

## **A Review on Mineral Imbalances in Grazing Livestock and Usefulness of Soil, Dietary Components, Animal Tissues and Fluid Analysis in the Assessment of these Imbalances**

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**Abstract:** This article briefly describes common mineral imbalances affecting grazing ruminants at farms, their mineral requirements, factors affecting these requirements and different methods of detection of mineral status of the animal. The benefits and limitations of mineral analyses of soil, forage, animal tissues, and fluids samples for the purpose of identifying and preventing mineral disorders of grazing animals are discussed. The role of minerals as buffers, in immunity, as antioxidant and their bioavailability from various sources have also been described.

**Key words:** Mineral imbalances, doil dietary, fluid analysis

### **Introduction**

The detection of mineral element deficiencies or excesses involves clinical, pathological, and analytical criteria as well as response from specific element supplementation. Clinical signs of mineral deficiencies along with soil, water, plant, and animal tissue analyses have all been used with varying degrees of success to establish mineral deficiencies and toxicities. The most reliable method to confirm mineral deficiencies is response derived from specific mineral supplementation. However, supplementation studies are costly in time and resources if conducted with adequate control and assessment (McDowell, 1985, 1987, 1997).

**Clinical and Pathological Evaluation:** Changes in animal appearance or level of production can often be an early indication of diet inadequacy. Where the nutritional abnormalities are acute, or severe, well-marked clinical and pathological stigmata appear making detection and correction relatively easy. As examples, severe or acute deficiencies of iodine (I), magnesium (Mg), and copper (Cu) and toxicities of selenium (Se) and fluorine (F) are often characterized by specific clinical signs, but nutritional disorders are often mild or marginal and expressed only as a vague unthriftiness (i.e., hair losing its sheen) or suboptimal growth, fertility, or productivity. Unfortunately, these changes are often nonspecific and indistinguishable from those resulting from inadequate energy-protein or vitamins, or from parasitism or toxic plants. Therefore, it often becomes necessary to resort to chemical analyses in order to adequately determine mineral insufficiencies (McDowell, 1987).

Clinical and pathological observations of animals have become essential diagnostic tools in the investigations of all mineral element deficiencies and toxicities. Since sudden death in the absence of clinical signs may also be a consequence of acute infections disease, it is important to conduct a postmortem examination for purposes of differential diagnosis. When evidence of gross pathology is supported by histological findings, it is often possible to present a tentative diagnosis of mineral element deficiency (Ullrey, 1983).

**Analysis of Water, Soil and Forage:** Mineral deficiencies and excesses have been established by soil, water, and plant analyses. Although highly variable, all mineral elements essential as dietary nutrients occur to some extent in water. Nevertheless, grazing livestock obtain the majority of their mineral requirements from forages that, under some conditions, are contaminated with soil.

Plants withdraw essential elements from the soil solution in quantities to satisfy their own requirements as well as satisfying many of the requirements of grazing livestock. Besides essential plant elements, plants also withdraw Se, Co, and I, which are essential for the grazing ruminant. The soil-plant relationship is direct in that the plant must obtain all mineral nutrients from the specific soil with which it has contact (Reid and Horvath, 1980).

In some instances, a soil survey can provide clues to potential livestock deficiencies. Soil concentrations of Co, Mo, and I reflect the plant's concentrations of these elements to a certain degree. However, numerous factors affect forage mineral uptake from soils, including yield of plant, stage of maturity, species and strain differences, climatic and seasonal conditions, chemical forms of minerals, and factors of the soil, including pH and degree of aeration of water logging. The concentration of a mineral in a soil in an uncertain guide to its concentration in the forage. Soil analysis, though useful for pasture fertilization, has been eliminated in some investigations because

of lack of direct relationship to mineral content of herbage growing on the soil (Van der Veen, 1973; Gitter *et al.*, 1975). For instance, plants growing on co-deficient soil may not necessarily be deficient in Co nor would a soil rich in Co necessarily yield plants with high levels of Co (Latteur, 1962; Davies, 1997; Reid and Horvath, 1980; McDowell *et al.*, 1989b). However, in the Netherlands, soil analysis is preferred to that of forage analysis to establish a Co deficiency [Netherlands Committee on Mineral Nutrition (NCMN), 1973].

Mineral analysis of the forage consumed by the grazing animal is basic to mineral status diagnosis (Ogebe and Ayoade, 1995). If mineral concentrations are below minimum requirements or above the minimum tolerance level, there is an immediate suggestion of a nutritional problem. However, relying on a forage mineral analysis to establish mineral status assumes that the sample is representative of what animals consume.

Additional disadvantages of forage element analyses to assess mineral adequacy is the difficulty of estimating forage intake and digestibility. The majority of mineral requirements is given in percentage or ppm (mg/kg), which assumes the expected consumption as estimated by dietary standards [i.e. National Research Council (NRC) or Agricultural Research Council (ARC)]. Unfortunately, commonly used dietary standards are based on temperate forage consumption data and, therefore, would over estimate the intake of minerals. It is generally accepted that tropical forages are less digestible than are temperate species, and, therefore, daily consumption by grazing ruminants is lower. For more accuracy, total grams of specific minerals consumed per day, and not forage concentrations, determine the true adequacy of a mineral. Likewise, relative adequacy based on forage mineral concentrations is dependent on interactions with other nutrient fractions, such as proteins, lipids, or other elements, that can greatly affect the availability of the respective elements for digestion, absorption, and retention (Egan, 1975; Abdel Rahim *et al.*, 1985 and Ammerman, 1995).

Forage analysis for certain trace minerals will be erroneously high due to the inherent problem of sampling forages free from contaminating soil. Mineral elements such as Ca, potassium (K), P, and Mo would not be greatly affected by soil contamination, since soil levels would be approximately equal to or less than plant material concentrations (Grace *et al.*, 1996 and Sheppard, 1998). In contrast, soil mineral levels of Co, Fe, I, sodium (Na), Mn and Se and, to a lesser extent, Zn and Cu, would be higher than forages, and even slight contamination caused by splashing rain could give an erroneously high impression of concentration of these elements (Healy, 1974). Mitchel (1963) indicated that soil contains 20-1000 times the Co and Fe content found in pastures grown on a particular soil.

**Examination of Tissues and Fluids:** Without question, forage analysis is a much better indicator of mineral status for ruminants than is soil analysis. Likewise, animal tissue-mineral concentrations are better indicators of the availability of minerals than are forage mineral analyses. Grazing livestock obtain part of their mineral supply from the consumption of water, soil, leaves, tree bark, etc. rather than entirely from forages. Livestock tissue-mineral concentrations, therefore, more accurately portray the contribution of the total environment in meeting the mineral requirements of grazing animals. As an illustration, neither the available Cu content of the soil nor the Cu content in the herbage show any positive relationship with the Cu status of the animal (Sutmoller *et al.*, 1966; Hartmans, 1970; Sutherland, 1980 and Minson, 1990). However, liver Cu concentrations of less than 25 ppm coincide with Cu deficiency signs in cattle (Sutmoller *et al.*, 1966 and Tokarnia *et al.*, 1961).

Animal tissue and fluid levels of minerals, in addition to concentrations of particular enzymes, metabolites, or organic compounds with which the mineral in question is associated functionally, are important indicators of mineral status. The diagnostic significance of tissue and fluid analysis is based upon evidence that mineral element deficiencies are ultimately reflected in subnormal concentrations of the element, in altered concentrations of related metabolites or in changed activities of affected enzymes. The concentrations of minerals in the tissues, or of their functional forms, such as thyroxine (I) and vitamin B12 (Co), must be maintained within narrow limits if the growth, health, and productivity of the animal are to be sustained. Departures from these normal limits, which are now well defined for most elements, therefore, constitute useful diagnostic indicators (Caple *et al.*, 1985; McDowell, 1985 and Wildeus *et al.*, 1992). A further valuable aspect of such tissue composition changes is that they frequently arise prior to the appearance of adverse clinical signs (Underwood, 1979).

Ideally, animal scientists would like to determine the mineral status of an animal by measuring the mineral content of one tissue that is readily available from a live animal (Conrad, 1978). Unfortunately, no mineral concentration of any one tissue or fluid will portray the status of all minerals. Blood, urine, saliva, milk, feces, and hair may be easily sampled, and even liver and bone may be routinely biopsied with a minimum of time and danger to the animal. Surgical techniques for the acquisition of biopsy samples of liver (Chapman *et al.*, 1963) and rib bone (Little, 1972, 1984) from cattle and sheep have been described illustrates the liver biopsy, and illustrates rib bone biopsy in cattle. Liver taken either by biopsy or from sacrificed animals is an excellent indicator of the status of certain trace elements (Judson and McFarlane, 1998) but bone is the preferred tissue for evaluating bone-forming minerals, particularly Ca and P (Williams *et al.* 1985).

