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Detection and Treatment of Mineral Nutrition Problems in Grazing Sheep

Editors: D.G. Masters and C.L. White



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Foreword

This book comprises selected papers presented at a workshop on mineral disorders in grazing sheep, which was held in Beijing in October 1995 as part of two research projects on mineral nutrition of sheep in China that have taken place over the last six years. The projects and workshop were funded by the Australian Centre for International Agricultural Research (ACIAR) and the Chinese Ministry of Agriculture.

Contributors were chosen for their sound practical knowledge of mineral deficiencies and toxicities in grazing sheep. Their contributions have produced a reference book designed to provide students, animal scientists, veterinarians, farmers and laboratory analysts with up-to-date, practical information on the detection and treatment of mineral disorders in grazing sheep. The emphasis is on extensive grazing systems, where treatment of mineral disorders may present special problems.

The projects involved scientists from the CSIRO Division of Animal Production in Perth, Western Australia, the Beijing Institute of Animal Science, the Inner Mongolia and Xinjiang Academies of Animal Science, the Lanzhou Institute of Traditional Chinese Veterinary Medicine, the Chifeng Institute of Animal and Veterinary Science and the AusAID-IDP China-Australia Research Project. Results of the projects plus a number of allied developing country studies are presented in a companion volume produced in ACIAR's Proceedings Series.

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Mineral Deficiency Problems in Grazing Sheep: an Overview

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Outline

Essential minerals

Naturally occurring mineral deficiencies in grazing sheep

Factors contributing to mineral deficiencies

Content of minerals in pasture and feed supplements

Soil type and conditions

Time of the year and stage of maturity of the plant

Use of fertilizer

Pasture species

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Soil and faecal ingestion

Efficiency of pasture use

Economics of mineral supplementation

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Minerals are essential for life, to fulfil the needs of growth and production and to replace quantities lost during the course of normal metabolism. Minerals participate in a range of biochemical reactions as components of enzymes and fulfil a structural and osmotic role in a number of animal tissues.

Essential Minerals

At present 19 of the naturally occurring mineral elements are known to be essential. A number of other minerals may be essential but the evidence is currently inconclusive (Table 1).

Deficiencies of many, but not all, essential minerals have been reported in grazing ruminants. However, for some of the minerals, requirements are extremely low relative to the concentrations of these minerals found in natural feedstuffs, and animal responses have been demonstrated only in the animal house under controlled condi-

tions using purified diets. For such elements, deficiencies in the field are unlikely.

In addition, some minerals listed in Table 1 are significant because they interfere with the use of other nutrients and not because they are deficient in herbage or feedstuffs. For example, high intakes of potassium decrease the absorption of magnesium and may increase the incidence of grass tetany induced by magnesium deficiency (Mayland and Grunes 1979). Similarly, high levels of molybdenum and iron reduce the availability of dietary copper and may induce a deficiency in that element (Dick 1953; Suttle et al. 1984; Grace and Lee 1990). Deficiencies of potassium, iron or molybdenum have not been reported in grazing sheep.

Some elements are also of practical significance because of their toxicity, or accumulation in animal tissues to concentrations unacceptable for human consumption. These include fluorine, mercury, cadmium

Table 1. The essential mineral elements for animals.

Major elements (or macrominerals)	Trace elements (or microminerals)	
Calcium ^a	Cobalt ^a	Nickel
Chlorine	Copper ^a	Arsenic
Phosphorus ^a	Iron ^a	Vanadium ^b
Magnesium ^a	Iodine ^a	Boron ^b
Potassium ^a	Manganese	Lithium ^b
Sodium ^a	Selenium ^a	Lead ^b
Sulfur ^a	Zinc ^a	Fluorine ^b
	Molybdenum ^a	Cadmium ^b
	Chromium	Tin ^b
	Silicon	

^a Important under grazing conditions.

^b Inconclusive evidence on essentiality.

and aluminium, in addition to some of the elements listed in Table 1 (see Howell, this publication).

Naturally Occurring Mineral Deficiencies in Grazing Sheep

Mineral deficiencies occur in all continents and cause decreased production of meat, wool, milk and lambs. In many areas, rearing sheep and cattle was impossible because a lack of minerals resulted in high mortality or a complete failure to grow and reproduce. These events often occurred even when pasture was plentiful (see Underwood 1981; Grace 1983; McDowell et al. 1993).

Mineral deficiencies may cause clinical disorders (Lewis and Anderson 1983) that have dramatic effects on health and survival of sheep, or marginal deficiencies that result in subtle and often undetected effects on productivity (Table 2). While severe deficiencies still occur, they are becoming less frequent due to improvements in identification and treatment. For example, copper deficiency, resulting in swayback in newborn lambs, was observed in New Zealand, Australia, Europe and the USA in the 1930s (see Underwood 1981) but use of copper-containing fertilizers and routine treatment of sheep born in copper-deficient areas has decreased the incidence of severe deficiency. Similarly, supplements of iodine, cobalt and selenium are often provided routinely in areas where there is a history of clinical deficiency.

Marginal or subclinical deficiencies are not so readily identified and frequently cause significant losses in production. For

example, in a series of experiments carried out in Australia over a 4-year period, Langlands and colleagues (Langlands et al. 1991a,b,c) reported increased fleece weights (3.8–7.5%), fibre diameter (2–2.5%), lamb survival (up to 12%), lamb birth weight (4%), weaning weight (10.7%) and wool production from lambs (8.6%) in response to selenium supplementation. During this 4-year period only two lambs exhibited overt signs of white muscle disease. The responses described are not specific for selenium-deficient sheep and are likely to go undetected except through experiments using untreated controls for comparison or by extremely perceptive animal managers. An additional complication is that responses are not necessarily consistent from year to year. Langlands et al. (1991b) observed increases in wool production from selenium supplements in three years out of four. Lee (1951) reported responses to cobalt in 8 of 13 years of experiments. Marginal deficiencies present the animal producers with the difficult problem of assessing mineral status in the sheep and of evaluating the economic benefits relative to costs of supplementation.

Factors Contributing to Mineral Deficiencies

Deficiencies are more likely to occur in grazing sheep dependent on locally grown pasture and feed supplements for all feed requirements. Under such conditions, both pastures and supplements are grown locally and where mineral deficiencies or imbalances exist in the soils, these are likely to be expressed in the grazing sheep.

Table 2. Summary of function and signs of severe and marginal mineral deficiencies and likely incidence of deficiencies.

Element	Function	Manifestation of deficiency		Likely occurrence of deficiency
		Severe	Marginal ^a	
Calcium	Mineralisation of bones and teeth. Nerve conduction. Muscle contraction. Blood clotting. Hormone secretion. Message transmission.	Lameness and stiffness of gait. Enlarged painful joints. Malformed teeth and jaws. Milk fever, including muscle tremors, general inertia, inappetence and death. ^b	Reduced growth. Reduced milk production.	High grain diet especially during drought. Increased requirements in lactation. Low calcium, acid soils in the tropics. Low vitamin D.
Phosphorus	Bone formation. Energy metabolism. Component of DNA, RNA. Acid–base balance.	Softening of bones, lameness. Depressed appetite. Abnormal appetite causing botulism.	Reduced growth. Reduced reproductive rates.	Low soil phosphorus. Prolonged dry period with dead pasture.
Sodium	Maintenance of osmotic pressure. Acid–base balance. Water metabolism. Rumen microbial growth.	Abnormal appetite, licking of soil. Depressed appetite. Loss of weight. Decreased milk production.	Abnormal appetite (pica). Reduced growth. Decreased milk production.	Tropical or dry arid climates. Rapidly growing or lactating sheep. High grain diets.
Sulfur	Component of amino acids methionine and cysteine. Component of biotin and thiamine. Sulfated polysaccharides in cartilage and joint lubrication. Removal and/or storage of toxic elements and compounds.	Reduced feed intake. Reduced dry matter digestibility. Reduced production of sulfur amino acids by rumen microbes. Possible accumulation of copper in animal tissues and copper toxicity.	Reduced feed intake. Reduced wool growth.	Low sulfur soils. Dry pastures.
Cobalt	Essential for the production of vitamin B ₁₂ by rumen microbes. Use of volatile fatty acids for energy (as vitamin B ₁₂). Recycling of methionine (as vitamin B ₁₂).	Loss of appetite. Loss of weight, reduced wool growth. Watery discharge from eyes. Anaemia. Poor reproductive performance. White (fatty) liver disease.	Reduced growth/ unthriftiness. Reduced wool production.	Occurs under range of soils and climatic conditions. Sandy or volcanic soils. Green lush pasture. Young growing or reproducing sheep.

Continued on next page.

Table 2. Cont'd.

Element	Function	Manifestation of deficiency		Likely occurrence of deficiency
		Severe	Marginal ^a	
Magnesium	Cofactor in many enzymes. Nerve conduction. Muscle contraction.	Tetany as indicated by muscle twitching, staggers and convulsions. High mortality. Depressed appetite.	Possible loss of condition. Possible reduced milk production.	Green pasture. Often but not always associated with lactation. High potassium, low sodium.
Copper	Mobilisation and transport of iron in the body. Collagen cross-linking in bone and elastic tissue. Pigmentation of hair and wool. Maintenance of myelin sheath around nervous tissue. Crimp formation in wool. Antioxidant.	Enzootic ataxia (swayback) as paralysis or staggering gait in newborn lambs. Bone fragility. Anaemia. Depigmentation of wool. Loss of crimp and tensile strength of wool. Reproductive disorders.		Leached low copper soils. Peat soils high in molybdenum. Use of molybdenum fertilizer.
Iodine	Component of thyroid hormones that are essential for energy and protein metabolism and for foetal growth and development.	Enlarged thyroid gland (goitre) Inability to withstand cold stress. Reduced foetal size and retarded brain development. A range of developmental disorders, wool loss.	Increased lamb mortality and thyroid size relative to body size. Small depression in wool and milk production.	Low soil iodine. Recent glaciation. Mountainous areas. Long distance from the sea. Low rainfall. Goitrogens in herbage.
Selenium	Detoxification of peroxides. Activation of thyroid hormone.	Lesions in heart and skeletal muscle (nutritional myopathy or white muscle disease). Increased mortality. Infertility in ewes. Induced thyroid hormone deficiency.	Reduced growth/illthrift. Reduced wool growth.	Green pasture. Low selenium in soil. High rainfall. Young growing or reproducing sheep. Low vitamin E.
Zinc	Cofactor in a large number of enzymes. DNA and protein synthesis.	Reduced feed intake. Reduced growth rates. Reduced wool growth and loss of crimp. Alopecia, parakeratosis.	Reduced growth rate. Reduced reproductive performance.	Low zinc soils. Dry pasture.

^a Marginal signs may also be seen during severe deficiency.^b Acute deficiency resulting from a rapid loss of calcium during milk letdown.

Content of minerals in pasture and feed supplements

Deficiencies of minerals usually occur within specific geographical regions. Soil types and climatic conditions will determine the types and amounts of minerals available for absorption by the pasture plants and other local plant species. However, there are many secondary factors that will contribute to the expression of potential mineral problems. These include: time of the year (stage of plant growth); amounts and types of fertilizer used; introduction of improved pasture species; short term climatic factors (recent rainfall and temperature); distance from the ocean; and amounts and types of supplementary feeding. These factors influence the supply of minerals to grazing sheep. Animal factors that influence requirements are equally important (White, this publication).

Soil type and conditions. The availability of minerals to plants depends on the concentration and the chemical form of these elements in the soil (Thornton 1983). The soils will initially resemble the parent rock in composition. But with older soils, elements may have been mobilised and redistributed through the soil profile and/or into neighbouring soil zones. Prolonged leaching of soils in high rainfall areas will increase the rate of mineral loss and redistribution. The process of soil formation varies with climate and this will also influence the concentration and form of minerals in the soil.

Other factors influencing availability of minerals from soils include: the nature of the soil solution (particularly pH); the amount

of organic material; drainage and waterlogging; and the proliferation of plant roots within the soil. These have been reviewed by Hannam and Reuter (1987). Use of soil mapping techniques to define areas of mineral deficiency in grazing animals have met with limited success due to the many interacting factors affecting availability and use of minerals (Lewis 1985).

Time of the year and stage of maturity of the plant. The effects of time of the year and stage of plant maturity on mineral concentrations in plants are interrelated. Concentrations of phosphorus, sodium, potassium, sulfur, iodine and zinc fall as the plant matures (Purser and Southey 1984; Minson 1990; Hendricksen et al. 1992). No consistent changes have been reported for calcium and magnesium, with some indications that concentrations either decrease or remain the same (Mayland and Grunes 1979). Consistent trends have not been reported for copper, selenium and cobalt.

In environments with extreme changes in season, pasture available for grazing may be dead and dry for extended periods of the year. These environments include the mediterranean climate, as found in much of southern Australia, where low rainfall and high temperatures exist for 4–6 months each year (Purser 1980), and the cold temperate environments, such as found in the pastoral regions of northern China, where low rainfall and extreme cold exist for 4–6 months each year (Masters et al. 1990). These conditions may cause marked fluctuations in the amount and availability of minerals to grazing sheep.

Table 3 shows the changes in mineral

concentrations in mixed pastures as they change from the green to the dry stage. Most elements show a decrease in concentration—selenium and iron are exceptions. Sheep grazing dry pastures may not consume sufficient minerals to meet the published requirements for a significant period during each year, and deficiencies may result. Deficiencies in phosphorus in grazing cattle in South Africa have been associated with low soil phosphorus together with a prolonged dry period (Underwood 1981). However, the low concentrations of minerals in dry pastures do not necessarily mean that signs of deficiency will result. The content of digestible energy and protein is also low in dry pas-

tures and so requirements for minerals may be reduced or deficiencies masked (White, this publication). The increased concentration of iron in dry pastures results (at least in part) from soil contamination. High intakes of iron may depress growth of young sheep (Wang 1995) or interfere with the utilisation of copper.

The green pasture phase is also associated with mineral problems. The concentrations of cobalt and selenium tend to be at their lowest during rapid pasture growth. Because plants do not require selenium for growth and the requirement for cobalt by the plant is less than that required by the ruminant, plants can display vigorous growth when little selenium or cobalt is

Table 3. Concentration of minerals in pasture during the green or dry phases in Western Australia and north-west China (mg/kg dry matter).

Element	Western Australia ^a			North-west China ^c		
	Green	Dry	% Change	Green	Dry	% Change
Calcium	8300	8715	+5	6500	4400	−32
Phosphorus	3800	1444	−62	1546	240	−85
Magnesium	—	—	—	1750	1380	−21
Potassium	28100	6182	−88	14900	2252	−85
Sodium	—	—	—	92	210	+130
Sulfur	3240	1458	−55	1900	830	−56
Copper	7.3	6.9	−6	6.8	4.4	−35
Iron	128	141	+10	903	2091	+57
Manganese	74	20	−73 ^b	67	67	0
Selenium	0.025	0.05	+100 ^b	0.03	0.06	+100
Zinc	43.3	18.6	−57	25.6	9.6	−63
Molybdenum	3.4	2.4	−30	2.1	2.1	0

^a Purser and Southey (1984).

^b Masters and White (1986).

^c Masters et al. (1993a,b) and Masters et al. (unpubl.).

available. Consequently during rapid pasture growth the available cobalt and selenium are diluted within the plant and the concentration of these elements is reduced (Hunter et al. 1982; Clark and Millar 1983) and deficiencies result (Hosking et al. 1986). In general, any factor that increases plant growth without changing the amount or availability of minerals in soil will result in depressed mineral concentrations in plants. Therefore, more widespread deficiencies may be expected as crop and pasture productivity increases.

Use of fertilizer. The use of fertilizer may raise mineral levels directly by providing an available source to the plant. Such fertilizers have been used successfully to increase concentrations of calcium, phosphorus, potassium, sulfur, magnesium, sodium, copper, iodine, zinc, selenium and cobalt in the plant. Simply because mineral fertilizers increase the concentration of an element in the plant does not necessarily mean that this is an economical method of supplementation. High rates of application to the soil may be needed to provide modest increases in concentrations in the plant. Judson and McDowell in this publication both advocate more direct methods of supplementation under such circumstances.

Fertilizer use may also indirectly affect mineral content of plants by changing factors such as soil pH, the availability of competing elements to the plant, the rate of pasture growth or pasture composition. Application of lime raises the pH of the soil and changes the solubility of several minerals. The availability of molybdenum and selenium increases as soil pH increases

whereas the availability of most of the cations including copper, zinc, cobalt, iron and manganese decreases (Williams 1977; Hannam and Reuter 1987).

Interactions between elements in the soil also influence the response to fertilizers. Application of potassium fertilizer markedly reduces the concentration of sodium, calcium and magnesium in plants. This leads to lower serum magnesium in sheep and increases their susceptibility to grass tetany (Mayland and Grunes 1979; Minson 1990). Overuse of molybdenum fertilizers to improve plant growth may result in reduced absorption of copper by the animal, and copper deficiency (Langlands et al. 1981). Application of phosphate or nitrogen fertilizers to increase the rate of plant growth may cause a dilution and reduced concentration of copper (Reuter et al. 1981), selenium and cobalt (Halpin et al. 1981) or may change mineral status of the grazing sheep through changes in the proportions of grass and legumes within a pasture.

Pasture species. The type of pasture species will influence mineral supply to grazing sheep. Temperate grasses and cereals generally contain less calcium, phosphorus, potassium, magnesium, iron, copper, zinc, molybdenum and cobalt but more selenium than temperate legumes (Reid and Horvath 1980; Underwood 1981; Minson 1990), but these differences are not consistent and the trends may be reversed in tropical pasture species. There are also plants termed 'accumulator' plants that contain high concentrations of some elements. Plants considered as weeds may also contain significantly different mineral

concentrations compared with grass or legume species grown on the same site (Gladstones and Loneragan 1970). Improvement of pastures that results in a change in the predominant pasture species, together with applications of fertilizer, will change the mineral content of pastures and may cause signs of deficiency in plants and/or grazing animals.

Supplementary feeds. Supplementary feeds may be conserved fodder such as silage and hay, or grain and pulses. Silage and hay, if prepared on-farm, will have a mineral composition similar to the pasture. Grains and pulses, however, contain different concentrations of minerals compared to the vegetative stage of the plant and also minerals in different relative proportions. The selection of supplement may exacerbate or alleviate potential deficiencies. For example, dry pasture and hay contain low concentrations of phosphorus and potassium while cereal grain and some grain legume seeds contain higher concentrations of phosphorus, both in absolute terms and relative to calcium and potassium. A deficiency in phosphorus on dry pastures is therefore unlikely if cereal grains or grain legume seeds are used as supplements either instead of or with conserved fodder. In environments that have prolonged dry periods and high levels of supplementary feeding with little pasture, calcium deficiency may occur (Langlands et al. 1967) due to low calcium intake together with the high phosphorus in cereal grains.

Recent rainfall and climatic events. Recent rainfall may also be important. Sele-

nium, cobalt and sodium are either not required for plant growth or only required in small amounts, and concentrations in plants decrease with increasing rates of plant growth. Low mineral concentrations in plants and increased incidence of deficiencies occur when seasonal conditions are favourable for plant growth. For example, in areas that support only annual species of pasture plants, selenium and cobalt deficiencies are more likely when there is an early start to the growing season, due to early rainfall and other conditions that are favourable to plant growth. Rainfall may also influence waterlogging of soil, and this may increase concentrations of cobalt, manganese and selenium in plants, although responses are not always consistent.

Location. Proximity to the ocean has a significant impact on minerals in plants, particularly sodium and iodine. Both sodium and iodine are carried on to pasture in sea spray or, in the case of iodine, through volatilisation from sea water. Iodine deficiency is usually found in mountainous regions, often with low rainfall and located a long distance from the sea. Sodium deficiency is more common in tropical or inland semi-arid climates.

Soil and faecal ingestion

Ingestion of soil and faeces by grazing sheep will influence mineral status. Healy (1967) reported that faeces from sheep grazing in New Zealand contained up to 50% soil (corresponding to 0.4 kg/day of ingested soil) when available feed for grazing was low. Estimates of the minerals

available from the soil indicated that substantial amounts of calcium, magnesium, phosphorus, manganese, zinc, iron, copper, molybdenum, cobalt and selenium are absorbed by the sheep. The amounts absorbed will depend on the type of soil.

Ingestion of soils high in iron depresses copper status, probably through an antagonistic interaction between copper and the iron supplied in the soil (Suttle et al. 1984). Yu et al. (1995) suggested that a lack of sodium in pasture caused increased soil ingestion in grazing sheep in China. This in turn resulted in increased intakes of the iron in the soil and depressed copper status.

Langlands et al. (1982) reported only small changes in selenium status and no changes in copper status when sheep were given approximately 100 g/day of either black clay or red sandy loam through a rumen fistula.

Less information is available on rates of consumption or availability of minerals from faeces. Peter et al. (1987) suggested, from visual observations on oesophageal samples collected when pasture availability was extremely low, that up to 50% of the

material ingested by the sheep was faeces. Faeces can contain a substantial amount of calcium, phosphorus, sodium, potassium, iron and zinc (Masters et al. 1993a,b). There is little information on the availability of minerals from faeces.

Efficiency of Pasture Use

A consistent sign of deficiency of many elements is a reduction in feed intake and in the efficiency of digestion and utilisation of ingested nutrients (Table 2). The reduction in intake will mean that a higher proportion of feed consumed will be used for maintenance of liveweight and non-productive processes such as thermoregulation, locomotion and excretion, and less will be available for liveweight gain.

With depressed intake under grazing conditions, stocking rate would need to increase to utilise all available pasture. This would result in lower liveweight gain per hectare (approximately 170 g/ha/day in the example shown, Table 4) with no change in total wool production. The diameter of wool fibres would be reduced as a

Table 4. Changes in productivity when intake of each sheep is depressed by 17% and stocking rate is increased by 17% to utilise all the pasture.^a

Sheep/ha	Feed consumed/day		Liveweight gain/day		Wool growth/day		Sheep costs/day ^b	
	kg/head	kg/ha	g/head	g/ha	g/head	g/ha	\$/head	\$/ha
5	1.43	7.15	197	985	8.7	43.5	3.00	15.00
6	1.19	7.14	136	816	7.2	43.2	3.00	18.00

^a Figures derived from GrazFeed (1993) computer program for 30 kg wether weaners, 12 months of age consuming green pasture. Pasture availability was adjusted within the program to derive intakes.

^b Costs in A\$ derived from shearing, drenching, ear tags, vaccines etc. (A. Herbert, Department of Agriculture, Western Australia, pers. comm.).

consequence of lower production per sheep. This would increase the value of wool per kilogram (partially offsetting the reduction in liveweight gain) in Australia, where diameter is a major determinant of wool price, but not in countries such as China, where all wool of less than 25 microns is considered fine wool (Longworth 1990).

A rise in the number of sheep per hectare increases the total costs for shearing, parasite control and other processes required for each individual animal. Therefore, overall production per hectare decreases but costs increase. Higher stocking rates will also contribute to damage to grasslands and pastures through soil compaction, trampling and erosion.

Lower efficiency of use of ingested nutrients results from deficiencies or imbalances of a number of elements including selenium, sodium, calcium/phosphorus (as a ratio), copper and zinc. This means that productivity may be decreased without a decrease in voluntary feed intake or that efficiency of use of nutrients may be decreased in addition to a reduction in intake (Underwood 1981).

Reduction in either intake or efficiency of use of ingested nutrients will therefore result in reduced liveweight and/or wool growth per unit of pasture available. In environments that experience prolonged periods of dry low quality grazing, any reduction in weight gains during periods of adequate or abundant feed availability will mean that sheep will be in poorer condition at the start of the dry period, have less body stores to buffer against the decrease in nutrient availability, have decreased ovulation rates

(Morley et al. 1978) and subsequently lower lambing percentages and increased mortality or a higher requirement for supplementary feeds.

Economics of Mineral Supplementation

Because the amount of minerals required by sheep is generally small relative to requirements of protein and energy, the cost of supplements is often low. This is particularly true for the trace elements that are required in milligram or microgram quantities each day. For example, Masters et al. (1995) estimated that the actual cost of copper and selenium, if supplied as copper sulfate and sodium selenate, is approximately 2.5 cents (Australian currency) per sheep each year. Use of these elements to correct marginal deficiencies in China, in the experiments described, had increased the value of production by 48 cents. There are extra costs associated with the incorporation of these elements into salt or other supplements. In countries where salt supplements are used routinely, or where sheep are tended continuously by herdsman, these costs would be small.

The potential return, even from correcting marginal deficiencies, is then many times the cost of the supplements. Economic analysis of this earlier research in China (M. Fearn, unpublished data) has indicated that an increase in wool production of 7% together with a 4% increase in weaning rate, achieved at a supplement cost of 19 cents per head each year, would result in a unit cost reduction of 4.8% for wool and 0.1% for meat.

When minerals are supplied as commercially prepared supplements marketed for sheep, costs will increase substantially but returns may still be high relative to costs. Edwards (1982) assessed the results of 14 selenium trials carried out in the high rainfall areas of Western Australia. Average results over all the trials showed that selenium supplementation increased body weight by 3.9%, wool growth by 5% and decreased mortality by 1.8%. Even with these relatively small responses, supplementing all sheep with commercial selenium pellets would have provided a return of more than 300% on funds invested.

Macromineral supplements are usually more expensive than the trace elements. This is primarily because these elements are required in gram quantities each day. Masters et al. (1995) reported that in northern China a 1-year supplement of micro-minerals costing 101 cents (Australian currency) resulted in a gross return of only approximately 50 cents. Therefore the economics of correcting marginal deficiencies may not warrant the use of multi-element supplements over prolonged periods. Where deficiencies exist, supplements need to be strategically used during the times of the year when sheep will respond. Where severe deficiencies exist or where the specific deficiencies are identified, returns will be sufficient to justify treatment.

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Understanding the Mineral Requirements of Sheep

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Outline

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Producers and advisers need to know the mineral requirements of sheep in order to formulate diets and predict and detect deficiencies. Published tables of recommended mineral requirements for sheep, such as those from the National Research Council in the USA (NRC 1985), the Agricultural Research Council in the U.K. (ARC 1980) and the Standing Committee on Agriculture in Australia (SCA 1990) are useful in determining whether a particular diet will meet an animal's requirements. There are some problems, however, especially when it comes to applying the values to grazing animals. For example, many published tables of requirements include a safety margin, the extra amount of which, in most circumstances, does not lead to increased production. In intensive production systems it is a relatively inexpensive task to add minerals above minimum requirement levels as an insurance policy. Under extensive grazing systems, however, it is usually difficult and costly to provide supplementary minerals, and so it is important to know whether the published tables of requirements represent minimum values or whether they include generous overallowances.

