mid-lactation (Suttle *et al.*, 1997). Mobilization of lead from a demineralizing maternal skeleton (p. 58) may have been responsible.

- Risks of lead toxicity to both mother and lamb may increase in early lactation.
- Conversely, factors that increase bone mineralization, such as dietary supplements of calcium and phosphorus, may reduce risk by capturing lead in an increased volume of newly formed bone.

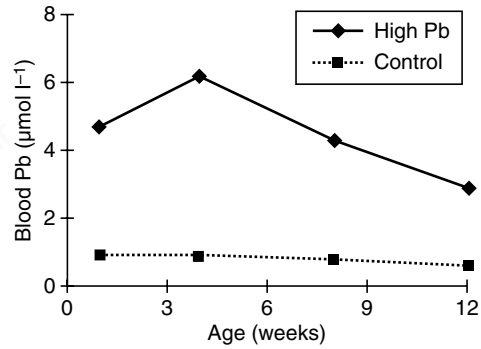

Fig. 18.9. Outbreaks of osteodystrophy in suckling lambs in former lead-mining areas are preceded by high blood-lead concentrations: efficient absorption of Pb from milk and ingested Pb-rich soil may be responsible (Moffat, 1993).

Tolerance of chronic exposure

In both acute and chronic toxicity, maximum tolerable oral doses of lead vary widely in all species and are determined by:

- developmental status;
- source;
- species; and
- dietary composition.

Pigs and poultry

Tolerance is minimal in quail chicks, in which 1mg Pb kg^{-1}DM, given from hatching, reduced egg production

at sexual maturity (Edens and Garlich, 1983). After sexual maturity, 10mg Pb kg^{-1}DM was required to retard growth and 200mg Pb kg^{-1}DM to lower egg production towards the end of lay. Tolerance was probably increased through export of lead in the eggshell and yolk: blood Pb was lower in laying hens than in cockerels given the same exposure (Mazliah *et al.*, 1989). Laying ducks tolerated the equivalent of approximately 120–240mg kg^{-1}DM (Jeng *et al.*, 1997). In weanling pigs, 10mg Pb kg^{-1}DM reduced growth rate (Fig. 18.1a) and harmed offspring of exposed sows with concurrent exposure to cadmium, tolerance was roughly halved (Fig. 18.1b).

- Reduced supplementation with Ca, P, Mn and Zn, recommended elsewhere in this book, may increase lead accretion and reduce tolerance.

Pre-ruminants and ruminants

- Tolerance cannot be adequately represented by a fixed concentration, either before or after weaning.

Bolus doses of PbAc that were lethal for milk-fed calves, were apparently harmless to weaned calves of the same age (Zmudzki *et al.*, 1984). Less-soluble sources such as $PbCO_3$, more representative of sources encountered on farms, were better tolerated before weaning[xv] and tolerance may be higher on milk replacers based on vegetable protein than on casein. Tolerance rises abruptly after weaning, with development of a fully functioning rumen. Weaned calves given 1g Pb kg^{-1}DM as $PbSO_4$ showed only a slight reduction in appetite and growth after four weeks (Neathery *et al.*, 1987) and weaned lambs showed no adverse effects after similar exposure for 6–12 weeks (Fick *et al.*, 1976; Pearl *et al.*, 1983): tolerance is probably reduced on diets lower in S.[xvi] In dairy cows, blood Pb peaked at 350 μg l^{-1} and sooner when exposure began during lactation than during gestation.

- Tolerance may be reduced during early lactation because less Pb is deposited in bone.

Tolerance of anthropomorphic sources of lead will be greatly influenced by dietary composition and presence of other PTE, notably Cd (Stark *et al.*, 1998). Reductions in atmospheric deposition of sulfur may have increased lead accretion, at a given level of lead contamination on pasture, since the last century.

Subclinical toxicity

Since negative associations between blood Pb and cognitive behaviour of children were first mooted in the 1960s, acceptable limits have fallen over tenfold to 50 μg l^{-1} (EFSA, 2010). Similar associations were sought in ewes and lambs. Oral exposure at 120 or 230 mg Pb day^{-1}, commencing one month prior to mating and continuing for 26 weeks and into lactation did not affect behaviour of offspring, despite increases in blood Pb from 64 to 302 μg l^{-1} (Van Gelder *et al.*, 1973). However, apparently similar exposure throughout gestation raised blood Pb in lambs to 250 μg l^{-1} and slowed learning behaviour (Carson *et al.*, 1974). In calves, normal age-related increases in ALAD were totally inhibited by as little as 0.65mg Pb kg^{-1}LW day^{-1} and accompanied by a rise in blood Pb to 911 μg l^{-1} but growth was unimpaired (Lynch *et al.* 1976b). Early reductions in ALAD were reported in cows given mixed sources of lead, equivalent to 100mg Pb kg^{-1} DM, without reducing liveweight or milk yield (Pinault and Milhaud, 1985). Increases in blood ZPP were also found, indicating that lead exposure had been sufficient to inhibit ferrochelatase activity and affect haem synthesis. Subclinical neurological effects of lead exposure may be attributable to haem-containing molecules, such as cytochromes, accumulating at learning centres in the brain (Moore *et al.*, 2006) and ionic substitution of Pb for ions essential to cell-signalling pathways (Flora *et al.*, 2019).

Livestock as sources of lead exposure for humans

Risk assessment poses essentially the same problems as those outlined for cadmium (p. 491). Lead does not accumulate in muscle during commonly encountered levels of exposure (Stark *et al.*, 1998) and there is no risk to humans from consuming carcase meat from livestock exposed to lead (Khalafalla *et al.*, 2011). There is, however, a small risk from consumption of offal and a greater risk that carcases may be rejected because lead levels in offal exceed national MAC (2 and 1mg kg^{-1} FW in liver and kidney, respectively, in Europe). Chronic exposure may raise kidney Pb above MAC. Simulation of high levels of ingestion (100g kg^{-1}DM) of Pb-rich, sludge-amended soils (containing 592 and 841mg Pb kg^{-1} DM) by lambs increased mean kidney Pb to 9.0 and 9.7mg Pb kg^{-1}DM (equivalent to nearly 2mg kg FM) (Brebner *et al.*, 1993), similar to values attained after feeding 100mg Pb kg^{-1}DM (as PbAc) (Fick *et al.*, 1976), albeit for a longer period (160 versus 84 days). Less lead accumulated in a parallel study but kidney and liver Pb were both linearly related to Pb

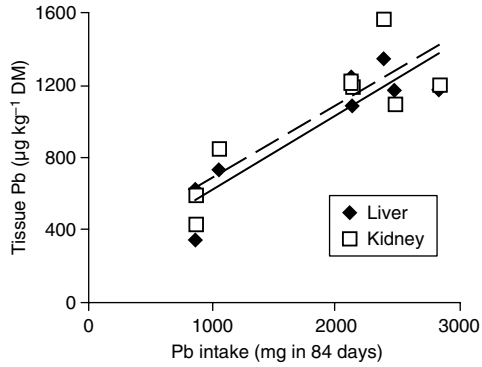

Fig. 18.10. Effects of cumulative lead intake from a sludge-amended soil on concentrations of lead in liver and kidney of lambs. (Data for two soils and two inclusion rates from Hill *et al.*, 1998b)

intake (Fig. 18.10). No increase in kidney Pb occurred either in cattle grazing on sludge-amended soils, containing 79–135mg Pb kg^{-1}DM, for eight years (Fitzgerald *et al.*, 1985) or in less-exposed ewes and lambs (Rhind *et al.*, 2005). The much higher AA_{Pb} in sewage sludge than in sludge-amended soils may be transformations in Pb speciation towards less biocactive forms (Zheng *et al.*, 2004) and implies that risks to consumers will be reduced by allowing adequate time for applied sludge to be washed into the soil.

Residual effects and bioremediation of exposure

Natural exposure will often be episodic rather than continuous and it is important to know what happens to tissue Pb when exposure ceases. The half-life of tissue Pb varies widely being shortest in the kidney and spleen (around 45 days), intermediate in blood, liver, muscle and heart (90 days) and longest in brain (150 days) in sheep. Bone Pb is exceptional in showing further substantial *increases* 45 days after cessation of exposure, but a fast decline thereafter (Pearl *et al.*, 1983).

- Simply transferring animals to uncontaminated pasture for a few months will reduce body Pb burdens.

Reducing exposure by bioremediation is a complex process (Rahman and Singh, 2020) beyond the scope of this book.

Mercury

The 1970s saw an explosion of interest in mercury toxicity following events in Minamata Bay, Japan. Industrial discharges of inorganic Hg (Hg_i) into the bay were converted to organic (methylated) mercury (Hg_o) by lower marine organisms and entered the human food chain in toxic amounts for a coastal community, heavily dependent on fish in the diet. Stringent restrictions were placed on MAC for food Hg (0.5mg kg^{-1}DM). Residual effects of long-standing exploitation of fungicidal and other toxic properties of mercury in agriculture also attracted attention. Methylation of mercury began with the origins of life on earth and is a fundamental property of anaerobic microorganisms, expressed in any environment, naturally or unnaturally endowed with mercury. The subject has been reviewed (Bernhoft, 2011) and only features pertaining to mercury toxicity in livestock and entry of mercury via edible animal products into the food chain will be considered here.

Sources

Mercury vapour is emitted by natural and anthropomorphic sources, enters the atmosphere and is returned to earth in soluble form in rainfall but some is inhaled. Soils contain little Hg (Table 18.2) and pastures and crops even less (<0.1mg Hg kg^{-1}DM). The exception is rice grown anaerobically, which accumulates methyl mercury, generated by bacterial activity in flooded and often contaminated soils (Jackson and Punshon, 2014). The principal source in normal animal diets is methyl mercury in fish meal. However, use of mercury-based fungicides for dressing cereal seed creates potentially hazardous levels (approximately 20mg Hg kg^{-1}DM) and such materials will cause toxicity if fed to cattle (Boyd, 1985) or other species. Accidental consumption of $HgCl_2$, intended for treatment of club root in brassica crops, has caused deaths in dairy heifers (Simpson *et al.*, 1997). The topical administration of Hg-containing treatments for skin infections has also resulted in deaths from mercury poisoning.

Metabolism

Absorption

Efficiencies of absorption and retention differed greatly between studies but effects of source, animal species and age are impossible to distinguish: AA_{Hgi} in weaned animals has varied from 1–3% (Kostial *et al.*, 1978) to 5–15% (Clarkson, 1987) but 45% of inherent feed Hg (5 µg kg^{-1}DM) was retained by laying hens and broilers (Zelenka and

Hedbavny, 2007). Very young, milk-fed mammals absorb Hg_i efficiently (Kostial *et al.*, 1978). Organic forms are fat-soluble and also absorbable, although rumen microbes can demethylate methylmercury (Kozak and Forsberg, 1979). An AA_{Hg} of 59% was recorded for radiolabelled methylmercury chloride in lactating dairy cows (Neathery *et al.*, 1974) and values as high as 90% have been reported for phenyl derivatives (Clarkson, 1987).

Distribution

Mercury has an affinity for sulfhydryl groups, which may explain its tissue distribution, toxicity and ability to interact with other micronutrients (Scheuhammer, 1987). Absorbed mercury is transported primarily in association with glutathione-rich erythrocytes, which are 20 times richer in Hg than plasma in exposed animals. In plasma, Hg binds to sulfhydryl groups in albumin. Mercury is avidly retained in tissues by similar associations but there is interconversion between Hg_i and Hg_o (NRC, 2005). Little ingested Hg appears in urine (1.1%) or milk (0.17%) and most (72%) is found in muscle (Neathery *et al.*, 1974). Affinity of Hg_i for cysteine-rich molecules, such as MT, can displace bound Zn and Cd (NRC, 2005) and MT probably affords protection from Hg_i but not Hg_o.

- Edible livestock products in Europe (Mastilović *et al.*, 2010) and China (Upadhyay *et al.*, 2019) contained far less Hg than respective MAC and are not a hazard for consumers.

Toxicity

Tolerance

Most incidents of poisoning involve exposure to Hg_o. When weanling pigs were given 0.5, 5.0 or 50mg Hg kg^{-1}DM as either Hg_o or Hg_i ,the least CH_3HgCl caused fatty liver degeneration and the highest was lethal after 3–4 weeks, whereas the Hg_i caused only subclinical (fatty liver) disorder after rearing to 82 kg LW (Chang *et al.*, 1977). Tolerance of Hg in raw fish was higher than anticipated at 3–8mg Hg kg^{-1}DM. No signs of toxicity were visible clinically or at *post mortem* and there were no teratogenic effects. Fish is rich in Se, which may attenuate the threat of Hg (Ganther *et al.*, 1972). Tolerance of poultry to Hg_o is said to be fivefold higher than for Hg_i (1 versus 0.2mg kg^{-1}DM) (NRC, 2005). Poultry are particularly susceptible to Hg_i toxicity: as little as 2 mg Hg_i kg^{-1}DM impaired testicular development in young quail and 8mg Hg kg^{-1}DM reduced egg fertility in young hens (Scheuhammer, 1987). Selenium supplements provided substantial protection from Hg_o and Hg_i toxicity in poultry and there is a mutual antagonism between these elements (El-Begearmi *et al.*, 1977). Concurrent exposure to Se and methylHg increased the binding of Hg to SelP (p. 372) in plasma. SelP is the major transporter for Hg and Se supplementation lowered plasma Hg and changed the tissue Hg distribution (Liu *et al.*, 2018). Vitamin E supplements also protect animals from mercury toxicity, suggesting cytotoxicity is partly caused by oxidative stress at the membrane level.

Symptoms

The symptoms of acute toxicity are similar in all farmed species exposed to Hg_o: anorexia, inability to drink, blindness, ataxia, hypersensitivity to people and sudden movements, recumbency and death. In heifers acutely poisoned by $HgCl_2$ (300–350mg kg^{-1}DM), clinical symptoms were bloody diarrhoea, excessive thirst, salivation, extreme depression and an unsteady gait (Simpson *et al.*, 1997). *Post mortem* examination usually reveals generalized congestion and haemorrhagic gastroenteritis. In chickens there is loss of appetite and signs of wing and muscle weakness; demyelination has also been reported (Scheuhammer, 1987). Subclinical effects on kidney and neurological function are of concern with regard to human health (Bernhoft, 2011) but unstudied in livestock

Diagnosis

Exposure to mercury is indicated by high values in hair (7.1), kidney (1.0) and liver (0.6mg Hg kg^{-1}DM) of pigs but not in the central nervous system after exposure to Hg_o (Chang *et al.*, 1977): higher concentrations have been found in clinically normal pigs, exposed to Hg_i. Tissue Hg must therefore be interpreted cautiously and in conjunction with pathological evidence. Exceedingly high kidney Hg was found in heifers poisoned with Hg_i (Simpson *et al.*, 1997). Diagnostic guidelines were suggested by Puls (1994).

Newer 'Essential' Elements: Hormesis or Artefact?

Evidence for 'essentiality' in goats

The suggestion that arsenic, of all elements, might be an essential dietary constituent for normal

development and health caused quite a stir when it was made at a conference in Dubrovnik in 1991. Female goat kids had been given a diet containing <35 μg As kg^{-1}DM and compared with a control group given 350 μg As kg^{-1}DM. Those not supplemented with arsenic grew less well, were less likely to conceive, had more abortions and produced less milk to nourish their young, which showed developmental abnormalities (Anke *et al.*, 1991a). The experiments were repeated 13 times, suggesting that arsenic was essential for goats on the chosen highly purified, roughage-free diet. However, in similar experiments with cadmium and nickel small supplements of both elements averted the same apparent symptoms of deprivation. In a companion paper (Anke *et al.*, 1991b), aluminium and vanadium were claimed to be essential on the same grounds. The final elements to be added to the essential list for goats were fluorine and rubidium in 1997 (Underwood and Suttle, 1999).

Evidence for essentiality in other species

Young rats maintained under rigorously clean conditions and fed highly purified diets containing <200 μg Pb kg^{-1} DM grew more slowly than a lead-supplemented group (Schwarz, 1974) and the finding was confirmed (Nielsen, 1996). Disturbances of iron metabolism and anaemia were recorded in Pb-depleted rats. Artificially reared piglets, given a diet containing 31 μg Pb kg^{-1}DM, gained 15% less weight than those supplemented with 800 μg Pb kg^{-1}DM and impaired lipid metabolism was suspected (Kirchgessner *et al.*, 1991).

The theory of hormesis

The concept that a PTE with no recognized function in mammals or birds can be essential to their development remains hard to accept, but Paracelsus, writing in the 16th century, observed that most toxic substances are beneficial when administered in small doses. This idea was developed into the concept of 'chemical hormesis' two centuries later by the German scientist Schulz (Stebbing, 1982). An attempt has been made to standardize dose–response curves by allowing for hormesis (Calabrese and Baldwin, 1998). Evidence for hormesis is much stronger for single-cell (e.g. yeast) cultures than for mammals because it is far easier to contrive and test a wide range of concentrations.

Microbes and hormesis

Adaptation to small doses of a potentially harmful stimulant may be beneficial to organisms faced with a toxic concentration (van der Woude *et al.*, 2005). The single-celled organisms that provide most of the evidence of hormesis developed in a primeval environment, rich in PTE, and may have evolved mechanisms for responding to PTE that are redundant in mammals. Those that contribute to the gut microbiome may respond to PTE in different ways, so that microbial populations in the gut are changed by small PTE supplements to the benefit of the host.

- Additions of Ni and Cd changed the archaea structure (balance between microbial species) in anaerobic culture (Wang *et al.*, 2019).
- OBE and PTE may improve animal health by affecting the gut microbiome.

Symbiotic relationships with gut microflora are particularly important in ruminant species like the goat, in which most novel 'essentialities' have been demonstrated. The highly purified, low-roughage diets necessary to deny access to rare elements may also establish abnormal microflora along the gut, including the caecum. The caecal microbiome may be affected by OBE and PTE in non-ruminants. Addition of PTE (and OBE) to such diets may affect microbial species in ways that benefit the host, without participating in any essential metabolic pathway in the host. For example, the oral, bactericidal effects of fluorine improve the health of children's teeth when added at low concentrations to drinking water (p. 498). In embarking on the goat studies, cited above, researchers faced a dilemma: they did not know how many undiscovered 'essential' elements lay ahead and took the precaution of adding nearly every element from the periodic table to their basal diet, from beryllium to zirconium. Responses of host and/or gut microbiome may have occurred through interactions between these little-studied PTE (and OBE).

Benefits from traces of potentially toxic elements

The evidence for essentiality of PTE cited so far has involved ultratrace concentrations in basal diets (μg kg^{-1}) and small supplements. Addition of 30mg Al kg^{-1}DM to the low-Al diet of day-old chicks increased growth rate by 22% over the first 18 days of life but no clinical abnormalities in unsupplemented chicks were reported (Carlisle and

Curran, 1993). Growth of pigs tended to be increased by adding 50mg Hg kg^{-1}DM, as $HgCl_2$, to the diet and was attributed to bactericidal activity (Chang *et al.*, 1977). Growth of broiler chicks was also improved by adding Cd (Bokori and Fekete, 1996) but the response may also have been pharmacological. Lead supplements increased egg production in hens in a long-term study but only by accelerating the reproductive cycle and increasing the overall time spent in lay (Mazliah *et al.*, 1989). When given in trace amounts (mg), PTE may have beneficial pharmacological effects that have nothing to do with essentiality.

Notes

[i] Adding >2g Al kg^{-1}DM (as $AlCl_3$) to the diet of lambs decreased appetite and phosphorus absorption, particularly on a low-P diet, but no clinical abnormalities developed during 2 months' exposure (Valdivia *et al.*, 1982). The interaction may involve the formation of insoluble and unavailable $Al_2(PO_4)_3$ in the gut (NRC, 2005). Neither hypophosphataemia nor poor bone mineralization were evident during long-term soil-ingestion studies with diets adequate in phosphorus (Suttie, unpublished data) or short-term studies with cattle (Allen *et al.*, 1986). Addition of 1.3g Al kg^{-1}DM as soil with high sorptive capacity for phosphorus, increased AA_P in lambs (Garcia-Bojalil *et al.*, 1988) but adsorbed P may have been retained on soil particles in the foregut.

[ii] In south-west England, values as high as 727mg As kg^{-1}DM were recorded in an area of 722 km^2 where arsenic occurs with other metals, such as tin and copper, in geological deposits (Thornton, 1996). Mining and smelting activities, dating back centuries, distributed those metals in the environment.

[iii] In a 191-day study with lambs, the relationship between liver Cd and dietary Cd, which ranged up to 60 mg kg^{-1}DM, was curvilinear (Doyle *et al.*, 1974). In a short-term (four-day) study with Japanese quail, the proportion of an oral Cd dose retained increased with size of dose (Scheuhammer, 1987) but may have reflected cytotoxicity at high doses (NRC, 2005).

[iv] With >40mg Cd kg^{-1}DM, symptoms of toxicity were similar to those of Zn deprivation, prevented by Zn supplementation (Powell *et al.*, 1964) and included loss of appetite, poor growth, retarded testicular development and parakeratosis in sheep; paradoxically, liver and kidney Zn increased (Doyle and Pfander, 1975). Later studies with lambs, using diets marginal in zinc, showed that growth was retarded by a less severe cadmium challenge (3.4mg Cd kg^{-1}DM) and prevented by a small zinc supplement (150mg Zn kg^{-1}DM) (Campbell and Mills, 1979; Bremner and Campbell, 1980): secondary zinc deprivation had been induced.

[v] When rats on a diet marginal in Cu (2.6mg kg^{-1}DM) were given one of four cadmium levels at each of three zinc levels, each antagonist was most inhibitory towards Cu when the other was *not* added (Campbell and Mills, 1974). Cadmium was by far the more potent antagonist, with a supplement of 6mg Cd kg^{-1}DM having the same inhibitory effect as 1000mg Zn kg^{-1}DM.

[vi] Uncontaminated pastures in England contained 2–16mg F kg^{-1}DM (mean 5.3) (Allcroft *et al.*, 1965). Lucerne hay from the USA, contained a median 2.0 mg but values ranged from 0.8 to 36.5mg F kg^{-1}DM (Suttie, 1969).

[vii] European and Asian sources of rock phosphate (RP) contained 3–4% Fe, whereas Pacific and Indian Ocean island deposits usually contained only half as much and were recognized as safe supplements for all livestock (Snook, 1962). High-fluorine RP injured livestock, when used over long periods (Phillips *et al.*, 1934): 10% of 168 samples of dairy feed in the USA contained >30mg F kg^{-1}DM, and some >200mg F kg^{-1}DM (Suttie, 1969).

[viii] Adding 30 mg F l^{-1} to drinking water for ewes raised plasma F from <0.03 to 0.15–0.64mg (7.9–33.7 µmol) l^{-1}; levels in the newborn lamb rose from 9.0 to 300 µg F l^{-1} and those in ewe's milk from 0.13 to 0.40 mg F l^{-1} (Wheeler *et al.*, 1985).

[ix] No abnormalities were reported when young female pigs were fed up to 450mg F kg^{-1}DM as NaF through two pregnancies (Forsyth *et al.*, 1972). Fluoride accumulated in their newborn and four-week-old offspring, particularly if the maternal diet was normal rather than high in Ca (5 rather than 12g Ca kg^{-1}DM), but there was no 'generation build-up'. Maximum safe dietary levels were 300–400 and 500–700mg F kg^{-1}DM as RP for growing chicks and laying hens, respectively (Gerry *et al.*, 1949). Similar tolerances were reported for growing female turkeys, but 200mg F kg^{-1}DM as NaF decreased weight gain in young male poults (Anderson *et al.*, 1955). Increased proportions of dietary fat enhanced growth-retardation in exposed chicks (Bixler and Muhler, 1960), possibly by delaying gastric emptying (McGown and Suttie, 1974).

[x] Mink are the most resilient species of farmed animal studied, tolerating approximately 600mg F kg^{-1}DM (as NaF) of a fish/liver/chicken diet for over a year: 1000mg F kg^{-1}DM caused unthriftiness, hyperexcitability and mortality in adult females and their offspring, as well as dental and skeletal lesions (Aulerlich *et al.*, 1987).