The organ, tissue, or fluid chosen for analysis varies with the elements, but estimations of whole blood, plasma or serum trace element, or enzyme concentrations have wide applicability and do not, of course, require sacrifice of the animal. The levels of certain mineral elements in hair or wool, urine, and even in milk are also of value in the

detection of deficiency or toxicity states, although individual variability can be very high and external contamination provides problems for trace element status evaluation (Szabu *et al.*, 1999).

Whole blood or blood serum or plasma is widely used for studies in mineral nutrition. Values significantly and consistently above or below "normal" concentrations or ranges provide suggestive but not conclusive evidence of a dietary excess or deficiency of particular minerals (Underwood, 1981; McDowell, 1985; Wildeus, 1992). Precautions must be taken in interpreting blood mineral data collected or prepared in less than optimum conditions. Factors responsible for elevations of serum of plasma mineral include stress, exercise, hemolysis, temperature and serum separation time (Fick *et al.*, 1979). These factors have often been difficult to control in studies in Latin America and Africa and have resulted in high serum P concentrations compared to extremely low levels of forage P (McDowell *et al.*, 1984).

**Analyses Most Indicative of Mineral Status:** Since mineral analyses are complicated and expensive, it is important to select and analyze the minimum number of plant and animal tissues (or fluids) that are more indicative of mineral status of ruminants illustrates analyses of considerable value in assessing specific mineral deficiencies and toxicities. The word "critical" is used to note a concentration in forages below (or above with excesses) what is considered the requirement. This assumes the expected consumption of dry matter. Critical animal tissue concentrations are levels below or above values associated with specific clinical signs as reported in the literature. Fifteen mineral elements of major importance have been selected for a brief discussion on methods of diagnosis of the status in the grazing ruminant. Excellent reviews on this subject are by the NCMN (1973), Miller and Stake (1974), Egan (1975), Underwood (1979) and Underwood (1981).

**Calcium and Phosphorus:** Visual signs of borderline Ca and P deficiencies are not easily distinguishable from other deficiencies. Inadequate intake of Ca will cause weakened bones, slow growth, low milk production and tetany (convulsions) in severe deficiencies. Signs of P deficiency are not easily recognized except in severe cases when fragile bones, general weakness, weight loss, emaciation, stiffness, reduced milk production, lowered reproduction and chewing of wood, rocks, bones and other objects may be noticed. Abnormal chewing of objects ("pica") may occur, however, with other dietary deficiencies as well. Bone chewing may lead to death as a result of botulism. One of the earliest biochemical measurements of P deficiency is a reduction in serum inorganic P (Underwood, 1981). Values consistently below 4.5 mg/100 ml in cattle and sheep are an indication of P deficiency. However, the NCMN (1973) did not consider serum inorganic P to be sufficiently sensitive to recommend it in diagnosing problems with cattle since forage analyses give earlier and more detailed information. Even though serum Ca does decline with deficiency, especially in some species and ages of animals, the homeostatic or physiological mechanisms regulating it are more effective than for P or for most other minerals (Underwood, 1981). Normal serum Ca concentration is 9-12 mg/100 ml.

Serum P is a good indicator of P status of ruminants only if stress factors, hemolysis, temperature and serum separation time can be strictly controlled. Due to the limitations of serum P, Cohen (1973) concluded that bone parameters (i.e., Ca, P and ash) provide a more reliable method of assessing Ca and P status of cattle. Collection of bone biopsy samples is being widely used as a survey technique to locate mineral deficiencies in tropical regions (McDowell *et al.*, 1984)

**Magnesium:** Hypomagnesemic tetany (grass tetany or staggers) is a metabolic disturbance most commonly occurring in adult cows and ewes, especially those that are lactating heavily and grazing lush grass pastures. It is manifested by irritability, tetany and convulsions, followed by death. In many instances, animals on pasture are found dead without illness having been observed.

The clinical signs of tetany are caused by inadequate Mg in serum and other extracellular fluids. Generally, lactating cows exhibiting tetany will have serum Mg levels below 1.0 mg/100 ml (Underwood, 1981). Dutch workers (NCMN, 1973) concluded that daily urinary Mg excretion is an earlier indicator of Mg supply than serum concentrations, with 2-10 mg/100 ml indicating inadequacy. The chance of hypomagnesemia increases as the Mg content of forage falls and as the product of K and crude protein contents of herbage increase (through reduced availability of dietary Mg) (NCMN, 1973)

**Potassium:** Potassium deficiency is characterized by nonspecific signs such as slow growth, reduced feed and water intake, lowered feed efficiency, muscular weakness, nervous disorders, stiffness and emaciation. Evaluation of K deficiency is difficult. Low serum K analyses have some diagnostic value for establishing deficiencies, but these may also be caused by malnutrition, negative N balance, gastrointestinal losses and endocrine malfunction. Reduced feed consumption appears to be an early sign of inadequate dietary K. Because reliable evaluations of a K deficiency are not available, dietary K concentration appears to be the best indicator of K status.

**Sodium:** The initial sign of Na deficiency is a craving for salt, demonstrated by the avid licking of wood, soil and

sweat from other animals and by drinking water. A prolonged deficiency causes loss of appetite, decreased growth, unthrifty appearance, reduced milk production and loss of weight. Because of its rapid reaction to deficiency long before clinical signs appear, the best criterion for assessment of Na status is the concentration of Na and K in saliva. Deficiency causes a fall in Na and a rise in K. Skydsgaard (1968) suggested the normal Na:K ratio in saliva to be from 17:1 to 25:1 and suggested that if it is between 10:1 and 15:1, Na deficiency can be suspected.

**Sulfur:** Signs of sulfur (S) deficiency have been described as loss of weight, weakness, lacrimation, dullness, depressed milk production and death. In a S deficiency, microbial protein synthesis is reduced and the animal shows signs of protein malnutrition. A lack of S also results in a microbial population that does not utilize lactate; therefore, lactate accumulates in the rumen, blood and urine. It is difficult to diagnose a deficiency, especially a borderline one. Serum sulfate levels have been suggested as an indicator of S deficiency, but blood lactate and dietary S levels may be the most reliable indicators of S status.

**Cobalt:** Clinical signs of Co deficiency in grazing ruminants are not specific. Deficient animals show a normocytic, normochromic anemia with a concomitant loss of appetite, retarded growth, general emaciation, rough hair coat and loss of milk production.

Both soil (extracted with acetic acid, 2.5%) and forage Co concentrations of less than 0.1 ppm are considered low. The levels of Co in the livers of sheep and cattle are sufficiently responsive to changes in Co intake to have value in the detection of Co deficiency, with liver vitamin B12 and even more reliable criterion. Values of 0.10ug vitamin B12/g wet weight or less are "clearly diagnostic of Co deficiency disease" (Underwood, 1979). Subnormal plasma glucose, vitamin B12 and elevated blood pyruvate levels are good indicators of Co deficiency in ruminants, together with loss of appetite (MacPherson *et al.*, 1976). While herbage and tissue analyses are helpful in diagnosing the deficiency, the definite proof is the prompt improvement in feed intake following the feeding of Co.

**Copper and Molybdenum:** Copper deficiency in cattle is characterized by poor growth, anemia, bone fragility, loss of hair color, diarrhea and myocardial fibrosis. Milk production and body condition are poor, fertility is low and calves may show congenital rickets. With sheep, demyelination of certain tracts in the fetal and neonatal nervous system results in incoordination, immobilization, blindness and death, with the disease known as swayback or enzootic ataxia. With sheep, bone fragility may occur and wool loses pigmentation and crimp (a condition known as steely wool).

In some regions, excessive dietary Mo and S induce a Cu deficiency (conditioned deficiency). Frank cases of Motoxicity occur on pastures containing an excess of the element and are characterized by a profuse scouring. The determination of Cu in the diet has limited diagnostic value and can, in fact, be seriously misleading unless other elements with which Cu interacts, including Mo and S in particular, are determined also. The criteria most widely used for Cu deficiency are the concentrations of Cu in liver and blood (Underwood, 1981). Plasma Cu can indicate a deficiency but does not reflect higher "marginal safety" liver storage (Hartmans, 1974). Copper status of cattle and sheep can be readily ascertained from serum ceruloplasmin activity, a Cu-containing enzyme. A high percentage of the plasma Cu exists as ceruloplasmin, with high correlations between serum Cu and ceruloplasmin activity reported (Miller and Stake, 1974).