This chapter summarises the various methods used to estimate the mineral requirements of sheep and highlights both the limitations and usefulness of such methods for identifying and preventing deficiencies in practical grazing situations.

What Is Meant by Requirement?

To meet all mineral requirements the minimum dietary supply must maintain

within healthy limits the concentrations and active forms of the mineral in all tissues including storage organs, and maintain the levels of the mineral in the product, such as milk.

Under practical farming conditions, where efficient production is the main aim, it is not always necessary to maintain storage levels because a reduction in this pool of minerals does not immediately affect production. Reduced productivity comes at the end of a series of events after dietary depletion of a mineral (Fig. 1). Levels within storage organs are the most sensitive to changes in intake of the nutrient, followed by levels in transport forms and finally enzyme activity and metabolic function. Once enzyme activity and metabolic processes are impaired then production falls and clinical signs appear. In most cases storage levels must be almost completely depleted before a measurable drop in production occurs. It follows from this that requirements are not a fixed value but vary with the criteria of adequacy adopted. For example, dietary copper deficiency in sheep is characterised as a decline in liver copper reserves from a normal range of between 1.6 and 4.7 mmol/kg dry matter (DM), to below 0.3 mmol/kg DM. At this point plasma copper concentration and plasma caeruloplasmin activity decline to about 20% of normal values. Within a month the first clinical signs appear, characterised as defects in wool.

The time to appearance of clinical signs depends on the severity of the deficiency and the mineral in question. Some elements, such as magnesium, are not stored to any extent in the body and need to be

supplied from the diet continually to prevent deficiency. Other elements, such as calcium and phosphorus, can be drawn from bone which offers a period of protection against production losses, albeit at the cost of fragile bones. Grazing sheep typically face environments where mineral supply varies greatly between seasons, and in these circumstances it is advantageous for the sheep to build up reserves in one season for use in another. Thus, although the diet may be technically deficient in minerals at certain times of the year, the supply of minerals from body reserves such as liver and bone can provide sufficient quantities to meet requirements for the short period of dietary deficiency.

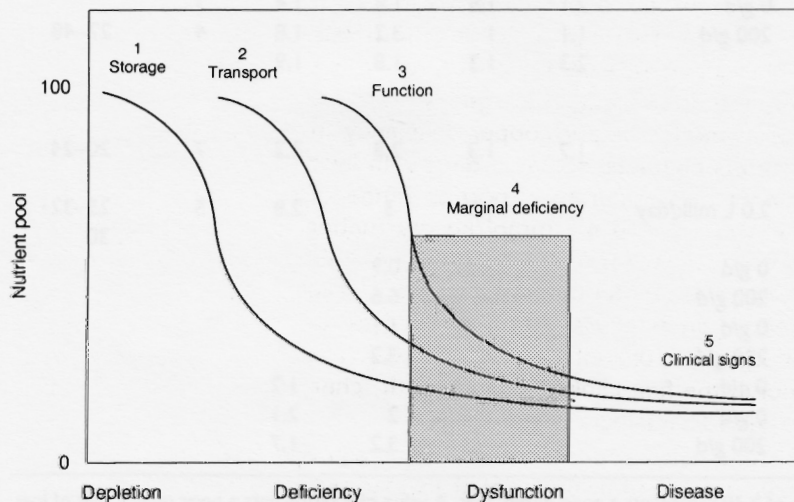


Figure 1. Temporal stages of deficiency leading to the appearance of deficiency signs (adapted from Suttle 1987).

What Factors Affect Requirements?

The dietary requirements for a mineral are influenced by both animal and dietary factors.

Animal factors

Genotypic/phenotypic differences. Animals of similar age, breed and physiological state in a common environment can show marked differences in their efficiency of mineral utilisation. For example, the fractional absorption of copper can vary from 0.042 to 0.112 (Suttle 1974). In grazing sheep, Wiener and Field (1970) reported heritable differences among three breeds and their crosses in susceptibility to sway-back and copper poisoning.

Age, physiological state and sex of the animal. Age can affect requirement through changes in absorption efficiency. For example, preruminating lambs have an 80% absorption efficiency for copper, whereas lambs with a functional rumen have only 3–5% (Underwood 1981).

Sex of the sheep affects mineral requirements through differences in growth rates and physiological functions. Rams grow faster than ewes and so require a greater daily supply of minerals; pregnant and lactating ewes require more minerals to meet body demands than non-pregnant females at maintenance (ARC 1980, see Table 1). Animals compensate for this by increasing absorption of most nutrients during late pregnancy and lactation.

Rate and type of production. Different production systems require different

Table 1. Published values for factorially derived estimates for minerals. Values are expressed as amounts per kg dry diet.

Source	Type of sheep	Type of diet	Weight (kg)	Weight gain/loss	Na g/kg	Mg g/kg	Ca g/kg	P g/kg	Cu mg/kg	Zn mg/kg
NRC (1985)	Replacement ewe lambs	M/Da = 10, 65% forage, 35% concentrate	30	227 g/d			5.3	2.2		
	Replacement ewe lambs	M/D = 10, 65% forage	40	182 g/d			4.2	1.8		
	Replacement ewe lambs	M/D = 8.8, 85% forage	50–70	115 g/d			3	1.6		
	Ewes	M/D = 8.4, 100% forage	70	10 g/d			2	2		
	Ewes, first third gestation	M/D = 8.4, 100% forage	70	30 g/d			2.5	2		
	Ewes, last third gestation	M/D = 8.8, 85% forage	70	180 g/d			3.5	2.3		
	Ewes, lactation	M/D=10, 65% forage	70	–25 g/d			3.2	2.6		
ARC (1980)	Castrate lambs	DMIb 0.31 kg/d, qc = 0.6	20	0 g/d	1.8	1.2	1.5	1.2		
		DMI 0.76	20	200 g/d	1.1	1.1	4.5	2.5	2	32–36
		DMI 0.53	40	0 g/d	2.1	1.5	1.8	1.4	3	
		DMI 1.23	40	200 g/d	1.1	1	3.2	1.8	4	27–48
	Ewes, single lamb, first third gestation	DMI 1.03 kg/d, q = 0.6	75		2.3	1.3	1.8	1.9		
	Ewes, last third gestation	DMI 1.4	75		1.7	1.3	2.8	2.2	7	20–24
	Ewes, lactation	DMI 2.2	75	2.0 L milk/day	1.4	1.5	3	2.8	5	25–32
	All classes									30
Minson (1990)	Castrate lambs	Unspecified	20	0 g/d			0.9			
			20	200 g/d			6.6			
			40	0 g/d			1.1			
			40	200 g/d			3.2			
SCA (1990)	Unspecified	M/D = 6		0 g/d			1	1.2		
		M/D = 10		0 g/d			2	2.1		
		M/D = 10		200 g/d			3.2	1.7		

^a M/D is metabolisable energy in MJ per kg dry matter. A value of ≥ 10 represents a good quality diet. A value of 6 represents a poor quality diet of low digestibility.

^b DMI = dry matter intake.

^c q = diet metabolisable energy/gross energy. The gross energy of most ruminant diets is between 18 and 19 MJ/kg.

amounts of minerals. Wool contains higher concentrations of sulfur, selenium and zinc than most other body tissues, and is particularly sensitive to deficiencies of these elements. Likewise, milk contains high levels of calcium and sodium, thereby increasing the requirement during lactation by almost 50% (AFRC 1991). The requirement expressed as the amount per kg of diet changes to a lesser extent during lactation than the requirement expressed as amount per day because of the increased feed intake associated with lactation. Nonetheless, high producing dairy cows are unable to meet their requirement for calcium from the diet and draw on calcium from bone (AFRC 1991). The concentration in milk of macro elements such as sodium, calcium, phosphorus and potassium remains relatively constant, so that these minerals can be spared under conditions of limited supply only by reducing milk production (McDowell 1992).

Rapidly growing sheep have a higher daily requirement for most minerals than sheep of similar age at maintenance by virtue of the demands for mineral accretion in growing tissues. Since higher growth rates are usually associated with diets of higher digestibility, requirements expressed on a dietary concentration basis need to be specified for each level of production and each level of metabolisable energy (Table 1). There is evidence, for calcium at least, that rapidly growing sheep compensate for the increased demand by having a higher efficiency of absorption than sheep at maintenance (Braithwaite and Riazuddin 1971).

Dietary factors

The form of mineral in the diet and the presence or absence of synergistic and antagonistic compounds and elements are of prime importance in determining whether or not the sheep meets its mineral requirements. Perhaps the best known interaction is between copper, molybdenum and sulfur. Figure 2, derived from equations of Suttle and McLaughlin (1976), shows that copper availability is a function of sulfur and molybdenum concentration in pasture. This interaction has a larger bearing on the copper status of sheep than does the copper concentration of pasture. A herbage concentration of 10 mg/kg copper can cause swayback in one area and copper toxicosis in another, simply by virtue of the differences in molybdenum and sulfur status of the herbage.

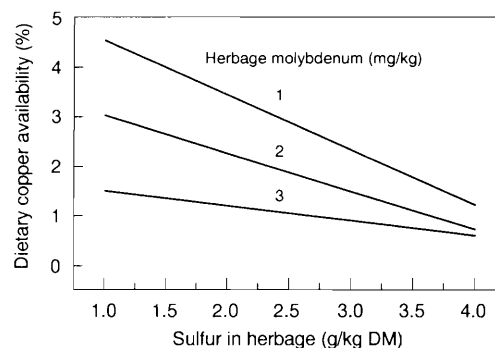


Figure 2. The effect of herbage concentrations of sulfur and molybdenum on the availability of copper to ruminants (from Suttle 1983).

Another notable example of an interaction is the effects of potassium fertilizer, protein,

sodium, calcium and fats on magnesium availability (Henry and Benz 1995) and potassium and protein on grass tetany (Mayland and Grunes 1979). Poor absorption of magnesium from forage is considered to be the most important factor causing magnesium deficiency (Wilson and Grace 1978), and the increased use of potassium fertilizer has a large effect on increasing the incidence of hypomagnesaemia (Grace 1983). The effects of pasture magnesium, potassium, sodium, calcium and protein on the incidence of grass tetany in cattle in the Netherlands are shown in Figures 3 and 4.

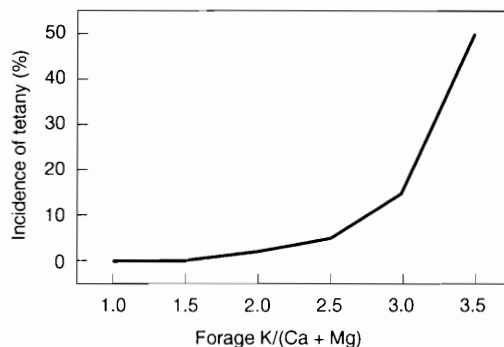


Figure 3. The effect of the pasture concentration of potassium, calcium and magnesium on the incidence of grass tetany in cattle in the Netherlands (Mayland and Grunes 1979; Kemp and 't Hart 1957).

In addition to interactions, the chemical form of the mineral is critical in determining availability. Availability of magnesium from ground magnesite and dolomite is less than half that from magnesium sulfate (Henry and Benz 1995). Heating magnesite pro-

duces magnesium oxide (MgO). If the temperature is too high (>1100°C, 'dead burnt') the availability is little different from magnesite, whereas at temperatures between 800 and 1100°C ('light burn' MgO) the magnesium availability is increased by a factor of three above unprocessed magnesite.

Calcium oxalates have low availability to sheep and they are present in high concentrations in some forages, particularly tropical grasses. Oxalates have been suggested as a cause of hypocalcaemia in Latin America (Kiatoko et al. 1978) and transit tetany in Australia (Minson 1990). Lucerne (*Medicago sativa*) contains 20–30% of calcium as calcium oxalate (Blaney et al. 1982).

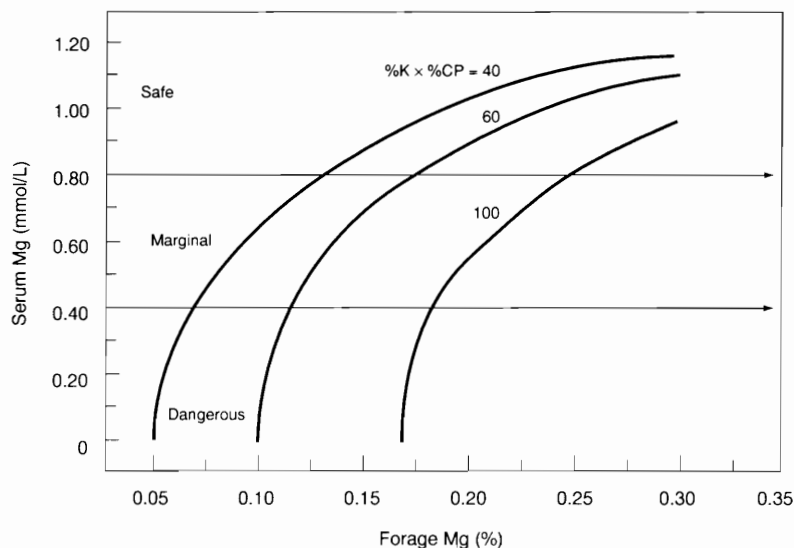


Figure 4. The effect of protein and potassium on serum magnesium concentration and on the risk of grass tetany in cattle (from Mayland and Grunes 1979). CP = crude protein.

Determining the Minimum Requirement for a Nutrient

Requirement is a function of some predetermined measure of response, be it enzyme activity, growth or wool production. The initial response of a deficient organism to increasing levels of mineral can be described using either a dose-response or Mitscherlich relationship (Figs 5, 6). The so-called clinical range is where signs of deficiency are easily recognisable. The marginal or subclinical range is where pathological signs of deficiency are not evident but production is impaired. Marginal deficiency constitutes the majority of cases. The critical value, seen on the shoulder of the curve in Fig. 5, describes the concentration of a mineral in a particular feedstuff or animal tissue at which growth or production are a predetermined proportion of maximum (e.g. 90%).

The critical value is determined experimentally with all other nutrients and conditions non-limiting and within the normal

physiological range. In practice, the critical value is expressed as a narrow range, to account for genotypic/phenotypic variability. In plants, for example, the critical zinc concentrations for growth of subterranean clover, lucerne and perennial ryegrass are 12–14, 16–20 and 10–13 mg/kg, respectively (Smith 1986). Measurements are made on a specific plant part during a specific growth phase. In the case of zinc the youngest open leaf of early vegetative growth is used. For sheep, critical values can be applied to diets but they are most useful when they refer to concentrations in the target tissue because this accounts for most of the variability associated with differences in availability.

From data using semipurified diets, the 95% critical values for zinc for growth of sheep are 6.7 $\mu\text{mol/L}$ in plasma (Fig. 6) or 10 mg/kg DM in the diet based on Mitscherlich relationships (White 1993). For wool growth they appear to be about 20% higher, but the data are limited to one experiment (White et al. 1994). In fact few estimates have been made of critical values for minerals for sheep because of the cost associated with the purified diets and the need for contamination-free animal facilities.

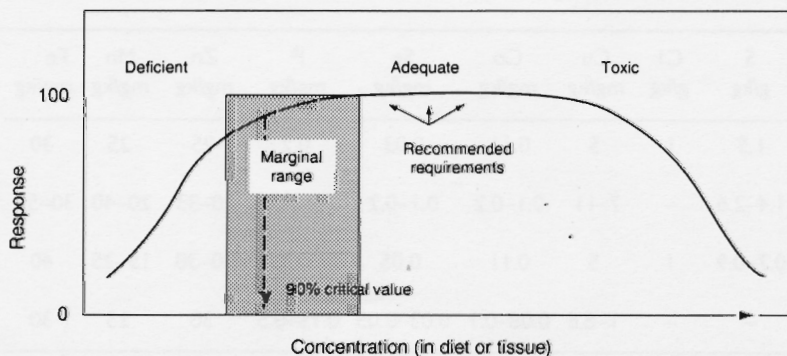


Figure 5. The dose-response relationship for minerals and animal production, showing the critical value.

Published Tables of Requirements

Inspection of Table 2 shows that some large differences exist between authorities with respect to recommended requirements. For example, the NRC (1985) requirements for selenium are between two- and seven-fold greater than those of other countries. Likewise, the Australian values (SCA 1990) for

phosphorus are significantly lower than those from other countries, and calcium requirements vary greatly between France (INRA 1978) and the U.K. (AFRC 1991) (Table 3).

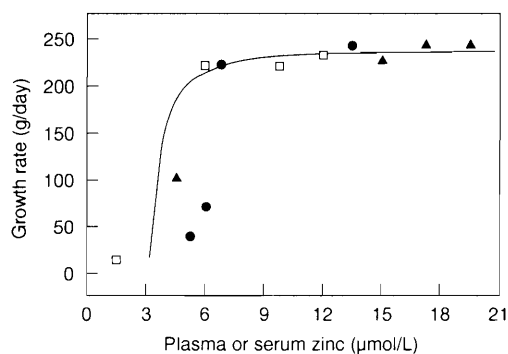


Figure 6. Mitscherlich relationship for plasma and serum zinc concentration versus growth rate of sheep using purified diets. Symbols for plasma zinc are □ White (1993) and ● Mills et al. (1967), and for serum zinc, ▲ Ott et al. (1965).

There are two main reasons for these differences. The first is the use of different absorption coefficients. The higher estimate of requirement for calcium by INRA (7.3 g/kg) versus the ARC (3.2 g/kg) (Table 3) is due mainly to the adoption of different values for absorption efficiencies—INRA using 0.45 and ARC 0.68. The second reason for different values for requirements between different authorities is the varying interpretations as to what the tables actually represent. The Australian (SCA 1990) values for phosphorus requirement are lower than others because they are considered to be minimum requirement values. The SCA (1990) attempted to estimate true minimum values and considered that those of the ARC (1980) and NRC (1985) were allowances rather than requirements because they included a safety factor.

In human nutrition a distinction is made between published tables of daily allowances and the minimum requirement of a

Table 2. Published values for dietary mineral requirements for sheep. Where a range is given the lower values are for maintenance and the higher for growth and lactation.

Source ^a	Na g/kg	K g/kg	Mg g/kg	Ca g/kg	P g/kg	S g/kg	Cl g/kg	Cu mg/kg	Co mg/kg	Se mg/kg	I ^b mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg
N.Z.	0.9	3.6	1.2	2.9	2	1.5	1	5	0.11	0.03	0.2	25	25	30
U.S.	0.9–1.8	5–8	1.2–1.8	2–8.2	1.6–3.8	1.4–2.6	–	7–11	0.1–0.2	0.1–0.2	0.1–0.8	20–33	20–40	30–50
Aus	0.7–0.9	5	1.2	1.5–2.6	1.3–2.5	0.7–0.9	1	5	0.11	0.05	0.5	20–30	15–25	40
U.K.	0.8–2.7 ^c	–	0.8–1.7	1.4–4.5	1.2–3.5	–	–	1–8.6	0.08–0.1	0.03–0.05	0.15–0.5	30	25	30

^a N.Z. = New Zealand (Grace 1983), U.S. = USA (NRC 1985), Aus = Australia (SCA 1990), U.K. = United Kingdom (ARC 1980).

^b Absence of goitrogens.

^c For diets of 11 MJ/kg metabolisable energy.

nutrient. Allowances are designed to meet the requirements of practically all people and so are set well above minimum requirements, usually at two standard deviations (Black 1986). This distinction is not so clear in animal nutrition. The NRC (1985) and INRA (1978) make no specific reference to safety margins, but apparently regard the values as allowances rather than minimum requirements (see AFRC 1991). The differences between estimates of dietary requirements are in fact mainly a reflection of differences in safety factors.

Table 3. Estimates of dietary calcium and phosphorus requirements.

Source	Ca ^a g/kg DM	P ^a g/kg DM
ARC (1965)	5.5	2.8
INRA (1978)	7.3	2.9
ARC (1980)	3.2	1.9
NRC (1985)	4.6	2.4
SCA (1990)	3.2	1.7
AFRC (1991)	3.2	2.9

^a For a 40 kg sheep at 200 g/day fed a roughage-based diet of approximately 10 MJ metabolisable energy (ME). Values are estimated where specific concentrations are not given.

Estimating Values of Requirements

Estimates of requirements in publications such as those shown in Table 2 have been derived in three ways:

1. by feeding animals with synthetic diets of known mineral concentration and measuring their response to different levels of mineral;

2. by supplementing practical diets, including pastures, that have a low mineral content and noting whether supplementation improves animal performance;
3. by factorial analysis.

Each method has advantages and disadvantages, which are summarised in Table 4.

Experiments using semi-purified diets

Animal house experiments using semi-purified diets are necessary to determine critical values of minerals, such as that shown in Figure 6 for zinc. In this type of work it is important to have more than three treatment levels, at least one of which is close to the critical value. The data can then be described either by a Mitscherlich or dose-response relationship and an estimate made of the critical value. Unfortunately, many experiments fail to meet these criteria and the results are of limited value for determining marginal or critical requirements.

Purified diets tend to be expensive, as are the animal facilities required to minimise contamination. For this reason there are few published data on the critical requirements for minerals, and other methods tend to be more frequently used to estimate minimum requirements.

Experiments using practical diets

In this type of experiment all animals are typically fed the same base diet or graze the same area, and a treatment group receives a single level of a mineral supplement. If the treated group performs better than the untreated group then the base diet is considered to be deficient. This approach

cannot define requirements accurately, but can indicate whether a certain level of mineral is adequate or not. However, when the results of several trials are combined, a pattern emerges defining the obviously deficient and adequate levels. An overlapping area of marginal deficiency may remain where responses to supplement are inconsistent or cannot be explained. Clarke et al.

(1985) have proposed a method of taking this uncertainty into account by plotting probability of response against a diagnostic value, such as blood selenium. This is shown in Figure 7 using stylised data on responses of calves to selenium supplements (taken from Langlands et al. 1989). At a blood selenium concentration of 0.25 $\mu\text{mol/L}$ there is a 70% chance of obtaining

Table 4. Advantages and disadvantages of various methods of estimating requirements.

Method	Advantage	Disadvantage
Animal house experiments with purified diets	Clearly defines requirements under a set of conditions where contamination is controlled. Suitable for detailed measurements of absorption and excretion using radioisotopes.	Cost. Experiments require expensive dietary ingredients and contamination-free animal facilities. Results may not apply to the practical situation due to the use of artificial diets.
Experiments using practical diets	Cheaper diets and animal facilities compared with using purified diets. Gives a useful indication of the deficient and adequate range of an element in a practical situation.	Difficult to produce a severe deficiency because of the natural diets. This limits the ability to define critical requirements accurately. Difficult to control and monitor contamination. Difficult to control variables (such as disease and weather) which may mask a possible response to the element under investigation. Limit on what metabolic information can be derived. Cannot use radioisotopes and it may be difficult to collect urine and faeces.
Factorial analysis	Results can be applied to any breed and production system.	Data requirements are high. It requires extensive basic information that can only be derived from animal house experiments. For several elements the variability associated with the input data make the output predictions of limited value.

a response of 10 g/day to a supplement, but only a 15% chance of obtaining a response of 90 g/day.

Both the purified diet approach and practical response trial suffer the disadvantage in that results apply only to that type of animal under those feeding conditions. To obtain a complete picture the experiments have to be repeated for all classes of live-stock under a wide range of conditions.

Experiments using practical diets require special controls. The experiments must be carried out using appropriate animals at an appropriate time of year (usually when production is highest) and using an appropriate experimental design. Ideally the sheep should be run together in the same paddock and a subset treated with minerals. This is

generally possible with trace elements such as cobalt and selenium because individual sheep can be treated within a control flock. The deficient control sheep should be monitored over time for contamination resulting from excreta from the treated sheep. It is also important to control for other more severe nutritional deficiencies by either checking status or supplying supplements. For example, it is likely that sheep will show a diminished or nil growth response to a mineral supplement if the energy intake is also limiting.

If macromineral deficiencies are suspected it is usually not possible to run the treated sheep within the control flock because of the difficulty of supplying gram quantities of minerals every day to individual grazing animals. In this situation sheep should be allocated to replicated plots or rotated through paddocks at frequent intervals to control for possible paddock differences. The latter method is likely to lead to contamination of the pastures consumed by deficient control sheep and this must be closely monitored. Experiments should be repeated over several years to account for variations in response due to annual differences in rainfall and pasture production. It is also preferable to use more than one genotype in a factorial design to account for genetic differences in requirements and response.

A major consideration in this type of experiment is the power of the design. In designing response experiments it is important to consider the expected size of response and the coefficient of variation (CV) of the parameter being measured. If too few animals are tested then the results

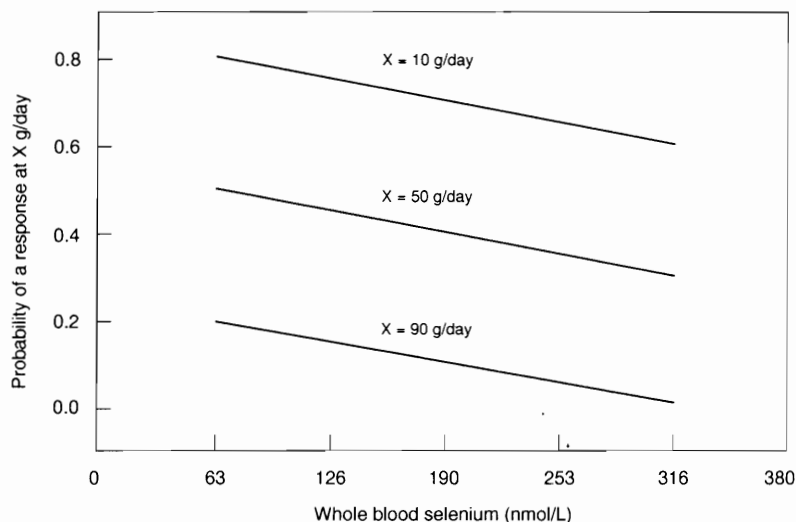


Figure 7. Probability of a response to a selenium supplement in calves at a given concentration of blood selenium. From Langlands et al. (1989).

can be misleading because of a type II statistical error—falsely accepting the null hypothesis. An example of the effect of sample size on the probability of rejecting the null hypothesis when it is false is given in Figure 8. For liveweight, if only 15 sheep per group are compared in a trial where the variance is 16 kg, then the probability of making a type II error is about 75% when treatment differences are 2 kg. Even with $n=30$ there is only a 50% chance of correctly detecting a true treatment response of 2 kg. To be 80% confident of not making a type II error a sample size of 70 sheep per group is required (Steel and Torrie 1981). The simplest way to reduce the number of animals without losing power is to reduce the variance, preferably by covariance adjustment using pretreatment measurements.