[xi] With cattle exposed to NaF: dairy cows tolerated 40 mg but signs of fluorosis appeared within 3–5 years at 50mg F kg^{-1}DM (Suttie *et al.*, 1957); with 7 years' exposure, beginning as young calves, tolerance was <30 mg F kg^{-1}DM (Shupe *et al.*, 1963b); male buffalo, tolerated between 30 and 60mg F kg^{-1}DM for 90 days, based on changes in plasma Ca (↑) and P (↓) at the higher level (Madan *et al.*, 2009). Tolerance towards F-contaminated hay will also be 30–60 mg (Shupe *et al.*, 1962) but

60–100 will probably be tolerated as RP, CaF_2 and phosphate-contaminated soil, and >100mg kg^{-1}DM as DFP, because of progressively lower availabilities.

[xii] Affected bones are often visibly enlarged and have porous, chalky, white surfaces instead of an ivory sheen. In cross-section, the shaft is often irregularly overgrown and lacking a normal compact appearance. These abnormalities can occur at any age and are not necessarily accompanied by dental lesions.

[xiii] Growth of rats, kept in plastic isolators to minimize atmospheric contamination, was improved by adding 2.5 mg F, as KF, to a purified diet containing <0.04mg F kg^{-1}DM (Schwarz and Milne, 1972; Milne and Schwarz, 1974) but others failed to confirm the finding (Maurer and Day, 1957; Doberenz *et al.*, 1964). Female mice on a diet containing 0.1–0.3mg F kg^{-1}DM for two successive generations became progressively infertile and severely anaemic, unless given 50–200 mg F l^{-1}, as NaF, in drinking water (Messer *et al.*, 1974): but others could not repeat the findings (Weber and Reid, 1974).

[xiv] A decrease in AA_{Pb} with increased duration of exposure and/or dietary Pb given up to 1g Pb kg^{-1}DM (Fick *et al.*, 1976) may have been caused by Pb becoming trapped by divalent metal transporter 1 in intestinal mucosa and subsequently sloughed into the gut, in the same manner as excess iron (p. 339): such reductions may only be seen with >200mg Pb kg^{-1}DM, added as a soluble salt combined, in ruminants given a diet low in S.

[xv] A dose of 5mg Pb kg^{-1}LW day^{-1} as PbAc killed milk-fed calves within seven days and as little as 1 mg caused mortality after longer exposure (Zmudzki *et al*., 1984). A less-soluble source, $PbCO_3$, added to a milk replacer, was tolerated at between 2.6 and 3.9mg kg^{-1}LW day^{-1} for 7–12 weeks (Lynch *et al*., 1976a, b).

[xvi] Rapid weight loss was reported in some lambs given 200mg Pb kg^{-1}DM after 6 weeks with a moderate sulfur intake (Quarterman *et al.*, 1977).

References

Abrahams, P.W. and Steismajer, J. (2003) Soil ingestion by sheep grazing the metal enriched floodplain soils of mid-Wales. *Environmental Geochemistry and Health* 25, 17–24.

Alfrey, A.C. (1986) Aluminium. In: Mertz, W. (ed.) *Trace Elements in Human and Animal Nutrition*. Academic Press, New York, pp. 399–413.

Allcroft, R., Burns, K.N. and Hebert, C.N. (1965) *Fluorosis in Cattle. 2. Development and Alleviation: Experimental Studies*. Animal Disease Surveys Report No. 2, Part 2. HMSO, London.

Allen, V.G., Robinson, D.L. and Hembry, F. (1984) Effects of ingested aluminium sulfate on serum magnesium and the possible relationship to hypomagnesaemic tetany. *Nutrition Reports International* 29, 107–115.

Allen, V.G., Horn, F.P. and Fontenot, J.P. (1986) Influence of ingestion of aluminium, citric acid and soil on mineral metabolism of lactating beef cows. *Journal of Animal Science* 62, 1396–1403.

Anderson, J.O., Hurst, J.S., Strong, D.C., Nielsen, H., Greenwood, D.A. *et al.* (1955) Effect of feeding various levels of sodium fluoride to growing turkeys. *Poultry Science* 34, 1147–1153.

Anke, M., Groppel, B. and Krause, V. (1991a) The essentiality of the toxic elements cadmium, arsenic and nickel. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals (TEMA 7), Dubrovnik*. IMI, Zagreb, pp. 11-6–11-8.

Anke, M., Groppel, B. and Krause, V. (1991b) The essentiality of the toxic elements aluminium and vanadium. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals (TEMA 7), Dubrovnik*. IMI, Zagreb, pp. 11-9–11-10.

Archer, F.C. and Hodgson, J.H. (1987) Total and extractable trace element content of soils in England and Wales. *Journal of Soil Science* 38, 421–431.

Arnold, W. (1988) Arsenic. In: Seiler, H.G. (ed.) *Handbook on Toxicity of Inorganic Compounds*. Marcel Dekker, New York, pp. 79–93.

Arnala, I., Alhava, E.M. and Kauranen, P. (1985) Effects of fluoride on bone in Finland: histomorphometry of cadaver bone from low and high fluoride areas. *Acta Orthopaedica Scandinavica* 56, 161–166.

Aulerlich, R.J., Napolitano, A.C., Bursian, S.J., Olson, B.A. and Hochstein, J.R. (1987) Chronic toxicity of dietary fluorine to mink. *Journal of Animal Science* 65, 1759–1767.

Bacon, J.R., Berrow, M.L. and Shand, C.A. (1992) Isotopic composition as an indicator of origin of lead accumulation in surface soils. *International Journal of Environmental Analytical Chemistry* 46, 71–76.

Bahri, L.E. and Romdane, S.B. (1991) Arsenic poisoning in livestock. *Veterinary and Human Toxicology* 33, 259–264.

Barrasso, R., Ceci, E., Stinga, L., Tantillo, G. and Bozzo, G. (2018) Presence of cadmium residues in muscle, liver and kidney of *Bubalus bubalis* and histological evidence. *Italian Journal of Food Safety* 7, 7684.

Becker, D.E., Griffith. J.M., Hobbs, C.S. and MacIntire, W.H. (1950) The alleviation of fluorine toxicosis by means of certain aluminum compounds. *Journal of Animal Science* 9, 647.

Beresford, N.A., Crout, N.M. and Mayes, R.W. (2001) The transfer of arsenic to sheep tissues. *Journal of Agricultural Science* 136, 331–334.

Bernhoft, R.A. (2011) Mercury toxicity and treatment: a review of the literature. *Journal of Environmental and Public Health 2012*, Article ID 460508. DOI:10.1155/2012/460508

Bixler, D. and Muhler, J.C. (1960) Retention of fluoride in soft tissues of chickens receiving different fat diets. *Journal of Nutrition* 70, 26–30.

Blakley, B.R. (1984) A retrospective study of lead poisoning in cattle. *Veterinary and Human Toxicology* 26, 505–507.

Blaxter, K.L. (1950) Lead as a nutritional hazard to farm livestock. II. Absorption and excretion of lead by sheep and rabbits. *Journal of Comparative Pathology* 60, 140–159.

Bokori, J. and Fekete, S. (1996) Complex study of the physiological role of cadmium. IV. Effects of prolonged exposure of broiler chickens to cadmium. *Acta Physiologica Hungarica* 44, 57–74.

Boyd, J.H. (1985) Organomercuric poisoning in fat cattle. *Veterinary Record* 116, 443–444.

Bramley, R.G.V. (1990) Cadmium in New Zealand agriculture. *New Zealand Journal of Agricultural Research* 33, 505–519.

Bratton, G.R. (1984) Laboratory diagnosis of lead poisoning in cattle: a reassessment and review. *Veterinary and Human Toxicology* 26, 387–392.

Bratton, G.R., Childress, M., Zmudski, J., Womac, C., Rowe, L.D. and Tiffany-Castiglioni, E. (1986) Delta aminolaevulinic acid dehydratase (EC 4.3.1.24) activity in erythrocytes from cattle administered low concentrations of lead acetate. *American Journal of Veterinary Research* 47, 2068–2074.

Brebner, J. (1987) The role of soil ingestion in the trace element nutrition of grazing livestock. PhD thesis, Imperial College, University of London.

Brebner, J., Thornton, I., McDonald, P. and Suttle, N.F. (1985) The release of trace elements from soils under conditions of simulated rumenal and abomasal digestion. In: Mills, C.F., Bremner, I. and Chesters, J.K. (eds) *Proceedings of the Fifth International Symposium on Trace Elements in Animals and Man*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 850–852.

Brebner, J., Hall, J., Smith, S., Stark, B., Suttle, N.F. and Sweet, N. (1993) Soil ingestion is an important pathway for the entry of potentially toxic elements (PTE) from sewage sludge-treated pasture into ruminants and the food chain. In: Allan, R.J. and Nriagu, J.O. (eds) *Proceedings of the Ninth International Conference on Heavy Metals in the Environment*, Vol. 1. CEP, Edinburgh, pp. 446–449.

Bremner, I. (1978) Cadmium toxicity. *World Review of Nutrition and Dietetics* 32, 165–197.

Bremner, I. and Campbell, J.K. (1980) The influence of dietary copper intake on the toxicity of cadmium. *Annals of the New York Academy of Sciences* 355, 319–332.

Brereton, N., Baxter, M. and Fernandes A. (2014) Analyses of cadmium, dioxins, furans and biphenyls in meat, liver and kidney from cattle. *Report for the UK Food Standards Agency (FS102047)*. Food Standards Agency, York, UK.

Burns, K.N. and Allcroft, R. (1962) The use of tail bone biopsy for studying skeletal deposition of fluorine in cattle. *Research in Veterinary Science* 3, 215–218.

Burns, K.N. and Allcroft, R. (1966) *Fluorosis in Cattle: Occurrence, Diagnosis and Alleviation*. Report of the Fourth International Meeting, World Association for Buiatrics, pp. 94–115.

Butler, E.J., Nisbet, D.I. and Robertson, J.M. (1957) Osteoporosis in lambs in a lead mining area. 1. A study of the naturally occurring disease. *Journal of Comparative Pathology* 67, 378–398.

Calabrese, E.J. and Baldwin, L.A. (1998) Hormesis as a biological hypothesis. *Environmental Health Perspectives* 106 (Suppl. 1), 357–362.

Campbell, J.K. and Mills, C.F. (1974) Effects of dietary cadmium and zinc on rats maintained on diets low in copper. *Proceedings of the Nutrition Society* 33, 15A–17A.

Campbell, J.K. and Mills, C.F. (1979) The toxicity of zinc to pregnant sheep. *Environmental Research* 20, 1–13.

Carlisle, E.M. and Curran, M.J. (1993) Aluminium: an essential element for the chick. In: Anke, M., Meissner, D. and Mills, C.F. (eds) *Proceedings of the Eighth International Symposium on Trace Elements in Man and Animals*. Verlag Media Touristik, Gersdorf, Germany, pp. 695–698.

Carson, T.L., Van Gelder, G.A., Karas, G.C. and Buck, W.B. (1974) Slowed learning in lambs prenatally exposed to lead. *Archives of Environmental Health* 29, 154–156.

Carter, D.R. and Beaupré, G.S. (1990) Effects of fluoride treatment on bone strength. *Journal of Bone Mineral Research* 5, S177–S184.

Casteel, S., Cowart, R.P., Weis, C.P., Henningsen, G., Hoffman, E. *et al.* (1997) Bioavailability of lead to juvenile swine dosed with soil from the Smuggler Mountain NPL Site of Aspen, Colorado. *Fundamental and Applied Toxicology* 36, 177–187.

Casteel, S., Evans, T., Turk, J., Basta, N., Weis, C., Henningsen, G. and Hoffman, E. (2001) Refining the risk assessment of metal-contaminated soils. *International Journal of Hygiene and Environmental Health* 203, 473–474.

Caulfield, M.J., Shi, L., Wang, S., Wang, B., Tobery, T.W. *et al.* (2007) Effect of alternative aluminum adjuvants on the absorption and immunogenicity of HPV16 L1 VLPs in mice. *Human Vaccines* 3, 139–145.

Chang, C.W.J., Nakamura, R.M. and Brooks, C.C. (1977) Effect of varied dietary levels and forms of mercury on swine. *Journal of Animal Science* 45, 279–285.

Chowdury, B.-A. and Chandra, R.K. (1987) Biological and health implications of toxic heavy metal and essential trace element interactions. In: *Progress in Food and Nutrition Science*, Volume II. Pergamon Press, Oxford, UK, pp. 55–113.

Clarkson, T.W. (1987) Mercury. In: Mertz, W. (ed.) *Trace Elements in Human and Animal Nutrition*, Vol. 7. Academic Press, New York.

Coppock, R.W., Wagner, W.C. and Reynolds, R.D. (1988) Migration of lead in a glass-lined, bottom-loading silo. *Veterinary and Human Toxicology* 30, 458–459.

Counotte, G., Holzhauer, M., Carp-van Dijken, S., Muskens, J. and Van der Merwe, D. (2019) Levels of trace elements and potential toxic elements in bovine livers: a trend analysis from 2007 to 2018. *PLOS ONE* 14(4), e0214584. DOI:10.1371/journal.pone.0214584

Cox, K.A. and Dunn, M.A. (2001) Aluminium toxicity alters the regulation of calbindin-D28k protein and mRNA expression in chick intestine. *Journal of Nutrition* 131, 2007–2013.

Cronin, S.J., Manoharan, V., Hedley, M.J. and Loganathan, P. (2000) Fluoride: a review of its fate, bioavailability, and risks of fluorosis in grazed-pasture systems in New Zealand. *New Zealand Journal of Agricultural Research* 43, 295–321.

Cunat, L., Lanhers, M.C., Joyeux, M. and Burnel, D. (2000) Bioavailability and intestinal absorption of aluminium in rats. *Biological Trace Element Research* 76, 31–55.

Dalgarno, A.C. (1980) The effect of low level exposure to dietary cadmium on cadmium, zinc, copper and iron content of selected tissues of growing lambs. *Journal of Science in Food and Agriculture* 31, 1043–1049.

Darwish, W.S., Gad, T.M. and Imam, T.S. (2017) Lead, cadmium and mercury residues in the edible offal of sheep and goat with a special reference to their public health implications. *International Journal of Advances in Research* 5 189–199.

Doberenz, A.R., Kurnick, A.A., Kurtz, E.B., Kemmerer, A.R. and Reid, B.L. (1964) Effect of a minimal fluoride diet on rats. *Proceedings of the Society for Experimental Biology and Medicine* 117, 689–693.

Domingo, J.L., Gómez, M. and Colomina, M.T. (2011) Oral silicon supplementation: an effective therapy for preventing oral aluminum absorption and retention in mammals. *Nutrition Reviews* 69 41–51

Doyle, J.J. and Pfander, W.H. (1975) Interactions of cadmium with copper, iron, zinc and manganese in ovine tissues. *Journal of Nutrition* 105, 599–606.

Doyle, J.J. and Spaulding, J.E. (1978) Toxic and essential elements in meat – a review. *Journal of Animal Science* 47, 398–419.

Doyle, J.J., Pfander, W.H., Grebing, S.E. and Pierce, J.O. (1974) Effect of dietary cadmium on growth, cadmium absorption and cadmium tissue levels in growing lambs. *Journal of Nutrition* 104, 160–166.

Edens, F.W. and Garlich, J.D. (1983) Lead-induced egg production in Leghorn and Japanese quail hens. *Poultry Science* 62, 1757–1763.

EFSA (2006) Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

EFSA (2008) Safety of aluminium from dietary intake: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC). *The EFSA Journal* 754, 1–34.

EFSA (2009) Cadmium in food: scientific opinion of the panel on contaminants in the food chain. *The EFSA Journal* 7, 980.

EFSA (2010) Scientific Opinion on lead. EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA Journal* 8, 1–147.

El-Begearmi, M.M., Sunde, M.L. and Ganther, H.E. (1977) A mutual protective effect of mercury and selenium in Japanese quail. *Poultry Science* 56, 313–322.

European Community (2002) Directive 2002/32/EC of the European Parliament and the Council. *Official Journal of the European Commission* L140/10–L140/21.

Exley C. (2016) The toxicity of aluminium in humans. *Morphologie* 100, 51–55.

Ezedom, T. and Asagba, S.O. (2016) Effect of a controlled food-chain mediated exposure to cadmium and arsenic on oxidative enzymes in the tissues of rats. *Toxicology Reports* 3, 708–715

Fick, K.R., Ammerman, C.B., Miller, S.M., Simpson, C.F. and Loggins, P.E. (1976) Effect of dietary lead on performance, tissue mineral composition and lead absorption in sheep. *Journal of Animal Science* 42, 515–523.

Finley, M.T. and Dieter, M.P. (1978) Influence of laying on lead accumulation in Mallard ducks. *Journal of Toxicology and Environmental Health* 4, 123–129.

Fitzgerald, P.R., Peterson, J. and Lue-Hing, C. (1985) Heavy metals in tissues of cattle exposed to sludge-treated pasture for eight years. *American Journal of Veterinary Research* 46, 703–707.

Flora, G., Gupta, D. and Tiwari, A. (2012) Toxicity of lead: A review with recent updates. *Interdisciplinary Toxicology* 5, 47–58.

Food Safety Committee of Japan (2019) Risk assessment report: aluminium ammonium sulfate and aluminium potassium sulfate (food additives). *Food Safety* 7, 79–82.

Forsyth, D.M., Pond, W.G. and Krook, L. (1972) Dietary calcium and fluoride interactions in swine: *in utero* and neonatal effects. *Journal of Nutrition* 102, 1639–1646.

Fox, M.R.S. (1987) Assessment of cadmium, lead and vanadium status of large animals as related to the human food chain. *Journal of Animal Science* 65, 1744–1752.

Fuge, R.G. and Andrews, M.J. (1988) Fluorine in the UK environment. *Environmental Geochemistry and Health* 10, 96–104.

Fullmer, C.S., Edelstein, S. and Wasserman, R.H. (1985) Lead-binding properties of intestinal calcium-binding protein. *Journal of Biological Chemistry* 260, 6816–6819.

Ganther, H.E., Goudie, C., Sunde, M.I., Kopecky, M.J., Wagner, P., Ott, S.-H. and Hoekstra, W.G. (1972)

Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. *Science* 175, 1122–1124.

Garcia-Bojalil, C.M., Ammerman, C.B., Henry, P.R., Littell, R.C. and Blue, W.G. (1988) Effects of dietary phosphorus, soil ingestion and dietary intake level on performance, phosphorus utilisation and serum and alimentary tract mineral concentrations in lambs. *Journal of Animal Science* 66, 1508–1519.

Gaskin, J.W., Brobst, R.B., Miller, W.B. and Tollner, E.W. (2003) Long-term biosolids application effects on metal concentrations in soil and Bermudagrass forage. *Journal of Environmental Quality* 32, 146–152.

Geeson, N.A., Abrahams, P.W., Murphy, M.P. and Thornton, I. (1998) Fluorine and metal enrichment of soils and pasture herbage in the old mining areas of Derbyshire, UK. *Agriculture, Ecosystems and the Environment*, 217–231.

Gerry, R.W., Carrick, C.W., Roberts, R.E. and Hauge, S.M. (1949) Raw rock phosphate in laying rations. *Poultry Science* 28, 19–23.

Grace, N.D., Loganathan, P., Hedley, M.J. and Wallace, G.C. (2003a) Ingestion of soil fluorine: its impact on the fluorine metabolism and status of grazing young sheep. *New Zealand Journal of Agricultural Research* 46, 279–286.

Grace, N.D., Loganathan, P., Hedley, M.J. and Wallace, G.C. (2003b) Ingestion of soil fluorine: its impact on the fluorine metabolism of dairy cows. *New Zealand Journal of Agricultural Research* 48, 23–27.

Hahn, P.H.B. and Guenther, W. (1986) Effect of dietary fluoride and aluminium on laying hen performance and fluoride concentration in blood, soft tissue, bone and egg. *Poultry Science* 65, 1343–1349.

Harvey, J.M. (1952) Chronic endemic fluorosis of Merino sheep in Queensland. *Queensland Journal of Agricultural Science* 9, 47–141.

Hemingway, R.G. (1977) Fluorine retention in growing sheep: a comparison between two phosphorus supplements as sources of fluorine. *Proceedings of the Nutrition Society* 36, 82A.

Hill, J., Stark, B.A., Wilkinson, J.M., Curran, M.K., Lean, I.J., Hall, J.E. and Livesey, C.T. (1998a) Accumulation of potentially toxic elements by sheep given diets containing soil and sewage sludge. 1. Effect of type of soil and level of sewage sludge in the diet. *Animal Science* 67, 73–86.

Hill, J., Stark, B.A., Wilkinson, J.M., Curran, M.K., Lean, I.J., Hall, J.E. and Livesey, C.T. (1998b) Accumulation of potentially toxic elements by sheep given diets containing soil and sewage sludge. 2. Effect of the ingestion of soils historically treated with sewage sludge. *Animal Science* 67, 87–96.

Hooser, S.B. (2018) Cadmium. In: Gupta, R.C. (ed.) *Veterinary Toxicology*, 3rd edn. Academic Press, pp. 417–421,

Houpert, P., Mehennaoni, S., Joseph-Enriquez, B., Federspiel, B. and Milhaud, G. (1995) Pharmokinetics of cadmium following intravenous and oral administration to non-lactating ewes. *Veterinary Research* 26, 145–154.

Jackson, B. and Punshon, T. (2014) Recent advances in the measurement of arsenic, cadmium, and mercury in rice and other foods. *Current Environmental Health Reports* 2, 15–24.

Jacob, K.D. and Reynolds, D.S. (1928) The fluorine content of phosphate rock. *Journal of Official Agricultural Chemists* 11, 237–258.

Jeng, S.L., Lee, S.J., Liu, Y.F., Yang, S.C. and Liou, P.P. (1997) Effects of lead ingestion on concentrations of lead in tissues and eggs of laying Tsaiya ducks in Taiwan. *Poultry Science* 76, 13–16.

Kennedy, S., Rice, D.A. and Cush, P.F. (1986) Neuropathology of experimental 3-nitro-4-hyroxyphenylarsonic acid toxicosis in pigs. *Veterinary Pathology* 23, 454–461.

Khalafalla, F.A., Ali, F.H., Schwagele, F. and Abd-El-Wahab, M.A. (2011) Heavy metal residues in beef carcasses in Beni-Suef abattoir, Egypt. *Veterinaria Italiana* 47, 351–361.

King, R.H., Brown, W.G., Amenta, V.C.M., Shelley, B.C., Handson, P.D., Greenhill, N.B. and Wilcock, G.P. (1992) The effect of dietary cadmium intake on the growth performance and retention in growing pigs. *Animal Feed Science and Technology,* 37, 1–7.

Kirchgessner, M., Plass, D.L. and Reichlmayr-Lais, A.M. (1991) Lead deficiency in swine. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals (TEMA 7), Dubrovnik*. IMI, Zagreb, pp. 11-20–11-21.

Kollmer, W.E. and Berg, D. (1989) The influence of a zinc-, calcium- or iron-deficient diet on the resorption and kinetics of cadmium in the rat. In: Southgate, D.A.T., Johnson, I.T. and Fenwick, G.R. (eds) *Nutrient Availability: Chemical and Biological Aspects*. Royal Society of Chemistry Special Publication No. 72, Cambridge, UK, pp. 287–289.

Kostial, K., Jugo, S., Rabar, I. and Maljkovic, T. (1978) Influence of age on metal metabolism and toxicity. *Environmental Health Perspectives* 25, 81–86.

Kozak, S. and Forsberg, C.W. (1979) Transformation of mercuric chloride and methyl mercury by the rumen microflora. *Applied Environmental Microbiology* 38, 626–636.

Krueger, G.L., Morris, T.K., Suskind, R.R. and Widner, E.M. (1985) The health effects of aluminium compounds in mammals. *CRC Critical Reviews in Toxicology* 13, 1–24.

Kumar, A. and Susheela, A.K. (1995) Effects of chronic fluoride toxicity on the morphology of ductus epididymis and the maturation of spermatozoa in the rabbit. *International Journal of Experimental Pathology* 76, 1–11.

Langlands, J.P., Donald, G.E. and Bowles, J.E. (1988) Cadmium concentrations in liver, kidney and muscle

in Australian sheep and cattle. *Australian Journal of Experimental Agriculture* 28, 291–297.