Chronic Cu toxicity signs in ruminants include suddenly depressed appetite, jaundice, blood in urine, sudden debility and death (NCMN, 1973). Copper content in the liver of poisoned cattle and sheep is always in excess of 700 ppm (NCMN, 1973).

**Flourine:** The most sensitive index of the toxic fluoride effect is the mottling, staining and excessive wearing of the permanent teeth formed during the time of excessive fluoride ingestion. Lameness caused by bony exostoses may result.

The remarkable capacity of the bone to sequester fluoride can be gauged from the fact that F concentrations in compact bone below 4500 ppm are considered to be innocuous, with a saturation point on the order of 15,000-20,000 ppm (Suttie *et al.*, 1958). Fluoride concentrations of tail bones represent a valuable means of detecting bovine fluorosis (Suttie, 1967). In cows, 20-30 ppm in the urine is considered to indicate borderline F toxicity and over 35 ppm of F to be indicative of systemic signs of toxicity (Shupe *et al.*, 1963). Carlson (1966) reports that 0.2 ppm of F can be considered a critical plasma concentration in young cattle.

**Iodine:** A simple I deficiency results in an enlargement of the thyroid gland (goiter), with newborn more likely to be affected than adults. Calves or lambs may be stillborn, weak and hairless, with irregular or suppressed estrus, or fetal development may be arrested with death, resorption, or abortion. Bulls may exhibit a decline in libido and a deterioration in semen quality.

Goitrogenic substances occur frequently and increase the animals' requirement for I. Consequently, the I content of the diet has limited value in diagnosing a deficiency problem. Serum protein-bound-I values in adult cattle less

than 3-4 ug/dl or total serum I values less than 5-10 ug/dl may indicate an inadequate I intake. Milk I levels less than 100 ug/dl are associated with inadequate intake (Hemken *et al.*, 1972).

**Iron:** Iron deficiency is rarely of practical concern for grazing livestock except when blood is lost from parasitic infestation or disease. Deficiency results in a hypochromic, microcytic anemia with low serum Fe, increased total serum Fe binding capacity and a decreased transferring saturation (Underwood, 1977). Low hemoglobin and hematocrit values are not sensitive indicators of early Fe deficiency stages because they do not occur until storage is depleted severely. Many consider percent saturation of transferring to be the most practical means of detecting Fe deficiency in its early stages (Underwood, 1977).

**Manganese:** Manganese deficiency in ruminants results in skeletal abnormalities, delayed estrus, reduced fertility, abortions and deformed young. Calves have deformed legs with "over-knuckling" and enlarged joints and grow poorly. Deficient heifers are slower to exhibit estrus and to conceive.

The Mn content of most body tissues is remarkably resistant to change with low intake (Underwood, 1977). However, some reduction occurs in liver, bones and hair. McDowell *et al.* (1984) concluded that a Mn deficiency can best be detected by the combination of liver (<6 ppm) and forage (<25 ppm) analyses and a toxicity is suspected when hair samples contain over 70 ppm Mn.

**Selenium:** The predominant Se deficiency disease in young ruminants is nutritional muscular dystrophy or white muscle disease. Affected lambs or calves exhibit difficulty in standing. White muscle disease can be diagnosed by gross and histological examination of the affected muscles. However, it is possible to measure serum glutamic-oxalacetic transaminase (SGOT) which is markedly elevated in calves and lambs with white muscle disease. The units of the enzyme per milliliter ranged from 295 to 3,460 for lambs and calves with white muscle disease and only 22-191 in normal animals (Blincoe and Dye, 1958).

The Se concentrations in the tissue of animals reflect the dietary Se level over a wide range from different to toxic intakes. The kidney and the liver are the most sensitive indicators of the Se status of the animal and the Se concentrations in these organs can provide valuable diagnostic criteria. The most widely used assessment of Se status is blood Se concentrations. Low blood Se is always found in Se-deficient conditions. A direct relationship between blood glutathione peroxidase (a Se-containing enzyme) activity and Se concentrations has been established. Since Se is deposited in all the tissues of the body, except fat, of animals consuming seleniferous feeds, high concentrations of the element provide indisputable evidence of an excessive intake.

**Zinc:** Early effects of Zn deficiency include reduced feed intake, growth rate and feed efficiency and skin disorders. Clinical signs include alopecia, general dermatitis of the neck and head, listlessness, reduced testicular growth, swollen feet and wounds that fail to heal properly.

Under experimental conditions, many biochemical changes have been identified in severely Zn-deficient animals (Miller and Stake, 1974). Those with the most promising diagnostic value are the Zn concentrations in plasma, hair and bone and alkaline phosphatase content of plasma or other tissues (Blackmon *et al.*, 1967). Serum of plasma Zn is greatly and quickly reduced in animals fed a severely deficient diet (Miller and Stake, 1974).

**Interactions:** One of the main factors affecting bio-availability of trace elements is interactions, both antagonistic and synergistic, between minerals. This is highlighted by the well-known wheel of interactions between minerals. However, such interactions are often not simple, direct or easily predictable. Judson and McFarlane (1998) pointed out the difficulty in applying Suttle and McLauchlan's formula (ARC, 1980) for predicting availability of dietary copper based on its interaction with molybdenum plus sulphur, because iron at concentrations of between 500 and 6000 mg/kg feed inhibits the availability of copper as well. To this could be added the other minerals interacting with copper, such as zinc, sulphur independent of molybdenum and cadmium. Such multiple interactions probably exist in the metabolism of most, if not all trace minerals. The different chemical forms and valence states of elements differ in bio-availability. Minerals in the organic form do not react to other ions in the same way as inorganic minerals. Consequently, a completely different scenario in terms of requirements and conditions for optimum supply exists where minerals present in organic compounds, are fed (McDowell, 1996). Differences in absorption of an element will exist when it is ingested as part of a diet or on an empty stomach, e.g. elements in drinking water (Mertz, 1995). This would be relevant especially in monogastric animals and hind-gut fermenters. Many non-nutritional factors can affect mineral metabolism, e.g. gastrointestinal parasites (Judson & McFarlane, 1998), genetic differences among breeds and individuals age, etc. (Suttle, 1994).

**Supplementation of Trace Minerals:** White (1996) pointed out that large differences exist between authorities with respect to recommended requirements, mainly because of the differences in absorption coefficient used and whether or not a safety factor has been included to meet the requirements of practically all animals. Recommended

requirements are thus well above minimum requirements, usually two standard deviations above the mean. It is obvious that it is impossible to state rigid requirements for livestock. Underwood and Suttle (1999) proposed the use of "marginal bands" to cover the range between adequate and inadequate. This permits the use to allow for the inevitable uncertainties surrounding precise mineral needs in a particular context. As the number of individuals falling within the marginal band increases, so does the likelihood of a significant proportion of the population benefiting from mineral supplementation. An important decision of the animal nutritionist is to decide which minerals and how much of each should be supplemented. Except for a knowledge on the mineral requirements of the animal, as much as possible information on the quantity of each mineral, including antagonists, supplied through the animal's feed and water should be obtained and be used.

A "shotgun" approach can be used when no information at all is available on the trace mineral supply situation in a specific area, attempts should be made to obtain such information. It seems advisable that the decision on the supplementation of trace minerals to grazing animals should be based on a knowledge of the mineral status of the animals in the specific region. General deductions can be made if the soil type, soil pH and type of vegetation are known (Judson and McFarlane, 1998). McDowell (1996) suggested that for most regions it would be appropriate to include selenium, unless toxicity problems have been observed; that iron could be supplemented in temperate region, but that both iron and manganese could be excluded in regions with acid soils. He recommended that, where internal parasitism is a problem, iron supplementation might be beneficial. It can be accepted that it is likely that animals in region with calcium rich soils may suffer from a deficiency of zinc and manganese. However, exceptions exist, e.g. in the areas of the world, where both manganese and limestone are mined, the soil and even the limestone may contain high concentrations of manganese (Van Ryssen, 1992). As the knowledge of the mineral nutritional situation in specific regions is expanding, regions can be mapped according to probable mineral status of the grazing animals there. The information for such maps can be arrived from soil mineral content, plant mineral concentrations or mineral status of the animals themselves, e.g. Old field (1999) put together all available information on selenium status worldwide. Van Tonder (1986) summarized the problem of copper toxicity in the Karoo; Boyazoglu (1976) attempted to identify mineral deficient areas based on the mineral concentrations in the livers of grazing animals; etc.