The factorial method

The factorial method for estimating requirements is based on energy and protein models and estimates the dietary need for a mineral by adding up the components of maintenance and growth or production and dividing by the coefficient of absorption. Net requirements (amounts/day) are defined as:

$$M_n = M_e + M_g + M_w + M_c + M_l.$$

M_e is mineral of endogenous origin lost in faeces and urine, and $M_{g,w,c,l}$ are minerals sequestered for liveweight gain, wool, conceptus and milk. This is represented schematically for phosphorus in Figure 9. Gross or dietary requirements are calculated by dividing net requirements by the coefficient of true absorption. This is the fraction of mineral ingested that can be absorbed by

the animal. True absorption is influenced greatly by the age of the animal, by the mineral status of the animal and diet, by the level of feed intake and by the presence of other interacting factors in the diet.

The main advantage of the factorial method is that mineral requirements can be calculated for any type and level of production. The main disadvantage is the paucity of accurate data, especially for absorption coefficients of the trace elements. This means that for some minerals precise estimates of minimum requirements cannot be made, and so safety margins are adopted which make the estimates of little practical value. Nonetheless, the factorial method is a powerful method for estimating mineral requirements and a summary table of

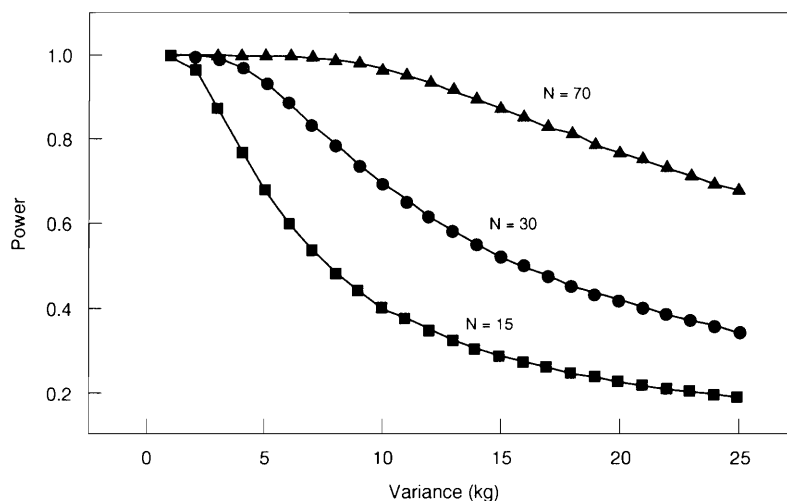


Figure 8. The effects of variance and sample size on the power of experimental design. The data are for three flocks of sheep (numbering 15, 30, or 70) weighing 40 kg and with a minimum detectable difference of 2 kg (5% of liveweight).

published requirement values is shown in Table 1.

The important thing to note is that the dietary need for minerals varies with the level of production and quality of the diet. Mineral requirements are higher for growth than maintenance, and higher for highly digestible diets than for diets of low digestibility (when expressed on a per kg of diet basis). For example, the recommended dietary concentration for calcium decreases by half when the metabolisable energy value of the diet falls from 10 to 6 MJ/kg DM (SCA 1990) at liveweight maintenance (Table 1).

In practice, a difference in growth between animals of the same age is usually due to a difference in diet quality and so mineral requirements for animals at maintenance should be assessed in relation to a low quality diet.

Conclusions

It can be seen that published estimates of requirements need careful interpretation and should not be applied to grazing sheep without qualification. Assessing mineral status by comparing analysed dietary con-

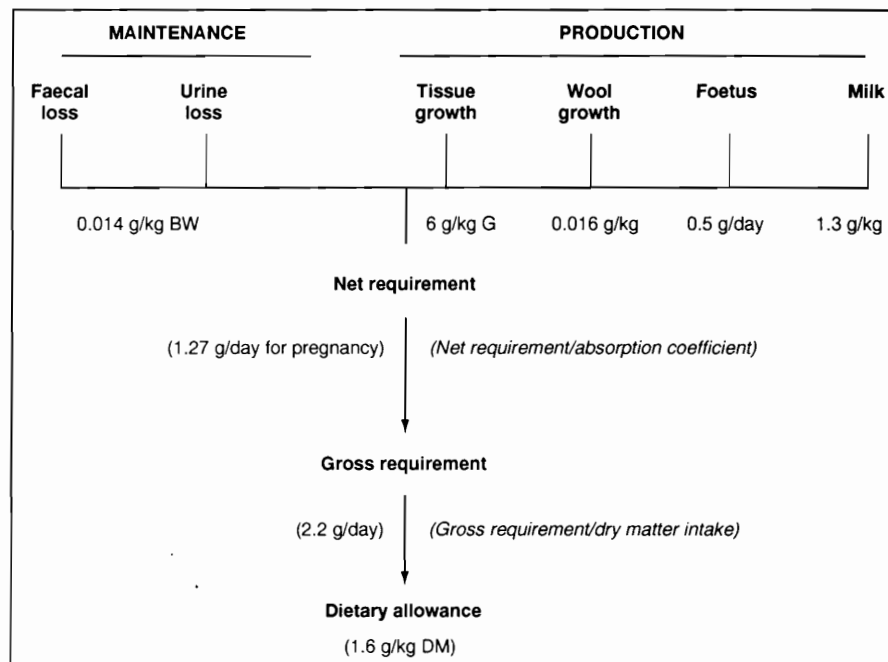


Figure 9. Factorial estimation of the phosphorus requirements of the pregnant ewe. The assumptions are that the ewe eats 1.4 kg DM and the absorption coefficient of phosphorus is 0.6. From Grace (1983).

centration with published estimates of requirement is of limited value in grazing situations because of the difficulty in knowing what the sheep selects and how much of the mineral the sheep absorbs. It follows that single estimates of requirement expressed as amounts per kg of diet need to be interpreted as recommendations only and should not be used in isolation to indicate whether a sheep is meeting its requirements for a mineral or not. An understanding of how the values were derived assists in applying the values in practice.

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Non-dietary Influences on the Mineral Requirements of Sheep

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Outline

Genetic variation in mineral metabolism

- Magnesium
- Phosphorus
- Copper

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- Copper
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- Phosphorus

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- Cold stress
- Other examples

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- Three-tier assessment system
- Dose-response trial

References

Natural selection within livestock populations exposed to mineral imbalances in local forages and crops has resulted in adaptations which protect most individuals from temporary deficiencies or excesses. Much has been written about the homeostatic and homeorhetic processes involved (e.g. Kirchgessner 1993) but less has been said about their genetic control or their influence on the incidence, diagnosis and control of disorders caused by mineral imbalance.

Such disorders are most likely to arise when sudden environmental or physiological changes cause abrupt changes in mineral requirements or when homeostatic mechanisms are not fully functional. The classical methods for studying mineral imbalances are of limited relevance to these disorders because experimenters usually rely on the establishment of a steady state. A refined diet, limiting with respect to a single mineral, has commonly been fed to individually-penned, castrated male or barren female animals which tolerate far

lower mineral intakes than those associated with disorders in the field.

Likewise, biochemical indices of mineral status in the bloodstream or tissues decline to limits rarely seen on farms before health is impaired. Since normal ranges for clinical biochemistry are also drawn largely from the results of indoor experiments, the basis for defining and controlling mineral-responsive disorders is unsound. The object of this paper is to bridge the gap between the indoor experiment and field observations by focusing on the marked influences of non-dietary factors on mineral requirements.

Genetic Variation in Mineral Metabolism

Variation in mineral status is often a striking feature of both experimental and field studies with ruminants. Since the variation is often repeatable and partly heritable (Table 1), it should influence the way mineral experiments are designed and eval-

Table 1. Magnitude (M) of 'genetic' variation in mineral status where M = highest value for breed (B), identical twin (T) or quadruplet (Q) divided by the lowest value.

Element	Species	Class	Parameter	M	Reference
Mg	Cattle	T	Availability ^a	3.3	Field and Suttle (1979)
Mg	Sheep	B	Availability ^a	1.3	Field et al. (1986)
P	Cattle	T	P in urine	12.3	Field and Suttle (1979)
P	Sheep	Q	P in urine	4.0	Field et al. (1986)
Cu	Sheep	B	Cu in liver	2.6	Woolliams et al. (1982)
Cu	Sheep	B	Cu in plasma ^b	2.5	Wiener et al. (1978)

^a Urine Mg ÷ intake of Mg.

^b Rate of repletion.

uated and also the way field problems are solved.

Magnesium

Students of magnesium metabolism in ruminants have long appreciated the need to remove repeatable individual variation when looking for treatment effects by the use of Latin Square designs (e.g. Suttle and Field 1967). Experiments with just three pairs of monozygotic twin cows have shown clear genetically controlled differences in the efficiency of magnesium absorption (Field and Suttle 1979). Awareness of such influences contributed to the Agricultural Research Council's decision (ARC 1980) to introduce a safety factor whereby dietary allowances of ruminants for magnesium were set 70% above estimated minimum requirements to cater for the most vulnerable individual. It would be surprising if practical magnesium deficiency problems in ruminants could not be reduced by genetic selection using a simple index such as the magnesium:creatinine ratio in urine of the sire. Significant differences in urinary magnesium excretion between the offspring of different sires have been reported in sheep with an estimated heritability of 0.60 (Field et al. 1986).

Phosphorus

Studies with sets of four identical, chimera-derived sheep showed that efficiency of phosphorus absorption ranged from 0.63 to 0.79 of phosphorus intake between sets, and variation within a wider population was estimated to be similar (Field et al. 1984).

The efficiency of phosphorus absorption in ruminants has a two-fold influence on phosphorus requirements because it determines the efficiency of phosphorus recycling via saliva and hence endogenous losses. The most efficient individual users of phosphorus were calculated to have phosphorus requirements about 30% below the ARC (1980) minimum requirement for ewes during lactation (Field 1981). As with magnesium, significant differences in urinary phosphorus excretion were found between identical twin heifers (Field and Suttle 1979) and between offspring from different rams (Field et al. 1986), and there was a four-fold difference between breeds (Field et al. 1986). The precision of experiments with phosphorus can also be improved by allowing for genetic variation in phosphorus metabolism (e.g. Field et al. 1984).

Copper

Differences in blood copper concentrations between breeds of sheep grazing the same pastures led to the discovery of substantial breed differences in the efficiency of copper absorption (Wiener et al. 1978; Woolliams et al. 1982). The heritability of the factor(s) controlling copper absorption were high enough for substantial increases in copper status to be achieved by sire selection for six male generations based on plasma copper concentrations at a young age (Woolliams et al. 1986a,b). Breeds that used copper efficiently, such as the Texel, were vulnerable to copper poisoning when housed (Woolliams et al. 1982) whereas those using copper inefficiently, such as the Scottish Blackface, were vulnerable to defi-

ciency when grazed (Woolliams et al. 1986a,b). The requirements of the Texel and Scottish Blackface probably differ by a factor of two or more (Suttle 1987). The messages are the same as for magnesium and phosphorus: allowing for initial variation in and the repeatability of copper status (e.g. use of initial liver biopsy concentrations and covariance analysis) improves the precision of experimentation; selection within or between breeds can solve problems of copper deficiency and excess (Woolliams et al. 1982, 1986a,b). However, selection for improved status for one mineral (e.g. magnesium) may result in lowered status of another, e.g. phosphorus (Field and Suttle 1979).

The presence of widespread, large genetic variation in mineral metabolism in populations is unique to ruminants and it is noteworthy that the rumen plays a vital role, directly or indirectly, in the absorption of each of the elements cited: for magnesium, the rumen is the principal site of absorption; for phosphorus, rumination is responsible for extensive phosphorus recycling via saliva; for copper, the rumen is the site of antagonisms which lower availability (e.g. Suttle 1987). Genetic variation may be linked to selection pressure arising from diverse nutritional conditions, endemic deficiency favouring the evolution of efficient users, and endemic toxicity the evolution of inefficient users of minerals. It would be surprising if there were not marked variations in mineral metabolism within species in many parts of the world, given the vast land areas and the diversity of agricultural systems used to grow sheep. Exploitation could be of particular value where supple-

mentation of the diet or stock presents economic (e.g. phosphorus) or practical (e.g. sodium) problems.

Impact of Nematode Infections of Pasture on Mineral Requirements

Pasture nematode infections increase mineral requirements and they are endemic in all pastoral systems.

Copper

In defining the role of continuous larval nematode infection in the pathogenesis of diarrhoea in a flock of Finnish Landrace (FL) sheep, the effects of discontinuous and continuous anthelmintic (albendazole) treatment were compared against no treatment. Effects of treatment on diarrhoea were small and late to develop (Suttle and McLean 1995) but plasma copper increased markedly in both young, non-reproducing females and lactating ewes (Fig. 1), suggesting that copper requirements had been increased by nematode infection. The initial hypocupraemia was unexpected since the pasture contained sufficient copper (8–10 mg copper/kg dry matter) to meet their requirements in the absence of antagonisms from molybdenum (<2 mg/kg DM) or iron (<1 g/kg DM) (e.g. ARC 1980). The fact that a continuous release bolus was more effective than drenching with anthelmintic once every 4 weeks suggests that larval infection was contributing to the impairment of copper metabolism. Furthermore, immune yearlings benefited as much as immunosup-

pressed ewes, suggesting that impairment can arise as a consequence of immunity as well as by physical damage to a poorly protected gastro-intestinal mucosa. Low

egg output in the ewes (median count 200 eggs/g fresh weight) suggests that only low worm burdens are needed to impair copper metabolism.

The quantitative impact of parasitic infection on copper requirements cannot be gauged from these studies but some interesting comparisons can be made. The repletion rates in yearlings were equivalent to absorption rates of 0.79 and 1.40 mg copper/d (from Suttle 1974) for those given drenches and boluses, respectively, i.e. a 77% improvement in availability from combating the continuous larval challenge. The faster repletion rate of hoggets over ewes grazing the same pasture confirms that lactation increases the copper requirement in terms of copper concentration in the pasture. In an earlier experiment with the FL flock it was noted that the same dose of cupric oxide particles (CuOp) given to two similar groups of lambs, grazing adjoining fields, protected one from hypocupraemia for at least 150 days and the other for only 60 days (Suttle and Brebner 1995). The poorly protected group was more prone to diarrhoea and had an abnormally high plasma pepsinogen concentration (1.1 v 0.8 U/L) (U = units) indicating abomasal parasitism. In previous experiments, a similar reduction in efficacy of CuOp was obtained by adding 3 mg molybdenum and 3 g sulfur/kg to the diet of ewes (Suttle 1981). Thus the impact of infection was equal to that of known potent antagonists of copper absorption.

Others have reported positive effects of anthelmintic treatment (e.g. Judson et al. 1985) and negative effects of abomasal parasitism (Bang et al. 1990) on liver

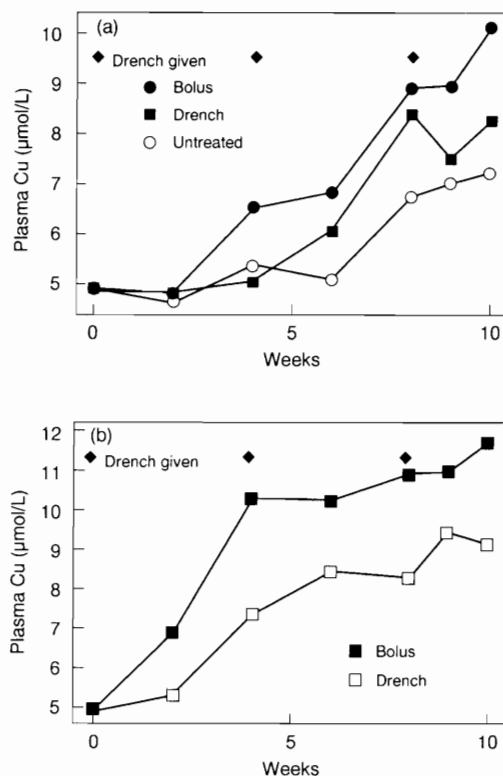


Figure 1. (a) Repletion of plasma copper in lactating ewes was hastened by the administration of anthelmintics, whether given in a slow-release bolus or as a drench. Diamond symbols indicate the times of drenching. (b) Comparison of the repletion rates of plasma copper in yearlings treated with bolus or drench. Diamonds indicate the times of drenching.

copper reserves in sheep. The new results show that continuous larval infection can induce copper deficiency in sheep grazing pastures which would normally meet their copper requirements and at the same time decrease the efficacy of a common remedy, CuOp. These effects probably reflect a decrease in copper absorption but impaired entero-hepatic recycling of copper may contribute by increasing faecal endogenous losses of copper. The net effect may be to more than halve the effective supply of copper from the pasture or the oral supplement.

Sodium

Periodically, lambs from the above naturally infected flock developed extremely low salivary sodium:potassium ratios indicative of sodium depletion (Suttle and Brebner 1995; N. Suttle and M. Herrero, unpubl.). Sodium deficiency was less pronounced in seasons when pasture larval infection was low (Suttle 1994a) and it was common to find relationships between saliva sodium:potassium or potassium, faecal DM (g/kg FDM) and/or plasma pepsinogen (PP, U/L) of the following kind:

$$K = 22.9 + 4.5 PP - 0.47 FDM \quad (r = 0.34; 82 \text{ df})$$

These associations suggested that abomasal parasitism and diarrhoea had induced a sodium deficiency. This view is supported by evidence that although anthelmintic treatment could raise sodium status, a drug whose efficacy is lowered by abomasal parasitism (albendazole) was relatively ineffective (Suttle et al. 1996).

As with copper, the pastures contained

sufficient sodium (0.1–0.2 g/kg DM) for lambs by standards (ARC 1980) which have been criticised as being far too high (Michel 1985). The criticisms are based on experiments with penned sheep not subjected to continuous larval nematode infection in which hormonally-induced adaptations conserve sodium and prevent ill-health at sodium inputs far below the published standards (Michel et al. 1988). An obvious explanation is that physical damage to the intestinal mucosa by parasites limited the ability to conserve faecal sodium. However, compensatory absorption of sodium should have occurred in the large intestine, which is usually unaffected by parasitism. The complete explanation may be found in other contrasts between indoor and pasture experiments—the former involve feeding dry diets low in potassium and in these circumstances sheep reduce their extracellular fluid volume by drinking less to maintain sodium concentrations in the extracellular fluid. When water is consumed principally in the food (grass) and that food is rich in potassium, which increases water intake (Suttle and Field 1967), homeostatic mechanisms may be far less effective, particularly in abomasal parasitism which causes large increases in sodium flow at the duodenum (Wilson and Field 1983).

Phosphorus

Anthelmintic treatment did not affect plasma phosphorus in the FL flock but homeostatic mechanisms probably mobilised extra phosphorus from the skeleton and were not compromised by infection of

the gut. Measurements of phosphorus flows along the gastrointestinal tract in parasitised sheep have shown that the efficiency of absorption of dietary phosphorus is reduced, as is that of salivary phosphorus (Wilson and Field 1983; Bown et al. 1989). The combined effect of these changes in one study was to increase estimated phosphorus requirements by 65% (Table 2). Parasitic infections of the gut reduce greatly the phosphorus content of the skeleton in lambs (e.g. Sykes et al. 1979) and the impact of nematodiasis on phosphorus deficiency might be critical if, prior to larval challenge, skeletal mineral reserves are depleted through reduction of dietary protein or calcium and by lactation.

Cattle may be more vulnerable to nematode-induced phosphorus deficiency than sheep because they have less time to replenish skeletal reserves of minerals between lactations (e.g. Read et al. 1986). Studies in the tropics have shown that phosphorus deficiency is more likely to

occur in beef cattle in the wet season than the dry season on pastures of similar phosphorus concentration (Winter et al. 1990). Heavier infection by parasites in the wet season may have contributed to that seasonal difference.

Continuous infections of the gut thus increase requirements for a trace element (copper), an anionic (phosphorus) and a cationic (sodium) macromineral, and it would be surprising if they did not affect requirements for most minerals (magnesium may be an exception because it is principally absorbed from the rumen). The work of Judson et al. (1985) suggests that zinc and cobalt requirements are increased also by worm infections. Good nematode control is an essential part of any program to minimise mineral deficiencies in grazing livestock and a flexible approach must be maintained in extrapolating from mineral requirements determined in penned, worm-free animals to needs and risks of deficiency in field situations.

Table 2. Effects of intestinal parasitism by *T. colubriformis* on the efficiency (E) of phosphorus absorption and reabsorption in two sets of identical quadruplet lambs and average extrapolated phosphorus requirements for lactating ewes (75 kg liveweight and yielding 3 kg milk/d).

	E saliva P		E dietary P		P requirement (g/d)	
Infection	0	+	0	+	0	+
Set 1 ^a	0.76	0.60	0.60	0.39	7.9	12.5
Set 2 ^a	0.84	0.25	0.65	0.39		

^a Unpublished data from A.C. Field: see also Field (1981) and Wilson and Field (1983).

Effects of Demand on Critical Pathways

The factorial derivation of mineral requirements takes no account of internal, diurnal or day-to-day variations in demand. Instead, the desired input is that which matches average output and it is hoped that any short-term variation in demand can be met by redistributing the body's mineral reserves. With the possible exception of iodine, all minerals have multiple functions at diverse sites in the tissues. Two examples will be given where redistribution of the available body minerals may fail to protect a critical pathway upon which health may depend.

Exercise

It is common to allow for the effects of activity when calculating energy requirements for livestock (AFRC 1993). For example, activity adds 12% to the energy requirement of hill ewes carrying a single lamb in mid-pregnancy, when compared to housed ewes. The extra energy is consumed during oxidative metabolism and increases the demand on numerous mineral-dependent metabolic pathways. In a study of selenium deficiency in calves, Arthur (1988) fed a low selenium diet (0.01 mg/kg DM) to penned animals for several months without inducing white muscle disease (monitored via changes in creatine kinase (CK) in plasma). When those calves were turned out to graze, plasma CK increased, indicating leakage of the enzyme across damaged muscle membranes.

Turn-out was accompanied by simultane-

ous changes in diet and muscular activity, and to determine how these changes contributed to muscular damage the experiment was repeated with a group of penned animals given grass cut from the field. Only when the grass-fed calves low in selenium were turned out did plasma CK rise. Throughout both experiments the selenium-depleted calves maintained blood levels of activity of the selenium-dependent enzyme glutathione peroxidase, which would be regarded as grossly deficient (< 15 U/g Hb). Only when the extra demand of muscular activity was superimposed did muscle damage occur in the low selenium calves. The presence of polyunsaturated fats in the spring grass may have contributed to the development of myopathy but without exercise they were obviously harmless. It would be surprising if exercise did not also influence requirements for other nutrients affording protection against oxidative stress (e.g. copper in superoxide dismutase).

Cold stress

Selenium is essential to the activity of the deiodinase enzymes which influence thyroxine metabolism (Arthur 1993). Mammals contain two such enzymes, Types I and II, and activities of both are suppressed in selenium deficiency. For the first few weeks of life, brown adipose tissue (BAT) in newborn ruminants shows a high level of Type I activity and it is probably important for local thermogenesis and the export of active T3 from BAT to other sites. The enzyme is there presumably to protect the newborn from hypothermia to which it is

highly prone (e.g. lambs, Eales et al. 1982). Type II activity in BAT increases during cold stress in rats but the increased activity cannot be sustained in selenium-deficient animals (Arthur et al. 1991). Although the critical experiments have yet to be performed, the offspring of selenium-deficient livestock born outdoors in cold weather may be more vulnerable to hypothermia than those born indoors in a sheltered environment. In this case, survival is at stake and the benefits of ensuring an adequate selenium status could be particularly high.

Other examples

Other examples of abrupt increases in demand likely to put strain upon critical pathways are: the respiratory burst in phagocytes during microbial infection (selenium, Boyne and Arthur 1979; copper, Jones and Suttle 1981); the contraction of the uterine wall during parturition (selenium, Suttle 1992); transportation (organic chromium, Wright et al. 1994); weaning (copper, Suttle 1975).

Redistribution of Minerals within the Body

The ability of animals to accumulate reserves of minerals during times of plenty and to mobilise those reserves during times of scarcity lessens the need to meet mineral requirements on a day-to-day basis and partly explains the tolerance of livestock towards diets which 'on paper' are mineral deficient. A classic example is the mobilisation of calcium and phosphorus reserves from the skeleton. Less well known is that

around parturition the mobilisation of calcium appears to be obligatory and cannot be halted by putting even a gross excess of calcium into the diet (AFRC 1991). Livestock have 'learnt' to become less dependent on the hands that feed them.

Hypocalcaemia prevention in lactating cows

Genetic selection for high milk yields in dairy cattle has created a health problem (milk fever) caused by failure to meet the abrupt and enormous increase in calcium demand with the onset of lactation. The disease can be prevented by improving the net contribution of calcium from the skeleton to the extracellular fluid by increasing the 'acidity' or anion:cation balance of the diet (Block 1984; Goff et al. 1991; Phillippo et al. 1994). The 'acid' diet works by modulating one of the hormones (1,25 hydroxy-cholecalciferol) which controls calcium resorption from the skeleton (and calcium absorption). However, efficacy may depend on non-dietary factors. Susceptibility to milk fever increases with age because the absorption and partitioning of dietary calcium is changed by a reduction in receptors for 1,25 hydroxy vitamin D in the intestine and bone with age (Horst et al. 1990). Increasing the acidity of the diet of young (1st–3rd parity) cows did not increase their resistance to hypocalcaemia (Van Mosel et al. 1993). A switch from hay to the more acidic silage as roughage source should also reduce milk fever in aged cows.

Other species are less vulnerable than the dairy cow to hypocalcaemia immediately after parturition because the require-

ment for calcium in late pregnancy can equal that for lactation (e.g. sheep, AFRC 1991). Nevertheless, the feeding of acidic diets (e.g. in sheep, Abu Damir et al. 1990) or the culling of older animals may reduce the risk of disorders associated with hypocalcaemia in species such as the sheep, goat and yak.

Redistribution at parturition

While the skeleton is pre-eminently a store for calcium and phosphorus, it also contains most of the body magnesium and substantial fractions of its total sodium and zinc (Table 3). Obligatory resorption of bone prior to and after parturition must result in the release of significant amounts of these elements in terms of the total demand for lactation.