Leach, R.M. Jr, Wang, K.W. and Baker, D.E. (1979) Cadmium and the food chain: the effect of dietary cadmium on tissue composition in chicks and laying hens. *Journal of Nutrition* 109, 437–443.

Lee, J., Rounce, J.R., MacKay, A.D. and Grace, N.D. (1996) Accumulation of cadmium with time in Romney sheep grazing ryegrass–white clover pasture: effect of cadmium from pasture and soil intake. *Australian Journal of Agricultural Research* 47, 877–894.

Li, Y., Liang, C., Slemenda, C.W., Ji, R., Sun, S. *et al.* (2001) Effect of long-term exposure to fluoride in drinking water on risks of bone fractures. *Journal of Bone Mineral Research* 16, 932–939.

Linden, A., Olsson, I.-M., Bensryd, I., Lundh, T., Skerfving, S. and Oskarsson, A. (2003) Monitoring cadmium in the food chain from soil via crops and feed to pig blood and kidney. *Ecotoxicology and Environmental Safety* 55, 213–222.

Liu, Y., Zhang, W., Zhao, J., Lin, X., Liu, J. *et al.* (2018) Selenoprotein P as the major transporter for mercury in serum from methylmercury-poisoned rats. *Journal of Trace Elements in Medicine and Biology* 50, 589–595.

Lloyd, M.K. (1983) Environmental toxicity. In: Suttle, N.F., Gunn, R.G., Allen, W.M., Linklater, K.A. and Wiener, G. (eds) *Trace Elements in Animal Production and Veterinary Practice*. British Society of Animal Production Occasional Publication No. 7, Edinburgh, pp. 119–124.

Loganathan, P., Hedley, M.J., Wallace, G.C. and Roberts, A.H.C. (2001) Fluoride accumulation in pasture forages and soils following the long-term application of phosphorus fertilisers. *Environmental Pollution* 115, 275–282.

Loganathan, P., Hedley, M.J. and Grace, N.D. (2008) Pasture soils contaminated with fertilizer-derived cadmium and fluorine: livestock effects. *Reviews in Environmental Contamination and Toxicology* 192, 29–66.

Longhurst, R.D., Roberts, A.H.C. and Waller, J.E. (2004) Concentrations of arsenic, cadmium, copper, lead and zinc in New Zealand pastoral topsoils and herbage. *New Zealand Journal of Agricultural Research* 47, 23–32.

Lynch, G.P., Jackson, E.D. and Smith, D.F. (1976a) Responses of young calves to oral doses of lead. *Journal of Dairy Science* 59, 1490–1494.

Lynch, G.P., Smith, D.F., Fisher, M., Pike, T.L. and Weinland, B.T. (1976b) Physiological responses of calves to cadmium and lead. *Journal of Animal Science* 42, 410–421.

Madan, J., Puri, J.P. and Singh, J.K. (2009) Growth, feed conversion efficiency and blood profile of buffalo calves consuming high levels of fluoride. *Tropical Animal Health and Production* 41, 295–298.

Masters, D.G., White, C.L., Peter, D.W., Purser, D.B., Roe, S.P. and Barnes, M.J. (1992) A multi-element supplement for grazing sheep. II. Accumulation of trace elements in sheep fed different levels of supplement. *Australian Journal of Agricultural Research* 43, 809–817.

Mastilović, J., Žarko, Z.S., Kevrešan, S., Janić-Hajnal, E.P., Novaković, A.R. and Radusin, T.A. (2010) Occurrence and risk management of main toxic elements in food production chain. *Food and Feed Research* 1, 13–22.

Maurer, R.L. and Day, H.G. (1957) The non-essentiality of fluorine in nutrition. *Journal of Nutrition* 62, 561–573.

Mazliah, J., Barron, S., Bental, E. and Reznik, I. (1989) The effect of chronic lead intoxication in mature chickens. *Avian Diseases* 33, 566–570.

McCaughey, W. (2007) Inorganic and organic poisons. In: Aitken, I.D. (ed.) *Sheep Diseases*. Blackwell Scientific, London.

McClure, F.J. (1949) Fluorine in foods: survey of recent data. *Public Health Reports* 64, 1061–1074.

McDowell, L.R. (2003) Aluminium, arsenic, cadmium, lead and mercury. In: *Minerals in Animal and Human Nutrition*. Academic Press, New York, pp. 355–356.

McGown, E.L. and Suttie, J.W. (1974) Influence of fat and fluoride on gastric emptying time. *Journal of Nutrition* 104, 909–915.

Mehdi, A.W.R., Al-Soudi, K.A., Al-Jiboori, N.A.J. and Al-Hiti, M.K. (1983) Effect of high fluoride intake on chicken performance, ovulation, spermatogenesis and bone fluoride content. *Fluoride* 16, 37–43.

Messer, H.H., Armstrong, W.D. and Singer, L. (1974) Essentiality and function of fluoride. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Trace Element Metabolism in Animals – 2*. University Park Press, Baltimore, Maryland, pp. 425–437.

Milhaud, G., Cazieux, A. and Enriquez, B. (1984) Experimental studies on fluorosis in the suckling lamb. *Fluoride* 17, 107–114.

Mills, C.F. and Dalgarno, A.C. (1972) Copper and zinc status of ewes and lambs receiving increased dietary concentrations of cadmium. *Nature, London* 239, 171–173.

Milne, D.B. and Schwarz, K. (1974) Effect of different fluorine compounds on growth and bone fluoride levels in rats. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Trace Element Metabolism in Animals – 2*. University Park Press, Baltimore, Maryland, pp. 710–714.

Moffat, W.H. (1993) *Long term residual effects of lead mining on man and grazing livestock within a rural community in Southern Scotland*. PhD thesis, University of Edinburgh, pp. 152–214.

Moore, M.R, Goldberg, A. and Yeung, A. (2006) Lead effects on the heme biosynthetic pathway relationship to toxicity. *Annals of the New York Academy of Sciences* 514, 191– 203.

Morcombe, P.W., Petterson, D.S., Masters, H.G., Ross, P.J. and Edwards, J.R. (1994a) Cadmium concentrations in kidneys of sheep and cattle in Western Australia. 1. Regional distribution. *Australian Journal of Agricultural Research* 45, 851–862.

Morcombe, P.W., Petterson, D.S., Ross, P.J. and Edwards, J.R. (1994b) Soil and agronomic factors associated with cadmium accumulations in kidneys of grazing sheep. *Australian Veterinary Journal* 71, 404–406.

Morrison, J.N. and Quarterman, J. (1987) The relationship between iron status and lead absorption in rats. *Biological Trace Element Research* 14, 115–126.

Morrison, J.N., Quarterman, J. and Humphries, W.R. (1977) The effect of dietary calcium and phosphate on lead poisoning in lambs. *Journal of Comparative Pathology* 87, 417–429.

Motzok, I. and Branion, H.D. (1958) Influence of fluorine on phosphatase activities of plasma and tissues of chicks. *Poultry Science* 37, 1469–1471.

Müller, M., Anke, M. and Illing-Günther, H. (1998) Aluminium in foodstuffs. *Food Chemistry* 61, 419–428.

Neathery, M.W., Miller, W.J., Gentry, R.P., Stake, P.E. and Blackmon, D.M. (1974) Cadmium^{109} and methyl mercury metabolism, tissue distribution and secretion into milk in dairy cows. *Journal of Dairy Science* 57, 1177–1184.

Neathery, M.W., Miller, W.J., Gentry, R.P., Crowe, C.T., Alfaro, E. *et al*. (1987) Influence of high dietary deed on selenium metabolism in dairy calves. *Journal of Dairy Science* 70, 645–652.

Nielsen, F.H. (1996) Other trace elements. In: Filer, L.J. and Ziegler, E.E. (eds) *Present Knowledge in Nutrition*, 7th edn. International Life Sciences Institute, Nutrition Foundation, Washington, DC.

NRC (2005) *Mineral Tolerances of Animals,* 2nd edn. National Academy of Sciences, Washington, DC.

Oelschlager, W.K., Loeffler, K. and Opletova, L. (1970) Retention of fluorine in bones and bone sections of oxen in respect of equal increases in fluorine in the form of soil, flue dust from an aluminium reduction plant and sodium fluoride. *Landwirsch Forsch* 23, 214–224.

Olgun, O. (2015) The effect of dietary cadmium supplementation on performance, egg quality, tibia biomechanical properties and eggshell and bone mineralisation in laying quails. *Animal* 9, 1298–1303.

Oliver, D.P., Tiller, K.G., Conyers, M.K., Slattery, W.J., Alston, A.M. and Merry, R.H. (1996) Effectiveness of liming to minimise uptake of cadmium by wheat and barley grain grown in the field. *Australian Journal of Agricultural Research* 47, 1181–1193.

Olsson, I.-G., Jonsson, S. and Oskarsson, A. (2001) Cadmium and zinc in kidney, liver, muscle and mammary tissue from dairy cows in conventional and organic farming. *Journal of Environmental Monitoring* 3, 531–538.

Pace, L.W., Turnquist, S.E., Casteel, S.W., Johnson, P.J. and Frankeny, R.L. (1997) Acute arsenic poisoning in five horses. *Veterinary Pathology* 34, 160–164.

Pareja-Carrera, J., Mateo, R. and Rodríguez-Estival, J. (2014) Lead (Pb) in sheep exposed to mining pollution: implications for animal and human health. *Ecotoxicology and Environmental Safety* 108, 210–216.

Patra, R.C., Dwivedi, S.K., Bhardwaj, B. and Swarup, D. (2000) Industrial fluorosis in cattle and buffalo around Udaipur, India. *Science of the Total Environment* 253, 145–150.

Pearl, D.S., Ammerman, C.B., Henry, P.R. and Littell, R.C. (1983) Influence of dietary lead and calcium on tissue lead accumulation and depletion, lead metabolism and tissue mineral composition in sheep. *Journal of Animal Science* 56, 1416–1426.

Peirce, A.W. (1954) Studies on fluorosis of sheep. 2. The toxicity of water-borne fluoride for mature grazing sheep. *Australian Journal of Agricultural Research* 5, 545–554.

Pereira, V., Miranda, M., Sierra, J., Benedito, J.L. and López-Alonso, M. (2021) Toxic and essential trace element concentrations in different tissues of extensively reared sheep in northern Spain. *Journal of Food Composition and Analysis* 96, 103709.

Petersen, D.S., Masters, H.G., Spiejers, E.J., Williams, D.E. and Edwards, J.R. (1991) Accumulation of cadmium in sheep. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals (TEMA 7), Dubrovnik*. IMI, Zagreb, pp. 26-13–26-14.

Phillips, C., Gyori, Z. and Kovacs, B. (2003) The effect of adding cadmium and lead alone or in combination to the diet of pigs on their growth, carcase composition and reproduction. *Journal of the Science of Food and Agriculture* 83, 1357–1365.

Phillips, P.H. and Suttie, J.W. (1960) The significance of time in intoxication of domestic animals by fluoride. *Archives of Industrial Health* 21, 343–345.

Phillips, P.H., Hart, E.B. and Bohstedt, G. (1934) *Chronic Toxicosis in Dairy Cows due to the Ingestion of Fluorine*. Research Bulletin Wisconsin Agricultural Experiment Station No. 123.

Pinault, L. and Milhaud, G. (1985) Evaluation of subclinical lead poisoning in dairy cattle. *Veterinary Pharmacology and Toxicology* 61, 715–124.

Pinot, F., Kreps, S.E., Bachelet, M., Hainault, P., Bakonyi, M. and Polla, B.S. (2002) Cadmium in the environment: sources, mechanisms of biotoxicity. *Reviews in Environmental Health* 15, 299–323.

Powell, G.W., Miller, W.I., Morton, J.D. and Clifton, C.M. (1964) Influence of dietary cadmium level and supplemental zinc on cadmium toxicity in the bovine. *Journal of Nutrition* 84, 205–211.

Puls, R. (1994) *Mineral Levels in Animal Health*, 2nd edn. Sherpa International, Clearbrook, Canada.

Quarterman, J., Morrison, J.N., Humphries, W.R. and Mills, C.F. (1977) The effect of dietary sulfur and of

castration on lead poisoning in lambs. *Journal of Comparative Pathology* 87, 405–416.

Quarterman, J., Morrison, J.N. and Humphries, W.R. (1978) The influence of high dietary calcium and phosphate on lead uptake and release. *Environmental Research* 17, 60–67.

Rahman, Z. and Singh, V.P. (2019) The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. *Environmental Monitoring and Assessment* 191, 419. DOI:10.1007/s10661-019-7528-7

Rahman, Z. and Singh, V.P. (2020) Bioremediation of toxic heavy metals (THMs) contaminated sites: concepts, applications and challenges. *Environmental Science and Pollution Research International* 27, 27563-81. DOI:10.1007/s11356-020-08903-0

Ranjan, R. and Ranjan, A. (2015) Fluoride tolerance. In: *Fluoride Toxicity in Animals*. Springer Briefs in Animal Sciences. Springer, Cham, Switzerland. DOI:10.1007/978-3-319-17512-6_5

Rhind, S.J., Kyle, C.E. and Owen, J. (2005) Accumulation of potentially toxic metals in the liver tissue of sheep grazed on sewage sludge treated pastures. *Animal Science* 81, 107–113.

Rimbach, G., Pallaul, J., Brandt, K. and Most, E. (1996) Effect of phytic acid and microbial phytase on Cd accumulation, Zn status and the apparent absorption of Ca, P, Mg, Fe, Zn, Cu and Mn in growing rats. *Annals of Nutrition and Metabolism* 39, 361–370.

Robinson, D.L., Hemkes, O.J. and Kemp, A. (1984) Relationships among forage aluminium levels, soil contamination on forages and availability of elements to dairy cows. *Netherlands Journal of Agricultural Science* 32, 73–80.

Rodella, L.F., Ricci, F., Borsani, E., Stacchiotti, A., Foglio, E. *et al*., (2008) Aluminium exposure induces Alzheimer's disease-like histopathological alterations in mouse brain. *Histology and Histopathology* 23, 433–439.

Roholm, K. (1937) *Fluorine Intoxication*. H.K. Lewis, London.

Ross, E.A. (2000) Lead contamination of calcium supplements. *Journal of the American Veterinary Medical Association* 284, 1425–1429.

Ruliffson, W., Burns, L.V. and Hughes, J.S. (1963) The effect of fluoride ion on ^{59}Fe iron levels in the blood of rats. *Transactions of the Kansas Academy of Science* 66, 52.

Sasser, L.B. and Jarboe, G.E. (1980) Intestinal absorption and retention of cadmium in neonatal pigs compared to rats and guinea pigs. *Journal of Nutrition* 110, 1641–1647.

Scheuhammer, A.M. (1987) The chronic toxicity of aluminium, cadmium, mercury and lead in birds: a review. *Environmental Pollution* 46, 263–295.

Schwarz, K. (1974) New essential trace elements (Sn, V, F, Si): progress report and outlook. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Proceedings of the Second International Symposium on Trace Element Metabolism in Animals*. University Press, Baltimore, Maryland, pp. 355–380.

Schwarz, K. and Milne, D.B. (1972) Fluorine requirements for growth in the rat. *Bioinorganic Chemistry* 1, 331–336.

Seffner, W. and Tuebener, W. (1983) Antidotes in experimental fluorosis in pigs: morphological studies. *Fluoride* 16, 33–37.

Selby, L.A., Case, A.A., Osweiler, G.D. and Mayes, H.M. (1977) Epidemiology and toxicology of arsenic poisoning in domestic animals. *Environmental Health Perspectives* 19, 183–189.

Sell, J.L. (1975) Cadmium in the laying hen: apparent absorption, tissue distribution and virtual absence of transfer into eggs. *Poultry Science* 54, 1674–1678.

Shearer, T.R., Kolstad, D.L. and Suttie, J.W. (1978a) Bovine dental fluorosis: histologic and physical characteristics. *American Journal of Physiology* 212, 1165–1168.

Shearer, T.R., Kolstad, D.L. and Suttie, J.W. (1978b) Electron probe microanalysis of fluorotic bovine teeth. *American Journal of Veterinary Research* 39, 1393–1398.

Sherlock, J.C. (1989) Aluminium in foods and the diet. In: Massey, R.C. and Taylor, D. (eds) *Aluminium in Food and the Environment*. Royal Society of Chemistry Special Publication, London, pp. 68–76.

Shupe, J.L. (1980) Clinicopathologic features of fluoride toxicosis in cattle. *Journal of Animal Science* 51, 746–757.

Shupe, J.L., Miner, M.L., Harris, L.E. and Greenwood, D.A. (1962) Relative effects of feeding hay atmospherically contaminated by fluoride residue, normal hay plus calcium fluoride, and normal hay plus sodium fluoride to dairy heifers. *American Journal of Veterinary Research* 23, 777–787.

Shupe, J.L., Harris, L.E., Greenwood, D.A., Butcher, J.E. and Nielsen, H.M. (1963a) The effect of fluorine on dairy cattle. 5. Fluorine in the urine as an estimator of fluorine intake. *American Journal of Veterinary Research* 24, 300–306.

Shupe, J.L., Miner, M.L., Greenwood, D.A., Harris, L.E. and Stoddard, G.E. (1963b) The effect of fluorine on dairy cattle. 2. Clinical and pathologic effects. *American Journal of Veterinary Research* 24, 964–979.

Simpson, V.R., Stuart, N.C., Munro, R., Hunt, A. and Livesey, C.T. (1997) Poisoning of dairy heifers by mercurous chloride. *Veterinary Record* 140, 549–552.

Smith, G.M. and White, C.L. (1997) A molybdenum–sulfur–cadmium interaction in sheep. *Australian Journal of Agricultural Research* 48, 147–154.

Smith, R.M., Griel, L.C., Muller, L.D., Leach, R.M. and Baker, D.H. (1991a) Effects of dietary cadmium chloride throughout gestation on blood and tissue metabolites of primigravid and neonatal dairy cattle. *Journal of Animal Science* 69, 4078–4087.

Smith, R.M., Griel, L.C., Muller, L.D., Leach, R.M. and Baker, D.H. (1991b) Effects of long term dietary cadmium chloride on tissue, milk and urine mineral concentrations of lactating dairy cows. *Journal of Animal Science* 69, 4088–4096.

Snook, L.C. (1962) Rock phosphate in stock feeds: the fluorine hazard. *Australian Veterinary Journal* 38, 42–47.

Stark, B.A., Livesey, C.T., Smith, S.R., Suttle, N.F., Wilkinson, J.M. and Cripps, P.J. (1998) *Implications of Research on the Uptake of PTEs from Sewage Sludge by Grazing Animals*. Report to the Department of the Environment, Transport and the Regions (DETR) and the Ministry of Agriculture, Fisheries and Food (MAFF). WRc plc, Marlow, UK.

Stebbing, A.R.D. (1982) Hormesis – the stimulation of growth by low levels of inhibitors. *Science of the Total Environment* 22, 213–234.

Stoewsand, G.S., Telford, J.N., Anderson, J.L., Bache, C.A., Gutenmann, W.H. and Lisk, D.J. (2005) Toxicological studies with Japanese quail fed winter wheat grown on municipal sludge-amended soil. *Archives of Experimental Contamination and Toxicology* 13, 297–301.

Sullivan, T.W., Douglas, J.H. and Gonzalez, N.J. (1994) Levels of various elements of concern in feed phosphates of domestic and foreign origin. *Poultry Science* 73, 520–528.

Supplee, W.C. (1961) Production of zinc deficiency in turkey poults by dietary cadmium. *Poultry Science* 40, 827–831.

Suttie, J.W. (1967) Vertebral biopsies in the diagnosis of bovine fluoride toxicosis. *American Journal of Veterinary Research* 28, 709–712.

Suttie, J.W. (1969) Fluoride content of commercial dairy concentrates and alfalfa forage. *Journal of Agricultural and Food Chemistry* 17, 1350–1352.

Suttie, J.W. (1980) Nutritional aspects of fluoride toxicosis. *Journal of Animal Science* 51, 759–766.

Suttie, J.W. and Faltin, E.C. (1973) Effects of sodium fluoride on dairy cattle: influence of nutritional state. *American Journal of Veterinary Research* 34, 479–483.

Suttie, J.W. and Kolstad, D.L. (1977) Effects of dietary fluoride ingestion on ration intake and milk production. *Journal of Dairy Science* 60, 1568–1573.

Suttie, J.W., Miller, R.F. and Phillips, P.H. (1957) Studies of the effects of dietary NaF on dairy cows. 1. The physiological effects and the developmental symptoms of fluorosis. *Journal of Nutrition* 63, 211–224.

Suttie, J.W., Phillips, P.H. and Miller, R.F. (1958) Studies of the effects of dietary sodium fluoride on dairy cows. 3. Skeletal and soft tissue fluorine deposition and fluorine toxicosis. *Journal of Nutrition* 65, 293–304.

Suttie, J.W., Gesteland, R. and Phillips, P.H. (1961) Effects of dietary sodium fluoride on dairy cows. 6. In young heifers. *Journal of Dairy Science* 44, 2250–2258.

Suttie, J.W., Carlson, J.R. and Faltin, E.C. (1972) Effects of alternating periods of high- and low-fluoride ingestion on dairy cattle. *Journal of Dairy Science* 55, 790–804.

Suttle, N.F., Brebner, J. and Hall, J. (1991) Faecal excretion and retention of heavy metals in sheep ingesting topsoil from fields treated with metal rich sludge. In: Momcilovic, B. (ed.) *Proceedings of the Seventh International Symposium on Trace Elements in Man and Animals (TEMA 7), Dubrovnik*. IMI, Zagreb, pp. 32-7–32-8.

Suttle, N.F., Brebner, J., Stark, B., Sweet, N. and Hall, J.W. (1997) Placental and mammary transfer of lead and cadmium by ewes exposed to lead and cadmium enriched, sewage-sludge treated soils. In: Fischer, P.W.F., L'Abbé, M.R., Cockell, K.A. and Gilson, R.S. (eds) *Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals (TEMA 9), Banff*. NRC Research Press, Ottawa, pp. 168–170.

Tao, S. and Suttie, J.W. (1976) Evidence for a lack of an effect of dietary fluoride level on reproduction in mice. *Journal of Nutrition* 106, 1115–1122.

Thompson, D.J. (1980) Industrial considerations related to fluoride toxicity. *Journal of Animal Science* 51, 767–772.

Thornton, I. (1996) Sources and pathways of arsenic in the geochemical environment. In: Appleton, J.D., Fuge, R. and McCall, G.J.H. (eds) *Environmental Geochemistry and Health*. Geological Society Special Publication No. 113, London, pp. 153–162.

Tiffany, M.E., McDowell, L.R., O'Connor, G.A., Martin, F.G., Wilkinson, N.S., Percival, S.S. and Rabiansky, P.A. (2002) Effects of residual and reapplied biosolids on performance and mineral status of grazing steers. *Journal of Animal Science* 80, 260–269.

Underwood, E.J. and Suttle, F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Upadhyay, M.K., Shukla, A., Yadav, P. and Srivastava, S. (2019) A review of arsenic in crops, vegetables, animals and food products. *Food Chemistry* 276, 608–618.

Valdivia, R., Ammerman, C.B., Henry, P.R., Feaster, J.P. and Wilcox, C.J. (1982) Effect of dietary aluminium and phosphorus on performance, phosphorus utilisation and tissue mineral composition in sheep. *Journal of Animal Science* 55, 402–410.

Van Bruwaene, R., Gerber, G.B., Kirchmann, R. and Colard, J. (1982) Transfer and distribution of radioactive cadmium in dairy cows. *International Journal of Environmental Studies* 9, 47–51.

Van Bruwaene, R., Kirchmann, R. and Impens, R. (1984) Cadmium contamination in agriculture and zootechnology. *Experientia* 40, 43–50.

Van der Woude, H., Alink, G.M. and Rietjens, I.M. (2005) The definition of hormesis and its implications for *in vitro* and *in vivo* risk assessment. *Critical Reviews in Toxicology* 35, 603–607.

Van Gelder, G.A., Carson, T., Smith, R.M. and Buck, W.B. (1973) Behavioural toxicologic assessment of the neurologic effect of lead in sheep. *Clinical Toxicology* 6, 405–418.

VIDA (2007) *Table 3 in Veterinary Investigation Surveillance Report, 2007.* Veterinary Laboratories Agency, Weybridge, UK.