Laboratory and experimental techniques to measure mineral concentrations and enzyme activities improved, or the situation in an area might have changed. This could be due to the wider distribution of feeds between regions, which would cancel out regional problems, farming practices changed or mining activities and other forms of pollution could have changed the situation in an affected area. Examples of these are; acid rain and other forms of pollution could increase the occurrence of selenium and copper deficiencies near factories and power generating plant; copper pollution occurred in the vicinity of the copper mine; the recent occurrence of copper and selenium deficiencies in beef cattle in Natal was suggested to be the result of the exclusion of chicken litter as a winter supplement to the animals; etc. Furthermore, the contribution of water to the mineral intake of animals has largely been ignored and under estimated. Different animal species are becoming important, e.g. in game farming where parameters for mineral status are largely unknown, e.g. the Tsessebe seems to be prone to selenium deficiency. Indicators of mineral status it is important to ensure that the criteria used to try to establish the mineral status of animals would supply reliable results. Care must be taken not to waste money and time on collecting and measuring tissues and material which are worthless as indicators of mineral status (Van Ryssen, 1997). It is interesting to note that liver analyses, which have been used widely for all possible minerals, are considered worth analyzing only to determine the copper, selenium and vitamin B<sub>12</sub> status of animals. Even with the measurements suggested, it is important to interpret the result correctly, because some measurements are reliable only within certain limits of mineral intake or mineral status of the animal (Painter, 1996).

Arthur (1999) published a number of functional measures to predict the iodine and selenium status of the body. Analytical kits have been developed to monitor both individual antioxidant components and the overall status of the antioxidant system in the body (Randox Laboratories brochure). Techniques have been developed and are used also in human health studies where total oxidative damage, oxidative damage to lipids and proteins are measured (Aruoma, 1997). The developments are sure to be taken over in investigations on the trace nutrient status of farm animals and should contribute to the development of a better basis to predict the nutrient status of animals and to supply supplements accordingly.

**Common Mineral Disorders:** Signs of mineral disorders commonly observed in livestock at pasture together with predisposing causes of diseases are numerous. In a few instances, these disorders lead to clinical signs that are specific, such as grass tetany due to Mg<sup>2+</sup> deficiency and enzootic ataxia (swayback) in lambs due to Cu<sup>2+</sup> deficiency. In most instances, however, the signs are not specific to any one deficiency and may only be evident as an ill thrift condition. Economically, these non-specific disorders are of considerable significance because production is depressed and in marginal deficiencies may go unnoticed and uncorrected (Judson and McFarlane, 1998).

**Major Minerals:** Phosphorus and S deficiencies are potentially diseases of economic significance in the more productive areas but are only occasionally seen, presumably because of frequent applications of fertilizers and the use of grain and hay supplements. In the pastoral areas, P deficiency is a major problem affecting cattle (McCosker and Winks, 1994): it is generally assumed that sheep at pasture are not susceptible to P deficiency. Live weight and reproductive responses in cattle to P supplementation have usually been observed in the wet season in the northern areas and not in the dry season when pasture P is usually at its lowest concentration: nitrogen (N) and available energy intakes are often the primary nutrients limiting responses to P supplementation when cattle are on dry pasture. Livestock in northern Australia are also at risk of S and  $\text{Na}^+$  deficiencies (Gartner *et al.*, 1980; Winter and McLean, 1988). Sodium deficiency is suspected in cattle in restricted areas of South-Eastern Australia, especially on pastures receiving K fertilizers (Harris *et al.*, 1986).

It was generally assumed that livestock at pasture were unlikely to be at risk of  $\text{Ca}^{2+}$  deficiency because when herbage was low in  $\text{Ca}^{2+}$  it was likely to be low in other nutrients whose nutritional effects would overshadow any inadequacy in  $\text{Ca}^{2+}$  (Underwood, 1981). However acute hypocalcaemia is a common problem in dairy cows in early lactation (Harris 1981; Judson and McFarlane, 1998) and in South-Eastern Australia in pregnant ewes lambing in winter and spring (Grant *et al.*, 1988). It is suspected that an imbalance of the major cations and anions of the diet predisposes lactating cows and pregnant ewes to hypocalcaemia. Chronic calcium deficiency causing reduced growth rate has also been reported in sheep fed grain and little roughage during drought (Peet *et al.*, 1985). Magnesium deficiency (grass tetany) usually occurs in lactating cows grazing grass-dominant pastures in late autumn and in winter in south-eastern Australia (leaver 1972; Harris *et al.*, 1983; Lewis and Sparrow, 1991). The disorder is uncommon in sheep and is not found in cattle grazing tropical pastures.

**Grass Tetany:** Grass tetany (Hypomagnesaemia) in lactating cows can result from reduced feed intake but it is often the outcome of interactions between several dietary constituents which reduce the availability of  $\text{Mg}^{2+}$ . The disorder is usually observed in cows on lush green pasture or cereal crops of high N content ( $>40$  g/kg Dm) and with  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Na}^{2+}$  concentrations of less than 2.0, 3.0 and 1.5 g/kg DM, respectively and  $\text{K}^+$  concentrations above 30 g/kg DM (Mayland and Grunes, 1979; Caple and West, 1993); the high  $\text{K}^+$  concentrations may also predispose the cow to metabolic alkalosis and increase the risk of hypocalcaemia (NRC, 1989).

Pasture analyses can assist in the resolution of factors likely to be important in reducing Mg utilization at specific locations and permit recommendations of the most appropriate management strategies to prevent the disorder. It is found that the incidence

of grass tetany increased markedly in cattle when the  $\text{K}^+ : (\text{Ca}^{2+} + \text{Mg}^{2+})$  ratio in herbage, expressed on an equivalence basis, was above 2.2. These findings were supported in a survey in winter of cattle on annual and perennial grass species on a range of soil types in the south-east of South Australia (Lewis and Sparrow, 1991). Lewis and Sparrow (1991) proposed that the  $\text{K}^+ : (\text{Ca}^{2+} + \text{Mg}^{2+})$  ratio in soil, calculated on an equivalent basis for the soil-extractable cations, was also indicator of risk with values above 0.07-0.08 associated with grass tetany in cattle.

The  $\text{K}^+ : (\text{Ca}^{2+} + \text{Mg}^{2+})$  ratio may not be appropriate for pastures of high  $\text{Ca}^{2+}$  content or for pastures where the high N content may have an adverse effect on  $\text{Mg}^{2+}$  utilization. A nomogram has been prepared in the Netherlands which predicts blood serum  $\text{Mg}^{2+}$  concentration in cattle from  $\text{Mg}^{2+}$ ,  $\text{K}^+$  and crude protein concentrations in the pasture (Mayland and Grunes, 1979); the nomogram shows that estimated serum  $\text{Mg}^{2+}$  decreases as the  $\text{Mg}^{2+}$  content of the pasture decreases and as the product of the  $\text{K}^+$  and crude protein content increases. This approach has been used to rank the tetany hazard of forages in the USA (Mayland *et al.*, 1976; Judson and McFarlane, 1998).

**Milk Fever:** Milk fever (Parturient paresis) in dairy animals is caused by a temporary imbalance between  $\text{Ca}^{2+}$  availability and high  $\text{Ca}^{2+}$  demand following the onset of lactation (Oetzel, 1996). Calcium leaves the extracellular fluid to enter the mammary gland faster than it can be replaced by intestinal  $\text{Ca}^{2+}$  absorption or bone  $\text{Ca}^{2+}$  resorption (Goff and Horst, 1993). Despite much research, milk fever incidence has remained steady in the United States at 8 to 9%. Milk fever is an economically important disease and can reduce the productive life of a dairy cow by 3.4 years. Each case of milk fever leads to a loss of \$334 to the producer by way of treatment charges and milk loss (Horst *et al.*, 1997). If left untreated, about 60 to 70% of cows die.