Magnesium. In milk fever, hypocalcaemia is usually accompanied by hypomagnesaemia which may indicate a parallel shortfall in magnesium mobilisation from the skeleton. Using a pyrophosphate analogue to inhibit bone resorption, Matsui et al. (1994) decreased plasma magnesium from

14.5±0.4 to 12.0±1.2 mg/L in dry sheep given a diet very low in magnesium for 5 days. However, an increase in diet acidity did not prevent a post-parturient fall in plasma magnesium in dairy cows (Phillippo et al. 1994). Gardner (1973) increased the rate of removal of magnesium from the skeleton of sheep by peritoneal dialysis against calcium-free medium but the effect was not reflected by a change in plasma magnesium. Thus, magnesium may have been redistributed by a change in acid:base balance of the diet but not seen as a change in plasma magnesium.

Sodium. There appears to be a shift in the set-point for salivary sodium:potassium from 18 to 32 associated with lactation in sheep (Morris and Peterson 1975). The mechanism is unclear but the purpose may be to trap sodium released from the skeleton in the recycling pool of sodium in the rumen and facilitate its transfer to offspring via the sodium-rich milk. Marked reductions in body sodium after lactation have been reported in hill ewes (Field et al. 1968) and such redistribution of body sodium may explain the tolerance of ewes to sodium

Table 3. Amounts of mineral which may be mobilised from the skeleton during lactation in sheep and the proportion of the total lactational demand which they would sustain.

	Na (g)	Mg (g)	Ca (g)	P (g)	Zn (mg)
Total ^a in skeleton	18.4	10.6	659	280	237
Amount mobilised for lactation	3.8	2.2	135 ^b	5.7	49
Proportion ^c (%) of yield sustained	40	69	465	248	181

^a From Grace (1983).

^b From Braithwaite (1983): amounts of other elements calculated pro rata.

^c Assuming a total lactational yield in the Merino of 18 kg milk containing 0.4 g Na, 0.18 g Mg, 1.6 g Ca, 1.3 g P and 1.5 mg Zn/kg.

depletion (e.g. Michel et al. 1988). Impairment of sodium recycling by gastrointestinal parasites may limit the effectiveness of this adaptive mechanism.

Zinc. The tolerance of the in-lamb ewe to zinc depletion (e.g. Masters and Moir 1983) may depend also partially on the distribution of body zinc. If mobilisation of one-fifth of the skeleton's major minerals (calcium and phosphorus) released a similar proportion of bone zinc, as much as 50 mg zinc could be released, enabling the secretion of zinc-rich colostrum and supplementing the less efficient dietary supply route for zinc. By reducing the mineralisation of bone (e.g. Sykes et al. 1979), gastrointestinal parasitism may reduce the skeletal reserve of zinc.

Assessment of Mineral Status

The influence of 'non-dietary' factors on the mineral status of livestock, whether they be related to infection, upsurge in demand or redistribution of reserves, must affect the approach to the diagnosis, anticipation and prevention of disorders. Relationships between mineral composition of the whole diet or of accessible parts of the animal (plasma, erythrocytes, liver biopsy and rib biopsy) and health are bound to be imprecise.

Three-tier assessment system

Uncertainties should be recognised by using a three-tier system to classify the mineral status of both diets and animals with a 'marginal' band to separate the 'deficient' from the 'normal' mineral status (e.g.

Clarke et al. 1985; Suttle 1994b; see also Tables 2 and 3 in Paynter in this publication). The approach is not new but adoption has not been consistent, and it is not used in the U.K. at present. Adoption of such a system will slow down the rate at which a diagnosis is made but greatly improve the precision of that diagnosis and greatly reduce the number of recorded diagnoses of 'mineral deficiencies'.

Dose-response trial

The best diagnosis of a mineral deficiency is obtained when specific mineral supplements improve health or productivity (Suttle 1994b). However, there is room for considerable improvement in the way that such responses are sought. Failure to significantly improve group performance by a given treatment could arise because the mineral deficiency was: (1) irreversible; (2) not fully reversed; (3) not affecting the whole group; (4) not the only factor limiting performance.

Factors that need to be considered in dose-response trials include: (1) the deleterious effect of the deficiency on milk yield in the dam cannot be overcome by treating suckling animals; (2) bolus treatments may be regurgitated; (3) flocks and herds are rarely uniformly affected; (4) there may be more important influences on production than mineral deficiency such as genetic potential, infectious disease, supply of digestible organic matter. To overcome such problems, the improved dose-response trial needs: sustained alleviation of deficiency; removal of as many 'non-mineral' constraints on performance as

possible; use of information on prior performance (Joyce and Brunswick 1975) and subsequent mineral status (by covariance analysis) to reduce or interpret within-group variability in performance. Careful comparison of biochemical indices of mineral status with improvement in performance will justify and extend the three-tier approach to assessment described above.

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Diagnosis of Mineral Deficiencies

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Outline

Soil indicators of mineral status

- Sampling
- Analysis
- Interpretation

Dietary indicators of mineral status

- Sampling
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Animal indicators of mineral status

- Sampling
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References

Mineral deficiencies in sheep often present as part of a multifactorial disease problem. In diagnosing these deficiencies, it is essential to consider them as part of an overall differential disease diagnosis, along with the potential for infectious, genetic, metabolic and non-mineral dietary contributions to a disease condition. Clinical signs presenting in the animal should be considered foremost. These clinical signs should be compared to the well documented clinical effects associated with the various mineral deficiencies (Underwood 1977). This examination should consider whether the signs appear to be acute or chronic in form, what tissues are predominantly affected and the age or class of animal affected. For example, an apparent ataxic condition confined to lactating mature ewes may suggest possible calcium or magnesium deficiency. In contrast, an ataxic condition confined to young lambs may indicate copper or selenium deficiency.

Where clinical signs are relatively specific, the possibilities in the differential diagnosis may be greatly simplified. Examples include white muscle disease associated with selenium and/or vitamin E deficiencies, and goitre associated with iodine deficiency. Where signs are non-specific (e.g. ill thrift) a number of mineral and non-mineral factors may need to be considered in the differential diagnosis. This includes the presence of subclinical deficiencies, where reduced animal production may be transient and associated with few, if any, specific signs.

In determining mineral nutrition of animals, response to treatment is often pro-

posed as the definitive diagnosis. However, carefully controlled response trials cannot always be conducted on-farm, due to the seasonal nature of mineral deficiencies and the time frames required to reach a diagnosis. Therefore, an approach based on measurement of specific indicators of mineral nutrition and the likely response to supplementation may be more beneficial (Grace 1983; SCA 1990). Using this approach, an indicator of mineral nutrition should be recognised for what it represents—a reflection of overall nutrition for that mineral. It may not accurately reflect the actual biochemical impairment which leads to a specific disease lesion.

This is well demonstrated in the case of copper, which is required for activity of a number of enzymes in most tissues. Each copper enzyme in each tissue has a different rate of depletion or repletion with changes in copper intake. Rates of depletion also vary with the mechanism by which a deficiency is induced, i.e. simple or thiomolybdate, with some copper enzymes in some tissues being affected by thiomolybdates far more than others (Paynter 1984). Decreases in plasma copper concentration reflect decreases in plasma caeruloplasmin activity, which in turn reflect decreases in liver copper reserves and the capacity of the liver to synthesise this enzyme (Paynter 1986). Plasma copper is therefore highly correlated with depletion of liver copper reserves, but is only indicative of the changes that may be occurring with other copper enzymes in other tissues.

In assessing mineral status, the terms marginal and deficient are often used to differentiate sub-optimal mineral intakes.

These terms are defined as follows:

- *deficient*—indicates depleted reserves associated with impairment of biochemical or physiological processes, and responses to treatment are expected;
- *marginal*—indicates reduced reserves, and production responses to treatment may be observed.

In using these definitions, it should be noted that a deficient level of an indicator should not be taken to indicate that clinical disease is present. Onset of clinical disease is often triggered by other dietary or non-dietary factors; the mineral deficiency is a predisposing requirement to the clinical disease condition. In a diagnostic sense, an example is white muscle disease and selenium nutrition. White muscle disease should not be diagnosed solely on the basis of low concentrations of blood selenium or glutathione peroxidase; elevated plasma creatine kinase activities should be used to determine the presence of significant muscle damage.

In the remainder of the review, the relative merits and uses of various indicators in soils, diets and tissues for determining mineral status in sheep are discussed.

Soil Indicators of Mineral Status

Soil indicators have limited direct applicability in determining mineral nutrition of grazing animals, their principal use relating to defining requirements for plant growth (Grace 1983; Hosking et al. 1986). Specific soil tests are of most use where there is a high correlation of the soil indicator with the mineral supply to the plant, over a range of

soil types and plant species. Few indicators, apart from phosphorus, potassium, and possibly sulfur, fit this category, and even these are restricted to periods when plants are actively growing.

Soil classifications based on type and pH may provide limited general information on mineral availability. Similarly, associations of local soil types with a specific mineral problem may be useful in estimating the extent of a problem at a local level. Granite derived soils and leached sands may be low in minerals such as copper and selenium. Highly acid soils may have low availability of the major cations and molybdenum but be high in manganese. In contrast, alkaline soils may have low availability of manganese, zinc and cobalt (Grace 1983; Hosking et al. 1986) and high availability of molybdenum (Leech and Thornton 1987), and are unlikely to produce selenium deficiency (Reuter 1975).

Sampling

For assessment of the major nutrients in relation to pasture requirements, soil cores of the top 10 cm soil profile are commonly used. The final sample for each area usually comprises 15–30 cores, representing each soil type. These samples may be then air dried (max. 40°C) prior to subsampling for further analysis (Rayment and Higginson 1992).

Analysis

As many of the analytical procedures used for soils are based on extractable (rather than total) mineral concentrations, results are highly method-specific. Rayment

and Higginson (1992) have documented individual standard methods of analysis commonly used for Australian soils.

Interpretation

Interpretation of soil results is highly dependent on the analytical method used. In general, soil analytical measurements for macrominerals are calibrated against plant responses, rather than any specific animal responses, and any extrapolations to animal nutrition are based on animal dietary requirements. Soil measurements of trace minerals have a generally poor correlation to animal requirements (Hosking et al. 1986).

Dietary Indicators of Mineral Status

For many minerals, determination of total dietary mineral concentrations provides a useful overall indicator of the *adequacy* of mineral nutrition to the animal (Table 1). Dietary analysis also forms the basis for understanding the seasonal nature of mineral deficiencies in grazing sheep, and may be the preferred measure of mineral nutrition to the animal where direct animal indicators are of limited value or where dietary interferences in availability to the animal are minimal. Examples include calcium, sodium, potassium, manganese and zinc (SCA 1990).

It should be emphasised that while dietary measurements of these and other minerals may not accurately correlate with their clinical deficiencies, they do form a strong basis for screening for adequacy of these

minerals and eliminating them from a differential diagnosis in the animal. Where other dietary factors may interact and affect availability of a mineral to the animal, dietary analysis is important in defining the extent of these interactions and, in some cases, the most appropriate form of treatment. However, for all minerals where dietary interactions may occur, a diagnosis of deficiency should be based, where possible, on animal rather than on dietary measurements.

Sampling

All dietary sources, including pastures, supplements and water, should be accounted for when dietary mineral intakes are measured. This may not be achievable with sheep on free choice diets where selective intakes of dietary components may occur, particularly where these dietary components are highly variable in composition and palatability. In these situations, pasture samples should be representative of those being selected by the animal and the area being grazed, and also reflect stocking rate. Separation of pasture samples into the main species, e.g. clovers and grasses, allows the assessment of seasonal variations in mineral concentrations within and between species composing pastures, and enables predictions of mineral nutrition to be made between seasons on the basis of pasture sward composition. This is particularly important where large seasonal species variations occur in pasture composition at marginally deficient sites.

Analysis

Analytical methods used for determining dietary minerals are principally based on the determination of total mineral concentrations. A number of methods and instruments is available for single or multi-element assays. All assays should be verified by the use of appropriate standard reference materials, which are readily available commercially.

Interpretation

Minimum dietary mineral requirements for sheep are shown in Table 1. Note that these values are approximate only, even for those minerals where availability may not be affected by other dietary factors. Their principal use should be to determine the level of adequacy of minerals. When dietary indicators are used to assess mineral nutrition of animals, the reserves available in the animal should be considered. Where animal reserves are minimal, deficiency may be induced within a few days. Magnesium is an example. In contrast, where there are considerable reserves or conservation of nutrients by the animal, deficiency may not be reached for several months. Examples include calcium, sodium, cobalt and selenium (SCA 1990).

For minerals essential for rumen function, particularly sulfur, dietary estimates of requirements may be improved by the inclusion of dietary nitrogen (SCA 1990). This inclusion is particularly relevant when the nitrogen content of the diet is outside that seen with green pasture-based diets, as occurs with senescent pastures and diets supplemented with nitrogen. More complex

dietary interactions may occur with magnesium and copper availabilities in diets. For example, dietary sodium, potassium, nitrogen and phosphorus have all been implicated in affecting magnesium nutrition (SCA 1990). Critical values for magnesium availabilities from diets and soils have been proposed that empirically account for at least some of these interactions (Kemp and 't Hart 1957; Lewis and Sparrow 1991). For copper, where the main interferences in uptake are associated with increased dietary molybdenum and sulfur concentrations, critical values for copper availability can be calculated (Suttle and McLauchlan 1978) which appear to closely correlate to copper nutrition in the sheep (Givens and Hopkins 1978; Paynter, unpubl.).

Considerable differences may occur in mineral concentrations of different pasture species. Direct extrapolation of results from one pasture species to others is not possible. Examples of pasture species mineral variations include: the large differences in sodium concentrations measured in natrophiles (sodium-tolerant plants that include many perennial grasses) and natrophobes (sodium-intolerant plants that include fescue, lucerne, and sorghums) (Smith et al. 1983); the lower magnesium, molybdenum and sulfur concentrations in many subterranean clover cultivars compared to other clover species (Evans et al. 1990); and the decreased copper availability in cruciferous species (Merry et al. 1983) and in annual grasses versus clovers (Paynter 1989).

Superimposed over all these dietary measurements are the variable effects of soil intake with sheep grazing at pasture (Grace 1983). In particular, soil ingestion

may be a major source of cobalt and iodine (SCA 1990). Direct assessment of these elements in the animal is therefore recommended.

Table 1. Minimum dietary mineral requirements for sheep (dry matter basis).

Mineral	Unit	Dietary requirement ^a
Calcium ^b	g/kg	1.5–2.6
Phosphorus ^b	g/kg	1.3–2.5
Magnesium ^{b,c}	g/kg	1.2
	K/(Ca + Mg)	<2.2
Potassium ^b	g/kg	5.0
Sodium ^b	g/kg	0.7–0.9
Sulfur ^b	g/kg	2.0
	g/g N ^b	0.08
Cobalt ^b	mg/kg	0.07
Copper ^d	mg available Cu/kg	0.24
Iodine ^b	mg/kg	0.5
Iron ^b	mg/kg	40
Manganese ^b	mg/kg	15–25
Selenium ^b	mg/kg	0.05
Zinc ^b	mg/kg	20–30

^a Lower values for maintenance. Upper values or single values are for rapidly growing/lactating sheep.

^b Values derived from SCA (1990).

^c Critical ratio derived from Kemp and 't Hart (1957).

^d Estimated from copper, molybdenum, sulfur concentrations (Suttle and McLauchlan 1978).

Animal Indicators of Mineral Status

Direct animal measurements potentially offer the best indicator of mineral nutrition in the sheep, as they account for dietary selection and the variable uptake and availability of minerals. Indicators of mineral nutrition based on tissue or body fluid

analyses are now available for the majority of minerals shown to be essential and commonly affecting nutrition of the sheep. Most of these indicators have been well calibrated against production or clinical responses to treatment, over a range of environmental conditions. For many minerals, several different indicators are available for assessment of nutrition; each has advantages and disadvantages, depending on the situation (Tables 2 and 3).

This review does not cover all these indicators extensively. For this, the reader is referred to reviews by Grace (1983), Hosking et al. (1986), Caple and Halpin (1985) and SCA (1990). Instead, the indicators listed below are those found by this reviewer to be currently in use for practical diagnostic purposes for those minerals principally affecting production. For these reasons, animal indicators of manganese status are not covered. Blood manganese concentrations are very low and other tissues show relatively small changes with deficiency (Paynter 1987).

Similarly, while determination of mineral concentrations in faeces potentially provides an indication of mineral intake from all dietary sources including soil, and overcomes dietary sampling problems associated with selective intakes, final faecal concentrations are dependent on feed digestibility and may not account for true availability of minerals from complex sources such as soils. These two major variables alone limit the defining of critical faecal concentrations as direct measures of mineral nutrition in the animal.

Several mineral indicators used for sheep show a relatively wide range of values in

their correlation with dietary uptake. Others have a limited range of application, principally relating to nutrition below adequacy. Examples of the former include blood selenium concentrations or glutathione peroxidase activities with selenium nutrition, plasma vitamin B₁₂ with cobalt nutrition, plasma sulfate with sulfur nutrition, liver copper with copper nutrition and urine

sodium and magnesium as possible indicators for sodium and magnesium. Examples of the latter include plasma copper, zinc, magnesium and phosphate, all of which plateau with adequacy due to homeostatic mechanisms such as urinary thresholds or controls on enzyme synthesis. Applicability of each of the major indicators is shown in Tables 2 and 3.

Table 2. Summary of significant macro-mineral indicators in the sheep.

Indicator	Units ^a	Range			Applicability
		Deficient	Marginal	Adequate	
Calcium					
plasma Ca ^b	mmol/L (mg/L)	<1.25 (<50)	1.25–2.12 (50–85)	2.12–2.87 (85–115)	Metabolic deficiency
eye fluid Ca ^b	mmol/L (mg/L)	<1.0 (<40)	–	1.0–2.5 (40–100)	Post mortem
urine Ca ^c	μmol/mosmol	–	<1	>1	Single urine samples
Magnesium					
plasma Mg ^b	mmol/L (mg/L)	<0.33 (<8)	0.33–0.74 (8–18)	0.74–1.44 (18–35)	Suitable <48 hours post mortem Single urine sample
eye fluid Mg ^d	mmol/L (mg/L)	<0.5 (<12)	–	0.5–1.44 (12–35)	
urine Mg ^c	μmol/mosmol	<1	1–2	>2	
Phosphorus					
plasma inorganic P ^b	mmol/L (mg/L)	–	<1.3 (<40)	1.3–2.7 (40–85)	
faecal P	mmol/kg DM (g/kg DM)	<64.5 (<2)	–	>64.5 (>2)	
Sodium					
urine Na ^c	μmol/mosmol	<1	1–10	>10	Single urine sample
parotid saliva Na/K ^{c,e}	mmol/L:mmol/L	<5	5–14	>14	
Sulfur					
plasma inorganic S ^b	mmol/L (mg/L)	<0.3 (<10)	0.3–0.8 (10–25)	0.8–1.9 (25–60)	

^a Alternative units with corresponding values are shown in parentheses.

^c Caple and Halpin (1985).

^e SCA (1990).

^b Paynter (unpublished data).

^d Lincoln and Lane (1985).

Sampling

Samples taken for analysis must be representative of the presenting disease or the flock. In a disease investigation, samples

should be taken from both affected and normal animals. If the sampling is for monitoring purposes, the number of sheep to be sampled depends on the expected normal variance for the indicator being measured.

Table 3. Summary of significant trace mineral indicators in the sheep.

Indicator	Units ^a	Range			Applicability
		Deficient	Marginal	Adequate	
Cobalt plasma B ₁₂ ^b	nmol/L (µg/L)	0.18 (<0.25)	0.18–0.52 (0.25–0.7)	>0.52 (>0.7)	sheep > 10 weeks old
Copper plasma Cu ^c	µmol/L (mg/L)	<4.7 (<0.3)	4.7–9.4 (0.3–0.6)	>9.4 (>0.6)	Dietary Mo <8 mg/kg DM Lambs > 1 week old
plasma caeruloplasmin ^c	U/L	<5	5–40	>40	Lambs > 1 week old
red cell SOD ^{c,g}	U/g Hb	<200	200–400	>400	Extent of adequacy
liver Cu ^c	µmol/kg wet (mg/kg wet)	<80 (<5)	80–240 (5–15)	>240 (>15)	
Iodine plasma T ₄ ^g lamb/ewe ^{b,d}		<1	1–2	>2	Lambs <2 weeks old
milk I ^{b,d}	µmol/L (µg/L)	<0.6 (<80)	–	>0.6 (>80)	Dead lambs
thyroid/body weight ^{b,d}	g/kg	>0.4	–	<0.4	
Selenium whole blood Se ^b	µmol/L (µg/L)	<0.25 (<20)	0.25–0.76 (20–60)	>0.76 (>60)	
whole blood GSHpx ^{d,g}	U/g Hb	<30	30–50	>50	
plasma Se ^{b,e}	µmol/L (µg/L)	<0.15 (<12)	0.15–0.51 (12–40)	>0.51 (>40)	
Zinc plasma Zn ^b	µmol/L (mg/L)	<6.1 (<0.4)	6.1–9.2 (0.4–0.6)	>9.2 (>0.6)	

^a Alternative units with corresponding values are shown in parentheses.

^c Paynter (1986).

^e Paynter et al. (1993).

^g SOD = superoxide dismutase, T₄ = thyroxine,
GSHpx = glutathione peroxidase.

^b SCA (1990).

^d Hosking et al. (1986).

^f Whelan et al. (1994).

For blood selenium or glutathione peroxidase, the minimum is 5–7 animals (Paynter et al. 1993). A minimum of 10 animals is suggested for blood copper, vitamin B₁₂ (Grace 1983) and for urine sodium and magnesium (Caple and Halpin 1985). For monitoring purposes, samples should preferably be taken at the time of year when lowest nutrition of a mineral is expected.

Prior to sampling, sheep should not be subjected to extended periods of starvation. This has been shown to affect the concentrations of several mineral indicators including plasma vitamin B₁₂ (Millar et al. 1984) and plasma phosphate (SCA 1990). Age of the sheep being sampled also may have an effect. Plasma vitamin B₁₂ concentrations are normally low in preweaned lambs; weaned or adult sheep should be sampled to assess flock cobalt nutrition (Hosking et al. 1986). Similarly, plasma copper and caeruloplasmin are normally lower in neonatal lambs than in older lambs or ewes, but not red cell copper superoxide dismutase (Paynter 1986). In the sheep there are also significant differences between serum and plasma copper concentrations and caeruloplasmin activities, as caeruloplasmin is sequestered into the clot during clot formation (Paynter 1982).

Contamination associated with sampling can be a major problem where minerals are assayed directly, particularly at trace concentrations. Sample collection tubes and associated processes should be checked to ensure that contamination is negligible. Faecal contamination of urines may present major errors in urinary magnesium and sodium determinations (Caple and Halpin 1985). Post collection treatment and

storage of samples should also be considered. Haemolysis may interfere with colorimetric endpoint assays; the subsequent hydrolysis of red cell phosphate esters may increase free phosphate concentrations in plasma.

Analysis

Many of the major mineral assays suitable for assessment of mineral nutrition in sheep are now well defined analytically, with commercial kit methods available for most. In addition, commercial quality control materials, suitable for use in both internal and external quality assurance programs, are now available for most blood, urine and tissue mineral assays. Analytical methods and results should be verified by using these materials.

Where there is a high correlation between two indicators in assessing nutrition of a mineral, the indicator used is often determined on the basis of suitability of equipment and expertise available. The assay of glutathione peroxidase activity or total selenium in whole blood, and caeruloplasmin activity or total copper in plasma are examples. In each case, there is a high correlation between indicators (Caple et al. 1980; Paynter 1986). Each has advantages or disadvantages in relation to stability, contamination, equipment requirements etc., and indicators should be chosen which most suit these particular requirements.

Haemolysis in samples can be a major source of interference in some colorimetric endpoint assays. This potential interference should be removed by appropriate sample pretreatment or appropriate sample blank-

ing in the assay. Potential interference in vitamin B₁₂ assays by inactive analogues of vitamin B₁₂ is not a significant factor in sheep plasma. Only minor amounts of analogues appear to be absorbed and present in the plasma of sheep relative to that measured with cattle (Halpin et al. 1984).

In the determination of mineral concentrations in single urine samples, variations in mineral concentrations associated with changes in water intake may be greatly reduced by the inclusion of a measure of total urine solute concentration, using either osmolality or specific gravity in the determination (Caple and Halpin 1985).

Interpretation

Summaries of mineral indicators in the sheep and the ranges associated with deficient and marginal nutrition of these minerals are shown in Tables 2 and 3. It is emphasised that the values in these tables are approximate. Correlations and calibrations of mineral indicators with field treatment response trials are often compromised by the large seasonal variations apparent with mineral nutrition (Hosking et al. 1986). Few indicators, particularly in trace minerals, have been extensively calibrated against production responses under true steady-state equilibrium conditions.

Thus, Whelan et al. (1994), on the basis of their field responses in wool growth in sheep, suggest that critical values for whole blood and plasma selenium concentrations may be 0.76 and 0.50 $\mu\text{mol/L}$, respectively, somewhat higher than the values derived in previous reviews (e.g. Hosking et al. 1986;

SCA 1990). However, using the correlation established between glutathione peroxidase activity and selenium concentrations in whole blood of sheep (Caple et al. 1980), 0.76 $\mu\text{mol/L}$ is similar to that established independently using glutathione peroxidase as an indicator of selenium nutrition (Paynter et al. 1993). In the practical sense, the between- and within-seasonal variations in these indicators with sheep grazing at pasture overshadow these apparent differences in defining deficient or marginal nutrition.

For several minerals, the phase of deficiency, i.e. depletion or repletion, can be determined at a single sampling time point. This determination is possible where indicators for the same mineral have different turnover rates. For copper, red cell copper or red cell superoxide dismutase provide a longer term reflection of copper intake than plasma copper. Simultaneous measurement of both these indicators can be used to determine if an animal is in a depletion, repletion or steady-state phase of copper nutrition (Paynter 1986).

A similar approach is possible for determining selenium nutrition. Whole blood selenium concentrations and glutathione peroxidase activities reflect selenium intake from several months previously. In contrast, plasma selenium concentrations respond more rapidly to selenium intake (SCA 1990; Paynter et al. 1993).

Interpretation of results should always reflect the seasonal nature of mineral deficiencies. For mineral indicators well correlated with dietary mineral intake over a wide range, the expected mineral nutrition through successive seasons can often be

broadly predicted. This is not possible for indicators with a range of applications largely confined to nutrition below adequacy, and interpretation of these indicators should be confined to the period of sampling only.