Waegeneers, N. Pizzolon, J.-C., Hoenig, M. and De Temmerman, L. (2009a) The European maximum level for cadmium in bovine kidneys is in Belgium only realistic for cattle up to 2 years of age. *Food Additives and Contaminants: Part A*, 26, 1239–1244.

Waegeneers, N. Pizzolon, J.-C. Hoenig, M. and De Temmerman, L. (2009b) Accumulation of trace elements in cattle from rural and industrial areas in Belgium. *Food Additives and Contaminants: Part A*, 26, 326–332.

Wang, T., Li, Z., Chen, X. and Long, X.-E. (2019) Effects of nickel and cobalt on methane production and methanogen abundance and diversity in paddy soil. *PeerJ* 7, e6274. DOI:10.7717/peerj.6274

Weber, C.W. and Reid, B.L. (1974) Effect of low-fluoride diets fed to mice for six generations. In: Hoekstra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. (eds) *Trace Element Metabolism in Animals – 2*. University Park Press, Baltimore, Maryland, pp. 707–709.

Wheeler, S.M., Brock, T.B. and Teasdale, D. (1985) Effects of adding 30 mg fluoride/l drinking water given to pregnant ewes and their lambs upon physiology and wool growth. *Journal of Agricultural Science, Cambridge* 105, 715–726.

Wilkinson, J.M., Hill, J. and Livesey, C.T. (2001) Accumulation of potentially toxic elements in the body tissues of sheep given diets containing soil and sewage sludge grazed on grassland given repeated applications of sewage sludge. *Animal Science* 72, 179–190.

World Health Organization (2000) Lead. In: *Safety Evaluation of Certain Food Additives and Contaminants. Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food Additives Series 44*. WHO, Geneva, pp. 273–312.

World Health Organization (2001) Cadmium. In: *Safety Evaluation of Certain Food Additives and Contaminants. Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food Additives Series 46*. WHO, Geneva, pp. 247–305.

Zelenka, J. and Hedbavny, J. (2007) Effect of age upon retention of mercury in chickens with a different growth rate. *Veterinarni Medicina* 52, 547–551.

Zheng, G., Chen, T.B., Gao, D. and Luo, W. (2004) Dynamic of lead speciation in sewage sludge composting. *Water Science and Technology* 50, 75–82.

Zmudzki, J., Bratton, G.R., Womac, C. and Rowe, L.D. (1984) The influence of milk diet, grain diet and method of dosing on lead toxicity in young calves. *Toxicology and Applied Pharmacology* 76, 490–497.

Zou, Y., Zhang, C., Ju, X., Wang, Z., Wu, Y. *et al*. (2019) Effect of removing cadmium with citric acid on the physicochemical and microstructure properties of rice bran. *Food Control* 98, 290–296.

19 Predicting Mineral Responses

The purpose of this chapter is to bridge the gaps in knowledge that complicate assessments of mineral deprivation and the extent to which they limit livestock production.

Uncertainties

For most minerals – major and trace – there are uncertainties regarding:

- effective supply (i.e. amount of mineral ingested, absorbed and utilizable);
- minimal demand (unavoidable mineral losses at zero balance);
- rates of mobilization of body stores after abrupt changes in supply and demand;
- rate-limiting metabolic pathways, when stores have been exhausted; and
- correlations of biochemical and genomic criteria with rate-limiting pathways.

Role of Dose–response Trials

The soundest diagnosis of mineral deprivation is provided by a clinical or production response to supplements of the mineral or minerals thought to be lacking (Suttle, 1987; McDowell, 1992; Judson, 1996). Guidelines for the design and interpretation of supplementation trials have been laid down to make fuller use of resources and data (Clark *et al.*, 1985; Grace, 1994; Underwood and Suttle, 1999), but can still be improved upon. Countless trials have been destined to confirm the null hypothesis before any data have been recorded because of inadequate numbers and failure to consult a statistician before starting (Sorzano and Parkinson, 2019). The essential requirements for a good supplementation trial are given below. Provision of minerals in excess of requirement does not improve reproductive performance and may be harmful (Rooke *et al.*, 2008).

Design of Dose–response Trials

Control groups

Benefits from supplementation cannot be gauged unless sufficient numbers of animals are left unsupplemented as controls (e.g. Suttle and Phillippo, 2005). 'Before and after treatment' comparisons are notoriously unreliable because any spontaneous recoveries (e.g. improved coat or fleece condition in spring; improved lamb growth with acquisition of immunity to gut parasites), new constraints (e.g. drought) or concurrent changes in husbandry (e.g. use of natural instead of artificial insemination) can give false-positive and false-negative results.

Control bias

Mineral supplementation *per se* constitutes a husbandry change and leaving animals uninjected may indirectly benefit treated cohorts, with whom they share a nutrient supply: if mineral deprivation causes loss of appetite, treated cohorts have access to more food. For example, if lack of dietary Co is limiting lamb growth by restricting appetite and insufficient milk and/or pasture is available to satisfy all appetites, individually treated (parenteral or oral) lambs will have access to more feed than if all lambs were treated. Thus, if one member of a suckling twin pair or half of the lambs in a flock grazed at high stocking rate is left untreated, treated animals may outperform untreated controls partly by default: the response to treatment may be exaggerated. Simply withdrawing poorly performing controls from trials on welfare grounds (e.g. Grace *et al.*, 2003; Gruner *et al.*, 2004) biases the statistical outcome. Treating such animals and either using them as a subgroup or ignoring their data but retaining them in the flock will reduce bias. Such 'control bias' can be reduced by leaving only a small fraction (e.g. 10%) of the flock untreated or by treating both members of a pair of suckling

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twins while leaving a minority of pairs untreated (e.g. Suttle and Small, 1993; Suttle, 2017). On experimental stations, treated and control groups can be housed or grazed separately in replicated groups (Masters and Peter, 1990; Whelan *et al.*, 1994) but this tactic is generally impractical under farm-trial conditions. Animals should be allocated at random within the available population, taking into account any parameter that might influence the measured response (e.g. initial liveweight (LW) or plasma enzyme activity). Selection of animals of low mineral status to maximize chances of obtaining a response (e.g. lactating cows of low Se status; Knowles *et al.*, 1999) may exaggerate responsiveness of the whole flock or herd. However, lack of response in the selected population will put beyond doubt the likelihood of the whole herd responding positively.

Adequate Group Size

The group size needed to demonstrate statistically significant differences in performance can be calculated from previous records of variability in the chosen criterion. Whether variables are continuous (e.g. LW) or discrete (e.g. survival), the appropriate analysis (e.g. analysis of variance or chi-square test) can be run on simulated datasets. The effect of group size on probability of discounting as non-significant a 5% improvement in LW with different variability is illustrated in Fig. 19.1. A group size of 250 was needed in an experiment with dairy cows to detect a 10% improvement in milk yield when no account was taken of pre-trial yield (Wu and Satter, 2000). Table 19.1 illustrates the importance of group size on attainment of statistical significance for a discrete variable such as calf mortality or ewe infertility. Even when frequency is high (40%) and halved by hypothetical treatment, groups of >40 are needed for statistical significance by chi-square test (>3.8 for P <0.05 with one degree of freedom). When event frequency is low but still improvable (mortality halved from 10% to 5%) groups of >200 are needed to prove the point.

Proportional hazards models are increasingly used but are not infallible: they can lessen the impact of small population size by assuming that, once an event has occurred, the affected individual, had it survived, had an equal chance of succumbing to another disease or hazard in the future. Such models have been applied to bovine infertility data (Black and French, 2004) but may have exaggerated treatment benefits by notionally returning 'early conceivers' to a pool with a lower chance of becoming fertile.

Small improvements can be economically significant when mineral deprivation is marginal (Kumagai and White, 1995). Economic significance is heightened with discrete, all-or-nothing variables. For example, a non-significant (P >0.05) increase of 5% in the calf or lamb crop every other year would be welcomed by most farmers and would repay the outlay on treatments faster than a consistent significant 5% improvement in growth. However, a non-significant improvement stands a one in ≥20 chance of occurring by chance.

A small but significant improvement in ewe fertility, recorded in each of 1072 ewes on six farms, to parenteral Se was obtained in Ireland, despite a mean blood Se (recorded in sub-samples) well above normal on all farms and absence of significant responses within farms (Munoz *et al.*, 2009).

- Under farm-trial conditions, use of whole flocks and pooling data between flocks is commended.

Avoidable constraints

Group sizes are constrained by budgets, flock or herd size, available grazing area or housing space, handling facilities and the need to return stock to their farm environment without undue disruption.

Table 19.1. A hypothetical illustration of group sizes needed to obtain a significant improvement in incidence of a disorder (e.g. mortality or infertility) with a particular treatment (for P <0.05, chi-square must be >3.84).

	Hypothetical group		Group size					
	Control	Treated	10	20	40	80	160	320
	Incidence (%)		Chi-square					
Experiment A	40	20	0.95	1.91	3.81	7.62	15.24	30.48
Experiment B	10	5	–	0.36	0.72	1.44	2.88	5.76

However, it may be neither necessary nor advisable to return stock on gathering day. Allowing an overnight fast might reduce errors in weighing and blood analysis due to recent ingestion of food, while increasing the number of individuals sampled and recorded in a working day. The number of variables and individuals recorded, need only be sufficient to confirm or deny responsiveness. Fewer numbers are usually needed to attain statistical significance for biochemical than performance criteria. Expensive tests (e.g. for methylmalonic acid) can be retrospectively restricted if there is no prior production response (to cobalt). There are increasing pressures to reduce the number of animals used in experiments on welfare grounds. 'Restricted' or 'licensed' procedures (e.g. blood sampling) need be applied to only a small proportion of those subjected to unrestricted farm procedures (e.g. weighing).

Accurate Measurement

Statistical significance is determined by the ratio of variation due to fixed effects (e.g. of treatment, litter and pen) to unaccountable, random variation. Random variation can be reduced by rigorous attention to the detail and consistency of procedures and observations. Under statutory pressure, principles of good experimental and laboratory practice are increasingly adopted. While some requirements may seem pedantic, it takes little time to regularly check the accuracy of both field (e.g. weigh scales) and laboratory equipment (e.g. for analyses) results against known standards and to adjust results accordingly. Electronic recording and data transference is more reliable than the human ear, hand and memory. All statistical inputs should be double-checked and attention given to outliers, routinely 'flagged' by modern statistical packages. The discarding of biologically impossible weight gains, for example, is duty not deceit; so, too, is the re-analysis of duplicate samples with statistically verified discordance.

Repetition

Repetition of observations and trials provides a safeguard against diurnal, individual, day-to-day, seasonal and year-to-year variations. Awareness of diurnal fluctuations is a prerequisite for deciding and adhering to an optimum time of observation (e.g. before or after feeding, morning or afternoon milking). If the sequence of a prolonged set of samplings is repeated (e.g. in individually penned animals), the sequence might usefully be recorded and entered as a variable in the statistical model. Repeated subsampling of a small sentinel subgroup allows seasonal trends to be followed, but relationships to

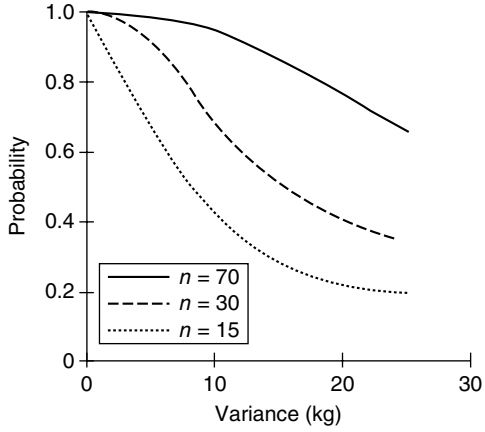

Fig. 19.1. Minimum acceptable group sizes for dose–response trials can be calculated from variances of response traits and the anticipated size of benefit from treatment. Here, the assumed benefit is one of 2 kg in liveweight gain in lambs; unless variance is low, large groups are required to correctly reject the null hypothesis. (From White, 1996)

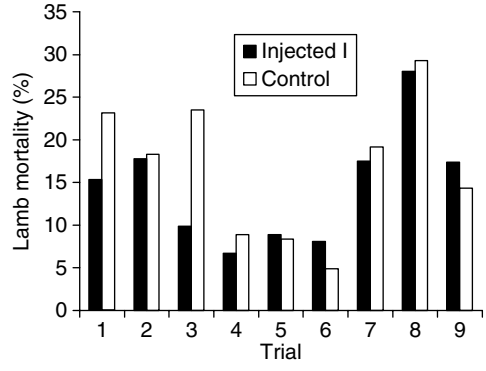

Fig. 19.2. Importance of sample size in dose–response trials: lamb survival improved following parenteral supplementation of ewes with iodine, pre-mating, on three out of nine farms (from Clark *et al.*, 1998): with large groups (500) a small improvement was significant in trial 4; relationships mean iodine (I) status was poor but hardly surprising, with only ten ewes or lambs sampled on each farm; iodine status was improved by supplementation on only one in eight farms.

health or performance in the main group may be disappointing (Fig. 19.2). Values recorded for the same animal on several occasions are not independent but can be analysed statistically as 'repeated measures' (St-Pierre and Jones, 1999), allowing removal of individual differences from variance, while increasing the likelihood of procuring significant treatment effects and interactions, evident as group X time interactions. Repeated observations (e.g. body weights on successive days) are increasingly used to improve reliability of the beginning and end-point measurements in an experiment. In heavily fleeced sheep, for example, liveweight (LW) can be increased by several kilograms by heavy rainfall prior to a particular weighing.

Seasonality

Seasonality impacts most farm trials by affecting growth of crops and pastures, animal physiology, even life-cycles of pathogens. Dividing an experiment into periods does not necessarily identify true season effects; waning efficacy of treatment and approach to maturity can masquerade as effects of 'season'. Awareness of seasonality can usefully influence timing of treatment and method of supplementation. When different treatments are applied sequentially, responses can become confounded with seasonal influences. For farmers, repeatability of responses to supplementation from year to year is of paramount importance. A classic example of year-to-year variation in response to a mineral supplement was provided by Lee (1951) in early studies of responses of 'coast disease' to cobalt supplementation in South Australia: growth was improved in only eight out of 13 years, and severity of deprivation varied in 'responsive' years from marginal growth depression to 100% mortality at the same site. In a sequential study of growth responses in lambs to Cu, Co and Se in successive years, significance of Cu insufficiency may have been underestimated by year-to-year fluctuations, with mean plasma Cu being highest in the year that Cu was under test (Suttle, 2017). Year-to-year variation is exploited commercially when tales of the 'good years' are more readily told than those of the 'bad years' by sellers of mineral supplements.

Using Individual Variation (Covariance)

Marginal mineral deficiencies are characterized by irregular performance rather than consistent failure and variation may be only partly attributable to mineral status (Table 19.2). Instead of being regarded as a nuisance, individual variation can be integrated into the design of experiments through covariance analysis. Use of covariance can reduce group size, redefine diagnostic limits and facilitate cost–benefit analysis. More can be done than simply pairing or stratifying the population initially according to a production parameter such as LW or milk yield. Before a growth trial even begins, a correlation between initial LW (i.e. past growth) and mineral status is worth looking for, although trends would only emerge if growth had been restricted by the last two factors listed in Table 19.2 or if maternal performance had been restricted by deprivation of the same mineral that their offspring lacked. Similar approaches can be taken with all parameters of production. Fig. 19.3 illustrates the variation found in a small experimental herd of dairy cows and the futility of conducting an experiment without allocating such animals according to pre-trial performance and past lactation records.

If pre-treatment performance has been restricted by mineral deprivation, variation in subsequent performance should be reduced by using initial ranking as a covariate: the relationship should be *negative* (i.e. poorest initial performers show greatest

Table 19.2. Seven possible reasons for poor growth in a group of young growing animals on first inspection, listed in order of probability.

Order	Reason for poor growth
1	Too little digestible, utilizable non-mineral nutrients provided by the diet
2	Maternal undernutrition (in the case of newly weaned or suckled offspring)
3	Parasitic, microbial or viral infection
4	Environmental stressors (e.g. wind, temperature, water supply)
5	Low genetic potential
6	Too little of one mineral in the diet
7	Too little of two or more minerals in the diet

response to supplementation). In lactating cows on an alfalfa diet, low in Na, individuals with the poorest yields prior to treatment showed the largest responses to Na, while previously good yielders failed to respond (Joyce and Brunswick, 1975). Use of conception rate records from previous matings as covariates may increase the chances of detecting and quantifying effects of suspected mineral deprivation on fertility, but this tactic has rarely been used. Significantly different relationships between trial performance and mineral status have been found *within* treated or untreated groups whose means did *not* differ significantly. In Table 19.3, influences of Cu supplementation on early lamb growth only became significant when initial LW was used as a covariate. The population distribution probably contained a 'tail' of responsive animals that alone benefitted from supplementation: differences in regression were not found later in the season (6–12 weeks) when growth was slower and the supplement delivered less Cu (Suttle, 2002, 2017). Such populations are marginally responsive and a cost–benefit analysis can be applied to ascertain whether all, none or part of the population merits treatment.

If individual performance prior to treatment was restricted by factors other than supply of the supplemented mineral (reasons 1–5 in Table 19.2), relationships with subsequent performance are likely to be *positive* if the influence continues (e.g. poor milk supply, poor genotype, chronic infection)

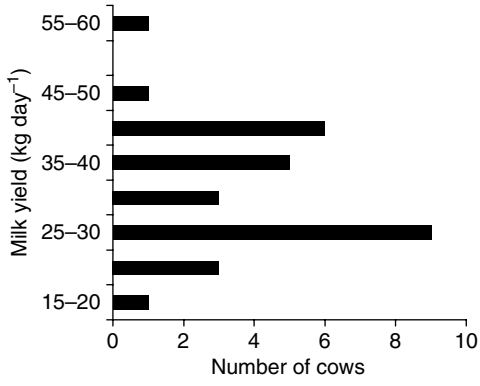

Fig. 19.3. An example of pre-trial variation in milk yield in a herd of multiparous Holstein cows: unless such cows are allocated to treatments on the basis of past lactation performance, significant responses to a mineral supplement are unlikely to be found (Voelker *et al.*, 2002).

but *negative* when it does not (e.g. weaning, acute infection), due to compensatory growth. In all cases where final performance is *proportional* to performance prior to treatment, simply calculating improvement by difference (e.g. LWG) will be *less* effective than covariance analysis.

Non-linear relationships

Few biological relationships are linear over the complete range of two covariates. In the series of trials to which Fig. 19.4 refers (Suttle, 2017), no significant growth responses to parenteral Se were found, but contrasting relationships with initial LW indicated that treatment made a difference on some farms. Whereas initial and final LW of untreated lambs were linearly correlated, the relationship in Se-treated lambs was disrupted (Fig. 19.4b). Curvilinear relationships within groups between LW and serum vitamin B_{12} have been used to confirm the marginal range for serum vitamin B_{12} in lambs (Grace *et al.*, 2003).

- Covariance analysis should always be open to the possibility that a relationship may be non-linear.

Diagnostic Use of Group Responses

Relationships between mean improvements in group performance and mean mineral status in dose–response trials have been used to predict responses to treatment on 'unknown' farms from the same index of status. For example, the relationship between plasma B_{12} and average LW benefit

Table 19.3. Treatment with copper oxide (CuO) needles did not improve mean liveweight gain (LW G) of 25 lambs compared to their untreated twins on molybdeniferous pasture (50–60mg Mo kg^{-1}DM) but within-group regressions of LWG on initial LW after six weeks differed. (Unpublished data from trials described by Suttle, 2017)

		Regression (kg units)	
CuO	LWG (g day^{-1})	Intercept (± SD)	Coefficient (± SD)
0	173[a]	8.1[b] (± 2.19)	0.95[d] (± 0.162)
+	191[a]	1.1[c] (± 2.27)	1.51[e] (± 0.168)
	21.6 (SED)		

SD, standard deviation; SED, standard error of the difference
Means with different superscripts within columns differ significantly.

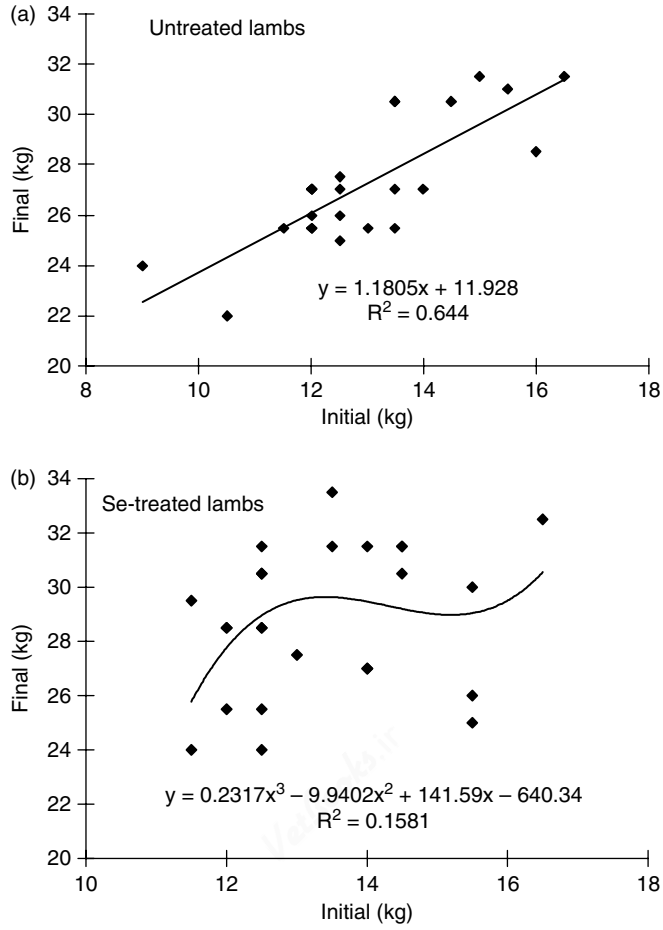

Fig. 19.4. Relationship between initial and 12-week liveweight (LW) of individual suckling lambs in the last year of three years in which the possibility of, first, copper then cobalt and, finally, selenium deprivation was examined on numerous Scottish hill farms (Suttle 2017). Parenteral selenium injections did not improve mean liveweight gain on any of 11 farms but clearly affected growth on the farm illustrated in the graph.

after treating lambs with Co or B_{12} was used to improve interpretation of plasma B_{12} and assess the economic benefit of treatment (Fig. 19.5). However, different conclusions values were correlated (p.239) (Grace *et al.*, 2003). The exponential nature of the relationship arises partly because more individuals show larger benefits as plasma B_{12} declines; however, it may also partly reflect control bias (p. 516): a similar approach has been used with Se (Fig. 19.6). The distribution of responses confirms the need for marginal bands for biochemical criteria in diagnosis (p. 41). In Fig 19.6, as blood Se declined linearly below 316 nmol (25 µg) l^{-1}, so the probability of obtaining a growth response in calves increased but large responses (90 g day^{-1}) remained improbable. The same research group used a 'bent-stick' model to predict responses in wool growth from blood Se (Langlands *et al.*, 1991). These simple strategies allow the animal to be the arbiter of 'deprivation' and begin to address *all* of the uncertainties set out at the start of this chapter.

Selective Supplementation

It is not always necessary or preferable to conduct trials with groups that are representative of the whole herd or flock. In New Zealand, Se supplements rarely improve milk yield, despite a generally low blood and herbage Se (Wichtel, 1998) but did

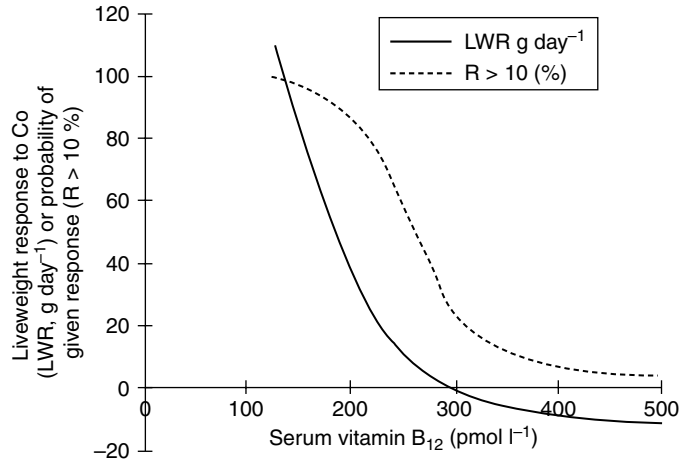

Fig. 19.5. Collation of data from cobalt supplementation trials with lambs in New Zealand allowed the interpretation of serum and liver B_{12} concentrations to be given an economic base (Clark *et al.*, 1985, 1989) but that base varies within and between countries.