Aged cows are at the greatest risk of developing milk fever. Heifers almost never develop milk fever. Older animals have a decreased response to dietary  $\text{Ca}^{2+}$  stress due to both decreased production of  $1,25\text{-(OH)}_2\text{D}_2$  and decreased response to the  $1,25\text{-(OH)}_2\text{D}$  target tissues of cows with milk fever may have defective hormone receptors and the number of receptors declines with age. In older animals, fewer osteoclasts exist to respond to hormone stimulation, which delays the ability of bone to contribute  $\text{Ca}^{2+}$  to the plasma  $\text{Ca}^{2+}$  pool. The aging process is also associated with reduced renal  $1\alpha$ -hydroxylase response to  $\text{Ca}^{2+}$  stress, therefore reducing the amount of  $1,25\text{-(OH)}_2\text{D}$  produced from 25-OHD (Goff *et al.*, 1991).

Special  $\text{Ca}^{2+}$  and P supplementation is required for high-producing dairy cows to prevent parturient paresis.

Parturient paresis can be prevented effectively by feeding a prepartum diet low in  $\text{Ca}^{2+}$  and adequate in P. Prepartal low- $\text{Ca}^{2+}$  diets are associated with increased plasma parathyroid hormone (PTH) and  $1,25\text{-(OH)}_2\text{D}_2$  and  $1,25\text{-(OH)}_2\text{D}_3$  concentrations during the prepartal period. These increased PTH and  $1,25\text{-(OH)}_2\text{D}$  concentrations resulted in "prepared" and effective intestinal and bone  $\text{Ca}^{2+}$  homeostatic mechanisms at parturition that prevented parturient paresis.

Supplemental vitamin D has been used to prevent parturient paresis in dairy cows for a number of years. Treatment with high levels of vitamin D has been successful, but toxicity problems have sometimes resulted and for some animals, the disease has been induced by treatment. Hodnett *et al.* (1992) used a combination of  $25\text{-(OH)}\text{D}_3$  plus  $1\alpha$ -hydroxycholecalciferol to reduce parturient paresis in dairy cows fed high dietary Ca. The incidence of the disease was reduced from 33 to 8%.

Anion-cation balance of prepartum diets (sometimes referred to as acidity or alkalinity of a diet) can also influence the incidence of milk fever (Gaynor *et al.*, 1989; Horst *et al.*, 1997; Pehrson *et al.*, 1999; Vagnon and Oetzel, 1998). Diets high in cations, especially  $\text{Na}^+$  and  $\text{K}^+$ , tend to induce milk fever, but those high in anions, primarily  $\text{Cl}^-$  and  $\text{S}$ , can prevent milk fever. The incidence of milk fever depended on the abundance of the cations  $\text{Na}^+$  and  $\text{K}^+$  relative to the anions  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ . This concept is now generally referred to as the cation anion difference (CAD). Because most legumes and grasses are high in  $\text{K}^+$ , many of the commonly used prepartum diets are alkaline. There are large variations in the mineral content of roughages fed on different farms and that the mineral content of grass. And consequently, the CAD of a diet can be significantly altered by different types of fertilization (Pehrson *et al.*, 1999). Addition of anions to a prepartal diet is thought to induce in the cow a metabolic acidosis, which facilitates bone  $\text{Ca}^{2+}$  resorption and intestinal  $\text{Ca}^{2+}$  absorption (Horst *et al.*, 1997). Diets higher in anions increase osteoclastic bone resorption and synthesis of  $1, 25\text{-(OH)}_2\text{D}_3$  in cows (Goff *et al.*, 1991). Both of these physiologic processes are controlled by PTH. Workers at the Rowett Research Institute (Abu Damir *et al.*, 1994) have also recently reported that  $1,25\text{-(OH)}_2\text{D}_3$  production is enhanced in cows fed acidifying diets.

Collectively, these data suggest that a major underlying cause of milk fever is metabolic alkalosis, which causes an inability of cow tissues to respond adequately to PTH (Horst *et al.*, 1997). This lack of response in turn reduces the ability of the cow to draw on bone  $\text{Ca}^{2+}$  stores and production of the second  $\text{Ca}^{2+}$ -regulating hormone,  $1,25\text{-(OH)}_2\text{D}$ , which is needed for active transport of  $\text{Ca}^{2+}$  within the intestine. The presumption is that metabolic alkalosis somehow disrupts the integrity of PTH receptors on target tissues. Low CAD diets prevent metabolic alkalosis, increasing target tissue responsiveness to PTH, which controls renal  $1\alpha$ -hydroxylase and resorption of bone calcium.

Several options exist regarding methods for the control of milk fever (Horst *et al.*, 1997). The current understanding of the CAD concept suggests that milk fever could be managed more effectively if dietary  $\text{K}^+$  as reduced (Goff and Horst, 1997). Calcium chloride has been used to reduce blood pH (Dhiman and Sasidharan, 1999; Schonewille *et al.*, 1999). This reduction is beneficial but excessive oral calcium chloride can induce metabolic acidosis (Goff and Horst, 1994), which can cause inappetence at a time when feed intake is already compromised. Dietary acidity can be monitored via the pH of urine, which should be below 7.5. Calcium propionate treatment has been beneficial in reducing subclinical hypocalcemia in all trials and reduced the incidence of milk fever in a herd having a problem with milk fever (Goff *et al.*, 1996 and Pehrson *et al.*, 1998). Commercial preparations of HCl mixed into common feed ingredients as a premix could offer an inexpensive and palatable alternative to anionic salts as a means of controlling the incidence of milk fever in dairy cows (Goff and Horst, 1998).

Treatment of milk fever returns serum Ca concentration to the normal range and must be carried out at the earliest possible opportunity to avoid muscular and nervous damage of downer cows. This is facilitated by maintaining close surveillance over cows that have calved in the preceding 72 h. Calcium borogluconate is most commonly used, with  $\text{Mg}^{2+}$  added to the injectable  $\text{Ca}^{2+}$  preparation when hypomagnesemia is in evidence. The produce is preferably administered intravenously for rapid response, but subcutaneous administration permits slow absorption of the  $\text{Ca}^{2+}$  ion and may lessen the danger of cardiac arrest.

Trace Mineral: For sheep and cattle in southern Australia,  $\text{Se}^{2+}$  deficiency is a serious problem in all states particularly in regions of high rainfall (>500 mm annually) and  $\text{Co}^{2+}$  deficiency is of importance in Western Australia and South Australia and in restricted areas of Tasmania and Victoria. Copper deficiency occurs in all states,  $\text{Zn}^{2+}$  deficiency is of concern in Western Australia and I deficiency occurs in defined areas of Victoria, Tasmania and New South Wales (Caple and McDonald, 1983; Hosking *et al.*, 1986 and Judson *et al.*, 1987). Only one field case of  $\text{Mn}^{2+}$  deficiency in grazing animals has been documented, associated with infertility of ewes at Moorlands in South Australia (Egan, 1972).

Trace element deficiencies found or suspected of limiting livestock productivity in northern Australia include  $\text{Cu}^{2+}$  deficiency over extensive areas of Queensland and the Northern Territory (Gartner *et al.*, 1980; Murphy *et al.*, 1981; Wesley-Smith and Schlink 1990),  $\text{Co}^{2+}$  deficiency along the coastal areas of Queensland (Nicol *et al.*, 1983 and Rosbrook *et al.*, 1992) and in restricted areas of the Northern Tablelands of New South Wales (Duncan *et al.*, 1986), Se deficiency in the central and coastal areas of Queensland and I deficiency in north-central Queensland (Knights *et al.*, 1979).



The potential magnitude of the trace element problem in affecting livestock productivity can be derived from a survey of South Australian cattle in 1989-91 (Koh *et al.*, 1993). During this period livers for trace element assay were collected from more than 9000 cattle slaughtered in commercial abattoirs. This survey showed that 22% of the cattle were at risk of  $\text{Cu}^{2+}$  deficiency, 19% of  $\text{Se}^{2+}$  deficiency and 6% of  $\text{Co}^{2+}$  deficiency. In some regions more than 50% of the cattle were estimated to be at risk of  $\text{Cu}^{2+}$  or  $\text{Se}^{2+}$  deficiency. Animals most susceptible to trace element deficiencies are young rapidly growing animals and animals during their first pregnancy and lactation. Sheep appear to be more susceptible to  $\text{Se}^{2+}$  and  $\text{Co}^{2+}$  deficiency and less susceptible to  $\text{Cu}^{2+}$  deficiency than cattle.