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Trace Element Supplements for Sheep at Pasture

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Outline

Oral supplements

- Drenches
- Copper oxide particles
- Intraruminal pellets
- Large intraruminal boluses
- Intraruminal devices of variable geometry

Injectable supplements

Indirect methods of supplementation

- Fertilizers and foliar sprays
- Mineral licks and blocks
- Water medication

Detrimental effects and interactions

Concluding comments

Acknowledgments

References

Various methods are available for supplying trace elements to grazing sheep. A survey of stock owners in South Australia showed that injections were regarded as the most convenient means of administering trace elements to grazing animals (Judson and Taylor 1993). Soil or plant treatments, mineral licks or blocks, drenches and intraruminal pellets are also convenient means of providing trace elements.

Once the need for a trace element supplement has been established (Paynter, this publication), the choice of treatment will be influenced by a number of factors including the likely duration of the deficiency, additional management procedures required, the cost of the supplement and whether the pasture may also respond to the trace element. Table 1 gives a summary of the common supplements used to correct or prevent trace element deficiencies in sheep. Figure 1 shows some of the delivery systems available.

Oral Supplements

Drenches

Drenches are generally cheaper than other supplements but they are usually short-acting, particularly for elements such as zinc and manganese (Egan 1972) which the animal does not have the capacity to store in physiologically significant quantities. In practice, the inconvenience of frequent treatments can be reduced if the trace element can be given with other supplements such as an anthelmintic. However, the reliance on infrequent anthelmintic drenches as the sole vehicle may result in variable and transient responses to supple-

mentation and such responses also may be impaired by the anthelmintic (Suttle et al. 1988).

Cobalt drenches are short-acting although the animal can store vitamin B₁₂, the physiologically active form of cobalt, in the liver. The major limitation is the poor retention of the cobalt in the rumen to permit its incorporation into the vitamin by the rumen microorganisms. For young rapidly growing sheep, weekly drenches of cobalt were needed to maintain optimum growth rate but larger and less frequent doses of cobalt were not as effective (Stewart et al. 1955; Lee and Marston 1969).

Although the liver of sheep can readily store selenium and copper, the maximum single oral dose is limited by consideration of toxicity. Sodium selenate and sodium selenite are widely used as selenium supplements and both are equally effective in raising blood selenium concentrations when given orally or subcutaneously, although the latter method is more effective (Meads et al. 1980). Most of the selenium in a salt given by mouth is reduced in the rumen to elemental selenium which is unavailable. The suggested dose of selenium for sheep, based on blood analysis rather than growth rate, is 0.1 mg/kg liveweight and the duration of effect has varied from 0.5–3.0 months (Hosking et al. 1986; Tasker 1992). The variation in estimates of the effective life of the supplement is due in part to the severity of the deficiency and to the method of assessment, whether responses to the supplement are measured in plasma or whole blood and the concentration of selenium accepted as indicative of adequate status (Langlands et al. 1990b).

Figure 1. Delivery systems for trace elements. The scale shown is centimetres.

Intraruminal pellets



(a) Selenium pellets (10 g) containing 5% by weight elemental selenium in an iron matrix.



(b) Cobalt pellets (10 g) containing 30% by weight cobaltic oxide in an iron matrix.



(c) Salt encrusted cobalt pellets recovered from the rumen of sheep.



(d) Demonstration of the use of an applicator in administering pellets to sheep.



(e) Steel grub screws (10 g) are given orally with selenium or cobalt pellets to reduce salt deposition on the pellet.



(f) An applicator used to administer cobalt and selenium pellets, steel grub screws and copper oxide capsules to sheep.

Copper oxide particles



(g) Copper oxide particles (2.5 g) in a soluble capsule. The particles are composed of a mixture of cupric and cuprous oxide coating a copper core.

Figure 1. Cont'd

Large intraruminal boluses



- (h) Controlled-release glass boluses (35 g) for use in sheep. The bolus contains by weight 13.4% copper, 0.5% cobalt and 0.3% selenium in a soluble glass matrix.



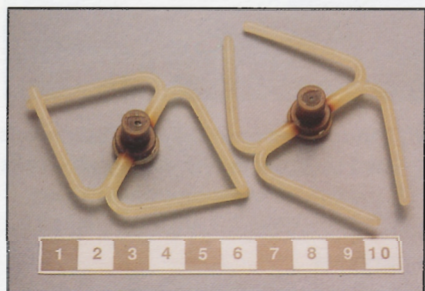
- (i) Sustained-release rumen bolus (80 g) for cattle. The bolus is composed of a compressed mixture of copper, cobalt, selenium, manganese, zinc, iodine and vitamins A, D and E. The round end of one bolus has been removed to show the 25 g high density weight.

Injectable supplements

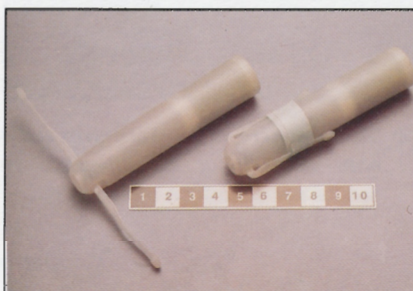


- (l) Barium selenate powder suspended in a viscous carrier (50 mg selenium/mL) suitable for subcutaneous injection.

Intraruminal devices of variable geometry



- (j) Iodine capsules for use in the rumen of sheep. Regurgitation of the capsule is prevented by arms on each side of capsule.



- (k) The Laby device developed for the sustained delivery of substances in the rumen of sheep. It consists of a plastic barrel which contains the substance and a spring loaded plunger, and folded wings that spring outward after dosing to prevent it leaving the rumen.



- (m) Solution of vitamin B₁₂ (2 mg/mL) administered subcutaneously to sheep.

Oral doses of copper, usually as copper sulfate, were evaluated in early studies as a means of preventing copper deficiency in sheep (Cunningham 1949; Lee 1950; Dunlop 1951). Copper drenching is not effective in increasing liver copper stores since the rapid absorption of significant quantities of copper is toxic (Howell, this publication). There is little evidence to suggest that drenching with other forms of soluble copper is significantly more effective than copper sulfate in correcting copper inadequacy in sheep.

Copper oxide particles

Dewey (1977) showed that a single oral dose of 10 g cupric oxide needles (laboratory reagent) when given to sheep increased liver copper concentrations. The needles which were retained in the alimentary tract for at least one month lodged in the walls of the abomasum, and in this acidic environment copper was readily released and subsequently stored in the liver. Copper oxide particles, a cheaper form of the supplement, are produced commercially from copper wire. The particles, which are about 3 mm long, 1 mm in diameter and have a specific gravity of about 6.4, are composed of a mixture of cupric and cuprous oxide coating a copper core (Judson et al. 1984). Oral doses of the particles (Langlands et al. 1986a) or the needles (Suttle 1987) are an effective means of providing copper to sheep. Single oral doses of the particles raise liver copper reserves of sheep for 6–12 months even when sheep are on diets of moderately high molybdenum and sulfur content (Langlands et al. 1986a; McFarlane

et al. 1991). The effectiveness of the supplement is due in part to the relative inertness of the copper oxide particles in the reticulo-rumen, the slow clearance of the dose from this segment of the gut and the subsequent retention of the dose in the abomasum.

The response in liver copper to copper oxide supplement is affected by a number of factors including the breed of sheep and diet (Suttle 1987), nematode infestation (Bang et al. 1990) and physical properties of the particles (Judson et al. 1984, 1991a). Merino sheep tolerate single doses of between 16 and 32 g of copper oxide particles without signs of copper toxicity, although British breeds of sheep appear to have a lower tolerance to copper oxide needles (Suttle 1987). The suggested doses of copper oxide particles for sheep (see Table 1) are based on studies with Merino sheep which show that these doses should not raise liver copper above the level permitted for human consumption (Langlands et al. 1986b). Although the recommended doses are higher for cupric oxide needles in the United Kingdom (Anon. 1984), these higher doses for the needles do not appear to be warranted for sheep in Australia (Judson et al. 1985).

Recent studies with sheep at pasture (G.J. Judson and P.J. Babidge, unpubl.) have shown for wire particles 1.5–12 mm long and 0.5–2 mm in diameter that the diameter and density of the particle were the most important physical characteristics which determined their retention in the alimentary tract: retention was unaffected by the length of the particle. Particles with a diameter of 0.5 mm and a specific gravity of 6.1 or less were poorly retained.

Table 1. Dose rate and duration of effect of supplements used for the prevention and correction of trace element deficiencies in sheep at pasture.

Supplement and suggested dose (dose/animal or kg liveweight)	Duration of effect ^a	Comments
<i>Copper</i>		
Copper sulfate drench		
9 mg Cu/lamb	2–3 days	Risk of toxicity if dose exceeded, not recommended.
10 mg Cu/kg	1 month	
Diethylamine cupro-oxyquinoline sulphonate		
0.5 mg Cu/kg subcutaneous injection	< 2 months	Rapidly mobilised from injection site, risk of toxicity if dose exceeded
Copper glycinate		
1 mg Cu/kg subcutaneous injection	> 6 months	Slight tissue reaction at injection site.
Copper calcium ethylenediaminetetraacetic acid		
1 mg Cu/kg subcutaneous injection	> 6 months	As for copper glycinate.
Copper methionate		
4 mg/kg subcutaneous injection	> 6 months	Slowly mobilised, low risk of toxicity, tissue reaction at injection site.
Copper oxide particles and needles		
1.25 g/lamb oral in soluble capsule	6 months	Little risk of toxicity.
2.5 g/sheep oral in soluble capsule	6–12 months	
<i>Selenium</i>		
Sodium selenate or selenite		
0.1 mg Se/kg drench	1–3 months	Risk of toxicity if dose exceeded, especially if given by injection: selenate is the preferred supplement.
0.1 mg Se/kg subcutaneous injection	2–4 months	
Barium selenate powder		
1.0 mg Se/kg subcutaneous injection	> 12 months	Low risk of toxicity, persists at injection site, slight tissue reaction.
Heavy selenium pellet, 5% w/w selenium		
1 x 10 g pellet orally	> 12 months	Suitable for weaned sheep, 1 or 2 grub screws given with pellet may be beneficial.
<i>Cobalt</i>		
Cobalt sulfate or chloride drench		
7 mg Co/sheep	1 week	Optimum growth rate with weekly drench, monthly drench prevents clinical signs of deficiency.
250 mg Co/sheep	1 month	

Continued on next page

Continued on next page.

Table 1. Cont'd.

Supplement and suggested dose (dose/animal or kg liveweight)	Duration of effect ^a	Comments
<i>Cobalt — cont'd</i>		
Vitamin B ₁₂ , 2 mg hydroxocobalamin/mL 2 mg/sheep subcutaneous injection	2 months	Ensure aqueous preparation injected well under the skin to reduce leakage to skin surface.
Heavy cobalt pellet, 30% w/w cobaltic oxide 1 × 10 g pellet orally	> 12 months	As for selenium pellet.
<i>Iodine</i>		
Potassium iodide or iodate drench 200 mg l/ewe	2–3 months	Drench given to ewes 2 and 1 month before lambing.
Iodised oil, 475 mg l/mL 475 mg l/sheep subcutaneous injection	2 years	Recommended for use in severe iodine deficient areas.
Iodine capsule, 1.4 g iodine 1 capsule orally/sheep	> 3 years	Commercial product to be released in 1996.
<i>Manganese</i>		
Manganese sulfate drench 6 mg Mn/kg	1 week	Approximate dose and duration of effect.
<i>Zinc</i>		
Zinc oxide or sulfate drench 3–10 mg Zn/kg	1 week	As for manganese.
<i>Multi-elements</i>		
Soluble glass bolus, 13.4% Cu, 0.5% Co, 0.15% Se		
1 × 16 g bolus orally to lambs	6 months	Approximate effective life of supplement.
1 × 33 g bolus orally to sheep	8 months	

^a The duration of effect will depend on the severity of the deficiency.

These studies suggest that increasing the specific gravity and mass of the copper oxide particles by oxidising the surface of 2 mm instead of 1 mm copper wire particles would improve the retention of the particle in the alimentary tract and, presumably, increase the effectiveness of this source of copper.

Oral doses of cupric oxide or cuprous oxide powder to sheep at pasture were found to be poorly retained in the alimentary tract and resulted in a transient increase in liver copper reserves (Judson et al. 1989a; Langlands et al. 1989). In these studies the grain size of the powder used was usually less than 80 microns. In other studies powder of larger grain size (250–350 microns) was retained in the alimentary tract for up to 13 days in penned sheep and markedly increased the liver copper reserves (Cavanagh and Judson 1994). It was suggested that the use of the larger grains of copper oxide powder may be a cheap supplement for preventing seasonal copper deficiencies in sheep.

Intraruminal pellets

The continuous supply of cobalt in the rumen of sheep provided by a heavy pellet was devised by Dewey et al. (1958). This method was also effective in providing a continuous supply of selenium to sheep (Kuchel and Buckley 1969). Early prototypes of the selenium pellet (10 g pellet containing 5% by weight elemental selenium in an iron matrix) lasted for at least 4 years (Hunter et al. 1981). However, studies with the commercially prepared selenium pellets showed that the effective life of the

pellet was variable, from less than 1 year to almost 8 years (Wilkins and Hamilton 1980; Hunter et al. 1981; Langlands et al. 1990a).

The amount of selenium in the pellet is probably the major factor determining the effectiveness of the pellet—increasing the selenium content of the pellet increases its longevity (Donald et al. 1993; Langlands et al. 1994). The effective life of a pellet containing 15% by weight elemental selenium was about 4 years whereas the life of the commercial pellet, containing 5% selenium, was about 2 years (Langlands et al. 1990a). Two pellets containing lower selenium concentrations were not as effective as a single pellet containing double the quantity of selenium (Langlands et al. 1994). The grain size of elemental selenium also influences the effectiveness of the pellet (Hudson et al. 1981; Peter et al. 1981a; Donald et al. 1993); pellets containing coarse grains of elemental selenium were more effective in releasing biologically active forms of selenium than pellets containing fine grains (<10 µm). Hudson et al. (1981) suggested that the pellet operated as a voltaic cell, the elemental selenium in the presence of iron being reduced to iron selenide with the release of hydrogen selenide (elemental selenium is biologically inert) (Langlands et al. 1990b).

Initial studies showed that the effective life of the cobalt pellet (10 g pellet containing 60% by weight cobaltic oxide in an iron matrix) was greater than 5 years (Dewey et al. 1969). In the late 1970s the cobaltic oxide content of the commercially available pellet was reduced from 60 to 30% due to the increasing cost of the cobaltic oxide, and this also increased retention by raising

the specific gravity of the pellet. The effective life of the pellet containing the lower cobalt content was found to vary from 1 to 3 years: the source of the cobaltic oxide used in the pellet appears to be a major determinant of the effective life (Judson et al. 1995). The effective life of this pellet in preventing phalaris staggers, a disorder in sheep responsive to cobalt (Lee et al. 1957), is not known.

Problems of regurgitation and deposition of calcium phosphate salts have been encountered with heavy pellets (Millar and Andrews 1964). It has been suggested that pellets are more likely to become encrusted with calcium salts when ruminal pH rises to between 7 and 8 (Owen et al. 1960) or after extended periods of sheep grazing lush pasture growth (Langlands et al. 1990a). Salt deposition can be reduced by the introduction of a steel grub screw with the pellet (Lee and Smith 1976) and in instances of severe salt deposition the administration of a second grub screw may be beneficial (Judson et al. 1995). Dewey et al. (1969) reported that the abrasive action of the grinder also increased the release of cobalt from pellets given to penned sheep. The heavy pellet appears to be well retained by Merino sheep (Lee and Smith 1976) but this high retention has not always occurred with other breeds of sheep (Millar and Andrews 1964; Poole and Connolly 1967).

Other high density pellets investigated include a copper pellet (Bray 1994) and a zinc pellet (Masters and Moir 1980). The zinc pellet, comprising 5 g zinc shot and 5 g iron filings, released about 10 mg zinc daily for about 7 weeks. The daily release rate for zinc is more than 10 times that required for

cobalt or selenium, implying that the effective life for the zinc pellet will be shorter than the cobalt and selenium pellets. The principle of the copper pellet is based on contact electrolysis, a process which occurs when one metal is corroded through aqueous contact with another metal of higher electromotive force. Bray (1994) has suggested this technique may be suitable for long-term supply of copper and perhaps zinc.

Large intraruminal boluses

The density and overall size of a pellet are probably the most important properties expected to influence retention in the rumen (Dewey et al. 1958). The heavy intraruminal pellets have a specific gravity of about 6. For boluses of lower density, retention is aided by increasing the size of the bolus; increasing the size is also important if a greater release rate of the active constituent is required, as is the case with the slow-release magnesium boluses developed for use in sheep and cattle (Davey 1968). These boluses consist of 86% by weight magnesium, 12% aluminium and 2% copper. To improve retention in the reticulo-rumen, the boluses are weighted with iron shot dispersed throughout the matrix of the bolus. The sheep bolus weighs about 35 g and is designed to erode in the rumen to release the magnesium; the effective life of the bolus appears to be less than 5 weeks (House and Mayland 1976).

Boluses of glass have been developed to carry trace elements. The solubility of the glass can be controlled by altering the major constituents—calcium, phosphorus and sodium ions. Telfer et al. (1984) reported

that oral dosing of a soluble glass bolus was an effective means of providing an approximately constant supply of additional trace elements to the grazing animal for many months. The glass bolus was retained in the reticulo-rumen and dissolved at a controlled rate in the ruminal fluid: the trace elements incorporated in the glass matrix were released as the glass dissolved. A bolus, containing by weight 13.4% copper, 0.5% cobalt and 0.3% selenium, was developed for use in sheep and cattle in the United Kingdom (Knott et al. 1985). Two sizes of bolus were developed for sheep—17 g for lambs and 35 g for weaned sheep. The boluses were effective for up to 6 months in lambs and 12 months in older sheep (Care et al. 1985; Carlos et al. 1985).

Because the surface of the bolus dissolves, coating of the pellet was not encountered. The glass boluses (density of about 3) were larger than the intraruminal pellets (density about 6) to ensure retention in the reticulo-rumen. Problems encountered with glass boluses included surface deterioration during packaging and fragmentation of the bolus in the rumen (Judson et al. 1988; Millar et al. 1988). The bolus was withdrawn from the British market in 1986 but has been replaced by a reformulated bolus (S.B. Telfer, pers. comm.) prepared by pressing ground glass containing copper and cobalt with sodium selenate. The bolus is heated to fuse the glass particles together with the sodium selenate bound into the spaces between the glass, and to prevent surface deterioration prior to use the bolus is sealed in foil. The trace element content of the reformulated bolus is unaltered except for selenium which

has been reduced from 0.3 to 0.15%. The lamb bolus weighs about 16 g and the sheep bolus 33 g and, according to commercial claims, the effective life of each bolus is 6 and 8 months, respectively.

Lawson et al. (1989) developed a sustained-release rumen bolus for cattle. It is cylindrical with one rounded end and is composed of a compressed mixture of copper, cobalt, selenium, manganese, zinc and iodine, and vitamins A, D and E. The surface, apart from the flat end, is coated with a polymer resin. Loss of active material occurs from the exposed flat end of the bolus in the reticulo-rumen. The coating progressively breaks off to maintain a constant exposed surface area. To improve retention of the bolus a 25 g high-density weight was incorporated into the round end of the bolus (Parkins et al. 1994b). The recommended dose is two 80 g boluses for cattle over 150 kg liveweight. The reported release rate of most of the elements (Hemingway et al. 1993) is similar to the minimum dietary allowances for young cattle; the release rate of copper of 138 mg/day appears excessive for young cattle when on diets of high available copper (SCA 1990). The effective life of the supplement is 8 months, although evidence for this has only been substantiated for selenium (Allan et al. 1993; Parkins et al. 1994b). An experimental prototype has been developed for use in sheep (Parkins et al. 1994a).

Intraruminal devices of variable geometry

A range of intraruminal devices (Laby 1980; Graham 1989) has been developed

for the controlled delivery of substances in the rumen of sheep and cattle. The release of the active substance is by diffusion, dissolution or by a galvanic effect. The magnesium bolus is an example of the last process. It consists of two half cylinders of magnesium alloy hinged together with rubber. Following administration the half cylinders open out to prevent regurgitation, the magnesium being released by electrolytic action over a period of about 90 days. The magnesium bolus is available commercially for the prevention of magnesium deficiency in cattle.

The iodine capsule employs a diffusion process to supply iodine to sheep (Mason and Laby 1978; Ellis and Coverdale 1982). Solid iodine, which is contained in a polyethylene envelope, is in equilibrium with iodine vapour which diffuses through the plastic at a constant rate. The plastic offers enough resistance to the diffusion of the vapour to limit its escape to an amount sufficient to meet the iodine needs of sheep for up to 6 years (P. Costigan, pers. comm.); the maximum duration of effectiveness of a single injection of iodised poppy seed oil is about 2 years (Statham and Koen 1982). Regurgitation of the capsule is prevented by arms on each side of the capsule which spring out once in the rumen. This device is due for release commercially in the near future.

The osmotic pump also uses a diffusion process. The pump has been shown to release physiologically significant quantities of selenium in cattle for at least 30 weeks (Campbell et al. 1990). The pump consists of a flexible reservoir that contains one hole to allow the sodium selenite matrix to

escape. The reservoir is surrounded by a saturated osmotic agent that is contained within a rigid semi-permeable outer casing. As ruminal fluid enters the rigid outer layer, the expanding osmotic agent squeezes the flexible inner reservoir, delivering the selenium at a constant rate.

The Laby device employs a dissolution process. It consists of a plastic barrel housing tablets of the active constituent and a spring loaded plunger. The device is fitted with folded wings that spring outward after oral dosing to prevent it from leaving the reticulo-rumen. The barrel is open at one end to expose the surface of the tablet to the ruminal fluid. The surface in contact with the fluid forms a gel and the expulsion rate of the gel from the barrel is equal to the rate of entry of the ruminal fluid to give a continuous rate of delivery of the active constituent. The composition of the tablets can be varied to incorporate inert material which permits a break in the delivery of the active substance. The Laby capsule has been developed as a faecal marker capsule using chromic oxide for estimating feed intake of grazing ruminants (Ellis et al. 1982) and for the sustained delivery of an anthelmintic (Anderson et al. 1980). Small amounts of sodium selenate incorporated in the anthelmintic matrix have been successful in preventing selenium inadequacy in lambs for at least 26 weeks (Grace et al. 1994). The Laby device has been used experimentally in sheep for the controlled delivery of a number of other trace elements including copper, cobalt, manganese and zinc at rates sufficient to meet their requirements for 12 weeks (Panggabean et al. 1984).

Injectable Supplements

Supplements vary from those that are rapidly mobilised from the injection site to replenish physiological stores to those that are constrained by a variety of chemical and physical methods at the injection site to provide a slow or sustained-release of the element. Considerable attention has been given to the development of copper preparations which can be given in non-toxic amounts to provide long-term protection against deficiency (Allen and Moore 1983; Allen 1987). A number of the organic complexes of copper have been evaluated and are available for use in sheep: they include copper di-ethylamine oxyquinoline sulphate (CuDOS), copper glycinate, copper methionate and copper-calcium ethylene diamine tetraacetic acid (CuCaEDTA).

These complexes differ in the rate the copper is translocated from the injection site to the liver, and it appears that this rate of mobilisation is directly related to the toxicity of the complex and its efficacy in correcting copper deficiency and inversely related to the severity of tissue reaction at the injection site (Camargo et al. 1962; Suttle 1981). Copper methionate is the safest complex, CuDOS the most toxic and the other two complexes are intermediate in their toxicity. Sheep appear to tolerate doses up to about 13 mg copper/kg liveweight when given as copper methionate (Ishmael and Howell 1977) but toxicity has been observed with doses of 0.7 mg copper/kg when given as CuDOS (Mason et al. 1984). The suggested therapeutic doses of these complexes for sheep differ markedly (see Table 1).

Methods used to provide a slow and sustained release of selenium subcutaneously have included the administration of sodium selenate or selenite in a viscous carrier (Hidiroglou et al. 1971) or in a soluble glass pellet (Allen et al. 1981) and of barium selenate in a viscous carrier (Kuttler et al. 1961). Barium selenate powder suspended in a viscous carrier has been developed commercially for use in livestock (Cawley and McPhee 1984). Single subcutaneous doses of barium selenate provide a slow, sustained release of selenium in sheep for 2-4 years (Overnes et al. 1985; Judson et al. 1991b).

The recommended dose of selenium of 1 mg/kg liveweight is 10 times the dose recommended for sodium selenate or selenite, the more soluble forms of selenium. Selenium when given as a single subcutaneous dose of 0.5 mg/kg liveweight as sodium selenate to sheep can result in selenium residues in tissues above those recommended for human consumption, but such an accumulation of selenium residues is unlikely to occur with barium selenate injections when given at the recommended dose, except at the injection site (Archer and Judson 1994a,b).

Significant quantities of vitamin B₁₂ are sold in southern Australia for subcutaneous administration to livestock for the correction and prevention of cobalt deficiency. The commercial product is a mixture of hydroxocobalamin (80%) and cyanocobalamin (20%). A major limitation with this form of therapy is the short duration of effect—up to 2 months in young sheep when given at the suggested dose of 1 mg (Hogan et al. 1973; Hannam et al. 1980). The

vitamin is relatively non-toxic but larger doses are unlikely to increase the effective life.

When given subcutaneously as an aqueous solution of either 2 mg hydroxocobalamin or 2 mg cyanocobalamin to young sheep, the vitamin was rapidly mobilised and a significant proportion of the dose excreted in the urine within 6 hours (see Figure 2; Judson et al. unpubl.). The hydroxocobalamin, which is less readily excreted and more effective in raising liver vitamin B₁₂ reserves than cyanocobalamin, is the preferred analogue (see Figure 3). A depot vitamin B₁₂ was developed for use in

humans (Bastrup-Madsen et al. 1983). It is a cyanocobalamin-tannin complex suspended in a sesame oil aluminium-monostearate gel. Studies with this product have shown that it was no more effective than the aqueous preparation of vitamin B₁₂ in raising the vitamin B₁₂ status of lambs at pasture (Judson et al. 1989b) or in penned sheep fed a cobalt deficient diet (Figure 3; Judson et al. unpubl.).

Indirect Methods of Supplementation

Fertilizers and foliar sprays

The minimum concentrations (mg/kg dry matter) of trace elements in legume-based pastures for optimum growth of pasture plots are about 0.04 for cobalt, 5 for copper, 0 for iodine, 15–20 for manganese, 0 for selenium and 20–25 for zinc (Reuter and Robinson 1987). The respective minimum dietary allowances for sheep are 0.11, 5, 0.5, 15–20, 0.05 and 20–30 (SCA 1990). From this comparison it appears that if sheep are at risk of copper and zinc deficiency then pastures may also respond to the trace element supplementation. In such instances the use of trace element fertilizers may be the most economical means of meeting the needs of both plant and animal.