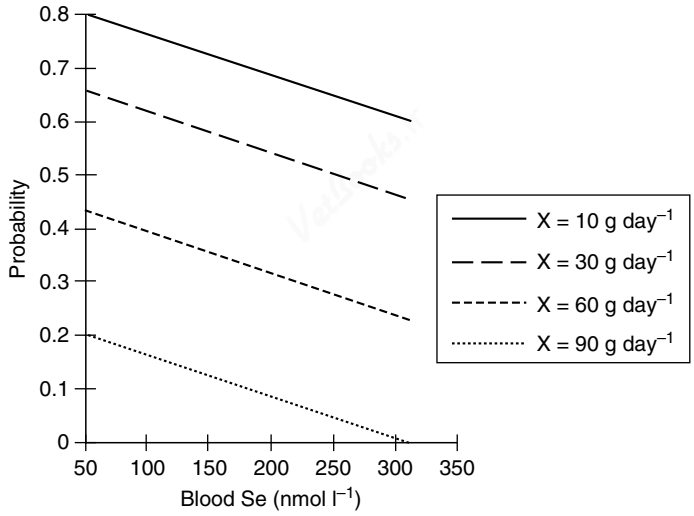

Fig. 19.6. The collation of data from large numbers of dose–response trials can allow prediction of benefits from supplementation in other areas from a common measure of trace element status. Here, the probability of different growth responses in cattle is predicted from blood selenium (Langlands *et al.*, 1989).

so when 10% of a herd with the lowest blood Se were treated, with herd mean blood Se and pasture Se both normal (Knowles *et al.*, 1999): cows were allocated to treatment according to previous milk yield, increasing the chances of achieving a positive response. The strategy raises difficult questions:

- Can responsiveness be delineated by current measures in marginally deficient herds, without costing more than blindly treating all animals?
- Is variance a better indicator of responsiveness than the mean value?
- Would plasma GPx or SelP assay more accurately distinguish the Se-responsive 'tail' in the population?

Understandably, manufacturers of mineral supplements and aids to digestion test their products in animal models that are most likely to show benefits. The one-day-old chick is a popular model for demonstrating responses to minerals because large numbers can be mustered and multifactorial designs accommodated. However, immaturity of the digestive system may mean that longer-term benefits in productivity are exaggerated. The capacity of phytase supplements to free phytate P from cereals and vegetable protein sources may have been exaggerated by studies with chicks in the first three weeks of life (Fig. 4.13). The model is popular with manufacturers of phytase supplements, but misleading to environmentalists.

Optimal Treatments

The choice of treatments for diagnostic trials is governed by different criteria from those that a farmer might routinely use and there are various options to be considered. Where possible, animals should be supplemented individually rather than as a group to avoid variable intake of supplement (p.47). The product used experimentally may be more expensive than that used in practice to guarantee efficacy throughout the period of risk but should present no risk of excess. Injections of vitamin B_{12} were preferred to cobalt bullets for raising plasma B_{12} status in growth-response trials in Scotland, but the chosen dose (1 mg) was inadequate when repeated in lambs that had grown from 20 kg to 30kg between doses (Suttle, 2017). Plasma B_{12} declined steadily in untreated lambs and responses were more likely to have been obtained after the second dose, had it been adequate. Slow-release injections of vitamin B_{12} are now available (p. 246) and would be more suitable for such field trials. Failure to scale Se injections to LW may have restricted growth in the lightest lambs in Fig.19.4b: scaling doses to body weight in growing animals is, therefore, recommended.

- Diagnosis by mineral-response only works with treatments that achieve sustained alleviation of deficiency, without causing harm and the possibility of multiple deficiencies is covered.

A decision to provide more than one mineral can only be guided by evidence from previous analyses of soil, diet, blood and tissues. Given the uncertainty of the respective relationships with animal performance (p. 516), the case for a pilot 'blunderbuss' (multi-supplement) approach (measuring the combined impact of all mineral deficiencies) is strong (White *et al.*, 1992: Kumagai and White, 1995) but several difficulties arise with multiple treatments:

- no diagnosis can be reached because the responsive element remains unknown;
- part of the flock or herd must remain unsupplemented as controls, creating risk of multiple deprivation, an unattractive prospect to participating farmers;
- uptake of a free-access mineral mixture, the common method of multiple supplementations, may vary substantially within treated groups (p. 47);
- minerals not needed may be provided in excess and limit performance; and
- the pilot trial may be taking place in an atypical, 'unresponsive' year.

The 'clustered treatment' compromise

A compromise was reached in assessing the extent to which trace element deficiencies were limiting lamb growth on 'improved' hill pastures in Scotland (Suttle, 2017). The possibility that dietary I, Mn and Zn were lacking was discounted from historical data and field experience. Possible deprivation of three elements, Cu, Co and Se, was covered with three treatment groups: fully supplemented (A); deprived of one mineral (B); and unsupplemented (C). Then, A–C would give the total response to Cu+Co+Se and B–C the minimal response to the selectively withdrawn mineral. By removing Cu, Co and Se in successive years, each element would be tested and the average response to triple treatment over three years would be apparent. Hardly any positive responses to treatment were found, although examination of responses on particular farms revealed suggestions that intervention with Cu (Table 19.3) and Se (Fig 19.4) affected performance on some farms. In retrospect, mistakes were made in applying the cluster model, chief of which was to apply treatments to lambs before weaning rather than ewes, when the latter were arguably most at risk (Suttle, 2017). Some workers have provided salt for control groups and looked for increments in performance to multiple minerals in all possible combinations but responses to individual elements were so small that only combined supplements gave significant responses (McDowell *et al.*, 1982; Masters and Peter, 1990).

Re-examining unresponsive populations

Failure of any treatment to improve performance does not rule out the possibility of future response. Other causes for poor performance that prompted the quest for mineral responses should be investigated, following the suggested algorithm (Appendix 1): lack of digestible organic matter and chronic parasitic infections are the most likely alternative causes of ill-thrift in grazing young livestock. If such constraints are identified and removed, mineral supplies may become truly limiting at the new, increased level of performance. However, prior evidence of marginal mineral status should be obtained before re-embarking on costly and laborious supplementation trials. Multiple treatment with trace elements by injection without prior knowledge of mineral status in dairy cows (p. 49) has given inconsistent results (Stokes *et al*., 2017, 2019) and yields no useful diagnostic information: any responses are more likely to be attributable to adjuvant effects rather than nutritional responses.

New Insights from Old Data: Meta-analysis

The last decade has seen some useful advances made through meta-analysis of past experiments on calcium (p. 80), phosphorus (p. 138) and zinc (p. 434) nutrition of pigs and poultry. More useful, practical information can be gained from such studies by excluding results from extremely low mineral inputs, obtained with highly purified diets in vain attempts to quantify minimum faecal endogenous losses (p. 6), data of entirely academic interest. Meta-analysis has been used to assess the average response of lactating dairy cows to supplements of organic trace elements, which were claimed to have raised daily milk yield by 0.95 kg and advanced first conception by 13.5 days (Rabiee *et al*., 2010), but the contrast was often with different cocktails of inorganic trace elements rather than unsupplemented cows. Half of the 21 studies had the same lead author and most had not been peer-reviewed. Meta-analyses in non-ruminants have shown no advantage from supplementation with chelated zinc (p. 425). Neither inorganic nor organic trace elements seem likely to improve either milk yield and fertility in cows already receiving more than their minimum requirements for the supplemented elements.

New Insights from New, Genomic Criteria

Analysis of the way genes respond to insufficient trace element supplies from the diet offer the prospect of better definition of status and requirement but have so far generally confused rather than illuminated the issue. Adaptive changes in gene expression to trivial changes to mineral supply are legion (p. 2) – it is what genes do. Supplying minerals to optimize gene expression is a recipe to oversupply and not without hazard (Fe, p. 336; Mn, p.358). This branch of nutrition is in its infancy and there is much to learn. However, breakpoint analysis of genomic responses along rate-limiting pathways holds great promise (Brugger *et al*., 2021).

References

Black, D.H. and French, N.P. (2004) Effects of three types of trace element supplement on the fertility of diary herds. *Veterinary Record* 154, 652–658.

Brugger, D., Hanauer, M., Ortner, J. and Windisch, W.M. (2021) The response of zinc transporter gene expression of selected tissues in a pig model of subclinical zinc deficiency. *Journal of Nutritional Biochemistry* 90, 108576.

Clark, R.G., Wright, D.F. and Millar, K.R. (1985) A proposed new approach and protocol to defining mineral deficiencies using reference curves: cobalt deficiency in young sheep is used as a model. *New Zealand Veterinary Journal* 32, 1–5.

Clark, R.G., Wright, D.F., Millar, K.R. and Rowland, J.D. (1989) Reference curves to diagnose cobalt deficiency in sheep using liver and serum vitamin B_{12} levels. *New Zealand Veterinary Journal* 37, 7–11.

Clark, R.G., Sargison, N.D., West, D.M. and Littlejohn, R.P. (1998) Recent information on iodine deficiency in New Zealand sheep flocks. *New Zealand Veterinary Journal* 46, 216–222.

Grace, N.D. (1994) *Managing Trace Element Deficiencies*. New Zealand Pastoral Agricultural Research Institute, Palmerston North, New Zealand, pp. 63–68.

Grace, N.D., Knowles, S.O., Sinclair, G.R. and Lee, J. (2003) Growth responses to increasing doses of microencapsulated vitamin B_{12} and related changes in tissue B_{12} concentrations in cobalt-deficient lambs. *New Zealand Veterinary Journal* 51, 89–92.

Gruner, T.M., Sedcole, J.R., Furlong, J.M., Grace, N.D., Williams, S.D., Sinclair, G.R. and Sykes, A.R. (2004) Changes in serum concentrations of MMA and vitamin B_{12} in cobalt-supplemented ewes and their lambs on two cobalt-deficient properties. *New Zealand Veterinary Journal* 52, 17–128.

Joyce, J.P. and Brunswick, I.C.F. (1975) Sodium supplementation of sheep and cattle. *New Zealand Journal of Experimental Agriculture* 3, 299–304.

Judson, G.J. (1996) Trace element supplements for sheep at pasture. In: Masters, D.G. and White, C.L. (eds) *Detection and Treatment of Mineral Problems in Grazing Sheep*. ACIAR Monograph No. 37, Canberra, pp. 57–80.

Knowles, S.O., Grace, N.D., Wurms, K. and Lee, J. (1999) Significance of amount and form of dietary selenium on blood milk and casein selenium concentrations in grazing cows. *Journal of Dairy Science* 82, 429–437.

Kumagai, H. and White, C.L. (1995) The effect of supplementary minerals, retinol and α-tocopherol on the vitamin status and productivity of pregnant Merino ewes. *Australian Journal of Agricultural Research* 46, 1159–1174.

Langlands, J.P., Donald, G.E., Bowles, J.E. and Smith, A.J. (1989) Selenium concentration in the blood of ruminants grazing in northern New South Wales. 3. Relationship between blood concentration and the response in liveweight of grazing cattle given a selenium supplement. *Australian Journal of Agricultural Research* 40, 1075–1083.

Langlands, J.P., Donald, G.E., Bowles, J.E. and Smith, A.J. (1991) Subclinical selenium insufficiency. 1. Selenium status and the response in liveweight and wool production of grazing ewes supplemented with selenium. *Australian Journal of Experimental Agriculture* 31, 25–31.

Lee, H.J. (1951) Cobalt and copper deficiencies affecting sheep in South Australia. Part 1. Symptoms and distribution. *Journal of Agriculture, South Australia* 54, 475–490.

Masters, D.G. and Peter, D.W. (1990) Marginal deficiencies of cobalt and selenium in weaner sheep: response to supplementation. *Australian Journal of Experimental Agriculture* 30, 337–341.

McDowell, L.R. (1992) *Minerals in Animal and Human Nutrition.* Academic Press, New York.

McDowell, L.R., Bauer, B., Galdo, E., Koger, M., Loosli, J.K. and Conrad, J.H. (1982) Mineral supplementation of beef cattle in the Bolivian tropics. *Journal of Animal Science* 55, 964–970.

Munoz, C., Carson, A.F., McCoy, M.A., Dawson, L.E.R., Irwin, D., Gordon, A.G. and Kilpatrick, D.J. (2009) Effects of supplementation with barium selenate on the fertility, prolificacy and lambing performance of hill sheep. *Veterinary Record* 164, 265–271.

Rabiee, A.R., Lean, I.J., Stevenson, M.A. and Socha, M.T. (2010) Effects of organic trace minerals on milk production and reproductive performance in lactating dairy cows: a meta-analysis. *Journal of Dairy Science* 93, 4239–4251.

Rooke, J.A., Dwyer, C.M. and Ashworth, C.J. (2008) The potential for improving physiological, behavioural and immunological responses in the neonatal lamb by trace element and vitamin supplementation of the ewe. *Animal* 2, 514–524.

Sorzano, C.O.S. and Parkinson, M. (2019) *Statistical Experimental Design for Animal Research*. Available at: https://ceua.fmrp.usp.br/wp-content/uploads/sites/195/2019/09/Statistical-experiment-design-for-animal-research.pdf (accessed 30 May 2021).

Stokes, R.S., Ralph, A.R., Mickna, A.J., Chapple, W.P., Schroeder, A.R., Ireland, F.A. and Shike, D.W. (2017) Effect of an injectable trace mineral at the initiation of a 14 day CIDR protocol on heifer performance and reproduction. *Translational Animal Science* 1, 458–466.

Stokes, R.S., Ireland, F.A. and Shike, D.W. (2019) Influence of repeated trace mineral injections during gestation on beef heifer and subsequent calf performance. *Translational Animal Science* 3, 493–503.

St-Pierre, N.R. and Jones, L.R. (1999) Interpretation and design of non-regulatory on-farm trials. *Journal of Animal Science* 77 (suppl. 2J), 177–182.

Suttle, N.F. (1987) The absorption, retention and function of minor nutrients. In: Hacker, J.B. and Ternouth, J.H. (eds) *Nutrition in Herbivores*. Academic Press, Sydney, Australia, pp. 333–362.

Suttle, N.F. (2002) Meeting the copper requirements of ruminants. In: Garnsworthy, P.C. and Cole, D.J.A. (eds) *Recent Advances in Nutrition – 1994*. Nottingham University Press, Nottingham, UK, pp. 173–188.

Suttle, N.F. (2017) Challenges for needs for mineral supplementation in livestock. In: Singh, B.R., McLaughlin, M.J. and Brevik, E.C. (eds) *The Nexus of Soils, Plants, Animals and Human Health.* Catena-Schweizerbart, Stuttgart, Germany, pp. 105–110.

Suttle, N.F. and Phillippo, M. (2005) Effect of trace element supplementation on the fertility of dairy herds. *Veterinary Record* 156, 155–156.

Suttle, N.F. and Small, J.N.W. (1993) Evidence of delayed availability of copper in supplementation trials with lambs on molybdenum-rich pasture. In: Anke, M., Meissner, D. and Mills, C.F. (eds) *Proceedings of the Eighth International Symposium on Trace Elements in Man and Animals, Dresden*. Verlag Media Touristik, Gersdorf, Germany, pp. 651–655.

Underwood, E.J. and Suttle, F. (1999) *The Mineral Nutrition of Livestock*, 3rd edn. CAB International, Wallingford, UK.

Voelker, J.A., Burato, G.M. and Allen, M.S. (2002) Effects of pretrial milk yield on responses of feed intake, digestion and production to dietary forage concentration. *Journal of Dairy Science* 85, 2650–2661.

Whelan, B.R., Barrow, N.J. and Peter, D.W. (1994) Selenium fertilizers for pastures grazed by sheep. II. Wool and liveweight responses to selenium. *Australian Journal of Agricultural Research* 45, 875–886.

White, C.L. (1996) Understanding the mineral requirements of sheep. In: Masters, D.G. and White, C.L.

(eds) *Detection and Treatment of Mineral Problems in Grazing Sheep*. ACIAR Monograph No. 37, Canberra, pp. 15–30.

White, C.L., Masters, D.G., Peter, D.W., Purser, D.B., Roc, S.P. and Barnes, M. (1992) A multi-element supplement for grazing sheep. I. Intake, mineral status and production response. *Australian Journal of Agricultural Research* 43, 795–808.

Wichtel, J.J. (1998) A review of selenium deficiency in grazing ruminants 2: towards a more rational approach to diagnosis and prevention. *New Zealand Veterinary Journal* 46, 54–58.

Wu, Z. and Satter, L.D. (2000) Milk production and reproductive performance of dairy cows fed two concentrations of phosphorus for two years. *Journal of Dairy Science* 83, 1052–1063.

20 Minerals and Humans

So far, mineral nutrition has again been considered almost exclusively from the viewpoint of livestock production, the primary concern being to avoid constraints of either inadequate or excessive mineral supplies on production. Here, developments at the interface between mineral nutrition in animals and humans are considered: the particular relationship between mineral nutrition of crops and human health has been reviewed since the last edition (Martínez-Ballesta *et al*., 2010) but there are additional facets at which either the mineral nutrition of livestock affects humans or human activity affects the mineral nutrition of livestock:

- the impact of the mineral nutrition of livestock on the environment;
- the impacts of climate change on mineral nutrition of livestock and of livestock production on climate change;
- manipulating the composition of edible products to enhance market value;
- the impact of regulations to reduce hazards to humans on animal production; and
- applying advances in assessing mineral status in humans to animals.

On the last point, specific chapters have highlighted examples of diagnostic aids in regular use in human medicine that may be of use in assessing iron (p. 329) and zinc (p. 421) status in animals.

The 'Mineral Footprint'

Most of the minerals consumed by livestock are voided in urine and faeces and the proportion increases as the margin of mineral supply over requirement increases. Three driving forces lead to overprovision of minerals on farms:

- farmers generally follow a precautionary principle, based on the belief that their stock cannot get too much of or too many essential minerals;
- suppliers of minerals and compound feeds exploit that belief, while following a similar one, anxious to avoid all risk of their product failing to optimize production;
- farmers and suppliers lack specialized knowledge of mineral nutrition and lack confidence in estimates of requirement.

Thus, excessive mineral supplies pollute the environment and the chosen or default policy for mineral nutrition on a given farm leaves behind a 'mineral footprint' (e.g. Hristov *et al.*, 2006, 2007). This legacy provokes regulatory and legislative reactions that will increasingly constrain animal production (e.g. Cd, p. 491) (Meyer and Mullinax, 1999).

Maximum permissible and residual limits

The European Community (EC) and national authorities (e.g. Food and Drug Administration (FDA)) set maximum permitted (MPL) or residual (MRL) levels for mineral concentrations in foodstuffs and foods to protect livestock, consumers and/or the environment, following similar precautionary principles to those adopted by producers. For essential minerals, MPL are set at levels several times greater than minimum requirements of livestock; feed compounders add minerals to attain concentrations close to the MPL, amplifying the 'precautionary principle' by regarding MPL as *optimal* rather than the intended *maximally acceptable* levels *of last resort*. Table 20.1 compared practice in 2009 for supplying minerals to ruminants in the UK with mineral requirements and MPL at the time. Mineral provision from concentrates alone exceeded requirement for nearly all elements and classes of livestock, yet an unsupplemented mixed ration would have met most minimum requirements. Overprovision of minerals for dairy cows with minerals in the UK has continued (Sinclair and Atkins, 2015) and remains a common

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Table 20.1. Mineral supplementation practice in Europe (from responses to a livestock feed industry questionnaire in 2009; A. Packington, personal communication) indicated widespread application of precautionary principles: trace mineral provision via complementary feeds generally exceeded the highest minimum requirement (values in *italics*): contribution of minerals from forages (assumed to constitute 50% of total rations) is ignored though often sufficient to meet requirements without supplementation. Maximum permissible concentrations for Cu, I and Zn in Europe were reduced in 2016.

Contribution to whole ration from complementary feed	Dairy (milking)	Calf rearer	Beef	Ewe	Minimum requirement[a]	MPL[e]
Calcium (g kg^{-1}DM)[c]	4.0 *(5.3)*	5.5 *(8)*	7 *(11)*	6.5 *(4.3)*	3–*11*	
Phosphorus	2.5 *(2.8)*	2.5 *(4.4)*	2.5 *(2.7)*	3 *(3.2)*	1–*4.4*	
Copper (mg kg^{-1}DM)[d]	20	10	10	0	5–*28*	30 (17)[b]
Cobalt	0.25	0.25	0.25	0.5	0.05–*0.15*	2.2
Manganese	10	30	30	40	15–*25*	170
Iodine	2.5	2.5	2.5	2	0.1–*1*	2
Selenium	0.4	0.25	0.25	0.35	0.05–*0.15*	0.6
Zinc	70	40	40	50	15–*40*	120

[a]The average requirement is skewed towards the lower limit of the range, the maximum rarely applies throughout a cycle of production and temporary dietary shortfalls can usually be tolerated.
[b]Addition of copper to sheep feeds is forbidden in European Community countries and a lower MPL is set (for sheep than cattle) to lower risks of copper poisoning.
[c]Concentration contributed by the complementary feed, including minerals added to it.
[d]Concentration contributed exclusively by the supplementary trace minerals in complementary feeds.
[e]MPL are stated on an 'as fed' or 88% DM basis and have been divided by 0.88 to allow a comparison with provision and requirement on a 100% DM basis.

policy for pigs and poultry. Many trace elements are potent oxidants, capable of inducing deleterious changes in rations, particularly during prolonged storage at high temperatures.

- MPL, set to protect the environment, have the opposite effect in practice – raising mineral concentrations in compound feeds far above livestock needs and enlarging mineral footprints, while possibly depleting livestock of other nutrients.

Anomalies in maximum permitted levels

As currently employed, MPL ignore large genetic and dietary effects on mineral needs and tolerances amongst animals. An MPL can sometimes either protect environments at the expense of livestock or protect the majority of livestock at the expense of the minority. Cattle can maintain adequate liver Cu without supplementation of total mixed rations (TMR) and additions of copper at the MPL can raise liver Cu to hepatotoxic concentrations (Hunter *et al.*, 2013), beyond the MRL for consumers. Addition of Cu to sheep diets is prohibited to protect breeds that absorb copper hyper-efficiently (e.g. Texels; Suttle *et al.*, 2002; Suttle, 2012) from chronic copper poisoning (CCP). Even without supplementation, it is hard for feed compounders to keep dietary Cu low enough to protect vulnerable breeds and a cocktail of antagonists (Fe, Mo, S and Zn) were routinely added to products to reduce risk of CCP. However, MPL for Zn was lowered by the EC (2003) directive from 350 to 150mg kg^{-1}DM on environmental protection grounds, increasing risk of CCP in vulnerable breeds via the 'antagonist cocktail' route (p. 282). The constraint on Zn could be offset by increasing the MPL for added Mo in concentrates from 2 to 3mg kg^{-1}DM, but this might put breeds that absorb copper inefficiently (e.g. Scottish Blackface) at risk of insufficiency.

- An evidence-based policy would set MPL in terms of a Cu:Mo ratio of >4.0, rather than separately for Cu and Mo and limit inclusion rates for permitted feeds to, say, 60% of TMR.
- Adding a further tier to regulations, a maximum desirable limit (MDL), based on satisfactory mineral allowances (p. 12) would remind farmers and feed compounders that the MPL is a warning against excess, *not* a nutritional target.