The requirement of an animal for a trace mineral is based on the assumption that all other minerals and interfering components are present at specific required levels. Any deviation would change the requirement for the specific mineral. Underwood and Suttle (1999) stated that: "Ideally diagnostic limits (like dietary requirements) should be set on the assumption that the supply of other nutrients is non-limiting". White (1996) pointed out that the critical value for a mineral requirement is determined experimentally with all other nutrients and conditions non-limiting and within the normal physiological range. Trace element requirements are a function of some predetermined measure of response (White, 1996). However, the criteria and critical values accepted as requirements are continuously changing because of the following reasons (Corah, 1996):

(i) The dramatic improvement in productivity of livestock because of the genetic improvement of farm animals and refinements in management, health control and nutrition;

(ii) Major advances in technology which led to new insight in the role and function of micronutrients, e.g. their role in the immune system of the animal.

(iii) Major advances in dissipating technology and transmitting information and in tracing of new information.

According to Mertz (1995) the use of new analytical methods to induce trace element deficiencies and the development of reliable "trace" analyses enable nutritionists and toxicologists to apply much stricter criteria than in the past of develop criteria for trace element requirements. As a consequence, estimates of nutrition requirements rose and those of toxicological limits declined. Determination of a requirement will be affected also by the prevailing conditions or antagonists at the test site. In New Zealand, compared to other countries, relatively low levels of selenium supplementation overcome a deficiency of the mineral (Grace and Clark, 1991). Requirements and blood selenium concentration as a criterion of deficiency are lower than in countries such as the United States. This lower need for selenium could be due to high levels of vitamin E in the pasture or a low challenge to selenium dependent functions in the animal. There are numerous potential sites for trace elements to affect immune function. Virtually every aspect of immunity involves  $\text{Zn}^{2+}$ . A nutritional deficiency of  $\text{Zn}^{2+}$  is consistently associated with increased morbidity and mortality (Kincaid, 1999). The immune response to many pathogens cause a rapid decline in blood  $\text{Zn}^{2+}$ , perhaps a 50% drop within a few hours. Zinc deficiency is associated with reduced phagocytosis and killing by macrophages. Zinc deficiency results in a decrease in blood lymphocyte population (Fraker and King, 1998) and atrophy of the spleen and thymus. The responsiveness of T-lymphocytes to mitogens (Droke *et al.*, 1993) and the cytokines (Tanaka *et al.*, 1990) is inhibited in zinc-deficient animals. Zinc also is important in B-cell activation. Calves fed zinc methionine had greater antibody response against bovine herpesvirus (Spears *et al.*, 1991). Zinc as Zn methionine has reduced somatic cell counts (SSC) about 22% in some trials (Kincaid *et al.*, 1999).

Copper deficiency reduces the number of circulating T cells, B cells and neutrophils. Impairment of bactericidal activity can occur early in the development of  $\text{Cu}^{2+}$  deficiency in cattle and sheep. Copper-deficient sheep had increased mortality from bacterial infection (Chew, 2000). Antibody titers to *Brucella abortus* and proliferation responses to concanavalin A and soluble antigen-stimulated mononuclear cells were lower in  $\text{Cu}^{2+}$ -deficient heifers (Cerone *et al.*, 1995). Ceruloplasmin, an acute-phase protein, did not have the normal post-inoculant (bovine herpesvirus-1) increase in  $\text{Cu}^{2+}$ -deficient calves (Arthington *et al.*, 1996).

Selenium deficient animals have impaired bactericidal activity. Selenium, as part of the enzyme glutathione peroxidase, protects the cytosol against peroxides produced during respiratory burst. There may be an increase in reactive oxygen species in Se deficiency and this could affect immune responses (Kincaid, 1999). Accordingly, cows supplemented with  $\text{Se}^{2+}$  have neutrophils in milk with increased intracellular kill of bacterial and reduced extracellular hydrogen peroxide concentrations. Mean plasma selenium concentration was inversely correlated with bulk tank (Weiss *et al.*, 1990).

**Mineral Toxicities:** Animals may become exposed to toxic concentrations of minerals in a variety of ways including through the feed, drinking water, ingestion of soil and by administration as a therapeutic substance (Howell, 1996). The more commonly observed toxicities in the grazing animal include fluorosis in sheep and cattle in Northern Australia and  $\text{Cu}^{2+}$  toxicity in sheep in Southern Australia. Chronic fluorosis causing dental lesions, lameness and ill thrift in livestock has been associated with the consumption of artesian waters with high F (> 5 mg/L) and the feeding of P supplements of high F content (Jubb *et al.*, 1993). Significant quantities of F may accumulate in livestock on pasture receiving heavy applications of P fertilizer (Mason *et al.*, 1989). In these situations the intake

of soil may be the significant source of the ingested F since plant uptake of soil  $\text{Fe}^{2+}$  appears to be low (McLaughlin *et al.*, 1997). Some P supplements and fertilizers also contain significant levels of cadmium (Cd) and although their use may not be detrimental to the grazing animal may result in Cd concentrations in offal and meat exceeding the maximum permitted level for human consumption (Murphy *et al.*, 1992 and Morcombe *et al.*, 1994). Hosking *et al.*, (1986) have described 2 forms of chronic  $\text{Cu}^{2+}$  toxicity affecting sheep in Victoria. The main form occurs in sheep ingesting heliotrope. The resulting pyrrolizidine alkaloid damage to the liver from the heliotrope is associated with excessive accumulation of  $\text{Cu}^{2+}$  in the liver; a similar situation exists in Western Australia for sheep grazing lupin stubble infected with the hepatotoxin phomopsin (Allen *et al.*, 1979). The other form of chronic  $\text{Cu}^{2+}$  toxicity occurs in sheep on pasture of high subterranean clover content (Hosking *et al.* 1986). The toxic pastures generally have  $\text{Cu}^{2+}$  concentrations of 10-20 mg/kg DM and low Mo concentrations ( $<0.2$  mg/kg DM). Signs of toxicosis include jaundice, presence of haemoglobin in urine and dull and lethargic appearance. Molybdenosis in cattle resulting in scours, reduced growth rate, weight loss, infertility and harsh dry coats can occur on pastures of high Mo content. The signs are associated with induced  $\text{Cu}^{2+}$  deficiency. Acute selenosis resulting in rapid death has been observed in sheep grazing a highly seleniferous area in North-West Queensland; the area supported vegetation with  $\text{Se}^{2+}$  concentrations as high as 4000 mg/kg DM. Manganese toxicity has been suspected in sheep grazing lupins in Victoria but not on pasture with  $\text{Mn}^{2+}$  concentrations in excess of 5900 mg/kg DM (Hosking *et al.*, 1986) It is possible that high  $\text{Fe}^{2+}$  concentrations in these pastures may have reduced the toxic effects of excess  $\text{Mn}^{2+}$  because of the mutual antagonism between the 2 elements during absorption.

**Dietary Interactions:** One of the main factors affecting bio-availability of elements is interactions, both antagonistic and synergistic, between minerals. This is highlighted by the well-known wheel of interactions between minerals. However, such interactions are often not simple, direct or easily predictable. Judson and McFarlane (1998) pointed out the difficulty in applying Suttle and McLaughlan's formula (ARC, 1980) for predicting availability of dietary copper based on its interaction with molybdenum plus sulfur, because iron at concentrations of between 500 and 6000 mg/kg feed inhibits the availability of copper as well. To this could be added the other minerals interacting with copper, such as zinc, sulfur independent of molybdenum and cadmium. Such multiple interactions probably exist in the metabolism of most, if not all trace minerals.

The different chemical forms and valence states of elements differ in bio-availability. Minerals in the organic form do not react to other ions in the same way as inorganic minerals. Consequently, a completely different scenario in terms of requirements and conditions for optimum supply exists where minerals present in organic compounds are fed (McDowell, 1996). Differences in absorption of an element will exist when it is ingested as part of a diet or on an empty stomach, e.g. elements in drinking water (Mertz, 1995). This would be relevant especially in monogastric animals and hind-gut fermenters. Many non-nutritional factors can affect mineral metabolism, e.g. gastrointestinal parasites (Judson and McFarlane, 1998), genetic differences among breeds and individuals (Suttle, 1994), age, etc. A synergistic or complementary role between vitamin E and selenium in glutathione peroxidase is well documented. This interaction does not displace the requirements for each nutrient, but high vitamin E levels in pastures may lower the dietary requirements for selenium (Judson and McFarlane, 1998). It is hypothesized that vitamin E and selenium form part of an integrated system of antioxidants which protect the body against harmful end-products of metabolism, inflammatory reactions and foreign pollutants, in general called "free radical" (Aruoma, 1997). The antioxidant system comprises of several components. Preventative antioxidants, e.g. caeruloplasmin (Cu), metallothioneine (Cu), albumin (Cu), transferrin (Fe), ferritin (Fe) and myoglobin (Fe), preventing the formation of new "Reactive Oxygen Species" (ROS), such as  $\text{O}_2$ , OH and other non-radical oxygen derivatives such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hypochlorous acid (HOCl).b Scavenging antioxidants which remove ROS, once formed, thus preventing radical chain reactions: They consist of enzymes such as superoxide dismutase ( $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ) glutathione peroxidase ( $\text{Se}^{2+}$ ), glutathione reductase, catalase, metalloenzymes and small molecules such as glutathione, ascorbate (vitamin C), tocopherol (vitamin E), bilirubin, uric acid, carotenoids and bioflavonoids (Radox Laboratories Brochure). Although individual antioxidants do not necessarily act against each kind of free radical, their protection of the body is a combined action of all, where one may alleviate the need for another.