The persistence of trace elements applied to soils can vary markedly and depend on interactions between soil conditions, the trace element applied and placement of the fertilizer in the soil (Hannam and Reuter 1987). The residual value of applied zinc can vary from 2 to 10 years on calcareous

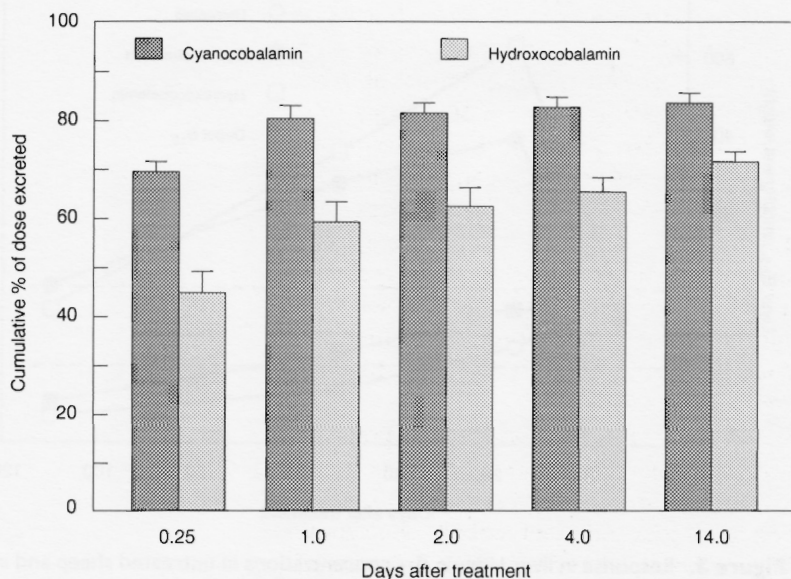


Figure 2. Mean values (with their standard deviations) for the cumulative excretion of ⁵⁷Co in urine, expressed as a percentage of the dose given, from sheep given subcutaneous injections of 2 mg cyanocobalamin-⁵⁷Co (three sheep) or 2 mg hydroxocobalamin-⁵⁷Co (four sheep).

soils and greater than 10 years on acid soils (R.J. Hannam, pers. comm.). The residual value of copper applied to soils can be considerable. For example, a copper dressing of 2 kg/ha to mineral soils provided adequate copper for pasture and sheep production for at least 23 years (Hannam et al. 1982). However, responses in copper concentrations in pasture to copper topdressing appear to plateau at concentrations of about 8–10 mg/kg dry matter (McFarlane et al. 1990). Therefore, where copper deficiency in sheep is induced by excess levels of dietary molybdenum, sulfur or iron, then direct supplementation of the animal may be more effective than copper topdressing. Plant analysis for copper and the interfering agents is valuable in deciding the most appropriate means of treatment.

In areas where sheep are at risk to cobalt or manganese deficiency the topdressing of these elements to soils, as a means of providing these elements to the animal, is not generally encouraged because the residual effect of applied cobalt or manganese is usually short and variable due to the ability of soils to adsorb these elements (Adams et al. 1969; Reuter et al. 1988). Cobalt misting of the pasture is an alternative means of providing cobalt to the animal. The cobalt is applied in strips across the grazed site but regular applications are required during inclement weather. However, when the cobalt is applied with a wetting agent at a rate of about 10 g cobalt/ha to leafy pasture it can prevent phalaris staggers and cobalt deficiency in sheep for up to 3 months (J.D. McFarlane, pers. comm.). Foliar sprays containing one or more trace elements applied to herbage before cutting for hay or

silage can raise the trace element concentrations in feed supplements for livestock.

Selenium topdressing of pasture was first used in New Zealand in the early 1980s (Watkinson 1994); the maximum permitted was 10 g/ha of selenium as sodium selenate. Although there was some concern about the risk of selenium toxicity to stock, Watkinson reported that there had been no cases of toxicity after 10 years of topdressing pasture with sodium selenate granules mixed with fertilizer. Studies by Whelan and Barrow (1994) have shown that in a mediterranean environment a single application

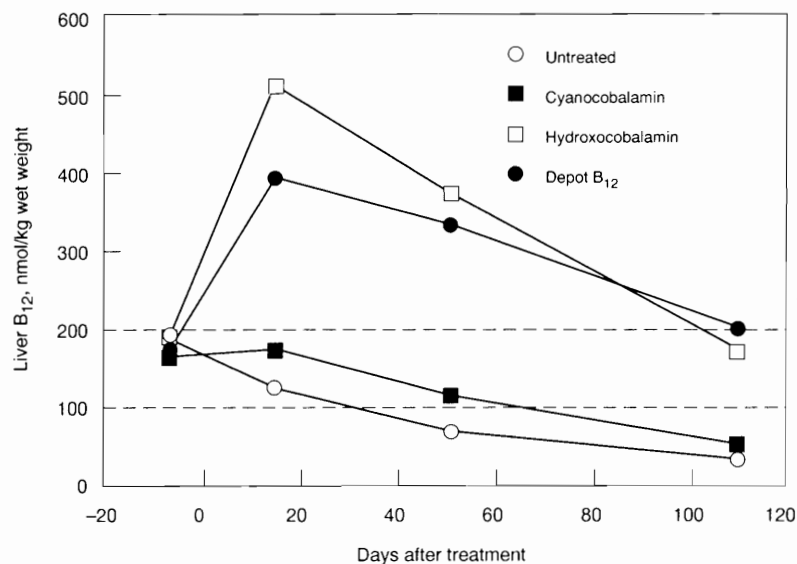


Figure 3. Response in liver vitamin B₁₂ concentrations in untreated sheep and in sheep given 2 mg vitamin B₁₂ subcutaneously as cyanocobalamin, hydroxocobalamin or depot B₁₂. Mean values are for four sheep, except for the cyanocobalamin treatment (three sheep). The least significant difference for all comparisons ($P = 0.05$) was 120. Liver vitamin B₁₂ concentrations (nmol/kg wet weight) > 200 adequate, 100–200 marginal, and < 100 deficient.

of the slow-release fertilizer, barium selenate, at 10 g selenium/ha maintained adequate blood selenium concentrations in sheep for 4 years, whereas a single application of sodium selenate fertilizer at the same rate was effective for 15 months. Selenium fertilizer containing a mix of the slow-release form of selenium as barium selenate and quick-release selenium as sodium selenate has been developed. To reduce costs only part of the grazed area needs to be top-dressed, since sheep do not have to graze selenium topdressed pasture continuously in order to maintain an adequate selenium status (Millar and Meads 1987).

Mineral licks and blocks

Free-access minerals are often convenient and simple for providing one or more minerals to sheep (McDowell, this publication). The ideal supplement is one that is consumed by all livestock and will provide only those elements, in non-toxic amounts, which are insufficient in pasture to meet the needs of the animal. As it is often impractical to determine those minerals which are limiting productivity, mineral licks and blocks have been produced to meet the perceived additional needs of selected minerals for livestock under a range of environmental and management conditions.

White et al. (1992) have developed a loose mineral mix containing all the known essential minerals for sheep on dry pasture in a mediterranean environment. Their initial findings indicate that the supplement is readily consumed and has increased productivity. In such instances the element(s)

limiting production should be identified and supplied instead of the whole suite of elements. In areas where the trace element deficiency is associated with deficiencies of one or more major elements, the use of a complete mineral mix may be the most appropriate form of supplementation (McDowell, this publication). It is unrealistic to expect that the mix will meet the additional trace element needs of sheep on dry pasture in a variety of regions. The limitation of using a single mix for different regions has been shown for copper. The supplement failed to correct copper inadequacy of sheep on dry pasture of moderately high molybdenum levels (Good et al. 1992).

Water medication

Water medication may prove an effective means of providing trace elements to all sheep during dry seasons in areas where all water supplies are controlled. In the more extensive grazing areas of northern Australia water medication is a recommended method of providing phosphorus to cattle during the dry seasons (McCosker and Winks 1994). Water medication for providing cobalt or copper to cattle has also been successful when using a dispenser to meter the addition of the trace element to the water trough (MacPherson 1983). The use of slow-release tablets of copper or cobalt, designed to be placed in water troughs, was found to be ineffective as a means of providing these elements to cattle (MacPherson 1983)—most of the copper released from the tablet was retained in the sludge at the bottom of the trough. A slow-release

tablet of selenium, however, was shown to markedly increase the selenium status of cattle, but of concern was the risk of selenium toxicity if the water selenium concentration cannot be maintained at the intended level (MacPherson 1983). The addition of trace element supplements to water of high salt content, particularly calcium, may result in precipitation of the supplement.

Detrimental Effects and Interactions

Although supplements are given to provide physiologically significant quantities of the element in non-toxic amounts to the animal there have been instances of adverse effects from supplementation. Depressed live-weight gains were recorded in lambs given 2 g instead of 1 g copper oxide particles (Langlands et al. 1986b). Depressed live-weight and fleece weight were observed in young sheep at one of three sites given an injection of 12 mg copper as CuDOS (Hannam et al. 1982) and reduced tensile strength of wool occurred in sheep given injections of 50 mg copper as CuCaEDTA (Masters and Peter 1990). Depressed growth rates due to parenteral injections of copper have also been observed in young beef cattle of marginal copper status (Black 1982). Clinical signs of copper toxicity were not observed in these studies, although the possibility of transient sub-clinical copper toxicity cannot be discounted.

Increased weight loss of ewes associated with lambing was observed when ewes were given parenteral injections of 3 mg selenium every 2–3 months (Quarterman et al.

1966). It was suggested that the selenium supplement was adversely affecting the pregnant ewe during periods of cold stress. Increased somatic cell counts in milk from dairy cows of marginal selenium status have been reported when cows were given a subcutaneous injection of barium selenate (Whelan et al. 1992).

Interaction between trace element supplements can reduce the efficacy of the supplement or result in adverse effects on animal health. The efficacy of copper oxide particles in raising liver copper levels in cattle was shown to be reduced when the particles were administered with an intra-ruminal selenium pellet (Koh and Judson 1987). Intra-muscular injections of selenium have been reported to enhance the toxic effects of oral copper sulfate supplements in sheep (Hussein et al. 1985).

Chronic selenium poisoning was observed in dairy cows, previously given two intra-ruminal selenium pellets (30 g pellets containing 10% by weight elemental selenium in an iron matrix), when given two high density magnesium pellets (Gill 1993). It was suggested that an electrochemical reaction between the two supplements may have caused a sudden and massive release of selenium from the pellets—cattle can tolerate single oral doses of multiple selenium pellets without signs of selenium toxicity (Judson and McFarlane 1984; Wilson et al. 1991). An increased release of available selenium from high density pellets has been recorded when the selenium pellet was given to sheep with another heavy pellet containing zinc, zinc/iron, zinc/nickel or iron (Peter et al. 1981b). In practice, heavy pellets of selenium and cobalt are often

given together, yet this combination has little effect on the release of available selenium.

Concluding Comments

Our experience in South Australia has shown that even the most vigilant stock owners can become complacent about ensuring livestock productivity is not limited by a mineral deficiency. Part of this complacency can be attributed to marginal deficiencies, which are not readily identified, and to the seasonal and year-to-year variation in the appearance of a deficiency. This variation was shown by Lee (1951) in an area where sheep were at risk to cobalt deficiency. Sheep were unthrifty as a result of cobalt deficiency in only 8 of the 13 years of the study, and the severity of the deficiency varied from a marginal depression in growth rate of the lambs to 100% mortality of the lambs. In many instances a marginal trace element deficiency may go undetected yet it may result in up to a 10% depression in growth rate and fleece weight (Judson et al. 1987).

It is important to encourage stock owners to adopt recommended methods for the prevention of trace element and mineral disorders by identifying areas where stock are at risk, by recognising factors contributing to the disorders, by demonstrating the cost-benefits of supplementation and making available appropriate diagnostic tests for the detection of the disorders.

The ideal supplement is one that fits easily into management procedures while providing enough of the element for the

period required for the least cost and without risk of undesirable side effects or residues in the animal. There is an ongoing need to monitor the efficacy of the supplement in preventing trace element disorders. For example, changes in the composition or source of constituents used in high density intraruminal pellets can significantly reduce the effective life of these supplements. Supplements for parenteral use, particularly those such as barium selenate which are restrained at the injection site (Archer and Judson 1994b), should be restricted to subcutaneous administration and to sites of the animal not sold for human consumption.

Multi-element boluses have considerable appeal to stock owners as a simple means of preventing a number of trace element deficiencies. They have the potential to deliver a number of trace elements, including zinc and manganese, at rates to meet the needs of sheep for many months. Although the use of such supplements containing selenium and copper in areas where sheep are adequate in these elements may pose little risk of toxicity there is the risk that the copper and selenium concentrations in the liver of these animals may be above the maximum permitted level for human consumption (Judson et al. 1988; Langlands et al. 1990a; Masters and Peter 1990).

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Free-choice Mineral Supplements for Grazing Sheep in Developing Countries

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Outline

Factors affecting consumption of mineral mixtures

- Soil fertility and forage type
- Season
- Energy and protein supplements
- Individual requirements
- Salt content of drinking water
- Palatability
- Availability of fresh minerals
- Physical form of minerals

Biological availability of mineral sources

Selecting a free-choice mineral supplement

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Evaluation

Manufacture of mineral mixes

References

For direct supplementation to sheep, the obvious advantage of the various methods of single or limited element supplementation (e.g., pellets or injections) is the almost certain assurance that each animal is receiving the needed element(s), compared to the voluntary consumption of a free-choice (free access) method where intakes of individual animals cannot be well controlled. The major disadvantage of single or limited element administration to grazing livestock in most parts of the world is that there are other minerals (e.g., phosphorus and sodium) that cannot be administered in this way (McDowell 1992). In most sheep grazing regions of the world there also will be deficiencies of sodium and phosphorus which must be corrected, and then use of a mixture of salt, calcium, phosphorus and trace elements offered free-choice is the probable system of choice. Cost is an additional disadvantage of the pellets or injections, and elements provided in a free-choice mixture would generally be cheaper.

In most countries where sheep are raised farmers attempt to meet mineral requirements of their flocks through use of free-choice dietary minerals. Sheep are particularly fond of salt and consume considerably more per kg of liveweight than do cattle (sheep consume about 5 times more salt per kg liveweight than cattle.) This method of providing minerals would seem most appropriate in China. Masters et al. (1995) noted that many farms in northern China currently use a salt supplement and research indicated that all sheep in northern China should be given salt regularly. Salt provides the vehicle for provision of other

minerals, and would have the advantage of not requiring change in the traditional methods of sheep management. An additional advantage for a free-choice salt-based mixture is that the salt can serve as a vehicle for providing anthelmintics (e.g. phenothiazine) or growth promoters.

There are definite disadvantages to the free-choice mineral supplementation method, the major concern being lack of uniform consumption by animals. Observations indicate that some animals may consume double their needs while others may eat practically none of a supplement. Using the free-choice method does provide some minerals that are not required, which may result in higher supplementation costs. Also, sometimes an injectable mineral may be preferred to dietary sources to counteract antagonism. In areas of severe molybdenum toxicity, injections of copper compounds are often the preferred method of administration, since the primary site for copper and molybdenum interaction is the gut.

Intakes of free-choice mineral mixtures by grazing ruminants are highly variable and not related to mineral requirements (McDowell 1985). Most mammals exhibit little nutritional wisdom and will select a palatable but poor quality diet in preference to an unpalatable, nutritious diet, even to the point of death. Some researchers have noted daily consumption of salt-based supplements in sheep to be as low as 2-3 g/animal (Money et al. 1977; Ullrey et al. 1978) while White et al. (1992) in Australia reported average consumption of 29 g for a salt-gypsum mixture. A number of researchers report daily mineral consumption

for sheep of between 10 and 15 g. Frequently, a proportion of animals in a flock make little or no use of free-choice mixture; hence, intake of the supplement varies greatly between animals. Ullrey et al. (1978) reported that salt consumption varied widely in ewes (2–14 g/day) and lambs (1.5–19 g/day). The between-animal variation from the Australian study was 22–35 g/day, with variability in intake greatest at shorter time periods of less than 6 months (White et al. 1992). After 6 months the between-sheep variability in intake was estimated to be less than two-fold.

Factors Affecting Consumption of Mineral Mixtures

Factors that affect the consumption of mineral mixtures have been listed by Cunha et al. (1964), Cunha (1987) and McDowell (1985, 1992).

Soil fertility and forage type

Usually, the higher the level of soil fertility, the lower the consumption of minerals. Sheep on low-quality or overgrazed pastures consume more mineral supplements.

Season

Mineral intake is often greatest during the winter or dry season when forages stop growing, lose green colour and become high in fibre and lignin and low in digestibility and mineral availability.

Energy and protein supplements

The kind and level of protein–energy supplementation will influence mineral supplement intake. Protein and energy supple-

ments that also provide minerals will decrease both the need and desire for free-choice minerals.

Individual requirements

Growth rate, percentage of lamb crop, quantity and quality of wool and milk production influence mineral needs. Added requirements of gestation and lactation increase mineral needs and, thereby, consumption. The higher the level of productivity, the more important an adequate level of mineral intake.

Salt content of drinking water

Naturally high salt concentration of drinking water decreases mineral supplement intake. Livestock have a natural craving for salt. However, if that desire is fulfilled from drinking water high in salt, grazing livestock will consume less or none at all of a free-choice mineral mixture based on salt. Where naturally occurring salt content of water is high, mineral supplements cannot be based on salt and should be reformulated with other palatability stimulants such as cottonseed meal and molasses.

Palatability

Ruminants have no particular desire for the majority of minerals, with the exception of common salt. Common salt, because of its palatability, is a valuable 'carrier' of other minerals. If mixtures contain 300–400g/kg salt or more, they are generally consumed on a free-choice basis in sufficient quantities to supply supplementary needs of other minerals. When bonemeal and salt were

mixed together, bonemeal consumption increased eight-fold (Dew et al. 1954). Many reports testify to the beneficial effects of bonemeal in free-choice supplements. Improperly processed bonemeals can emit an unpleasant odour, which reduces consumption. Also, there is a danger of botulism and other disease conditions that can be transmitted from inadequately processed bonemeal.

Palatability and appetite stimulators such as cottonseed meal, dried molasses, dried yeast culture and fat help achieve more uniform consumption. Some of these products not only give the supplement a dust-free, moist, and free-flowing character, but also provide energy and protein. Ingredients that increase palatability must be used in moderation, or they will cause overconsumption.

Availability of fresh minerals

Previous diet or access to mineral supplements is a factor affecting short-term consumption of minerals. When sheep have been deprived of salt for any length of time, and then get access to it, they may overindulge and suffer salt poisoning (treatment—access to water). Under these conditions, they will consume 2–20 times the normal daily quantities of minerals until appetite is satisfied.

Mineral feeders for sheep should be constructed so that 1) they cannot be easily pushed over, 2) sheep cannot get into them and 3) they have a roof to protect the minerals from rain. Rainproof mineral feeders help increase mineral intake by preventing caking, moulding, and blowing away during windy weather. The choice of palatability or

appetite stimulators is important when considering the keeping value of a supplement. Cornmeal is a good appetite stimulator when included in a mineral mixture but is more easily fermentable than a proteinaceous product such as cottonseed meal. The use of 200–400 g/kg salt prevents moulding and blowing.

Mineral feeders will be used more frequently by sheep if they are located near water tanks, shaded loafing areas and areas of best grazing. Mineral feeders should be constructed low enough so that lambs can also consume minerals. They should be located on dry ground accessible for checking and servicing throughout the year. Mineral boxes should be filled often and not allowed to get empty. Keeping the mineral supply fresh increases its consumption.

In some regions with vast grazing areas, there are great difficulties in locating feeders so that animals have constant access to minerals. This is a particular problem where animals graze over large areas with no central location for drinking water. As with the water source, the location of the mineral supplement will affect the movement of the sheep in the grazing area and so the siting of both of these can be used in managing the grazing.

Physical form of minerals

Intakes of loose salt-based supplements by sheep are substantially higher than where the same material is offered in a compressed form (mineral block). For sheep consuming both loose and block forms of salt, intake was 7.2 times greater for the loose form and 2.3 times greater in

a second experiment compared to block forms (Rocks et al. 1982). Likewise, individual variation in intake from week to week by wethers was considerably less among groups offered loose vs block salt mixtures; a coefficient of variation of 58% for a loose salt mix and 115% for a salt block. Apart from a greater uniformity in intake, the use of loose instead of compressed salt has a substantial advantage in cost per unit.

Mineral blocks can be developed on the basis of degree of hardness to take into consideration rainfall, humidity and other environmental conditions. Rain will dissolve too soft a block causing mineral losses, and yet livestock experience difficulty consuming enough of a hard block to fulfil mineral requirements. If the animals remain only a limited time in the vicinity of mineral blocks, then excessive block hardness will result in reduced mineral consumption. Providing a supplement in block form has the advantages of convenience and much greater resistance to rain and dew. Also, the control of excessive intakes by the use of blocks may be a significant advantage.

Biological Availability of Mineral Sources

The bioavailability and percentage of mineral elements in some inorganic sources commonly used in mineral supplements are shown in Table 1. These variations in bioavailability of sources must be taken into consideration when evaluating or formulating a mineral supplement. Calculations are required to account for both the amount of element in mineral salts as well as bioavailability.

Excellent reviews on the significance of chelates and complexes for the feed industry have been prepared (Nelson 1988; Spears et al. 1991). Spears et al. (1991) concluded that the use of certain organic trace mineral complexes or chelates in ruminant diets has increased performance (growth and milk production), carcass quality and immune responses, and decreased somatic cell counts in milk compared with animals fed inorganic forms of the mineral. Trace minerals sequestered as amino acid or polysaccharide complexes have the highest biological availability and also have a higher stability and solubility. These mineral forms also have a lack of interaction with vitamins and other ions and are effective at low levels. In cases where there is high dietary molybdenum, copper in chelated form would have an advantage over an inorganic form as it may escape the complexing that occurs in the digestive system between copper, molybdenum and sulfur (Nelson 1988).

Some studies have shown no benefit from chelated and complexed minerals, but most have shown positive responses when compared to inorganic sources. Spears (1989) showed that zinc-deficient lambs retained zinc from zinc-methionine better than from zinc oxide. Zinc in the form of zinc-lysine fed to sheep resulted in the highest levels of metallothionein in liver, pancreas and kidney compared to other zinc sources, thus indicating a more bioavailable source of zinc (Rojas et al. 1995). Dietary requirements for minerals may be greatly reduced by the addition of chelating agents to animal diets, but cost to benefit relationships need to be established.

Table 1. Percentage of mineral element and relative bioavailability.^a

Element	Source compound	Element in compound (%)	Bio-availability
Calcium	Steamed bonemeal	29.0 (23–37)	High
	Defluorinated rock phosphate	29.2 (19.9–35.7)	Intermediate
	Calcium carbonate	40.0	Intermediate
	Soft phosphate	18.0	Low
	Ground limestone	38.5	Intermediate
	Dolomitic limestone	22.3	Intermediate
	Monocalcium phosphate	16.2	High
	Tricalcium phosphate	31.0–34.0	—
	Dicalcium phosphate	23.2	High
	Hay sources	23.3	Low
Cobalt	Cobalt carbonate	46.0–55.0	— ^b
	Cobalt sulfate	21.0	— ^b
	Cobalt chloride	24.7	— ^b
Copper	Cupric sulfate	25.0	High
	Cupric carbonate	53.0	Intermediate
	Cupric chloride	37.2	High
	Cupric oxide	80.0	Low
	Cupric nitrate	33.9	Intermediate
Iodine	Calcium iodate	63.5	High
	Ethylenediamine dihydroiodide	80.0	High ^c
	Potassium iodide, stabilised	69.0	High
	Cuprous iodide	66.6	High
Iron	Iron oxide	46.0–60.0	Unavailable
	Ferrous carbonate	36.0–42.0	Low ^d
	Ferrous sulfate	20.0–30.0	High
Magnesium	Magnesium carbonate	21.0–28.0	High
	Magnesium chloride	12.0	High
	Magnesium oxide	54.0–60.0	High
	Magnesium sulfate	9.8–17.0	High
	Potassium and magnesium sulfate	11.0	High

Continued on next page.

Table 1. Cont'd.

Element	Source compound	Element in compound (%)	Bio-availability
Manganese	Manganous sulfate	27.0	High
	Manganous oxide	52.0–62.0	High
Phosphorus	Defluorinated rock phosphate	13.1 (8.7–21.0)	Intermediate
	Calcium phosphate	18.6–21.0	High
	Dicalcium phosphate	18.5	Intermediate
	Tricalcium phosphate	18.0	—
	Phosphoric acid	23.0–25.0	High
	Sodium phosphate	21.0–25.0	High
	Potassium phosphate	22.8	—
	Soft phosphate	9.0	Low
	Steamed bonemeal	12.6 (8–18)	High
Potassium	Potassium chloride	50.0	High
	Potassium sulfate	41.0	High
	Potassium and magnesium sulfate	18.0	High
Selenium	Sodium selenate	40.0	High
	Sodium selenite	45.6	High
Sulfur	Calcium sulfate (gypsum)	12.0–20.1	Low
	Potassium sulfate	28.0	High
	Potassium and magnesium sulfate	22.0	High
	Sodium sulfate	10.0	Intermediate
	Anhydrous sodium sulfate	22.0	—
	Sulfur, flowers of	96.0	Low
Zinc	Zinc carbonate	52.0	High
	Zinc chloride	48.0	Intermediate
	Zinc sulfate	22.0–36.0	High
	Zinc oxide	46.0–73.0	High

^a From Ellis et al. (1988).^b Critical tests not done, but source effective.^c Some liberation of free iodine when mixed with trace elements.^d Some samples are fairly high in availability—but not as available as ferrous sulfate.

Selecting a Free-choice Mineral Supplement

Though it has been found that grazing live-stock do not balance their mineral needs perfectly when consuming a free-choice mixture, there is often no other practical way of supplying mineral needs under grazing conditions. As a low cost insurance to provide adequate mineral nutrition, a modified 'complete' mineral supplement should be available free-choice to grazing sheep. A 'complete' mineral mixture usually includes salt, a low fluoride-phosphorus source, calcium, cobalt, copper, iodine, and zinc. Selenium could be included as many sheep-grazing regions are selenium deficient, however, selenosis is also a problem in some areas so caution is necessary. Often sulfur should be included, being more important for sheep than cattle. Additional elements which may be required include magnesium, potassium and manganese. Most frequently, iron is not required, with grazing sheep often consuming excesses. The modified 'complete' mineral mixture needs to be flexible and readily modified as new information suggests different needs or possible excesses.