Copper and zinc footprints on pig and poultry farms

Addition of 250mg Cu kg^{-1}DM, as $CuSO_4$, to pig and poultry rations to stimulate growth (p. 285) created

Cu-rich excreta, local dispersal of which increased soil extractable (DTPA) Cu (e.g. Payne *et al.*, 1988). Although plant uptake of Cu showed little or no increase, the soil microbiome might be affected and copper may enter the food chain via soil ingestion in mixed farming systems. A similar problem arose with dietary Zn, used at 'pharmacological' concentrations (up to 3g kg^{-1}DM, as ZnO) to control diarrhoea in early weaned pigs (p. 435). A survey of liver Cu and Zn in cattle in north-west Spain found a positive correlation between liver Cu and density of pig-rearing units (Lopez-Alonso *et al.*, 2000): liver Zn was not raised but is a poor indicator of exposure (p. 418). Both practices were progressively constrained on environmental protection grounds in Europe, the most recent MPL for Cu being 25mg Cu Zn kg^{-1}DM for all pigs (EFSA, 2016) and 150, 120 and 100mg Zn kg^{-1}DM for pigs, turkeys and poultry, respectively (EFSA, 2014). The feed industry began investigating mechanisms responsible for the response and searching for eco-friendly, alternative treatments, including Cu and Zn compounds that stimulated growth at lower concentrations: nanoparticulate sources showed most promise but none was likely to stimulate growth at the new MPL.

Copper footprints on dairy farms

In a situation typical of that pertaining on many UK farms, Cu supplements were being used on a grand scale in a Scottish dairy herd, taking dietary Cu in a TMR for pregnant cows closer, at 35mg kg^{-1}DM, to the MPL rather than to requirement (5–10mg Cu kg^{-1}DM) for much of the year (Table 20.2). Calves were reared on a milk replacer, with 10 mg added Cu kg^{-1}DM, a common industry practice that tracked the MPL but ignored the very high A_{Cu} from milk (p. 260). Replacement heifers were weaned onto a ration containing the current EC MPL of 30mg Cu kg^{-1}DM but also supplemented with coccidiostat that would defaunate the rumen and greatly increase A_{Cu}: dry and lactating cows were treated equally generously. Cumulative overprovision led to calves being born with excess liver Cu (Table 20.2). Calf mortality was high but fell while Cu was progressively removed from all rations, without reducing plasma and liver Cu below normal. The ignorance of feed formulators of important details of copper nutrition, the eagerness of farmers to follow the precautionary principle at every opportunity and weaknesses of the MPL system were leaving behind a colossal 'copper footprint' as tonnes of copper were annually dispersed around farms in policies unlikely to benefit herd health. Bovine copper toxicity is causing continuing concern from the UK (Kendall *et al.* 2015; Suttle, 2016), to New Zealand (Grace and Knowles, 2015).

Table 20.2. Simultaneous routes of copper supplementation employed in a UK dairy herd and the hepatotoxic concentrations that were a cause for concern in newborn calves (actual copper requirements are given in parentheses) (Hunter *et al.*, 2013).

	Cu (mg kg^{-1}DM)
Dry cow	30–50 (5–10)
Milking cow	40–60 (5–10)
Calf milk replacer	10 (1–2)
Calf rearer	20 (5–8)
Liver Cu in newborn calves	13–17 mmol kg^{-1}DM

Phosphorus footprints

Among major minerals, the problem posed by overfeeding phosphorus (P) has long been recognized (Joengbloed and Lenis, 1998). Farmers and feed compounders regularly include more P than national authorities recommend (e.g. dairy cows, Hristov *et al.*, 2006; pigs, NRC, 1994; turkeys, Godwin *et al.*, 2005). Furthermore, the recommendations themselves are generally excessive (p. 153). The problem is accentuated by overfeeding Ca (e.g. in dairy cows, Chapuis-Lardy *et al.*, 2004) and the fact that excess P is excreted in highly labile forms, particularly on dairy farms (Toor *et al.* 2005; McDowell *et al.*, 2008). Concurrent overfeeding of Ca changes the nature of the P footprints by decreasing P absorption in all farmed species, as P is diverted from urine to faeces. Survey data for the UK (Table 20.1; Sinclair and Atkins, 2015) confirmed that P was generally being overfed to most classes of ruminant that received complementary feeds. New, lower estimates of P requirement in Europe (p. 146) and the USA (NRC, 2016) are still too conservative.

Reducing the Phosphorus Footprint

Genetic manipulation of plants and microbes

Biotechnology was applied to the problem by improving the utilization of plant phytate phosphorus (*Py*P) in

pigs and poultry through 'genetic engineering'. A first generation of microbial and fungal phytases showed limited capacity to degrade *Py*P but a second generation, with improved pH optima and better stability in both pelleted ration and gut, increased AA_P (p. 141). In parallel studies, lines of genetically modified corn, low in *Py*P but not P, were developed and AA_P improved but the margin over normal corn was far greater in one pig study (Spencer *et al.*, 2000) than in another (Sands *et al.*, 2001) in which the feed was fed as a mash. Low-*Py*P barley (Veum *et al.*, 2002) and soybean (Dilger and Adeola, 2006) also provided more AA_P than normal varieties. However, low-*Py*P mutations of barley gave poor grain yields and there were problems to be overcome before they become commercially viable (Bregitzer and Raboy, 2006). A new approach was to focus on the low P rather than low phytate trait (Rose *et al.*, 2013). Success would have secondary benefits in raising the bioavailabity of several trace elements, for which *Py*P is an antagonist, and lower other mineral footprints (Cominelli *et al.*, 2020).

Genetic manipulation of animals

Pigs were genetically modified to express microbial phytase in saliva and utilized *Py*P better than conventional pigs (Golovan *et al.*, 2001). Poultry were similarly modified to overexpress a recombinant avian phosphatase with high activity towards *P*yP, a development that seemed to offer a 'golden bullet' solution to the problem of environmental pollution with P (Cho *et al.*, 2006). Research has progressed and the Yorkshire EnviroPig has been created to turn vision into reality, enabling pigs to be fattened without supplementary P (Meidinger *et al.*, 2013). Genetic modification of plants and animals sufficient to meet peak demands for AA_P would provide excess P at times of low demand (e.g. finishing pigs and laying hens) and simply re-route surplus P from the faeces to urine, increasing its lability. If the two technologies were concurrently and successfully adopted, the combination could lead to hyperexcretion of urinary P.

Dietary manipulation for pigs and poultry

There are many complementary ways of *immediately* reducing P footprints that do not require costly supplements and new technologies:

- partial replacement of corn with a 'low-P' feed (e.g. cassava, sugarbeet pulp) for animals with low phosphorus demands (e.g. finishing broilers, poults and pigs, hens);
- feeding grain by-products of the distillery and biofuel industries, which are of much higher 'phosphorus value' than the grains themselves (Pedersen *et al.*, 2007);
- reducing calcium supplements in rations to the minimum required (pp. 81–84);
- blending corn and/or soybean meal with cereals and by-products rich in phytase activity (e.g. laying hens, turkey poults) (p. 138);
- adding water to rations prior to feeding to stimulate endogenous phytase activity (p. 138) (Godwin *et al.*, 2005);
- avoiding pelleting, which lowers phytase activity in feeds (p. 138);
- substituting animal or insect protein for SBM, in which AA_P is far lower; and
- co-supplementing with carbohydrases and phytases, which increases AA_P (p. 148).

When inorganic P was partially replaced with wheat middlings during the growing period and all or most mineral and vitamin supplements withdrawn for the last 27 days of fattening, incidence of bone fractures did not increase (Shaw *et al.*, 2006). However, serum osteocalcin and pyridinoline, indicators of osteoblast activity and bone resorption were increased and bone density and metacarpal strength were both reduced, suggesting that a delicate balance was being struck. Addition of up to 30% wheat middlings should have allowed early reductions in both total P and Ca below the generous levels of the NRC (1994), fed prior to withdrawal: abrupt reductions from 6.1 to 3.8g Ca and from 5.2 to 3.4g P kg^{-1}DM for the last 27 days were neither wise and nor necessary.

Dietary manipulation in ruminants

It was suggested that dairy farmers in Ontario, Canada, could save $20 per cow and spare the environment 1.3 kt P per annum by reducing dietary P from 4.1 to 3.5g kg^{-1}DM (Kebreab *et al.*, 2008). A similar suggestion had been made to Idaho farmers (Hristov *et al.*, 2006), but both recommendations were conservative. No harm was done by a reduction to 2.1g P kg^{-1}DM in a pre-calving diet (Petersen *et al.*, 2005) and the calculations presented here (Tables 6.10 and 6.11) recommend 2.5g kg^{-1}DM: savings to both farmer and environment in the USA can be more than doubled! Not only

is the addition of inorganic P unnecessary on most dairy farms, but also its use may decrease degradation of *Py*P (McDowell *et al.*, 2008). Recent experiments investigating how little dietary P cows need have not gone far enough: it was predictable that 3.4g P kg^{-1}DM would be more than enough (Guo *et al.*, 2019).

Recycling phosphorus

A new approach to conserving the scarce P resource is to recycle instead of export P from farms (Margenot *et al.*, 2019) by recovering P from point sources of outflow. Recycling P requires a combination of engineering, agronomic and economic considerations, since speciation of exported P influences the cost-effectiveness of recycling P through crops and pastures. The first stage is to quantify the P cycle for a given farm, area (Wu *et al.*, 2014) or even country (e.g. France; Senthilkumar *et al.*, 2012) by Substance Flow Analysis techniques. The soil P balance in France had remained positive between 1990 and 2006 but then declined from +18 to +4kg P ha^{-1}, due largely to a reduction in use of P fertilizers. Reductions in livestock supplementation may be needed to effect further significant reductions.

Reducing Other Mineral Footprints

Feeding to minimum mineral requirements

All mineral footprints could be reduced by feeding minerals at levels that meet best estimates of minimum requirement (Table 20.1). Throughout this new edition, efforts have again been made to derive low average requirements and identify factors that increase them so that proportionate allowances can be made for 'adverse mineral circumstances'. Implementation of the proposed requirements would be assisted by changes in current commercial and legislative practice. Removal of all supplementary trace minerals from a fattening ration for pigs had only minor effects on performance and carcase quality: a few were even positive! (Shelton *et al.*, 2004).

- No progress has been made on the safety of withdrawing or reducing mineral supplements *per se*, because such studies do not attract commercial sponsorship.

Such reductions are consistent with the current drive for 'precision feeding'.

Chelated minerals – a false economy

Suppliers of chelated minerals leapt on the environmental protection bandwagon. It was argued that use of hyperavailable mineral sources would reduce mineral footprints by reducing dietary concentrations needed for optimal performance, while reducing mineral dispersal in animal wastes. If chelation was a guarantor of hyperavailability then this would indeed be a valid approach, but there is little or no evidence that it is (p. 48). Undaunted and assuming what remained to be proven, commercially inspired trials often fed 50% less mineral in organic than organic form and claimed a benefit of source for the inevitable 50% reduction in faecal mineral excretion: such 'win–win' designs provided the commercially desirable answer, while avoiding the crucial question: did the chelate supplement improve animal performance? For example, faecal Cu excretion by pigs given $CuSO_4$ at growth-stimulatory levels was compared to that of pigs given less chelated Cu (Veum *et al.*, 2004). Although faecal Cu was predictably reduced (Fig. 20.1), no difference in availability between sources was evident. Similar false comparisons were made with supra-nutritional inorganic and chelated Zn supplements (Hollis *et al.*, 2005; Mullan and D'Souza, 2005).

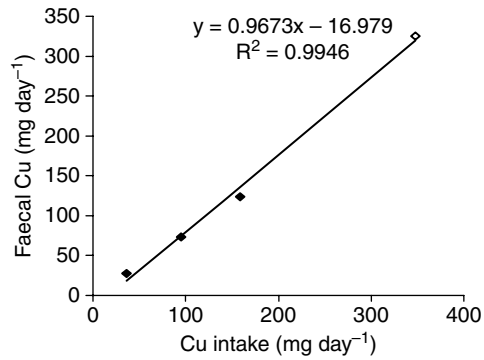

Fig. 20.1. A failed attempt to justify use of chelated copper (solid symbol) rather than $CuSO_4$ (open symbol) to stimulate pig growth and protect the environment: assuming that the chelate contained hyperavailable Cu, less was added and faecal Cu excretion reduced but the common relationship between intake and excretion of copper suggested the two sources provided equally available copper; the environment could be protected by simply feeding less $CuSO_4$. (Data from Veum *et al.*, 2004)

- Footprints from mineral sources must be compared over similar ranges of mineral concentrations.

Climate Change and Mineral Nutrition

Climate change and steps taken to ameliorate it will affect the mineral nutrition of livestock. Higher temperatures and heavier spring and autumn rainfalls are extending the grazing season in temperate zones. Mineral disorders associated with sudden (e.g. hypomagnesaemic tetany, p. 101) or prolonged exposure to lush pastures (hypocuprosis, p. 101) may be exacerbated. Infections of spring pasture with nematode larvae may occur earlier and be of increased severity, consequently depriving animals of sodium (p. 186). By contrast, shifting rainfall distribution from already arid zones will extend areas of salinity and aridity, with attendant problems for mineral nutrition (p. 197).

Ruminants are significant contributors of the 'greenhouse gas' methane to the environment and attempts are being made to manipulate the rumen microflora by pharmacological and genetic means. One avenue is to reduce methane output from protozoa by defaunating the rumen. Rumen protozoa generate sulfide in the rumen and defaunation will have secondary effects on mineral availability, both good and bad: availability of copper will increase, reducing the need to supplement but increasing the risk of copper poisoning, particularly in sheep; in the incident reported in Fig 11.16, lambs began life in a germ-free and protozoa-free environment. Toxicities of cadmium and lead may also be increased, since precipitation as insoluble sulfides is part-and-parcel of ruminants' tolerance of those elements (p. 499).

- Some methane inhibitors may affect synthesis of B vitamins, including vitamin B_{12}.

Another approach involves pharmacological inhibition of Ni-dependent methane coenzyme M (p. 468) but may be unacceptable in organic farming systems. The red seaweed *Asparagopsis taxiformis* contains two inhibitors of methane synthesis, bromoform and dibromochloromethane. Addition of 2.5 and 5g kg^{-1}DM to the diet of beef cattle inhibited methane output by up to 87% (Roque *et al*., 2021). However, *A. taxiformis* also contained 2270mg I kg^{-1}DM and added 5.7 and 11.7mg I kg^{-1}DM to the diet, nearly three and six times the proposed limit for cattle feeds in Europe (p. 316) and possibly sufficient to inhibit absorption of colostral immunoglobulins by the newborn calf (Fig. 12.13). European limits are set to limit exposure of humans to excess I from milk and could arguably be raised for beef cattle, since accumulation of I in edible meat is trivial (Roque *et al*., 2021).

Increased production of biofuel and other by-products

Cereals are increasingly grown for fuel production, increasing the supply of by-products such as dried distillers grain solubles (DDGS). Availability of *Py*P in DDGS is far greater than that in the unfermented grain (Whitney *et al*., 2006; Pederson *et al*., 2007). Inclusion of DDGS in pig and poultry rations can obviate the need to supplement with phosphate, a scarce resource. However, use of H_2SO_4 during production means that DDGS are high in S and their use for feedlot cattle heightens risk of sulfide poisoning (p. 213): secondary inhibition of copper absorption has yet to be fully explored (p. 222). Competition between animals and humans for scarce energy and protein sources is increasing the use of other by-products. Cassava peel and rapeseed meal are potential sources of goitrogens (p. 312), which are transferable to milk and of possible danger to animals and humans in low-Se and low-I areas.

Enhancing 'Mineral Value' of Foods for Humans

Minerals in edible livestock products now attract the accolade of 'functional foods' and are the focus of entire research institutes and dedicated journals. The life-sustaining properties of micronutrients in foods for humans has health and market value, which can be enhanced by mineral supplementation of livestock (Welch and Graham, 2004; Knowles *et al*., 2006; Rooke *et al*., 2010). The ability of selenium at supra-nutritional intakes to reduce susceptibility to carcinogens is now in doubt but other benefits from enhanced antioxidant capacity may exist (Mandour *et al*., 2020). Functional foods offer the agricultural industry opportunities to reverse a tide of adverse publicity on life- climate- and environment-threatening qualities of meat, milk and egg production. Lucrative markets for trace element supplements for humans testify to concerns that modern diets are inadequate. However, most claimed benefits, such as improved fertility, vitality and resistance to infection, remain as illusory as those claimed for chelated minerals in livestock nutrition. The

temptation to overprovide minerals is as strong in human as in animal nutrition and notes of caution are sounded here.

Enhancing thyroid function

Goitre is such a conspicuous and specific sign of iodine deprivation that its regional occurrence prompted interventions to increase dietary iodine intakes, sometimes with spectacular success. A high incidence of goitre in Denmark led to a nationwide scheme in 1988 to increase iodine intakes by arranging for table salt and bread to be iodized. Surprisingly, incidence of hypothyroidism rose, particularly in previously goitre-free areas (Laurberg *et al.*, 2006). It was discovered that daily iodine intakes varied widely from area to area due to variations in iodine supply from drinking water and some individuals from high-iodine areas were exposed to sufficient iodine to suppress thyroid activity. Iodine supply can be improved by other means, such as feeding supra-nutritional levels of iodine to the dairy cow, because there is a linear relationship between dietary I and milk I (Fig. 12.6). However, there are those who thought that milk I was already too high (Phillips *et al.*, 1988). Supplementation with Se in New Zealand did not improve thyroid status in New Zealand (Thomson *et al.*, 2005). Mass medication to improve micronutrient-dependent functions is fraught with problems because of variations in individual micronutrient intakes as well as vocal minorities who do not want either their food nor water supplies tampered with. Campaigns such as that against fluoridation are likely to be replicated.

Enhancing antioxidant status

Oxidant stress is alleged to be a contributory factor in many diseases (Surai, 2006). One component of antioxidant defences, glutathione peroxidase (GPX) activity, is enhanced simply by raising dietary Se (Lei *et al.*, 1998). Human Se intakes declined in the UK (Givens *et al.*, 2004) and were low in northern Europe, New Zealand and elsewhere (Rayman, 2005; Combs, 2001). Addition of Se to livestock rations was authorized in Sweden in 1980 and in the USA in 1985. Selenium fertilization of cereal crops was promoted in Finland from 1995. Biofortification and phytoremediation have been widely studied in China, where Se-responsive disorders in the human population are relatively common (Wu *et al.*, 2015). Supplementation of rations with sources rich in selenomethionine (SeMet), such as selenium yeast (SeY), has also been advocated because it raises milk (Fig. 20.2; Juniper *et al.*, 2006) egg (Fig. 20.3) and meat Se (Juniper *et al.*, 2008) more than the same amount of inorganic Sei but no advantage may be found in GPX activity. Antioxidant capacity at crucial intracellular sites, such as the pathogen-engorged phagocyte, may even be inferior, for reasons discussed earlier (p.se).

- Total tissue and blood Se provide inaccurate measures of functional Se status in consumers and are as unreliable for consumers as for animals (p. 395).

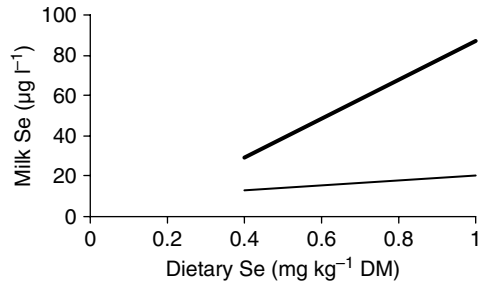

Fig. 20.2. When selenium yeast is added at high levels to the diet of dairy cows (bold line), the increase in milk Se is far greater than when the same amount of Se is added as selenite or a 'metalosate' (amino acid complex) (Givens *et al.*, 2004).

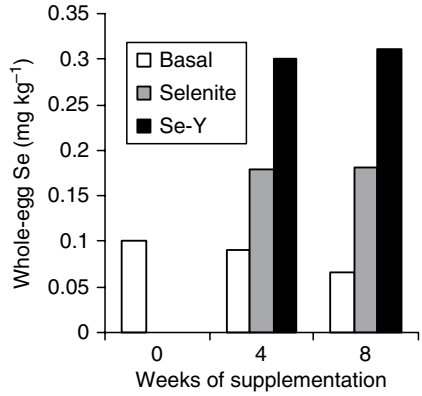

Fig. 20.3. The supplementation of the ration of laying hens with selenium yeast (Se-Y) at the maximum level permitted in the USA (+0.3mg kg^{-1}DM) raises the Se concentration in the whole egg threefold and is more effective than the same amount of Se added as selenite. Note that egg Se declines significantly with time when hens were fed a basal diet that contained 0.1mg Se kg^{-1}DM. (Data from Utterback *et al.*, 2005)

Selenium source, cancer prevention and treatment

Recent reviews of the relationship between dietary Se and cancer incidence cast doubt on the review relied on in the last edition (Rayman, 2005). Routine addition of Se to fertilizers in Finland from 1985 had raised Se intakes in the human population without decreasing cancer incidence, 30 years later (Alfthan *et al*., 2015). The current consensus is that there is no general relationship between dietary Se and cancer incidence (e.g. Chen *et al*., 2013; Vinceti *et al*., 2018) but a possible role in preventing prostate cancer and limiting metastasis (Chen *et al*., 2013).

- Relationships involving historic indices of status, such as blood and plasma Se, will probably have been disrupted by increased consumption of organic Se supplements (p. 388), elicited by heightened expectations of benefit.

Efficacy of chemotherapy and radiotherapy is enhanced by Se supplements and safer with organic than inorganic Se (Chen *et al*., 2013). Chemotherapy and radiotherapy cause intense oxidative stress, which may explain the complex interactions with Se status. Links between bowel cancer and Se status have also been investigated[ii] but benefits of SeMet (or SeY) over inorganic Se in this and other contexts seem unlikely. Indeed, efficacy of SeMet may be relatively poor when rates of protein accretion or secretion are high (e.g. realimented infants, adolescents and nursing mothers; Fig. 15.6). Selenium-accumulating plants with atypical Se speciation (e.g. certain brassicas and vetches) may be pharmaceutically superior, at high inputs, to SeMet. The association of value with a particular 'exotic' species would allow consumers to choose for themselves a dietary component that might increase carcinogen tolerance. The case for increasing milk, meat and egg Se by supra-supplementing livestock with organic Se is weakened by the latest consensus.

Enhancing iron status

Iron-deficiency anaemia is the most prevalent trace-element-responsive disorder of humans, affecting as many as 100 million people worldwide, most of them adult women or infants (Beard, 2008). The primary cause is low availability of iron from cereal sources, particularly maize (p. 334) and herein lies a major opportunity for improving the 'functional iron status' of human diets. Vegetable protein sources can be modified to lower their content of iron antagonists, notably phytate, or increase their content of available iron (White and Broadley, 2009). However, enhancing iron nutrition increases susceptibility to malaria (Prentice, 2008) and malaria risk must be greatly reduced before releasing 'high-iron genotypes' into populations at high risk of contracting the disease. One simple dietary remedy is to consume a vitamin C-rich drink or supplement prior to eating.

- Consuming vitamin C-rich milk from animals given green feeds may improve iron status.

Enhancing calcium status

Increasing attention is being given to the nutritive value of milks, other than cows milk, for human nutrition, taking account of the superior calcium and protein concentrations in milks from most other species (Table 2.4). A comparison of ewes and cows milk in growing rats has shown that the former increases trabecular bone formation (Burrow *et al*., 2019). Reindeer, camel and goat milk products may be useful in avoiding malnutrition in children. Given the prevalence of osteoporosis in the elderly, the therapeutic value of Ca-rich milks is worthy of study in this context.

Enhancing the Sensory Value of Foods

During the interval between slaughter and cooking, autolytic changes take place in meat that affect the taste, smell and visual appearance (organoleptic quality) of the product. The process is essentially one of oxidative deterioration and consequences such as change of colour (e.g. accumulation of metmyoglobin) (Ryu *et al*., 2005), exudation ('watery pork') and taint (Bou *et al*., 2005) can have a negative effect on retailer and consumer valuation of meat products. Manufacturers of mineral supplements with potential antioxidant properties, including zinc chelates and SeMet, were quick to search for evidence that feeding antioxidant minerals to livestock enhanced the sensory value of meat, measuring everything from pH and shear strength to 'tastiness' in blindfolded consumers: such responses had been found with antioxidant vitamin E supplements and when Se was given with vitamin E (Ryu *et al*., 2005). Improvements in storage characteristics following mineral supplementation alone have generally been small (Bou *et al*., 2005). For example, a supra-nutritional inorganic Se supplement produced only minor improve-

ments in oxidative stability of chicken meat (Ryu *et al.*, 2005). Raising dietary Se in a pig ration from 0.18 (high) to 0.48mg kg^{-1}DM (very high) reduced 'drip loss' significantly and more effectively when added as organic rather than inorganic Se (Mateo *et al.*, 2007). Whether a 0.8% reduction in drip loss, only one third of total drip loss, justified such 'medication' of pig rations is questionable and consumer reaction to the practice would need to be tested.