The form of the mineral and the presence of other minerals or organic constituents in the diet (Ammerman *et al.*, 1995) can markedly affect the requirements of dietary minerals. These interactions are difficult to quantify in the field and may involve 2 or more dietary constituents.

It is generally recommended that diets of livestock should have Ca:P ratios of about 1:1 to 2:1 (Underwood, 1981). Livestock will tolerate dietary Ca:P ratios of more than 10:1 without ill effect provided that the P intakes are adequate (Ternouth, 1990). The cation-anion balance of the diet can affect the ability of the dairy cow to utilize dietary  $\text{Ca}^{2+}$  and tissue  $\text{Ca}^{2+}$  reserves in meeting the demands of lactation.

Elements whose physical and chemical properties are similar may act antagonistically to each other (Ammerman *et al.*, 1995). For example, increasing the intake of dietary  $\text{Zn}^{2+}$  protects sheep against the onset of Cu toxicity (Bremner *et al.*, 1976) and it was suspected that  $\text{Cu}^{2+}$  deficiency in sheep grazing near an industrial smelter was probably induced as a result of excessive intakes of Cd and  $\text{Zn}^{2+}$  (Koh and Judson, 1986). Sulfur and  $\text{Se}^{2+}$  are

similar chemically and hence are potentially antagonistic in their uptake in plants and their utilization in animals (Pope *et al.*, 1979 and Abdel-Rahim *et al.*, 1985).

Dietary  $Mg^{2+}$  availability to stock is markedly affected by other dietary components, especially  $K^+$  (Dua and Care, 1995). High dietary levels of  $K^+$  and N will inhibit  $Mg^{2+}$  absorption from the rumen. Diets of low  $Na^+$  content increase dietary Mg requirements indirectly by raising the  $K^+$  concentrations in the rumen. Increased dietary levels of  $Ca^{2+}$  and of soluble carbohydrates may respectively increase and decrease the dietary  $Mg^{2+}$  requirements of livestock, whereas raised dietary P levels appear to lower the requirements for both  $Ca^{2+}$  and  $Mg^{2+}$ .

The inhibitory effects of Mo and S on  $Cu^{2+}$  availability have received considerable attention. Thiomolybdates, formed from Mo and S in the rumen, complex with  $Cu^{2+}$  making it unavailable to the animal. The quantitative assessment of the inhibitory effects of Mo and S on  $Cu^{2+}$  availability was undertaken by Suttle and McLaughlan (1976) using sheep given semi-purified diets. Difficulties have been encountered in applying this equation and other revised equations to predict  $Cu^{2+}$  availability to sheep and cattle in the field (Suttle, 1991). Part of the problem may be that no allowance is made for the inhibitory effect of Fe on Cu availability. Dietary Fe concentrations of 500-6000 mg/kg DM have been shown to reduce  $Cu^{2+}$  utilization in sheep and cattle (Towers and Grace 1983; Grace and Lee, 1990).

Tiffany *et al.* (2000) reported mineral status of cattle that grazed forage fertilized with too high Mo (12 or 33 ppm) containing biosolids for 176 days. Forage Mo uptake was low due to good drainage and acid soils. However, resulting forages contained high S which significantly reduced animal  $Cu^{2+}$  status (liver and plasma). High dietary S reduces  $Cu^{2+}$  absorption, possibly due to unabsorbable Cu sulfide formation, independent from its part in thiomolybdate complexes (Underwood and Suttle, 1999). If cattle are to graze pastures treated with high levels of biosolids where forages with low Cu status are grown,  $Cu^{2+}$  supplementation is essential.

Sulfur alone may also lower  $Cu^{2+}$  availability with the formation in the rumen of insoluble cupric sulfide. The significance of this effect of S on  $Cu^{2+}$  availability in the grazing animal is uncertain. It was suspected that sheep grazing pastures containing crucifers of high S content were at risk to induced  $Cu^{2+}$  deficiency (Merry *et al.*, 1983), however, Suttle (1983) found that brassica crops contained  $Cu^{2+}$  of higher absorbability than any other crop examined despite the high levels of S. Suttle (1983) suggested that the higher absorbability of  $Cu^{2+}$  may have been due to the low fibre content of the brassicas. It was through that fibre might limit  $Cu^{2+}$  absorption by irreversibly binding  $Cu^{2+}$  or indirectly by increasing the residence time of  $Cu^{2+}$  in the potentially hostile environment of the rumen.

Improved practices that lead to improved egg or milk production and growth rates will necessitate more attention to mineral nutrition. Mineral deficiencies, often marginal under low levels of production, are likely to become important and previously unsuspected nutritional deficiency signs may occur as production level increases. Specific mineral requirements are difficult to pinpoint since exact needs depend on chemical form and numerous mineral interrelationships. The chemical form of mineral elements varies greatly in amount of dietary minerals supplied and in biological availability (Judson and McFarlane, 1998; McDowell, 2002).

Mineral supplementation is much less important for livestock if energy protein requirements are inadequate (Van Niekerk and Jacobs, 1985). However, when energy and protein supplies are adequate, livestock gain weight rapidly, resulting in high mineral requirements. In some countries of the world during the dry winter season unsupplemented cattle and sheep grazing extensive grassland may lose 25-30% or more of their maximum summer body mass. It is therefore uneconomical and often of no benefit, to provide mineral supplements to grazing livestock, if the main nutrients which they are lacking are energy and (or) protein (McDowell, 1997).

**Factors Affecting Mineral Requirements:** Levels of requirements as well as thresholds of deficiency and toxicity vary with age, sex production level, activity level, species and genetic strain of the animal. Since it must be recognized that mineral requirements are to a large extent species and breed specific and can only be extrapolated from research with other species and breeds within limits or in a general way.

Significant species differences have been reported for  $Cu^{2+}$ , I, Mo, As, among other elements (Haenlein, 1980, 1991; Anke and Szentmihalyi, 1986; Devendra, 1989). In case of Mo, goats will tolerate more than 300 mg Mo/kg DM in feed intake, while sheep tolerate only 30 mg/kg DM and cattle will already suffer from diarrhea at 10 mg Mo/kg DM (Falke and Anke, 1987). In case of  $Cu^{2+}$ , toxicity symptoms are noted in sheep at 10-20 mg  $Cu^{2+}$ /kg DM feed intake, while cattle tolerate up to 100 mg  $Cu^{2+}$ /kg DM. Data are still needed but observations have indicated, that goats are tolerant of much higher  $Cu^{2+}$  levels than sheep (Anke and Szentmihalyi, 1986; Zervas *et al.*, 1989) In case of I, radioactively marked I showed that goats transfer 22 percent of diet I into milk vs. 8 percent in cows (Groppel *et al.*, 1988). Colostrum from normal goats also had much higher I contents (3662 nmol/l) than from normal cows (416 nmol/l). Thyroids from goats were lighter than from sheep on equal feed supplies of I, which may indicate that more I is available in sheep for synthesis of T4/T3 and that goats are more sensitive to low I supplies (Groppel *et al.*, 1989). Contents of less than 300 mcg I/kg DM white hair are indicative of insufficient I supplies for growing, pregnant and lactating Swiss goats, while for sheep the limit is 200 mcg I/kg DM white wool. Goat kids with less than 0.6 mg I/kg white hair, but calves with less than 1.8 mg I/kg black hair

during week one have probably an I deficiency (Groppe *et al.*, 1988)

Supplies of minerals are influenced by climate and soil on which feed plants grew, also by stage of maturity of the plants and its parts (Fielder and Heinze, 1985; Szentmihalyi *et al.*, 1985 and Kalac, 1986).  $\text{Cu}^{2+}$  contents in red clover have been reported to decrease from 13 to 8 mg/kg DM, in fescue grass from 11 to 6, in forage rye from 9 to 3, when sampled in different months of the year (Anke and Szentmihalyi, 1986). There are also many mineral interactions in the federation influencing net absorption (Haenlein, 1987).