Calcium, copper, or selenium, when in excess, can be more detrimental to ruminant production than any benefit derived by providing a mineral supplement. Copper requirements are greatly influenced by dietary concentrations of molybdenum and sulfur. With sheep, great care has to be taken to avoid over-supply of copper because of the extreme sensitivity of this species and even breed differences to copper toxicity. When the dietary copper

level falls below normal (5–8 mg/kg) or the dietary sulfur level is high (4 mg/kg), molybdenum intake as low as 1–2 mg/kg may prove toxic. Lactating ewes require 14–17 mg/kg copper when dietary molybdenum is greater than 3 mg/kg, but only 7–8 mg/kg when dietary molybdenum is less than 1 mg/kg (Suttle 1983). Thus, the exact level of copper to use in counteracting molybdenum or sulfur antagonism is a complex problem and should be worked out for each area.

Table 2 lists characteristics of a 'good' (complete or 'shotgun') mineral mix for sheep. A number of 'authorities' feel there is no justification for the use of 'shotgun' free-choice mineral mixtures that are designed to cover a wide range of environments and feeding regimens and that contain a margin of safety as an insurance against deficiency. These people feel that 'shotgun' mixtures are economically wasteful and can also be harmful. This viewpoint is valid for developed countries which have access to analytical laboratories which can easily determine mineral status for ruminants. However, this author believes that in developing countries or for countries with vast extensive grazing regions, 'shotgun' mixtures have been highly successful in improving ruminant productivity. There is little danger of toxicity or excessive cost in relation to the high probability of increased production rates for sheep from administering a complete 'shotgun' free-choice mineral mixture following the guidelines in Table 2.

Copper, selenium, iron and fluorine would be the minerals of most concern for toxicity to sheep. Even without mineral analyses,

the likelihood of toxicities of copper and selenium in specific regions would be suspected (e.g. clinical signs and high soil pH). Iron should be avoided in complete mineral mixtures unless evidence suggests otherwise, and safe sources of phosphorus principally used to avoid fluorine toxicity. In

conclusion, it is best to formulate free-choice mixtures on the basis of analyses or other available data. However, when no information on mineral status is known for a given region, a free-choice complete ('shotgun') mineral supplement is definitely warranted.

Table 2. Characteristics of a 'good' semi-complete free-choice sheep supplement.

An acceptable complete sheep mineral supplement should be as follows:

1. Contains a minimum of 3–5% total phosphorus. In areas where forages are consistently lower than 0.20% phosphorus, mineral supplements in the 5–7% phosphorus range are preferred.
 2. Has a calcium:phosphorus ratio not substantially over 2:1.
 3. Provides a significant proportion (e.g., about 50%) of the trace mineral requirements for cobalt, copper, iodine, and zinc.^a In known trace-mineral-deficient regions, 100% of specific trace elements should be provided.
 4. Sulfur should be provided if forages are consistently lower than 1.5 g/kg sulfur and/or if urea is fed. Due to wool growth, the nitrogen:sulfur ratio should not exceed 10:1.
 5. Magnesium and potassium would only be provided when a need is shown.
 6. Includes high-quality mineral salts that provide the best biologically available forms of each mineral element, and avoidance or minimal inclusion of mineral salts containing toxic elements. As an example, phosphates containing high fluorine should be either avoided or formulated so that breeding sheep would receive no more than 30–50 mg/kg fluorine in the total diet. Fertilizer or untreated phosphates could be used to a limited extent for finishing sheep or in combination with safe sources.
 7. Is sufficiently palatable to allow adequate consumption in relation to requirements.
 8. Is backed by a reputable manufacturer with quality control guarantees as to accuracy of mineral-supplement label.
 9. Has an acceptable particle size that will allow adequate mixing without smaller size particles settling out.
 10. Is formulated for the area involved, the level of animal productivity, the environment (temperature, humidity, etc.) in which it will be fed, and is as economical as possible in providing the mineral elements used.
-

^a For most regions it would be appropriate to include selenium, unless toxicity problems have been observed. Iron may be included in temperate region mixtures but often both iron and manganese can be eliminated for acid soil regions. In certain areas where parasitism is a problem iron supplementation may be beneficial.

An oral magnesium supplement is of value only during seasonal occurrences of grass tetany (Allcroft 1961). Outbreak of tetany occurs most frequently in nursing ewes shortly after they are turned out to pasture in the spring, with the highest incidence in the 4–5 weeks following lambing. Unfortunately, many commercial magnesium-containing, free-choice mineral supplements are often of little value for sheep because (1) they contain inadequate amounts of magnesium to protect against tetany during susceptible periods, and (2) provision of such supplements to normal animals during nonsusceptible periods is useless as a prophylactic measure, since additional magnesium will not provide a depot of readily available magnesium for emergency use. Some producers feed magnesium supplements about a month before the magnesium tetany season, to decrease the amount of magnesium needed daily during the susceptible period. Various combinations of magnesium oxide with salt, protein supplements, molasses, other concentrate ingredients, and other feeds have

been used to obtain optimal magnesium intakes. For special tetany-preventing mixtures, high levels of magnesium are required (e.g. 100–200 g magnesium/kg).

Data and Calculations for Mineral Supplement Formulation

To evaluate a free-choice mineral supplement, one needs an approximation of: 1) sheep requirements for the essential minerals, which include the age of the animals involved, stage of current production or reproduction cycle, and intended purpose for which the animals are being fed; 2) relative biological availability of the minerals in the sources from which they will be provided; 3) approximate daily intake per head of the mineral mixture and total dry matter that is anticipated for the target animals; and 4) concentration of the essential minerals in the free-choice mixture.

The calculations for free-choice mineral supplement evaluations are shown below.

Sample calculation for free-choice mineral supplement evaluation

$$\frac{\text{element in mineral mixture (g/g)} \times \text{daily intake of mineral mix (g)}}{\text{total daily dry matter intake (g)}} \times 100 = \text{element from mineral mixture expressed as percent of the diet.}$$

If, for example, copper in mineral mixture = 0.0008 g/g

Daily intake of mineral mixture (g) = 15

Total daily intake of dry matter (g) = 1800

then:

$$\frac{0.0008 \times 15 \times 100}{1800} = 0.00066\% \text{ or } 6.6 \text{ mg/kg}$$

If 8 mg/kg is considered the allowance, then 82.5% of the copper requirement would thus be supplied by this mixture.

Table 3 illustrates the estimated trace mineral requirements and percentages of each element required in a sheep mineral mixture to meet 25, 50 or 100% of the requirements. These figures are based on an estimated daily mineral consumption of 15 g. With less consumption, the mineral supplement should contain a higher percentage of each mineral. Likewise, a lower intake of dry matter would reduce the percentage of minerals required in the mixture. Each producer should determine mineral consumption for his flock and change products if higher consumption rates are required (e.g., increase the cottonseed meal from 5 to 10%).

Evaluation

Problems concerned with mineral supplementation programs in diverse world regions (McDowell et al. 1993) include:

1) insufficient chemical analyses and biological data to determine which minerals are required and in what quantities; 2) lack of mineral consumption data needed for formulating supplements; 3) inaccurate and/or unreliable information on mineral ingredient labels; 4) supplements that contain inadequate amounts or imbalances; 5) standardised mineral mixtures that are inflexible for diverse ecological regions (e.g., supplements containing selenium distributed in a selenium toxic region); 6) farmers not supplying mixtures as recommended by the manufacturer (e.g., mineral mixtures diluted 10:1 and 100:1 with additional salt; 7) farmers not keeping their animals continuously supplied with minerals; and 8) difficulties involved with transportation, storage, and cost of mineral supplements. Many of these problems are more related to developing regions, as in more developed countries there is better quality control of products.

Table 3. Percentage of trace minerals required in an adequate trace mineral supplement for sheep.^a

Element	Requirement ^b (mg/kg)	Percent in mixture for:		
		25%	50%	100%
Cobalt	0.1	0.0004	0.0007	0.0014
Copper	8	0.024	0.048	0.095
Iodine	0.8	0.003	0.005	0.01
Manganese	25	0.075	0.15	0.3
Zinc	25	0.075	0.15	0.3
Iron	40	0.12	0.24	0.48
Selenium	0.2	0.0007	0.0013	0.0026

^a This assumes an average consumption of 15 g/day of mineral mixture and 1.8 kg/day of total dry feed per animal. As an example, this could be equivalent to the needs of a 70 kg ewe during the last 4 weeks of gestation or last 4–6 weeks lactating suckling singles.

^b NRC (1985).

Responsible firms that manufacture and sell high-quality mineral supplements provide a great service to individual farmers. However, there are companies that are responsible for exaggerated claims of advertising and some that produce inferior products that are of little value—or worse, those likely to be of detriment to animal production. Table 4 provides an example of an inferior mineral mixture available in Latin America. This particular mineral supplement is recommended for cattle, sheep, pigs, and chickens. It is impossible to adequately meet requirements of both rumi-

nants and monogastric animals with the same mixture. This unbalanced mineral mixture, which is extremely high in calcium (294 g/kg) and low in phosphorus (18 g/kg), would likely be more detrimental to grazing sheep than having no access to supplemental minerals, and may actually contribute to a phosphorus deficiency.

Manufacture of mineral mixes

Mineral elements exist in many chemical forms including sulfates, carbonates, chlorides, oxides and organic forms (e.g., amino

Table 4. An inferior mineral mixture available for sheep in Latin America.^{a,b,c}

Element	Mineral dietary allowance	Amount in mixture	Allowance provided from mineral mixture	Proportion of allowance provided from mineral mixture (%)
Sodium chloride	5 g/kg	200 g/kg	1.7 g/kg	34.0
Calcium	4 g/kg	294 g/kg	2.5 g/kg	62.5
Phosphorus	2 g/kg	18 g/kg	0.15 g/kg	7.5
Magnesium	1.5 g/kg	32 g/kg	0.27 g/kg	17.8
Iron	40 mg/kg	8800 mg/kg	73.3 mg/kg	183.3
Zinc	25 mg/kg	200 mg/kg	0.16 mg/kg	0.6
Cobalt	0.1 mg/kg	20 mg/kg	0.16 mg/kg	60.0
Iodine	0.8 mg/kg	10 mg/kg	0.08 mg/kg	10.0
Copper	8.0 mg/kg	150 mg/kg	0.125 mg/kg	1.6
Manganese	25 mg/kg	750 mg/kg	0.62 mg/kg	2.5
Selenium	0.2 mg/kg	5 mg/kg	0.041 mg/kg	20.5

^a Modified for sheep: from McDowell et al. (1993).

^b Mineral mixture is recommended for cattle, sheep, pigs, and chickens. It is assumed that mineral consumption will average approximately 0.5% of the total dietary intake. This is based on an estimated intake of 15 g of mineral mixture for sheep and 1.8 kg of total dry feed per head daily.

^c Criticisms of mineral mixture are as follows: 1) mixture extremely low in phosphorus and exceptionally high in calcium (the calcium:phosphorus ratio is 16.4:1); 2) the supplement does not provide a significant proportion (i.e. 50%) of the trace mineral requirements of copper, iodine, zinc, manganese and selenium; 3) the majority of the iron is from ferric oxide, an unavailable form of this element; 4) since this diet contains 294 g/kg calcium and only 200 g/kg salt (NaCl), it is likely to be of low palatability.

acid complexes). The form chosen for use should depend on its biological value, cost, availability in the area, its stability and effect in the type of diet used and other functions. It is important to know what combinations of mineral salts to mix together to avoid their reacting with each other and causing adverse effects in a mineral mixture (Cunha 1987). Both loose and block mineral forms also need to be stored and fed under a wide range of weather conditions and under varying levels of rainfall or snow. A particular expertise is required to produce mineral blocks; if the block is too hard, consumption will be reduced, if not hard enough, the product will crumble. The user of mineral supplements must rely on the reputation and integrity of the mineral feed manufacturer. Safe, biologically available and palatable forms of the minerals, at a fair price, allow both the user and manufacturer to realise a profit from their use.

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Toxicities and Excessive Intakes of Minerals

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Outline

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- Miscellaneous minerals

Limitations for human consumption

Acknowledgments

References

Most of the information in this publication is about deficiencies of certain minerals. These minerals have to be present in the diet if animals are to develop, produce and survive. They are essential minerals. For many of the essential elements the amount required for survival and production is very small. They are required in trace amounts. They are known as the essential trace elements. Even though the elements are essential for health, the concentration of the element within the tissues must be kept within fairly narrow limits if normal body structure and function are to be maintained. Deviation from these limits may result in damage to the tissues.

The limits for the concentration of the element within tissues are determined by the efficiency of the homeostatic mechanisms of the animal. If the homeostatic mechanisms can no longer handle the concentration of the element present within the tissues, the metabolic processes within the cells are disturbed, the cells are damaged and the animal is no longer normal. This may result in overt clinical illness or it may result in subclinical disease manifesting as poor performance in such things as weight gain, fleece growth, the ability to carry a foetus to term or milk production. In addition the animal may have an enhanced susceptibility to other diseases such as those produced by toxins or infectious agents.

If the concentration of an essential element within an animal's tissues is reduced it is entirely predictable that the animal would no longer function normally and might show a drop in production or become clinically ill or die. It is not always understood that the development of poor

performance, ill health and possibly death may occur when the concentration of the element within the tissues is markedly increased. There can be no doubt that a little of an essential element in an animal's diet is good, but it is not true that a lot of that element in the animal's diet is even better. Thus there is the paradox of certain elements being both necessary for, and damaging to, the proper functioning of cells, tissues and animals. This has led to conflicting statements such as: 'toxic elements do not exist' or 'everything is poisonous'. The paradox of essentiality and toxicity is resolved once it is realised that it is the concentration of the element within the biological system that determines the type of reaction that occurs.

Particular concentrations of trace elements may result in three types of biological activity:

- 1) low concentrations at which the element is necessary for life;
- 2) higher concentrations at which the element may reduce the productive capacity of the animal and may increase susceptibility to inter-current disease;
- 3) concentrations at which a toxic clinical syndrome is clearly recognisable (Howell 1983).

Exposure and Interactions

The requirements for trace elements depend upon a variety of factors including the overall constitution of the diet, the stage of growth, magnitude of production, stage of pregnancy and the genetic makeup of the animal. Changes to any of these factors may influence whether a particular concen-

tration of a mineral is deficient, sufficient or toxic.

Animals may become exposed to toxic concentrations of minerals in a variety of ways, but the two most important by far are by administration as a therapeutic substance or by ingestion with the feed. Iatrogenic, or therapy induced, damage may occur following attempts to treat or prevent deficiency states and the therapy for copper deficiency in sheep is a good example. A great number of ways have been developed to administer copper so as to prevent the development of copper deficiency in ruminants and one of these ways involves the subcutaneous injection of copper complexes.

However, use of these copper complexes has sometimes been followed by the death of a variable proportion of the injected sheep. Some of these animals developed a haemolytic crisis and examination of their tissues revealed the changes of copper poisoning. It was found that the organic components of the complexes in which the copper was given markedly increased its absorption and toxicity (Ishmael et al. 1971; Mahmoud and Ford 1981).

Another example is provided from the New Zealand work on facial eczema which is seen as a disease of sheep and cattle grazing pastures infected with the fungus *Pithomyces chartarum*. This fungus produces an hepatotoxin called sporidesmin. The toxin damages the liver cells, resulting in photosensitisation, and the major damage is seen as inflammation and destruction of the skin of the head—hence the term ‘facial eczema’. The disease can be so severe that it results in death. It may be

prevented by the oral administration of zinc supplements. This treatment is widely used but has sometimes resulted in the development of zinc toxicity. The development of zinc toxicity seems to be particularly common when the zinc solution is given by drenching gun, as this may stimulate the rumeno-reticular groove and the dose may go straight into the abomasum from where it is absorbed quickly (Smith et al. 1979).

The most common way in which an animal becomes exposed to toxic concentrations of minerals is by ingesting them in the feed. This may be due to environmental contamination which most frequently arises when animals are grazing pastures close to smelters, mineral processing plants or old mine sites, or are consuming foods harvested from their vicinities. The two most commonly cited toxicities that arise in this way are those of fluorine and lead (Lloyd 1983) but copper toxicity in ruminants may also be due to industrial contamination of pasture (Gummow et al. 1991; Vrzgulova 1993).

Alternatively, the toxic concentration may have resulted from the incorrect formulation of a ration or mineral mix. This may have arisen from a clerical error such as having, within the formula, a decimal point in the wrong place, or by having an imbalance of elements, or by human error during mixing, or by environmental contamination of the feed, or a component of the feed. These may be simple mistakes, or they may arise due to ignorance of the variability of tolerance for these elements from one species to the next.

Consider a particular farming enterprise involving sheep and pigs. A mineral mix

designed to increase the production of pigs could work well for the pigs but fatalities are likely if the same mix is fed to the sheep. A concentration of 4 mg copper/kg of dry matter in the diet is adequate for growing pigs of up to 90 kg live weight (ARC 1967), but Braude and Ryder (1973) showed that supplementing the ration to contain 250 mg copper/kg dry matter greatly increased the liveweight gain and efficiency of feed use in pigs. Thus manufactured feed for pigs often contains these high concentrations of copper.

The recommended level of copper in the feed of sheep is 5 mg/kg dry matter (ARC 1965), yet Hartmans (1975) reported that diets containing more than 15 mg/kg dry matter were dangerous for sheep and cases of copper poisoning have been reported in sheep fed on mixed hay and concentrate diets containing less than 10 mg copper/kg dry matter (Hogan et al. 1968; Todd 1969; Buck 1970). Therefore it would be potentially disastrous to formulate a single mineral mix to be fed to both the species on this farm.

It is also potentially dangerous to change a single item in a diet without having due regard for the effects that this may have on other components of the diet. Underwood (1981) wrote 'The significance of overall dietary balance for the absorption and utilisation of mineral nutrients by animals cannot be overstressed'. Interactions between minerals can occur in the environment, in the feed, or in the alimentary tract and may significantly alter the biological availability of the trace element, its absorption from the gut, its transport and distribution within the body and its effective

biological life. The interactions between elements are complex and specific examples are given elsewhere in this paper.

The bioavailability of a mineral in the diet may depend upon such factors as its physicochemical form and the protein and vitamin content of the ration. This is particularly important in monogastric species where ascorbic acid reduces the toxicity of several elements and phytic acid binds copper and zinc and renders them less available for absorption (Davies and Nightingale 1975). A diet in which the main protein component is of plant origin, such as soya bean, cottonseed or sesame meal, will be high in phytic acid and will have a great capacity to bind copper and zinc. A similar concentration of copper or zinc in a ration in which the main source of protein is of animal origin will have the potential to be more toxic. This interaction with phytate has not been found in ruminants, due to breakdown of phytate by rumen micro-organisms.

Grazing animals may ingest differing concentration of minerals according to the stages of development of their pastures, for the mineral composition of plants varies between species, within species and at various stages of growth. Improving pasture through the application of fertilizer usually leads to changes in the botanical composition and may lead to substantial changes in the mineral content of mixed herbage. In times of drought animals will also be ingesting considerable quantities of soil and this will influence the intake of minerals (Howell 1983). It is also important to realise that some plants are capable of absorbing minerals from the soil even though the mineral

may be present in very small quantities or in a form relatively unavailable to other plants growing alongside.

This is true of the selenium accumulator plants such as *Astragalus racemosus* which will grow only in areas where there is a high concentration of selenium in the soil. There are more than 250 species of *Astragalus* growing in China (Cao et al. 1992). Selenium accumulator plants absorb forms of selenium which are not taken up by other plants. The selenium is incorporated into organic forms which are then available to grazing animals. These animals may develop selenium toxicity. In addition when the unconsumed parts of the plant die, the selenium within them is returned to the soil in forms which may be available to the non-accumulator plants. Thus the selenium content of the total plant population may increase.

Toxins other than minerals may play a significant role in mineral toxicity. This is the case when the presence of the mineral and the other toxin within the tissues leads to a synergistic toxic action. A good example is provided by a syndrome which is of economic importance in the Australian sheep industry. The syndrome is called hepatogenous copper poisoning or 'the Yellows' of sheep. Animals developing the Yellows become jaundiced, may develop a haemolytic crisis and may die. The syndrome is seen when sheep ingest both copper and pyrrolizidine alkaloids such as those found in senecio, echium and heliotrope plants.

It has been shown experimentally that the feeding of heliotrope alone produced liver damage but did not induce a greater accumulation of copper in the liver than that

seen in control sheep, nor did it produce significant clinical illness. However, the administration of copper to sheep at the same time as, or subsequent to, the ingestion of heliotrope markedly increased the accumulation of copper in the liver, resulted in significant liver damage in all sheep and clinical illness in the majority. The copper accumulation, tissue damage and clinical illness in these sheep was far greater than in sheep given the same dose of copper alone (Howell et al. 1991a,b, 1993).

There is increasing evidence that genetic factors influence the way in which animals absorb, transport and retain such elements as selenium, copper, iodine, zinc and iron (Wiener and Woolliams 1983; Wiener 1987). Most of this work has been done on sheep and it is possible to say that dietary amounts of copper that are adequate for some breeds will be inadequate for others and possibly will be inadequate for some. In studies of 231 samples of sheep liver, Meyer and Coenen (1994) found a range of concentrations of copper. From 198 of the samples from adult sheep 28% suggested copper deficiency and 21% suggested copper overload.

Sheep of the Heidschnuke breed comprised the main part of those with the lesser concentration; Merino and Texel sheep were predominant in the group with the elevated concentration. It has been known for some time that the Texel breed of sheep has a greater susceptibility to copper poisoning than other breeds (Luke and Wiemann 1970). Housing Texel sheep and feeding them grain-based concentrate feeds may well result in copper toxicity in situations that would not be detrimental to other breeds.

Diagnosis

Earlier in this paper it was stated that mineral toxicity may result in clinical disease, loss of production or increased susceptibility to other disease states. When clinical disease (such as fluorosis or chronic selenium toxicity or chronic copper poisoning) develops it is relatively easy to make a diagnosis. However, in other situations subclinical effects leading to loss of production make diagnosis more difficult and a considerable amount of production may be lost before a correct diagnosis is made. In attempting to make a diagnosis of mineral overload one must:

- look for a history of exposure;
- take notice of any clinical features;
- examine the morphological changes in material obtained by biopsy or at post-mortem examination;
- use appropriate ancillary biochemical tests;
- analyse the mineral content of diet, tissues and body fluids.

It must be stressed that in making a diagnosis, all of these factors should be taken into account. Considering only one category may lead to wrong decisions. Some of the tissue changes and clinical signs seen in mineral toxicities may be produced by a variety of other factors, so consideration should always be given to a differential diagnosis.

History of exposure

A history of exposure may be obvious if a specific problem has arisen in a number of animals that have grazed for some time down-wind of a smelter belching forth

clouds of smoke, gas and particles. In that situation making a diagnosis is assisted by the presence of a time factor, multiple animals involved with a similar syndrome, and visual evidence of contamination. However, one would still need to be mindful of all the other factors and take into consideration the differential diagnosis. Evidence of exposure is not obvious in the situation where human error has occurred in mixing or administering a mineral supplement, particularly if the supplement had previously been used in the correct amount. In this instance analysis of the food and the tissues of the animals will provide the most valuable clues.

Clinical features

When a clear-cut toxic syndrome develops the clinical features will be of great value, as they would be in fluorosis or chronic copper poisoning. However, if the degree or pattern of toxicity does not produce an overt clinical problem but results in subclinical problems such as loss of production, the diagnosis is much more difficult because the causes of production loss are many and varied. In this case it is essential to use all the parameters indicated above. In addition a response to treatment would be of value.

Morphological change

When one is involved with a flock problem it is often sensible and economically viable to sacrifice one—or better two or three—of the affected animals in order to look for morphological changes in all tissues. Such a procedure also enables the sampling of

multiple tissues for the analysis of their mineral content. If it is not possible to sacrifice animals, consideration should be given to taking a biopsy. Biopsy of the liver (Dick 1952; Donald et al. 1984) is relatively easy and safe to perform on sheep. Biopsy of bone is sometimes useful and a technique is illustrated by McDowell et al. (1983). Bone can also be obtained as a coccygeal vertebra by removing the end of the tail. These methods should provide sufficient tissue for morphological examination and chemical analysis. Morphological changes alone can strongly indicate a particular mineral toxicity, such as fluorosis and chronic copper poisoning. More often they will provide a valuable indication of which mineral requires further investigation.

Biochemical tests

Ancillary biochemical tests on body fluids such as blood will often indicate the organ involved but only rarely do they specifically identify the particular mineral. A decrease in the activity of δ -aminolaevulinic acid dehydratase in the red blood cells is found in cases of lead poisoning in sheep and is a reasonably specific indicator of lead overload. However the level of δ -aminolaevulinic acid in urine was found to vary too greatly to be of value as a diagnostic test (Rolton et al. 1978). Using blood samples from calves on a farm contaminated with lead-based paint Wada et al. (1993) found that estimation of free erythrocyte protoporphyrin concentration was a good indicator of lead contamination. Other tests such as those which indicate consistent increases in the activity of creatine kinase or glutamate dehydroge-

nase in the blood will indicate that changes are occurring in muscle and liver respectively but do not indicate the nature of the agent which produced the damage.

Analyses of diet, tissues and body fluids

Analysis of body fluids for concentrations of particular minerals is useful but should be combined with analysis of the feed and wherever possible of storage organs such as liver and kidney. It is obviously important to obtain the correct sample for analysis. Always try to ensure that the feed sample was taken from the food ingested by the animals during the development of toxicity and that the methods of sampling and transport have not caused contamination. Blood, urine, saliva and hair are frequently analysed because they can be obtained without killing the animal.

It should always be remembered that the homeostatic mechanisms will be endeavouring to keep the concentration of minerals in the blood within the normal limits. Nevertheless there can be great variations between individuals in the concentration of a particular element in the blood. For these reasons more information will be obtained from multiple blood samples from individuals than from single samples. If the problem arose from recent exposure, analysis of ingesta, faeces and urine might be of value.

If the diet in question had been consumed over a long period then the major storage organ for the particular mineral should be sampled. This will often be the liver. It should also be remembered that chemical analysis will give the amount present, but will not

indicate the bioavailability. For example, the concentration of copper in the blood and kidney from clinically normal but copper-loaded sheep dosed with tetrathiomolybdate to prevent copper poisoning will be much higher than in normal sheep (Howell and Kumaratilake 1990). However, much of the copper in the sheep given tetrathiomolybdate will be biologically unavailable.