Putting a price on functional and sensory value

Enhancement of functional or sensory value will usually increase production costs and will only be commercially sustainable if extra costs are more than recouped in the marketplace. Furthermore, enhanced functional value will have to be measurable and measured as part of quality-assurance schemes. Alternatively, national governments might sponsor production of 'functionally valuable' products if mass consumption was likely to produce cost-saving improvements in a nation's health. In either case, far more evidence of real health benefits is required before the enhancement of functional value becomes either a commercially viable proposition or an intervention policy. Major purchasers of meat who recognized superior keeping quality in produce from particular suppliers might be prepared to offer a premium price.

New regulations on additives to animal feeds

Given the need to avoid micronutrient excess as well as deprivation, there will be a need to regulate disparate attempts to improve the functional and sensory value of our foods. Trace mineral intakes from edible cuts of meat in Switzerland vary widely and early approval of Se supplementation in the USA probably contributed to threefold higher beef Se in US than Swiss produce (Gerber *et al.*, 2009). Some consumers are already eating – wittingly or unwittingly – bread, eggs and bacon enriched with organic Se, and milk on the breakfast table may also be enriched with Se.

- How can consumers know the adequacy of their Se intakes and when should those in the habit of self-medication stop taking Se-rich pills?

There is an urgent need for international regulatory standards to constrain powerful commercial interests that make substantial profits from selling mineral supplements for livestock that neither animals nor consumers need.

Conflict between Anti-nutritional and Nutritional Properties

Selenium and polyunsaturated fatty acids

Concern about the adverse effects of saturated fats in dairy and meat products on human health has stimulated interest in desaturating animal foods by feeding livestock more unsaturated fat. It is not difficult to produce softer, less saturated fat. In ruminants, unsaturated dietary fat can be protected from saturation in the rumen. However, high polyunsaturated fatty acid (PUFA) intakes increase the demand for vitamin E and increase the risk of Se-responsive disorders in livestock (p. 377). It is probably only a matter of time before high PUFA intakes are recognized as potentially harmful to human health, increasing the human requirement for selenium.

Phytate – friend or foe?

The enhanced utilization of plant P from cereals and protein sources, selected to be low in phytate, has been hailed as a breakthrough in terms of environmental protection from pollution, but are there possible unforeseen side-effects that could harm human health? Questions must be asked, since the benefits of *raising* phytate intakes for humans are being investigated. Dietary phytate supplements have anti-neoplastic activity against a range of tumours (Singh *et al.*, 2004; Zhang *et al.*, 2005) and protect tissues from calcification (Grases *et al.*, 2004). Phytate is also found in mammalian tissues and cell membranes, where incorporation into phospholipids may influence cell-signalling, angiogenesis (Vucenik *et al.*, 2004) and calcification of blood vessels (Joubert *et al.*, 2016). Populations that rely heavily on grains in their diets have low prevalences of bowel and certain other cancers.

- The extensive replacement of 'wild-type' cereals with low-phytate varieties would lower phytate intakes from 'western' diets with possible adverse effects on human health, including increased cancer incidence.

Conflict between Health Hazard Reduction and Animal Nutrition

The current approach in the context of food safety is one of hazard reduction rather than risk assessment; in other words, to lower the concentration of a potentially harmful mineral at all costs, rather than

to conduct a risk (to humans)–benefit (to livestock) analysis. Fear of excess iodine consumption by humans has restricted MPC in animal feeds to 2mg I kg^{-1}DM. While this is more than enough in rations low in goitrogens, it is close to the need for I in goitrogen-rich rations. Increased use of goitrogen-rich by-products by dairy farmers may reduce milk I, a major contributor of I to the human diet (p. 310).

Hazard labelling for mutagenic or carcinogenic but essential minerals

Toxicologists and politicians can and increasingly do take their roles as protectors of the human population to extremes. For example, identification of cobalt as a carcinogen (National Toxicology Program, 1998) led to the introduction of a new EC Regulation (No. 1272/2008) that required a skull-and-crossbones symbol to be placed on bags of feed containing >0.01% added Co, with a warning: 'May cause cancer by inhalation'; no concession is made for pelleted diets! The real risk, if any, is to those involved in the manufacturing process and not to the livestock farmer. The energetically and nutritionally inefficient option of using synthetic vitamin B_{12} to replace Co as a feed supplement was even considered. Evidence of carcinogenicity comes from experiments with mice exposed semi-continuously (five days a week) for months on end to an aerosol containing 3 mg Co m^{-3}, enough cobalt to keep an adult sheep nourished for a month! It is probably only a matter of time before other trace elements join the list of carcinogens, creating further difficulties for those who formulate and manufacture animal foodstuffs, with no credible impact on cancer risk.

Maximum residual levels for potentially toxic elements

The case for reducing human exposure to potentially toxic elements via the food chain once seemed unarguable (Satarug *et al*., 2000) but the idea that all elements, even Pb and Cd, are beneficial in small doses (p. 505) is causing debate amongst toxicologists that has yet to impinge upon those who set MPL and MRL. Could the drive to reduce human exposure to Pb and Cd eventually be harmful to health? On these and other matters there is a need to restore a balance, which until now has been determined entirely by simplistic 'hazard-reduction' models. MPL in feedstuffs should be based on the cumulative exposure likely to raise kidney Pb and Cd at slaughter, in growing and breeding animals, above MAC, rather than on a single concentration. The MRL in human kidney, one below which kidney function remained normal, was lowered to 50 µg Cd kg^{-1}FW and the acceptable daily Cd intake to 1µg Cd $kg^{-1}d^{-1}$ (Satarug *et al*., 2000) but translating that into an acceptable MRL for kidney Cd in livestock involves huge assumptions about human dietary preferences.

Permissible sources

In the wake of the BSE scare in the UK, use of animal protein sources in animal feeds was banned in Europe to protect consumers from a perceived health hazard. The ban has so far been only partially relaxed. The possibility of using meat and bone meal to reduce the need for supplementation with inorganic P is thus prohibited, although it could lessen environmental pollution from pig and poultry farms and enhance availability of Cu, Fe, Mn and Zn.

Conclusion

The need for dialogue between those whose priority lies in the domain of human health and those whose priority is animal nutrition has, arguably, grown since the last edition went to print. It remains the case that unless each side listens and learns from the other, problems of adequate but safe mineral nutrition for animals and humans are likely to increase in number and severity.

Notes

[i] Most of the additional Se in milk (Phipps *et al.*, 2008) and muscle (Juniper *et al.*, 2008) is present as SeMet. Partition of Se in the egg shifts from yolk to white when Se yeast is used instead of selenite (Leeson *et al.*, 2008). The 'success' of SeMet is attributable to its intact absorption and long half-life (Juniper *et al.*, 2008) but availability for GPX synthesis in supplemented animals is relatively poor.

[ii] Pre-neoplastic crypt abnormalities were induced more readily in colons of mice with genetically impaired selenoprotein expression than in wild-type mice and large selenium supplements gave partial protection (Irons *et al.*, 2006). Induction of GPX activity in Se-depleted, Caco-2 cells was faster with selenium added as selenite and methylselenocysteine than as SeMet (Zeng *et al.*, 2008). As in the nutritional context, SeMet may have been diverted from selenoprotein synthesis. SeMet

was no more effective than selenite for promoting DNA methylation, believed to be instrumental in protecting rats from colonic cancer (Davis *et al.*, 2000).

References

Alfthan, G., Eurola, M., Ekholm, P., Venäläinen, E.R., Root, T. *et al.* (2015) Selenium Working Group. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: from deficiency to optimal selenium status of the population. *Journal of Trace Elements in Medicine and Biology* 31, 142–147.

Beard, J.L. (2008) Why iron deficiency is important in infant development. *Journal of Nutrition* 138, 2523–2528.

Bou, R., Guardiola, P., Barroeta, A.C. and Codony, R. (2005) Effect of dietary fat sources and zinc and selenium supplements on the consumption and consumer acceptability of chicken meat. *Poultry Science* 84, 1129–1140.

Bregitzer, P. and Raboy, V. (2006) Effects of four independent low-phytate mutations on barley agronomic performance. *Crop Science* 46, 1318–1322.

Burrow, K., Young, W., Carne, A., McConnell, M., Hammer, N., Scholze, M. and Bekhit, A.E. (2019) Consumption of sheep milk compared to cow milk can affect trabecular bone ultrastructure in a rat model. *Food Functions* 22, 163–171.

Chapuis-Lardy, L., Fiorini, J., Toth, J. and Dou, Z. (2004) Phosphorus concentration and solubility in dairy faeces: variability and affecting factors. *Journal of Dairy Science* 87, 4334–4341.

Chen, Y.-C., Prabhu, K.S. and Mastro, A.M. (2013) Is selenium a potential treatment for cancer metastasis? *Nutrients* 5, 1149–1168.

Cho, J., Choi, K., Darden, T., Reynolds, P.R., Petite, J.N. and Shears, S.B. (2006) Avian multiple inositol polyphosphate phosphatase is an active phytase that can be engineered to help ameliorate the planet's 'phosphate crisis'. *Journal of Biotechnology* 1, 248–259.

Combs, G.F. (2001) Selenium in global food systems. *British Journal of Nutrition* 85, 517–547.

Cominelli, E., Pilu, R. and Sparvoli, F. (2020) Phytic acid and mineral biofortification strategies: from plant science to breeding and biotechnological approaches. *Plants* 9, 553. DOI:10.3390/plants9050553

Davis, C.D., Uthus, E.O. and Finley, J.W. (2000) Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and *in vivo* in rat liver and colon. *Journal of Nutrition* 130, 2903–2909.

Dilger, R.N. and Adeola, O. (2006) Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing pigs fed conventional and low-phytate soybean meals. *Journal of Animal Science* 84, 627–634.

EFSA (2014) EFSA/FEEDAP Panel on additives and products or substances used in animal feed: scientific opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. *EFSA Journal* 12, 3668. DOI:10.2903/j.efsa.2014.3668

EFSA (2016) EFSA/FEEDAP Panel on additives and products or substances used in animal feed: scientific opinion on the revision of the currently authorised maximum copper content in complete feed. *EFSA Journal* 14, 4563. DOI:10.2903/j.efsa.2016.4563

Gerber, N., Brogioli, R., Hattendof, B., Scheeder, M.R.L., Wenk, C. and Gunther, D. (2009) Variability in selected trace elements of different meat cuts determined by ICP-MS and DRC-ICPMS. *Animal* 3, 166–172.

Givens, D.I., Allison, R., Cottrill, B. and Blake, J.S. (2004) Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. *Journal of Science in Food and Agriculture* 84, 811–817.

Godwin, J.L., Grimes, J.L., Christensen, V.L. and Wineland, M.J. (2005) Effect of dietary phosphorus and phytase levels on the reproductive performance of large white turkey breeder hens. *Poultry Science* 84, 485–493.

Golovan, S.P., Meidinger, R.G., Ajakaiye, A., Cottrill, M., Wiederkehr, M.Z. *et al.* (2001) Pigs expressing salivary phytase produce low-phosphorus manure. *Nature Biotechnology* 19, 741–745.

Grace, N. and Knowles, S. (2015) Taking action to reduce the risk of copper toxicity in cattle. *Veterinary Record* 177, 490–491.

Grases, F., Perello, J., Prieto, R.M., Simonet, B.M. and Torres, J.J. (2004) Dietary myo-inositol hexaphosphate prevents dystrophic calcifications in soft tissues: a pilot study in Wistar rats. *Life Sciences* 75, 11–19.

Guo, Y.Q., Tong, B.X., Wu, Z.G., Ma, W.Q. and Ma, L. (2019) Dietary manipulation to reduce nitrogen and phosphorus excretion by dairy cows. *Livestock Science* 228, 61–66.

Hollis, G.R., Carter, S.D., Cline. T.R., Crenshaw, T.D., Cromwell, G.L. *et al.* (2005) Effects of replacing pharmacological levels of dietary zinc oxide with lower dietary levels of various organic zinc sources for weanling pigs. *Journal of Animal Science* 83, 2123–2129.

Hristov, A.N., Hazen, W. and Ellsworth J.W. (2006) Efficiency of use of imported nitrogen, phosphorus, and potassium and potential for reducing phosphorus imports on Idaho dairy farms. *Journal of Dairy Science* 89, 3702–3712.

Hristov, A.N. Hazen, W. and Ellsworth J.W. (2007) Efficiency of use of imported magnesium, sulfur, copper and zinc on Idaho dairy farms. *Journal of Dairy Science* 90, 3702–3712.

Hunter, A.G., Suttle, N., Martineau, H.M., Spence, M.A., Thomson, J.R., Macrae, A.I. and Brown, S. (2013) Mortality, hepatopathy and liver copper concentrations in artificially reared Jersey calves before and after reductions in copper supplementation. *Veterinary Record* 172, 46.

Irons, R., Carlson, B.A., Hatfield, D.L and Davis, C.L. (2006) Both selenoproteins and low molecular weight selenocompounds reduce colon cancer risk in mice with genetically impaired selenoprotein expression. *Journal of Nutrition* 136, 1311–1317.

Joengbloed, A.W. and Lenis, N.P. (1998) Environmental concerns about animal manure. *Journal of Animal Science* 76, 2641–2648.

Joubert, P., Ketteler, M., Salcedo, C. and Perello, J. (2016) Hypothesis: phytate is an important unrecognised nutrient and potential intravenous drug for preventing vascular calcification. *Medical Hypotheses* 94, 89–92.

Juniper, D.T., Phipps, R.H., Jones, A.K. and Bertin, G. (2006) Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood, milk, urine and feces. *Journal of Dairy Science* 89, 3544–3551.

Juniper, D.T., Phipps, R.H., Ramos-Morales, E. and Bertin, G. (2008) Selenium persistency and speciation in the tissues of lambs following the withdrawal of a high-dose selenium-enriched yeast. *Animal* 2, 375–380.

Kebreab, E., Odongo, N.E., McBride, B.W., Hanigan, M.D. and France, J. (2008) Phosphorus utilization and environmental pollution and economic implications of reducing phosphorus pollution from Ontario dairy farms. *Journal of Dairy Science* 91, 241–246.

Kendall, N.R., Holmes-Pavord, H.R., Bone, P.A., Ander, E.L. and Young, S.D. (2015) Liver copper concentrations in cull cattle in the UK: are cattle being copper loaded? *Veterinary Record* 177, 493.

Knowles, S.O., Grace, N.D., Knight, T.W., McNabb, W.C. and Lee, J. (2006) Reasons and means for manipulating the micronutrient composition of milk from grazing dairy cattle. *Animal Feed Science and Technology* 131, 154–167.

Laurberg, P., Jorgensen, T., Perrild, H., Ovesen, L., Knudsen, N. *et al.* (2006) The Danish investigation on iodine intake and thyroid disease, DanThyr: status and perspectives. *European Journal of Endocrinology* 155, 219–228.

Leeson, S., Namkung, H., Caston, L., Durosoy, S. and Schlegel, P. (2008) Comparison of selenium levels and sources and dietary fat quality in diets of broiler breeders and laying hens. *Poultry Science* 87, 2605–2612.

Lei, X.G., Dann, H.M., Ross, D.A., Cheng, W.-S., Combs, G.F. and Roneker, K.R. (1998) Dietary selenium supplementation is required to support full expression of three selenium-dependent glutathione peroxidases in various tissues of weanling pigs. *Journal of Nutrition* 128, 130–135.

Lopez-Alonso, M.L., Benedito, J.L., Miranda, M., Castillo, C., Hernandez, J. and Shore, R.F. (2000) The effect of pig farming on copper and zinc accumulation in cattle in Galicia (North-West Spain). *The Veterinary Journal* 160, 259–266.

Mandour, A.S., Samir, H., El-Beltagy, M.A., Abdel-Daim, M.M., Izumi, W. *et al.* (2020) Effect of supra-nutritional selenium-enriched probiotics on hematobiochemical, hormonal, and Doppler hemodynamic changes in male goats. *Environmental Science and Pollution Research International* 27, 19447–19460. DOI:10.1007/s11356-020-08294-2

Margenot, A.J., Kitt, D., Gramig, B.M., Berkshire, T.B., Chatterjee, N. *et al.* (2019) Toward a regional phosphorus (re)cycle in the US Midwest. *Journal of Environmental Quality* 48, 1397–1413.

Martínez-Ballesta, M.C., Dominguez-Perles, R., Moreno, D.A., Muries, B., Alcaraz-López, C. *et al.* (2010) Minerals in plant food: effect of agricultural practices and role in human health: a review. *Agronomy for Sustainable Development 30 Springer Verlag/EDP Sciences/INRA*, 30, 295–309.

Mateo, R.D., Spalholz, J.E., Elder, R., Yoon, I. and Kim, S.W. (2007) Efficacy of dietary selenium sources on growth and carcass characteristics of growing-finishing pigs fed diets containing high endogenous selenium. *Journal of Animal Science* 85, 1177–1183.

McDowell, R.W., Dou, Z., Toth, J.D., Cade-Menun, B.J., Kleinman, P.J.A., Soder, K. and Saporito, L. (2008) A comparison of the phosphorus speciation and potential bioavailability in feed and feces of different dairy herds using ^{31}P nuclear magnetic resonance spectroscopy. *Journal of Environmental Quality* 37, 741–752.

Meidinger, R.G., Ajakaiye, A., Fan, M.Z., Zhan, G.J., Phillips, J.P. and Forsberg, C.W. (2013) Digestive utilization of phosphorus from plant-based diets in the Cassie line of transgenic Yorkshire pigs that secrete phytase in the saliva. *Journal of Animal Science* 91, 1307–1320.

Meyer, D. and Mullinax, D.D. (1999) Livestock nutrient management concerns: regulatory and legislative overview. *Journal of Animal Science* 77 (Suppl. 2J), 51–62.

Mullan, B. and D'Souza, D. (2005) The role of organic minerals in modern pig production. In: Taylor-Pickard, J.A. and Tucker, L.A. (eds) *Redefining Mineral Nutrition.* Nottingham University Press, Nottingham, UK, pp. 89–117.

National Toxicology Program (1998) NTP toxicology and carcinogenesis studies of cobalt sulfate heptahydrate (CAS No. 10026-24-1) in F344/N and B6C3F1mice (inhalation studies). *National Toxicology Program Technical Report Series* 471:1, 1–268.

NRC (1994) *Nutrient Requirements of Poultry*, 9th edn. National Academy of Sciences, Washington, DC.

NRC (2016) *Nutrient Requirements of Beef Cattle*, 8th edn. National Academy of Sciences, Washington, DC.

Payne, G.G., Martens, D.C., Kornegay, E.T. and Lindemann, M.D. (1988) Availability and form of copper in three soils following eight annual applications of copper-enriched swine manure. *Journal of Environmental Quality* 17, 740–746.

Pedersen, C., Boersma, M.G. and Stein, H.H. (2007) Digestibility of energy and phosphorus in ten samples of distillers dried grains with solubles fed to growing pigs. *Journal of Animal Science* 85, 1168–1176.

Peterson, A.B., Orth, M.W., Goff, J.P. and Beede, D.K. (2005) Periparturient responses of multiparous Holstein cows fed different dietary phosphorus concentrations prepartum. *Journal of Dairy Science* 88, 3582–3594.

Phillips, D.I.W., Nelson, M., Barker, D.J.P., Morris, J.A. and Wood, T.J. (1988) Iodine in milk and the incidence of thyrotoxicosis in England. *Clinical Endocrinology* 28, 61–66.

Phipps, R.H., Grandison, A.S., Jones, A.K., Juniper, D.T., Ramos-Morales, E. and Bertin, G. (2008) Selenium supplementation of lactating dairy cows: effects on milk production and total selenium content and speciation in blood, milk and cheese. *Animal* 2, 1610–1618.

Prentice, A.M. (2008) Iron metabolism, malaria and other infections: what is all the fuss about? *Journal of Nutrition* 138, 2537–2541.

Rayman, M.P. (2005) Selenium in cancer prevention: a review of the evidence. *Proceedings of the Nutrition Society* 64, 527–542.

Rooke, J., Flockhart, J. and Sparks, N. (2010) The potential for increasing the concentrations of micro-nutrients relevant to human nutrition in meat, milk and eggs. *Journal of Agricultural Science* 148, 603–614.

Roque, B.M., Venegas, M., Kinley, R.D., de Nys, R., Duarte, T.L., Yang, X. and Kebreab, E. (2021) Red seaweed (Asparagopsis taxiformis) supplementation reduces enteric methane by over 80 percent in beef steers. *PLOS ONE* 16, e024782.

Rose, T.J., Liu, L. and Wissuwa, M. (2013) Improving phosphorus efficiency in cereal crops: Is breeding for reduced grain phosphorus concentration part of the solution? *Frontiers in Plant Science* 4, 444. DOI:10.3389/fpls.2013.00444

Ryu, Y.-C., Rhee, M.-S., Lee, K.-M. and Kim, B.-C. (2005) Effects of different levels of dietary supplemental selenium on performance, lipid oxidation and color stability of broiler chicks. *Poultry Science* 84, 809–815.

Sands, J.S., Ragland, D., Baxter, C., Joern, B.C., Sauber, T.E. and Adeola, O. (2001) Phosphorus bioavailability, growth performance and nutrient balance in pigs fed high available phosphorus corn and phytase. *Journal of Animal Science* 79, 2134–2142.

Satarug, S., Haswell-Elkins, M.R. and Moore, M.R. (2000) Safe levels of cadmium intake to prevent renal toxicity in human subjects. *British Journal of Nutrition* 84, 791–802

Senthilkumar, K., Nesme, T., Mollier, A. and Pellerin, S. (2012) Conceptual design and quantification of phosphorus flows and balances at the country scale: the case of France. *Global Biogeocheical Cycles* 26, GB2008. DOI:10.1029/2011GB004102

Shaw, D.T., Rozeboom, D.W., Hill, G.M., Orth, M.W., Rosenstein, D.S. and Link, J.E. (2006) Impact of supplement withdrawal and wheat middling inclusion on bone metabolism, bone strength and the incidence of bone fractures occurring at slaughter in pigs. *Journal of Animal Science* 84, 1138–1146.

Shelton, J.L., Southern, L.L., LeMieux, F.M., Bidner, T.D. and Page, T.G. (2004) Effects of microbial phytase, low calcium and phosphorus, and removing the dietary trace mineral premix on carcass traits, pork quality, plasma metabolites, and tissue mineral content in growing-finishing pigs. *Journal of Animal Science* 82, 2630–2639.

Sinclair, L.A. and Atkins, N. (2015) A survey of dietary levels of selected macro and trace elements fed on UK dairy farms. *Journal of Agricultural Science* 153, 743–752.

Singh, R.P., Sharma, G., Mallikarnjuna, G.U., Dhanalakshmi, S., Agarwal, C. and Agarwal, R. (2004) *In vivo* suppression of hormone-refractory prostate cancer growth by inositol hexaphosphate. *Clinical Cancer Research* 10, 244–250.

Spencer, J.D., Allee, G.L. and Sauber, T.E. (2000) Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. *Journal of Animal Science* 78, 675–681.

Surai, P.F. (2006) *Selenium in Animal and Human Health*. Nottingham University Press, UK.

Suttle, N.F. (2012) Control of hepatic copper retention in Texel ram lambs by dietary supplementation with copper antagonists followed by a copper depletion regimen. *Animal Feed Science and Technology* 173, 194–200,

Suttle, N.F. (2016) Reducing the risk of bovine copper toxicity. *Veterinary Record* 178, 196.

Suttle, N.F., Lewis, R.M. and Small, J.N. (2002) Effects of breed and family on rate of copper accretion in the liver of purebred Charollais, Suffolk and Texel lambs. *Animal Science* 75, 295–302.

Thomson, C., McLachlan, S., Grant, A., Paterson, E. and Lillico, A. (2005) The effect of selenium on thyroid status in a population with marginal selenium and iodine status. *British Journal of Nutrition* 94, 962–968.