Large differences have been observed between animals within a herd or flock in their susceptibility to mineral disorders. For example, a 50% mortality in young sheep on Co deficient soil is not uncommon on the coastal areas of the south-east of South Australia if treatment is withheld (Hannam *et al.*, 1980) yet many of the surviving sheep show no signs of ill health.

Genetic variation accounts for part of the differences between and within breeds of livestock in their requirements for a number of minerals including  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ , P,  $\text{Se}^{2+}$  and  $\text{Zn}^{2+}$  (Langlands *et al.*, 1980 and Suttle 1996). Utilisation of dietary  $\text{Cu}^{2+}$  varies markedly within and between breeds of sheep (Wiener and Woolliams, 1983) and recent evidence suggests that breed (Ward *et al.*, 1995) may also affect  $\text{Cu}^{2+}$  requirements of cattle. These differences in  $\text{Cu}^{2+}$  requirements appear to reflect genetic differences in the efficiency of  $\text{Cu}^{2+}$  absorption (Suttle, 1974).

Age of animal can affect requirements of minerals through changes in efficiency of absorption. In general, the young suckling animal (preruminant) absorbs most minerals more efficiently than the older animal (Standing Committee on Agriculture, 1990). Marked differences have been observed in the efficiency of absorbing  $\text{Cu}^{2+}$  and  $\text{Mg}^{2+}$ . In suckling animals about 90% of the Mg and 50-70% of the  $\text{Cu}^{2+}$  is absorbed whereas in older animals these values drop to about 10-20% for Mg and 5-6% for  $\text{Cu}^{2+}$ . Other dietary factors are thought to account for much of this drop in absorption efficiency although the major site of Mg absorption shifts from the intestine in the young to the rumen in the older animal.

Gender of animal affects susceptibility to mineral disorders probably through differences in growth rate and physiological function. Studies on growth rate responses to trace element supplementation have shown that wethers are more susceptible than ewes to Co deficiency (Shallow *et al.*, 1989) and steers are more susceptible than heifers to Se deficiency (Nelson and Miller, 1987) and possibly to other trace element deficiencies (Baumgurtel and Judson, 1998).

**Immunity and Antioxidant Role of Minerals Along with Vitamins:** Both trace minerals and vitamins play important roles in the health of cattle. For lactating dairy animals nutrient supplementation for trace minerals and vitamins go beyond correcting for deficiencies but are aimed rather at minimizing stress and optimizing production efficiency. Free radicals can be extremely damaging to biological systems (Padh, 1991). Also, phagocytic granulocytes undergo respiratory burst to produce oxygen radicals to destroy intracellular pathogens. However, these oxidative products can, in turn, damage healthy cells if they are not eliminated. Antioxidants serve to stabilize these highly reactive free radicals, thereby maintaining the structural and functional integrity of cells (Chew, 1995). Therefore, antioxidants are very important to immune defense and health of humans and animals.

Tissue defense mechanism against free-radical damage generally include vitamin C, vitamin E and  $\beta$ -carotene as the major vitamin antioxidant sources. In addition, several metalloenzymes which include glutathione peroxidase ( $\text{Se}^{2+}$ ), catalase (Fe), and superoxide dismutase ( $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Mn}^{2+}$ ) are also critical in protecting the internal cellular constituents from oxidative damage. The dietary and tissue balance of all these nutrients are important in protecting tissues against free radical damage. Both *in vitro* and *in vivo* studies show that these nutrients generally enhance different aspects of cellular and non-cellular immunity. The antioxidant function could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells. A compromised immune system will result in reduced animal production efficiency through increased susceptibility to diseases, thereby leading to increased animal morbidity and mortality (McDowell, 2002)

**Dietary Mineral Buffers:** Mineral buffers are included in animal diets to improve lactational performance, milk composition and a favourable acid-base balance. Buffers are used in the diets of dairy cows to combat milk fat depression. Downer and Cummings (1987) estimated that more than 50% of all dairy farms in the US may be using dietary buffers, such as  $\text{NaHCO}_3$ , for this purpose. Researchers have concluded that responses to dietary buffers occur via reduced ruminal acidity and subsequent improvements in systemic acid-base status, particularly during sudden ration changes.

Schneider *et al.* (1986) hypothesized that responses of lactating cows to dietary buffers are the result of both the  $\text{HCO}_3^-$  (buffering effect) and Na moiety solute effect). Ressel and Chow (1993) suggested that bicarbonates function not by increasing ruminal buffering capacity, but by increasing water intake, ruminal fluid dilution and flow of undegraded starch and by reducing ruminal propionate production (Staples and Lough, 1989). Metabolic acidosis is a complicating factor in a number of diseases that affect cattle, including ketoacidosis, lactic acidosis (grain overload), enterotoxigenic diarrhea of calves and some enteric diseases of adult cattle. Treatment with  $\text{NaHCO}_3$

i.v. or orally, is an effective method to restore blood pH to normal (Kasari, 1990; Roussel, 1990). Orally administered  $\text{NaHCO}_3$  and Na propionate were equally effective in correcting the acid-base balance of blood (Bigner *et al.*, 1997). Sodium propionate may be considered a more effective treatment of metabolic acidosis in diseases such as ketosis because the added propionate can serve as a source of glucose for the cow.

**Biological Availability of Mineral Sources:** There is considerable difference in the availability of a mineral element provided from different sources. The bioavailability and percentage of mineral elements in inorganic sources commonly used in mineral supplements have been reported (McDowell, 1999). These variations in bioavailability of sources must be taken into consideration when evaluating or formulating a mineral supplement.

Excellent reviews on the significance of chelates and complexes of minerals for the feed industry have been prepared (Nelson, 1988; Kincaid, 1989; Patton, 1990 and Spears *et al.*, 1991). Spears (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 Mo,  $\text{Cu}^{2+}$  in chelated form would have an advantage over an inorganic form as it may escape the complexing that occurs in the digestive system among Mo,  $\text{Cu}^{2+}$  and S (Nelson, 1988). Some studies have shown no benefit from chelated and complex minerals, but most have shown positive responses when compared to inorganic sources. Zinc and  $\text{Cu}^{2+}$  complexed with proteins or amino acids, such as methionine or lysine, tended to have an advantage over inorganic forms of trace elements when given to stressed cattle. Weaning weights were higher for zinc methionine and manganese methionine supplemented calves compared to control or oxide supplemented calves (Spears and Kegley, 1991). Herrick (1989) reviewed zinc\_methionine feeding in four dairy trials and concluded that the  $\text{Zn}^{2+}$  complex treated animals had lower somatic cell counts and higher milk yields than control cows. Kincaid *et al.* (1986) compared copper proteinate and copper sulfate in terms of their ability to increase copper status in calves fed a diet naturally high in Mo (3.1 ppm) and low in Cu (2.8 ppm). Calves fed  $\text{Cu}^{2+}$  proteinate had higher plasma (0.87 vs. 0.75 mg/l) and liver (325 vs. 220 ppm) Cu concentrations than calves supplemented with a similar level of  $\text{Cu}^{2+}$  from the sulfate from after 84 days. Zinc in the form of zinc lysine resulted in the highest levels of metallothionein in liver, pancreas and kidney compared to other  $\text{Zn}^{2+}$  sources, thus indicating a more bioavailable source of  $\text{Zn}^{2+}$  (Rojas, 1994). Copper lysine at 16 ppm  $\text{Cu}^{2+}$  was more beneficial or cattle that were borderline to deficient in  $\text{Cu}^{2+}$  status versus copper sulfate (Rabiansky *et al.*, 1999).

The supplemental form of  $\text{Se}^{2+}$  most widely used is the inorganic form of sodium selenite. An alternative organic  $\text{Se}^{2+}$  source derived from yeast has been developed whereby  $\text{Se}^{2+}$  is incorporated into the protein structure of growing yeast cells (Mahan, 1996). Dairy cattle have exhibited a higher glutathione peroxidase activity when the organic form of selenium is fed compared to when selenite was provided (Pehrson *et al.*, 1998). In the future, organic  $\text{Se}^{2+}$  will likely be an extremely important source of supplementation of this element.

Much more needs to be learned about the selectivity of chelating agents toward minerals, the kind and quantity most effective, their mode of action and their behaviour with different species of animals and with varying diets. 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.

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