It may be necessary to perform chemical analysis on more than one organ and to combine chemical analysis with morphological analysis. For example, two sheep may contain similar concentrations of copper in the liver, but if only one animal has necrosis of the centrilobular liver cells it is probable that this animal died of chronic copper poisoning and that the other died of something else. The diagnosis of chronic copper poisoning would be confirmed if it was found that the sheep with the necrotic liver cells also had elevated concentrations of iron and copper in the kidney, indicating that significant haemolysis had occurred.

Toxic Syndromes

In the toxic syndromes outlined below some indication is given of toxic concentrations but no attempt is made to give 'safe levels'. This is because of the interactions outlined above and also the variation of animal response that follows a single exposure or repeated exposures to toxic concentrations of a mineral. Table 1 summarises the findings.

Zinc

Domestic animals appear to have a considerable tolerance to diets containing high

levels of zinc. Calcium, copper, iron and cadmium have all been shown to interact in the processes of absorption and utilisation of zinc. Young animals seem to be more susceptible and Davies et al. (1977) concluded that suckling lambs were considerably more susceptible to zinc toxicity than were mature ruminants. They reported poor growth, low appetite and extensive kidney damage in suckling lambs maintained for 4 weeks on a milk substitute diet containing Toprina yeast with a content of 2065 mg zinc/kg dry matter. Renal damage was also seen in lambs fed a milk diet supplemented with an equivalent amount of zinc.

Pregnant ewes are more susceptible to zinc toxicosis than non-pregnant sheep (Campbell and Mills 1979) and this might be due to an increase in the efficiency of zinc absorption during pregnancy (Bremner 1979). Smith et al. (1979) have shown that the toxicity of zinc given to prevent the development of facial eczema was enhanced when zinc sulfate was given by drenching gun. Administration of zinc solutions by drenching gun resulted in increased concentrations of zinc in serum and organs, severe lesions in the abomasum and pancreas and even death, even though the same dose was apparently non-toxic when delivered by intraruminal intubation. These authors suggested that the administration of zinc solutions by drenching gun resulted in contact of zinc with the pharyngeal mucosa which resulted in stimulation of the reticular groove reflect and direct channelling of the solution into the abomasum. The resulting damage to the mucous membrane might also result in an increased rate of absorption.

Table 1. Indication of the concentrations, interactions, signs and tissues damaged by high concentrations of minerals (adapted from Howell 1983). This summary is not exhaustive; the text and the references cited in the text should be consulted.

Element	Species commonly affected	Toxic concentrations ^a	Interactions ^b	Signs	Tissue damage
Zinc	Sheep Cattle	33–100 mg Zn/kg body weight 750–2000 mg/kg DM in diet	Ca, Cu, Fe, Cd,	Reduced weight gains, death	Many tissues but, particularly pancreas, abomasum and kidney
Copper	Acute Lambs Cows	25–50 mg/kg body weight 200		Abdominal pain, diarrhoea	Alimentary tract
	Chronic Sheep Goats Calves	10–20 mg/kg 10–20 DM 115 in diet	Zn, Fe, Cd, Mo + S, Ascorbic acid	Inappetence, soft faeces, dark red urine, jaundice and death	Blood, liver, kidney, muscles, brain
Manganese	Sheep	400 mg/kg DM in diet	Fe	Reduced growth rate	—
Cobalt	Cattle Sheep	1 mg/kg body weight 4–10	Fe	Inappetence, loss of weight, occasionally death	—
Molybdenum	Cattle Sheep	20–100 mg/kg DM in diet	Cu and S, Zn, Pb, methionine, cystine	Inappetence, weight loss, diarrhoea, irregular oestrus cycles	Bones, joints, testes
Selenium	Cattle Sheep	5–30 mg/kg DM in diet	Dietary protein, As, Hg, Ag, Cu, Cd, S, methionine, vitamin E	Respiratory distress, diarrhoea, loss of weight, blindness, paralysis, loss of hair and hooves	Heart, liver, bones, joints, blood
Fluorine	Chronic Cattle Sheep	Yearly average in diet for cattle must be 40 mg F/kg DM or less, and intake must not exceed 60 for more than 2 months or 80 for more than 1 month	Al, Ca	Growth retardation, inappetence, weight loss, lameness	Teeth and bones

Continued on next page.

Table 1. Cont'd.

Element	Species commonly affected	Toxic concentrations ^a	Interactions ^b	Signs	Tissue damage
Arsenic	<i>Inorganic</i> All species	Varies as composition and solubility, etc.	Se	Acute gastroenteritis, death	Alimentary tract
Lead	All species	3 mg/kg DM in diet. 5–20 mg/kg body weight. Great variation according to source and species etc.	Ca, P, Fe, Cu, Zn, vitamin C	Anaemia, diarrhoea, constipation, hyper-excitability, tremor, ataxia, death	Liver, kidney, blood, alimentary tract, central and peripheral nervous system, bones

^a The figures given in this column provide, where possible, an indication of the doses and ranges at which signs of toxicity have been reported.

^b Symbols for elements: Ag, silver; Al, aluminium; As, arsenic; Ca, calcium; Cd, cadmium; Cu, copper; F, fluorine; Fe, iron; Hg, mercury; Mo, molybdenum; P, phosphorus; Pb, lead; S, sulfur; Se, selenium; Zn, zinc.

Inflammation of the abomasum and necrosis of the pancreas have been associated with the administration of high concentrations of zinc in order to prevent the development of facial eczema and lupinosis in sheep. Allen and Masters (1980) and Allen et al. (1983) have written comprehensive accounts of zinc toxicity in the ruminant. They described the changes seen in naturally occurring and in experimentally induced zinc toxicity in sheep and calves. Pathological changes were found in the pancreas, kidney, liver, rumen, abomasum, small intestine and adrenal gland. However, the pancreas was the only organ to be consistently affected and degeneration and regeneration often occurred together.

Many of the affected sheep developed anaemia. Elevated concentrations of zinc were usually found in the liver, kidney and pancreas. Noorduin et al. (1993) successfully used zinc to prevent chronic copper poison-

ing in sheep by giving doses of 35 mg zinc oxide/kg body weight on 5 days of the week, but lesions of zinc toxicity were found in the pancreas. Smith and Embling (1993) have shown that the pancreatic lesions are evident 7 days after treatment and take up to 4 weeks to develop fully.

Copper

Copper interacts metabolically with many other substances including zinc, iron, cadmium, molybdenum, sulfur and ascorbic acid. Therefore it is hazardous to give a maximum safe dietary level based on copper values alone. Sheep are particularly susceptible to copper poisoning and the increased susceptibility may be associated with several factors. Bremner (1980) has suggested that the greater susceptibility of sheep and calves to chronic copper poisoning could be related to their inability to

accumulate large amounts of copper as monomeric metallothionein in their livers. In addition they are often fed cereal-based rations which are low in molybdenum and sulfur and from which copper is more easily absorbed. It has been suggested that this may account for the marked increase in susceptibility to copper poisoning seen in housed sheep, which are commonly fed such rations (Todd 1972).

Acute poisoning with symptoms of acute gastroenteritis sometimes occurs but the most commonly encountered form of copper toxicity is known as chronic copper poisoning. This may be primary copper poisoning due to the ingestion of too much copper, phyto-genous copper poisoning due to the ingestion of plants with a high copper content with or without a low concentration of molybdenum, or hepatogenous copper poisoning where the liver is damaged by plant toxins prior to or in conjunction with the ingestion of copper.

Grazing sheep are relatively safe unless there has been a recent application of copper to the pasture. One of the first authentic reports of primary copper poisoning in sheep occurred when animals were allowed to graze in orchards after the fruit trees had been sprayed with copper sulfate solution to combat insect pests (Schaper and Lütje 1931). Poisoning with associated liver damage has been reported in sheep grazing pasture sprayed with copper-rich pig slurry (Ulsen 1972; Kneale and Howell 1974). However, holding the pig slurry in an anaerobic tank for 60 days made the copper unavailable to herbage (Kneale and Smith 1977). Animal excreta are rich in nitrogen and Suttle and Price (1976) suggested that

there would be a minimum risk of copper toxicity if substances such as molybdenum and sulfur were added to dried poultry waste in order to make it safe for use as a cheap nitrogen source for ruminants.

In Australia phyto-genous copper poisoning has been seen in sheep grazing pastures with a high content of the clover *Trifolium subterraneum* which may contain 10–15 mg copper/kg dry matter but in which the molybdenum content rarely exceeds 0.1–0.2 mg/kg dry matter. Such conditions greatly favour copper accumulation. Hepatogenous copper poisoning has occurred in grazing sheep when plants containing hepatotoxic pyrrolizidine alkaloids such as *Heliotropium europaeum*, were growing in the pasture. Care should always be taken to ensure that the feed for sheep does not have a high copper content and has a balanced copper:molybdenum:sulfur ratio (see White, this publication).

Mention has already been made in this paper of possible problems which may arise when sheep have access to diets prepared for pigs; on occasion diets prepared for cattle may also cause problems (Robles et al. 1993).

There are three phases of chronic copper poisoning, namely the prehaemolytic, haemolytic and posthaemolytic. The pre-haemolytic phase may last for weeks and during this time copper accumulates in the tissues of clinically normal animals. The haemolytic phase is the time of the so-called haemolytic crisis. Haemolysis may be induced by stress, occur suddenly and may be severe or mild. The animals may have a haemolytic anaemia, with haemoglobin-aemia, methaemoglobinaemia, haematuria

and jaundice. Severely affected animals may be dull and lethargic, pass soft faeces, lose their appetite and have an excessive thirst and the sclera may show the chocolate brown colour of methaemoglobin. The animals may die, or recover to move into the posthaemolytic phase, during which other bouts of haemolysis may develop. The principal sites of tissue damage are the liver and kidney but changes also occur in other organs. The condition in sheep has been reviewed by Howell and Gooneratne (1987).

Several workers have used the oral administration of ammonium molybdate (50–500 mg) and sodium sulfate (0.3–1 g) given daily for up to 3 weeks in attempts to reduce tissue levels of copper and to prevent the development of haemolysis in sheep flocks in which copper poisoning had been diagnosed (Ross 1966; Hogan et al. 1968; Kline et al. 1971). Tetrathiomolybdate was first used in experimental situations to treat and prevent chronic copper poisoning in sheep by administering the agent by the intravenous route (Gooneratne et al. 1981). Subsequently Humphries et al. (1988) successfully gave three subcutaneous injections of 3.4 mg tetrathiomolybdate/kg body weight, on alternate days, to successfully treat and prevent copper poisoning.

The use of 175–375 mg zinc/kg of feed in sheep fed diets high in copper may prevent the development of copper toxicosis (Bremner et al. 1976). Noordin et al. (1993) gave either 17 or 35 mg zinc oxide/kg body weight by mouth on 5 days per week to prevent the syndromes of copper, heliotrope and hepatogenous (heliotrope/copper) poisoning in sheep. Both dose regimens had a beneficial effect but the higher dose rate was

required to prevent the development of clinical signs, and significantly reduce liver injury and copper accumulation. At the higher dose rate pancreatic lesions were present.

Manganese

Manganese poisoning is rare, but in some New Zealand pastures manganese levels can be above 500 mg/kg dry matter. At these high manganese intakes the rate of growth of young sheep is significantly reduced (Grace 1973). A relationship between manganese, iron and haemoglobin formation has been demonstrated in lambs and pigs (Hartman et al. 1955; Matrone et al. 1959) and probably arises because of a mutual antagonism between manganese and iron during absorption.

Cobalt

Most animal species seem tolerant to high concentrations of cobalt. This is so in ruminants but some evidence suggests that cattle are less tolerant than sheep. The reports have usually indicated that the signs of excess in cattle were reduced appetite and weight loss, but Dickson and Bond (1974) reported five cases of fatal cobalt poisoning. A cardiomyopathy has been seen in man following the heavy consumption of cobalt-fortified beer but this change has not been reported in cobalt-overloaded animals.

Molybdenum

The tolerance of farm animals to toxic concentrations of molybdenum varies

according to the species, the chemical form of the ingested molybdenum and the copper status of the animal. The status of the diet with regard to protein, copper, sulfur, zinc, lead, methionine and cystine are also of importance. Pigs and horses appear to be more tolerant than sheep and cattle and it has been known for many years that horses may safely graze the molybdenum-rich so called 'teart' pastures which will produce marked diarrhoea in ruminants.

Exposure to high concentrations of molybdenum causes growth retardation, weight loss and anorexia in all species but diarrhoea was seen as a conspicuous clinical feature only in cattle. Within a few days of grazing 'teart' pastures containing 20–100 mg molybdenum/kg dry matter, cattle may scour profusely and develop harsh, staring coats. Treatment with copper sulfate at the rate of 2 g/day for cows or 1 g/day for young stock may effectively control the scouring (Ferguson et al. 1938).

Outbreaks of molybdenum toxicity resulting in severe diarrhoea in cattle have been reported following the discharge of waste waters containing molybdenum ore into the Nanle river (Li et al. 1994) and following the pollution of cultivated land by waste water containing molybdenum ore in Dayu county, Jiangxi province in China (Fan et al. 1983). Sheep were also affected and they lost wool and became anaemic. The animals recovered after copper sulfate was given as 'two doses of 1 g/45 kg body weight'. Changes in bones and joints have also been reported, as have difficulties in conception in cows and loss of libido in bulls. The latter may be associated with the bone and joint lesions but changes have

also been reported in the interstitial cells and germinal epithelium of the testes (see Underwood 1977).

Sharma and Parihar (1994) gave ammonium molybdate by mouth to young goats every day for 235 days—treatments were 0, 50 and 100 mg molybdenum/kg of feed intake on a dry matter basis. The concentrations in the blood of copper and caeruloplasmin were reduced. Clinical signs, which developed first in the 100 mg/kg group, were diarrhoea, anaemia, reduced body weight, weakness, unthriftiness, depigmentation of the hair and altered reproductive performance.

Many signs of molybdenum toxicity are associated with the induced hypocuprosis. When either ammonium molybdate or tetrathiomolybdate was given to pregnant guinea pigs, the concentration of copper in the liver was reduced and that of molybdenum was elevated. Death of the foetus was common in guinea pigs given the higher dose of thiomolybdate. This, together with degeneration of the pancreas, was thought to be due to induced hypocuprosis (Howell et al. 1994). High concentrations of molybdenum in the tissues have been associated with irregular oestrus cycles in cattle but not with damage to the foetus (Phillippo et al. 1985).

Selenium

The toxicity of selenium may vary according to the chemical form of the ingested selenium and the duration and continuity of intake. The toxicity of selenium may be decreased by increasing dietary protein, arsenic, mercury, silver,

copper, cadmium and sulfur compounds, linseed meal, methionine and vitamin E (Underwood 1981; Cooper 1987). Elemental selenium is well tolerated because of its insolubility and poor absorption. Selenides are less toxic than selenites or selenates and the forms of selenium found in plants appear to be even more toxic. It is worth noting that sodium selenite contains 1.93 times as much selenium as sodium selenate and this has contributed to accidental selenium poisoning in sheep (Kyle and Allen 1990).

It is probable that the majority of deaths of animals due to selenium toxicity result from miscalculation of dose or the use of incorrect preparations. Increasingly selenium is being added to injectable vaccines and anthelmintics. In this way it has become an integral part of the disease prevention program in many flocks. However, care should be taken to ensure that only appropriate doses are given to lambs. A dose of unsupplemented vaccine prepared for ewes will do no harm if given to lambs but lambs may well develop selenium toxicity if the vaccine also contained the dose of selenium suitable for ewes. Additional problems arise when animals receive selenium from two sources simultaneously (Bruere et al. 1990).

Selenium toxicity occurs primarily in cattle, sheep and horses in the so-called 'seleniferous areas', which occur in many parts of the world including China (Cheng et al. 1980). In these areas the soil may contain in excess of 500 mg/kg of selenium. Hou et al. (1993) reported selenosis in goats that had been fed maize grown in selenium-toxic land in Ziyang county, Shaanxi prov-

ince. The authors reported that a selenium content of blood over 2.5 $\mu\text{mol/L}$ can be regarded as an early diagnostic index of toxicity and that symptoms will appear when the concentration of selenium in blood and wool exceed 6.25 $\mu\text{mol/L}$ and 38 $\mu\text{mol/kg}$ respectively.

The toxic selenium intakes may arise from the ingestion of plants that have concentrated selenium in their foliage and seeds. Plants may be subdivided into obligate accumulators (which require high levels of selenium for their development), facultative accumulators and non-accumulators. Obligate accumulators, such as *Astragalus* species, are also known as indicator plants as they only survive in soils rich in selenium and may accumulate concentrations of over 1000 mg/kg selenium. These plants absorb selenium from the soil even though it is in forms relatively unavailable to other plant species. Within the plant, the selenium is converted into organic forms which are available to animals grazing the plants. When unconsumed parts of the plants die, the selenium is returned to the soil in forms available to non-accumulator plants. Facultative accumulators take up large amounts of selenium from soils containing normal concentrations of selenium. Non-accumulator plants take up large amounts of selenium only when they are growing in seleniferous soils.

The selenium toxicity syndromes can be classified into three types. These are, the acute, subacute ('blind staggers') and chronic ('alkali disease') forms. Acute toxicity results in respiratory distress, diarrhoea and death. Animals showing the 'blind staggers' syndrome may wander, stumble, have

impaired vision, paralysis, abdominal pain, salivation, grating of the teeth and respiratory distress. Animals with chronic selenium poisoning show lack of vitality and body condition, roughness of coat, sloughing of the hooves, articular lesions and lameness, changes in the myocardium, cirrhosis of the liver and anaemia. There may be loss of hair from the mane and tail of horses and body of pigs. It has been suggested (James et al. 1994) that 'blind staggers' is due to dietary factors other than selenium, such as sodium sulfate, and that the symptoms and lesions are those of polioencephalomalacia (Howell 1961).

Quin et al. (1994) gave six goats a diet containing 11 mg selenium/kg fed for 60 days. The animals showed depression, inappetence, loss of hair, pruritus, increase in heart rate and respiratory distress and they died as a result of respiratory failure. Lesions were found in lungs, liver, kidney, heart, brain, lymph nodes and spleen. The mechanisms underlying these toxic actions are poorly understood and it may be that alkaloids and other toxic substances in the plants may also have a part to play in the subacute and chronic forms (see Ewan 1978; Underwood 1981).

Fluorine

Most pasture plants, forages and grains have a low fluorine content. However, significant contamination can arise in pasture close to smelters and mineral processing plants (Lloyd 1983). Pasture contamination has also been reported due to fluorine contained in superphosphate fertilizers (O'Hara et al. 1982), excessive contamination of

water from boreholes (Botha et al. 1993) and following volcanic eruptions (Araya et al. 1993). East (1993) reported fluorine, calcium pyrophosphate and calcium orthophosphate poisoning in 41 out of 200 pregnant ewes after consumption of superphosphate fertilizer—the consumption of which was thought to be related to the lack of salt availability.

Poisoning may be seen as an acute episode of gastroenteritis, usually in pigs following the ingestion of an excessive amount of sodium fluoride, or as the chronic syndrome of fluorosis in ruminants, pigs and horses. Fluorosis is associated with the long term ingestion of contaminated feed. Suttie (1981) pointed out that the current tolerance level established for the continual ingestion of a soluble fluoride by young dairy cattle is 40 mg fluorine/kg dry matter in the total ration but recommended that the yearly average forage concentration should not exceed 40 mg fluorine/kg dry matter, and it should not exceed 60 mg fluorine/kg for more than 2 months or 80 mg fluorine/kg for more than 1 month. Fluorosis has occurred in Inner Mongolia, Xinjiang and other areas of China where pastures, forage and/or drinking water were contaminated by airborne residues from industrial processes or where the fluorine in soil was plentiful (Deng 1989; Guo et al. 1990).

Wang et al. (1992, 1994) examined goats that had been grazing pasture which was severely polluted by fluorine coming from a nearby industrial plant. They suggested that dry and cold weather conditions led to excessive concentrations of fluorine in the grass consumed by animals, resulting in the development of osteoporosis, and that

differences in fluoride and calcium availability during different seasons resulted in uneven abrasion of teeth and a shortened life span. Tooth wear decreased with increased protein supplementation.

The major changes in animals suffering from chronic fluorosis are seen in the teeth and bones. Animals exposed to excess fluorine before eruption of the permanent teeth develop pitted, mottled, incisors and abraded molars. There are changes in shape and colour of the teeth and fractures may occur. Thickenings, outgrowths and changes in shape of the jaw and long bones can occur in animals exposed at any age. These bones may appear to be chalky, rough and porous. Mineralisation of tendinous attachments may occur. Growth is often subnormal and weight loss is often associated with a reduction in milk production and fertility. The impairment of these facilities may, in some part, be due to reduced feed consumption brought about by the dental and skeletal lesions.

Arsenic

The toxicity of arsenic compounds varies considerably according to such factors as their mode of application, chemical composition and solubility. The trivalent form is much more toxic than the pentavalent form. For sheep, the average total lethal dose of arsenic is 3–10 g when given as arsenic trioxide and 0.2–0.5 g when given as sodium arsenite. Soluble preparations such as dips, weed killers and defoliant are particularly dangerous and many outbreaks of acute poisoning have resulted from their careless use. The clinical signs and lesions asso-

ciated with acute arsenic poisoning are those of an acute gastroenteritis.

The incidence of arsenical poisoning in farm animals has declined due to the marked reduction in the use of arsenical compounds. However, in recent years a large number of cases of arsenic poisoning has been reported in humans due to arsenic contamination of water from deep tube wells. It has been estimated that there are at least 50 000 such cases in Inner Mongolia (Pearce 1995) where sheep are also being affected (Lee, pers. comm.).

Lead

Lead ingestion is one of the most common causes of poisoning in the domestic animal. The common sources are lead-bearing paints, consumed directly or indirectly, e.g. in silage taken from lead-painted silos, metallic lead and soluble lead compounds in car batteries, golf balls or engine sump oil, industrial and mine effluents and emissions. The absorption and retention of ingested lead is greatly influenced by the dietary levels of calcium, phosphorus, iron, copper and zinc. Young ruminants and puppies appear to be affected by lead toxicity more commonly than other domestic animals, and young animals appear to be more susceptible than adults.

The symptoms and tissue changes of lead intoxication are those associated with anaemia, gastroenteritis and damage to the brain and peripheral nerves leading to a variety of behavioural signs, blindness and paresis (Wells et al. 1976). In 'acute' poisoning, death may occur after symptoms

have been seen for 2–3 days or less. In 'chronic' poisoning animals show the symptoms for several days before death occurs. Both syndromes probably result from the accumulation of lead over a period of time. A blue 'lead-line' at the junction of gum and tooth, due to the periodontal deposition of lead sulfide, may be seen in the dog and osteoporosis has been reported in lambs. Degenerative lesions may be present in liver and kidney and acid-fast intranuclear inclusions may be found during histological examination of these organs. Earlier in this paper mention was made of the usefulness in diagnosis of estimations of the activity of δ -aminolaevulinic acid dehydratase in red blood cells and of free erythrocyte protoporphyrin concentration.

Cadmium

Cadmium is one of the most toxic metals. Cadmium oxide and cadmium anthranilate have been used for the treatment of ascarid infestations in swine but, other than this, domestic animals are unlikely to be frequently exposed to high intakes of the metal. Its emetic properties would lessen the effects of ingesting a single large amount but chronic poisoning would follow the long-term exposure to environmental contamination. Cadmium has been introduced into the West Australian environment as a contaminant of fertilizers formulated using rock phosphate. Sheep and cattle grazing on fertilised and improved pastures accumulated cadmium in their tissues and concentrations exceeding the maximum permissible concentration (MPC) of 22 $\mu\text{mol/kg}$, set by the Australian National

Health and Medical Research Council, occurred in kidney and sometimes in liver (Langlands et al. 1988).

Cadmium has a long biological half life (Nordberg et al. 1985) and the concentration in tissues increases with age. However, the fastest uptake into the kidney occurs during the first few months after weaning (Lee et al. 1994). Hoggets grazing pastures on acidic soils and soils with a sandy-textured surface had higher cadmium concentrations in kidneys than those grazing on pastures on more alkaline soils or those with a more textured surface. Application of more than 100 kg/ha of phosphatic fertilizer during a 3-year period to loamy soils was associated with a high cadmium concentration in the kidney (Morcombe et al. 1994). The action of cadmium is influenced by its interaction with zinc, copper, selenium and ascorbic acid. Damage to a wide range of tissues has been reported following cadmium administration. Industrial contamination of the food and water supply in Japan has produced a syndrome of kidney damage and osteomalacia in humans called 'itai-itai' or 'ouch-ouch disease', presumably because of the severe pain associated with the syndrome. Many tissues may be damaged by cadmium overload but the most typical feature of chronic cadmium intoxication is marked kidney damage (Kawai et al. 1976). Cadmium-induced metallothionein has an apparently paradoxical role in the development of lesions of cadmium toxicity. It has been shown to protect against acute, cadmium-induced, testicular necrosis but the cadmium-metallothionein complex accumulates in the kidney and is thought to be

responsible for the subsequent renal damage (Nordberg et al. 1975).

Miscellaneous minerals

Toxicity in sheep is unlikely to arise from high concentrations within the tissues of minerals such as iron, iodine, potassium, sodium chloride and sulfur. Information on these topics has been reported by Seawright (1982), Howell (1983) and McDowell et al. (1983).

Limitations for Human Consumption

High concentrations of minerals are also toxic for humans and an increasing interest is being taken in the mineral content of agricultural commodities used for human consumption. Australia has established Maximum Permitted Concentrations (MPCs) for some minerals in some food commodities. MPCs are not health or safety levels but are set to be well below the amounts required to exceed the acceptable daily intake in human food. The MPCs established as $\mu\text{mol/kg}$ wet weight are: arsenic 13.4 (1.0 mg/kg) in the tissues tested; cadmium 22 (2.5 mg/kg) in edible offal other than liver, 11 (1.25 mg/kg) in liver and 1.8 (0.2 mg/kg) in meat muscle; copper 1575 (100 mg/kg) in edible offal other than ovine liver, 3150 (200 mg/kg) in ovine liver and 157 (10 mg/kg) in other tissues sampled; lead 7.2 (1.5 mg/kg) in the tissue tested. During 1992–1994 a National Residue Survey of Metals in Meat was carried out by the Bureau of Resource Sciences of the Australian Commonwealth

Department of Primary Industries and Energy. This survey found that 14.7% of sheep kidney samples and 11.3% of sheep liver samples exceeded the MPC for cadmium, and 4.5% of sheep liver samples exceeded the MPC for copper (Anon. 1995).

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