Toor, G.S., Cade-Munn, B.J. and Sims, J.T. (2005) Establishing a linkage between phosphorus forms in dairy diets, faeces and manures. *Journal of Environmental Quality* 34, 1380–1391.

Utterback, P.L., Parsons, C.M., Yoon, I. and Butler, J. (2005) Effect of supplementing selenium yeast in

diets of laying hens on egg selenium content. *Poultry Science* 84, 1900–1901.

Veum, T.L., Ledoux, D.R., Bollinger, D.W., Raboy, V. and Cook, A. (2002) Low-phytic acid barley improves calcium and phosphorus utilisation and growth performance in growing pigs. *Journal of Animal Science* 80, 2663–2670.

Veum, T.L., Carlson, M.S., Bollinger, D.W. and Ellersieck, M.R. (2004) Copper proteinate in weanling pig diets for enhancing growth performance and reducing faecal copper excretion compared with copper sulfate. *Journal of Animal Science* 82, 1062–1070.

Vinceti, M., Filippini, T., Del Giovane, C., Dennert, G., Zwahlen, M. *et al.* (2018) Selenium for preventing cancer. *Cochrane Database of Systematic Reviews*, Issue 1, article no. CD005195. *doi:10.1002/14651858.CD005195.pub4*

Vucenik, I., Passaniti, A., Vitolo, M.I., Tantivejkul, K., Eggleton, P. and Shamsuddin, A.M. (2004) Anti-angiogenic activity of inositol hexaphosphate (IP6). *Carcinogenesis* 25, 2115–2123.

Welch, R.M. and Graham, R.D. (2004) Breeding for micronutrients in staple food crops. *Journal of Experimental Botany* 55, 353–364.

White, P.J. and Broadley, M.R. (2009) Biofortification of crops with seven mineral elements often lacking in human diets: iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist* 182, 49–84.

Whitney, M.H., Shurson, G.C., Johnston, L.J., Wulf, D.M. and Shanks, B.C. (2006) Growth performance and carcass characteristics of grower-finishing pigs fed high-quality corn distillers dried grain with solubles originating from a modern Midwestern ethanol plant. *Journal of Animal Science* 84, 3356–3363.

Wu, H., Yuan, Z., Zhang, Y., Gao, L. and Liu, S. (2014) Life-cycle phosphorus use efficiency of the farming system in Anhui Province, Central China. *Resources, Conservation and Recycling* 83, 1–14,

Wu, Z., Bañuelos, G.S., Lin, Z.-Q., Liu, Y., Yuan, L., Yin, X. and Li, M. (2015) Biofortification and phytoremediation of selenium in China. *Frontiers in Plant Science* 6, 136. DOI:10.3389/fpls.2015.00136

Zeng, H., Botnen, J.H. and Johnson, L.K. (2008) A selenium-deficient Caco-2 cell model for assessing differential incorporation of chemical or food selenium into glutathione peroxidase. *Biological Trace Element Research* 123, 98–108.

Zhang, Z., Song, Y. and Wang, X.-L. (2005) Inositol hexaphosphate-induced enhancement of natural killer cell activity correlates with suppression of colon carcinogenesis in rats. *World Journal of Gastroenterology* 11, 5044–5046.

Appendix 1

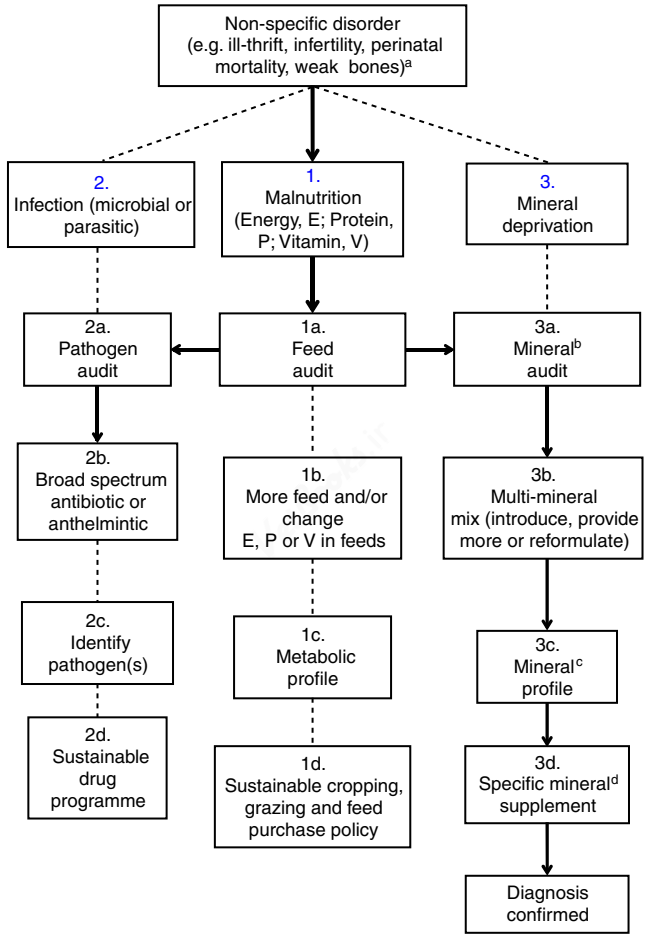

Fig. A1. A scheme for the differential diagnosis of mineral disorders with non-specific signs. Proceed vertically with a given line of investigation, starting with 1 and switching to 2 and 3 if the results are normal or negative.

[a]First rule out exotic agents, e.g. plant or industrial poisons.

[b]See Appendix 2 for guidance on macromineral audit.

[c]See Appendices 3 and 4 for guidance on profile interpretation.

[d]Some animals must be left untreated.

Appendix 2

Table A2. Mean macromineral composition (g kg^{-1}DM) of major feedstuffs sampled in the UK by the Ministry of Agriculture (standard deviations in parentheses) (MAFF, 1990). Diagnostic flow diagram (Appendix 1). Such data can be used to conduct a 'mineral audit'. Farm values that lie >1 standard deviation below the mean for that feed (or above for an antagonist such as K) strengthen the possibility of mineral imbalance; where coefficients of variation are high (e.g. Na) use median vales if possible. Use of equivalent data from a home country or county is encouraged (e.g. Kerr *et al*., 2008, for USA; Knowles and Grace, 2014, for NZ) but may be hard to find.

	Ca	Mg	P	K	Na	S	Ratio of S to N[a]
Roughages							
Grass – fresh (242)	5.4 (1.7)	1.6 (0.56)	3.0 (0.68)	25.8 (6.6)	2.5 (2.1)	2.2 (0.6)	0.088
Grass – hay (128)	5.2 (2.5)	1.4 (0.52)	2.3 (0.75)	20.7 (5.3)	2.1 (1.7)	–	–
Grass – silage[b] (180)	6.4 (2.0)	1.7 (0.54)	3.2 (1.7)	25.8 (6.8)	–	–	–
Grass – dried (112)	7.4 (4.9)	1.8 (0.47)	3.3 (1.1)	26.0 (8.0)	2.8 (1.6)	3.5 (0.3)	0.12
Lucerne – hay (5)	15.6 (1.8)	1.7 (0.27)	3.1 (0.66)	27.3 (5.0)	0.6 (0.07)	–	–
Lucerne – silage (8)	17.6 (3.4)	1.8 (0.23)	3.0 (0.35)	24.6 (4.0)	1.3 (0.95)	–	–
Lucerne – dried (50)	15.0 (2.6)	2.3 (0.65)	3.0 (0.8)	25.4 (8.3)	1.3 (0.8)	–	–
Clover silage (10)	16.7 (5.5)	2.3 (0.75)	3.1 (0.66)	27.4 (7.6)	0.3 (0.1)	–	–
Maize silage (26)	4.3 (2.0)	2.2 (0.69)	2.6 (1.2)	12.3 (4.1)	0.3 (0.2)	–	–
Fodder beet (10)	2.8 (2.4)	1.6 (0.30)	1.8 (0.3)	17.5 (4.8)	0.3 (0.4)	–	–
Kale – all varieties (10)	13.1 (1.4)	1.6 (0.2)	4.1 (0.4)	29.3 (3.2)	1.2 (0.6)	–	–
Straw – barley[c] (17)	3.8 (1.8)	0.7 (0.31)	1.1 (1.10)	16.0 (6.5)	1.8 (2.1)	2.0 (0.4)	0.33
Straw – oat (6)	3.9 (1.2)	0.9 (0.31)	0.9 (0.24)	17.9 (2.4)	1.8 (2.1)	2.0 (0.4)	0.19
Straw – wheat (70)	3.9 (1.1)	0.9 (0.9)	0.8 (0.35)	10.2 (3.7)	4.8 (0.6)	1.3 (0.4)	0.24
Concentrates							
Barley[d] (56)	0.9 (0.6)	1.2 (0.2)	4.0 (0.46)	5.0 (0.7)	0.3 (0.4)	1.5 (0.3)	0.073
Maize (16)	0.1 (0.1)	1.3 (0.1)	3.0 (0.3)	3.5 (0.2)	–	1.6 (0)	0.10
Oats[d] (27)	0.9 (0.2)	1.0 (0.1)[e]	3.4 (0.5)	5.0 (0.9)	0.2 (0.1)	1.9 (0.1)	0.11
Sorghum (5)	0.3 (0.3)	1.1 (0.1)	2.8 (0.5)	3.7 (0.14)	0.5 (0.0)	–	–
Wheat (37)	0.6 (0.2)	1.1 (0.1)	3.3 (0.4)	4.6 (0.4)	0.1 (0.06)	1.6 (0.08)	0.078
Cottonseed meal (9)	2.1 (0.21)	5.8 (0.4)	8.9 (1.1)	15.8 (0.8)	0.2 (0.06)	5.0 (0.32)	0.083
Fish meal, white (7)	56 (6.0)	2.3 (0.3)	38 (14.5)	10.2 (1.3)	11.2 (1.5)	–	–
Linseed meal (5)	3.4 (0.1)	5.4 (0.1)	8.7 (0.1)	11.2 (0.1)	0.7 (0.0)	5.0	0.083
Maize-germ meal (9)	0.2 (0.04)	2.1 (0.7)	2.8 (1.2)	–	–	4.9	0.046
Maize-gluten feed (22)	2.3 (3.6)	4.1 (0.7)	9.3 (1.2)	12.5 (2.7)	2.6 (1.4)	4.1	0.065
Palm-kernel meal (7)	2.4 (0.3)	3.0 (0.4)	6.2 (0.6)	6.9 (1.2)	0.2 (0.1)	3.2	0.118
Rapeseed meal (17)	8.4 (2.7)	4.4 (0.5)	11.3 (1.5)	14.3 (2.2)	0.4 (0.3)	16.9 (1.7)	0.26
Soybean meal[f] (12)	3.9 (1.6)	3.0 (0.2)	7.4(0.4)	25.0 (1.0)	0.16 (0.05)	4.6	0.058
Sunflower meal (6)	4.8 (1.4)	5.8 (0.5)	10.8 (1.9)	17.1 (1.8)	1.0 (1.2)	–	–
By-products							
Wheat bran (10)	1.1 (0.2)	6.2 (2.7)	12.6 (3.0)	–	–	–	–
Rice-bran meal (8)	0.9 (1.0)	3.8 (4.1)	17.4 (1.7)	5.6 (5.9)	0.24 (0.13)	–	–
Brewers grains (20)	3.5 (1.4)	1.7 (0.4)	5.1 (1.0)	0.6 (0.95)	0.3 (0.30)	3.0 (0.17)	0.084
Distillers grains[g] (7)	1.7 (0.32)	1.7 (0.5)	9.6 (0.8)	10.2 (0.8)	1.2 (1.2)	–	–

Continued

Continued.

	Ca	Mg	P	K	Na	S	Ratio of S to N[a]
Citrus pulp (13)	14.6 (2.4)	1.1 (0.5)	1.1 (0.1)	–	–	3.7	0.084
Sugarbeet pulp[h] (14)	7.6 (2.0)	1.1 (0.2)	0.8 (0.2)	18.2 (1.9)	–	–	–
Cassava meal (5)	2.2 (0.6)	1.4 (0.6)	0.9 (0.1)	8.1 (1.5)	0.6 (0.25)	18.1 (0.6)	0.127
Feather meal (5)	5.6 (0.1)	0.4 (0.04)	3.1 (0.1)	1.5 (0.0)	1.4 (0.0)	18.1	0.127

[a]The ratio for S to N is more important and the means far less variable than data for S (ratio standard deviation not given in database).
[b]Clamp
[c]Untreated
[d]Winter and spring
[e]Winter
[f]Extracted
[g]Barley
[h]Molasses

References

Kerr, B.J., Ziemer, C.J., Weber, T.E., Trabue, S. L., Bearson, B.L., Shurson, G.C. and Whitney, M.H. (2008) Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. *Journal of Animal Science* 86, 2377–2384.

Knowles, S.O. and Grace, N.D. (2014) A recent assessment of the elemental composition of New Zealand pastures in relation to meeting the dietary requirements of grazing livestock. *Journal of Animal Science* 92, 303–310

MAFF (1990) *UK Tables of the Nutritive Value and Chemical Composition of Foodstuffs*. In: Givens, D.I. (ed.) Rowett Research Services, Aberdeen, UK.

Appendix 3

Table A3. Marginal bands for the interpretation of macromineral concentrations in blood or bone biopsy samples from chronically deprived weaned ruminants. Mean values should normally exceed these bands; the nearer they are to the lower limit or the higher the proportion of individual values falling within the band, the greater is the likelihood of mineral deprivation. Simple marginal bands for dietary mineral concentrations cannot be used because of interactions between elements, notably K x Mg and K x Na.

			Plasma (mmol l^{-1})			
Animal	Ca	Mg	P	K	Na	Sulfate
Cattle	1.8–2.0	0.50–0.75	1.0–1.5	<2.5	124–135	<0.3
Sheep	1.8–2.0	0.60–0.75	1.0–1.5	2.4–4.0	140–145	<0.3
			Bone ash (g kg^{-1} DM)			
Calf/cow	500–600	<12[a]	500–600 (rib cortex)	–	–	–
Lamb	0.2–0.3	<7[a]	200–300 (vertebra)	–	–	–

[a]mg g^{-1} ash.

Appendix 4

Table A4. A summary[a] of marginal bands for diagnostic criteria indicating a possible risk of trace element disorder[b] in ruminants. Mean plasma and tissue values for a randomly sampled population falling within these bands indicate the probability of disorder, while 10% of individual values falling within the bands indicates possible benefits from supplementation, particularly if other possible causes of ill-thrift have been eliminated (see Appendix 1).

Sample	Cu	Co	I[c]	Se	Zn
Diet (mg kg^{-1}DM)	S:Cu, 500[d]–1000	0.05–0.10[e]	Summer, 0.10–0.15	0.025–0.05[e]	10–20
	Fe:Cu, 50[d]–100 Mo:Cu, 0.3[d]–1		Winter, 0.20–0.30		
Plasma (l^{-1})	Neonate, 2–4 µmol	B_{12}, 230–350 pmol[g]	Thyroxine, 40–60 nmol	250–500 nmol	–
	Others, 3–9[f] µmol	B_{12}, 336–500 pmol[g] MMA, 5–15 µmol	Thyroxine, 20–30 nmol Total iodine, 30–40 µg	150–250[d] nmol	6–9 µmol
Tissue (kg^{-1} FW)	Neonate liver, 260–1050	Liver B_{12}, 280–340 nmol	Thyroid weight, 0.4–0.9 g kg^{-1}LW	Liver, 800–900 nmol	Bone, 0.77–1.08 mmol kg^{-1} fat-free dry rib
	Others, 30–100 µmol		Thyroid I, 1.2–2.0 g kg^{-1}DM	Liver, 250–450[e]	
Other	Fleece/hair, 31–62 µmol kg^{-1} DM	Soil, extractable Co <0.4 mg kg^{-1}DM Soil, extractable Mn >1000 mg kg^{-1}DM Soil, pH <5 or >6 Milk B_{12}, 250–500 pmol l^{-1}	Milk I, 70–100 µg l^{-1}	Erythrocyte GPX 30–50 U g^{-1} Hb or 10–16 U ml^{-1} cells (assay temp. 37°C)	Fleece/hair, 1.15–1.54 mmol kg^{-1}DM

DM, dry matter; FW, fresh weight; GPX, glutathione peroxidase (an important indicator of functional selenium status, but bands depend on species and assay conditions; see Chapter 15).

[a]It is impracticable to cover in a single table the many factors that can affect the reliability of a single measure of trace element status. In sheep, for example, the interpretation of plasma selenium is different for barren and pregnant ewes (see Chapter 15) and trace element deprivation in early pregnancy may have residual effects on perinatal mortality and lamb growth that are not detectable from later sampling. Readers are advised to consult the relevant section in each chapter.

[b]For the diagnosis of iron and manganese deprivation, rarely encountered in weaned animals, see Chapters 13 and 14, respectively.

[c]Exposure to goitrogens is a major cause of iodine deprivation and may influence the reliability of biochemical markers of iodine and selenium status in ways that have yet to be investigated (see Chapters 12 and 15). The link between iodine and selenium metabolism may mean that more dietary selenium is needed in winter than in summer, as is the case for iodine.

[d,e]Species differences in susceptibility, cattle and sheep, respectively, being the ruminants most likely to suffer at relatively mild exposure to copper and cobalt or selenium deprivation. The high-yielding dairy cow may need higher plasma selenium than other animals to avoid selenium deprivation.

[f]Plasma must be used, not serum.

[g]Bovine assays often underestimate.

Appendix 5

Table A5. Common ingredients of mineral supplements in Europe and their mineral content prior to mixing. (From International Association of the Europjean (EU) Manufacturers of Major, Trace and Specific Feed Mineral Materials (EMFEMA), Brussels)

			Content (%)		
Mineral	Source	Formula	Theoretical	Actual	Other elements
Ca	Calcium carbonate	$CaCO_3$	40	37–39.5	
	Oyster shells	$CaCO_3$	40	*c*.37	
Mg	Magnesium oxide	MgO		50.5–52.0	
	Magnesium sulfate	$MgSO_4.7H_2O$		10.0	13 S
	Magnesium chloride	$MgCl_2.6H_2O$		12.0	35 Cl
	Magnesium hydroxide	$Mg(OH)_2$		36–38	
	Magnesium phosphate	$MgHPO_4.nH_2O$		24–28	13–15 P
Na	Marine salt	NaCl		39.2	
	Vacuum-dried salt	NaCl		38.9–39.2	
	Rock salt	NaCl		36.4	
	Bicarbonate	$NaHCO_3$		27.0	
	Sodium sulfate (anhydrous)	Na_2SO_4		32.0	22 S
P	'Di' calcium phosphate (anhydrous)	$CaHPO_4$		18–20.5	25–27 Ca
	'Di' calcium phosphate (dihydrate)	$CaHPO_4.2H_2O$		17–18.6	23–25 Ca
	'Mono-di' calcium phosphate	$CaHPO_4.Ca(H_2PO_4)_2.H_2O$		20–22	19.5–23.0 Ca
	'Mono' calcium phosphate	$Ca(H_2PO_4)_2.H_2O$		22–22.7	15–17.5 Ca
	Phosphate-containing $CaCO_3$	$Ca(H_2PO_4)_2.xH_2O + CaCO_3$		18	21 Ca
	Calcium–magnesium phosphate	$(Ca,Mg)PO_4.nH_2O$		18	15 Ca; 9 Mg
	Na–Ca–Mg phosphate	$(Na,Ca,Mg)PO_4.nH_2O$		17.5	7.5–8.5 Ca 4.5–5.5 Mg12.0–14.5 Na
	Monoammonium phosphate	$NH_4H_2PO_4$		26	11 N
	Monosodium phosphate	$Na_2PO_4.nH_2O$		24	20 Na
S	Calcium sulfate	$C_aSO_4.2H_2O$		18.5	23.3 Ca
Co	Cobaltous sulfate heptahydrate	$CoSO_4.7H_2O$		21	
Cu	Copper sulfate	$CuSO_4.5H_2O$		25	
Zn	Zinc sulfate	$ZnSO_4.H_2O$		35	
	Zinc oxide	ZnO		75	
Fe	Ferrous sulfate monohydrate	$FeSO_4.H_2O$		29.5–30.0	
	Ferrous sulfate heptahydrate	$FeSO_4.7H_2O$		19.5	
Mn	Manganous oxide	MnO		52–63	
	Manganous sulfate monohydrate	$MnSO_4.H_2O$		31–32	
Mo	Sodium molybdate dihydrate	$Na_2MoO_4.2H_2O$		39.5–40	I
	Calcium iodate monohydrate	$Ca(IO_2)_2.H_2O$		61.6	
	Sodium iodate	NaI		84.7	
	Potassium iodide (may be stabilized with stearates)	KI		76.5	
Se	Sodium selenite	Na_2SeO_3		45	
	Sodium selenate	Na_2SeO_4		41.8	

Appendix 6

Table A6. Chemical symbols, atomic numbers and atomic weights of elements of nutritional significance and their grouping in the periodic table.

Name	Symbol	Atomic number	Atomic weight	Group	Name	Symbol	Atomic number	Atomic weight	Group
Aluminium	Al	13	26.98	IIIb	Mercury	Hg	80	200.59	IIb
Arsenic	As	33	74.92	Vb	Molybdenum	Mo	42	95.94	VIa
Barium	Ba	56	137.34	IIa	Nickel	Ni	28	58.71	VIII
Boron	B	5	10.81	IIIb	Nitrogen	N	7	14.007	Vb
Cadmium	Cd	48	112.40	IId	Oxygen	O	8	15.9994	VIb
Calcium	Ca	20	40.08	IIa	Phosphorus	P	15	30.974	Vb
Carbon	C	6	12.011	IVb	Potassium	K	19	39.102	Ia
Cerium	Ce	58	140.12	IIIa	Rubidium	Rb	37	85.47	Ia
Caesium	Cs	55	132.91	Ia	Ruthenium	Ru	44	101.1	VIII
Chlorine	Cl	17	35.453	VIIb	Selenium	Se	34	78.96	VIb
Chromium	Cr	24	52.00	VIa	Silicon	Si	14	28.09	IVb
Cobalt	Co	27	58.93	VIII	Silver	Ag	47	107.870	Ib
Copper	Cu	29	63.54	Ib	Sodium	Na	11	22.9898	Ia
Fluorine	F	9	19.00	VIIb	Strontium	Sr	38	87.62	IIb
Hydrogen	H	1	1.0080	VIIb	Sulfur	S	16	32.064	VIb
Iodine	I	53	126.90	VIIb	Technetium	Tc	43	(99)	VIIa
Iron	Fe	26	55.85	VIII	Tin	Sn	50	118.69	IVb
Lanthanum	La	57	138.91	IIIa	Titanium	Ti	22	47.90	IVa
Lead	Pb	82	207.19	IVb	Tungsten	W	74	183.85	VIa
Lithium	Li	3	6.939	Ia	Vanadium	V	23	50.94	Va
Magnesium	Mg	12	24.312	IIa	Zinc	Zn	30	65.37	IIb
Manganese	Mn	25	54.94	VIIa					

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Note: Page numbers in **bold** type refer to **figures**
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Mineral Nutrition of Livestock

5th Edition

Neville F. Suttle

The fifth edition of this important book reviews recent advances in livestock mineral nutrition, updated throughout with new references that reflect the growing complexity of mineral metabolism. Major related themes covered include the assessment of the 'mineral value' of feeds, the false hopes placed on organic mineral supplements and limiting the 'mineral footprint' of livestock production to lower environmental pollution. Also discussed are new developments and concepts including:

- Salt tolerance and optimizing production in salt-rich environments.
- Use of phytase rather than phosphate supplements in pig and poultry rations.
- Demineralization of the skeleton during confinement.
- Recognition of sub-acute, clinical hypocalcaemia as a disorder in dairy cows.
- The assay of 'free' plasma B12 to assess cobalt status of cattle.
- Limitations of cell culture and ligated loop techniques for assessing bioavailability.

Following a clear and easy to reference structure, the book also considers potential pitfalls, such as misleading estimates of mineral requirements for growth, and misinterpretation of genomic markers for mineral requirements and bioavailability of supplements. An essential resource for researchers and students in animal nutrition, agriculture and veterinary medicine, this book also forms a useful reference for veterinary practitioners and those concerned with human nutrition and environmental protection.