Assessment of trace mineral status of ruminants: A review

R. L. Kincaid[1]

Department of Animal Sciences, Washington State University, Pullman 99164-6351

Abstract

An assessment of the trace mineral status of ruminants begins with clear objectives. These may be to determine whether a nutrient deficiency exists, to assess the prevalence of a deficiency, or to estimate the endogenous reserves of trace minerals. The sampling size needed for assessment is a function of the herd size, expected prevalence of deficiency or toxicity, desired confidence of the assessment tool, and the estimated standard deviation of the assessment criteria. The trace mineral status of animals is best described by concentrations in liver. Correlation coefficients between concentrations of trace minerals in blood and liver are highest in deficient animals because endogenous reserves are depleted. Concentrations of Zn, Cu, and Se in plasma also are affected by infection, stress, pregnancy, and erythrocyte hemolysis. Because erythrocytes in cattle have a 160-d life span, concentrations of trace minerals in whole blood change more slowly than those in plasma in response to changes in intakes of trace minerals. Improvements in sensitivity of blood measures to assess trace mineral status await determination of the most critical metalloenzyme activity. At present, metalloenzyme activities are seldom more useful than concentrations of minerals in plasma and often are impractical because of loss of activity in shipment to the laboratory. Perhaps the ultimate assessment tool is the response of animals to supplementation.

Key Words: Cattle, Sheep, Copper, Zinc, Selenium, Manganese

Introduction

The most common reason to assess the trace mineral status of ruminants is because performance is below expectation. Accordingly, the assessment is done to determine the presence or prevalence of nutrient deficiencies (or toxicities) within a population. Assessment also is done to evaluate efficacy of dietary supplementation or to compare available supplements.

A critical part of assessment is the determination of the most appropriate measurement criteria. Physiological functions are progressively affected by deficiencies. For example, loss of pigmentation occurs with intakes of Cu that are sufficient for pregnancy maintenance and hemoglobin formation. Pregnancy is not maintained by intakes of Cu that prevent anemia. Furthermore, the disruption to Fe metabolism caused by Cu deficiency does not occur until after most other clinical signs have appeared (Mills, 1987). However, economically important effects on performance and health of animals can be affected by trace element deficiencies even before clinical signs are evident. Thus, the assessment will fail if inappropriate criteria are selected. The purpose of this paper is to review various methods of assessment for trace element status of ruminants

Discussion

Methods of Assessing Status

Diet analyses provide useful supporting data if representative samples of all feeds can be obtained. Actual chemical analyses need to be performed and should include those elements with important interactions (e.g., Mo, S, and Fe). Tabular values and feed tags should not be relied on for estimating trace element intakes. Other useful diet information includes the forage type, processing method, the forage:concentrate ratio, addition of buffers or anionic salts, protein level, and protein solubility.

Blood measures are frequently used in assessment because they are significantly correlated to nutritional status of some trace elements (Claypool et al., 1975; Levander, 1986; Mills, 1987), and blood is less invasive to sample than liver. However, there are several limitations to blood analyses. Because red blood cells in cattle have a life span of about 160 d (Schlam, 1980), concentrations of minerals in whole blood often change slowly. Homeostatic control mechanisms can limit changes in concentrations of some trace minerals in plasma until endogenous reserves are substantially depleted (Miller, 1975). Also, careful handling of blood samples is needed to prevent hemolysis and contamination of plasma.

Liver is the organ that often represents the status of several trace elements in animals (McDowell, 1992). Ideally, biopsy samples are taken before and after treatments are applied. Other tissues do not consistently reflect trace mineral status. Analyses of minerals in milk and urine are seldom useful in mineral assessment because most cations in milk are actively transported into the mammary gland and concentrations are regulated (Miller, 1975). Anions, (e.g., Mo and I) in milk are exceptions and reflect dietary intake (Miller et al., 1975; Wittenberg and Devlin, 1988). Values of Se and I in milk and urine could have a role in indicating excess intakes if reference values were established. Likewise, values for minerals in hair, wool, and hoofs lack reference standards, are too slowly responsive to intakes, and can be easily contaminated.

Sampling Size

Before the number of animals to sample within a population can be determined, the goal of the assessment must be clear. The goal may be to estimate the mean concentration of a trace mineral in a tissue of a population, to estimate the prevalence of a deficiency or toxicity within a population, or to find if any deficient animals exist within a population. If the goal is to estimate the mean, the sample size (**n**) depends on the herd or group size (**N**) and the standard deviation (**SD**) of the estimate. Examples of changes in the needed sampling size were taken from a table developed by Hancock (1996) and are given below. To estimate the mean concentration of plasma Cu in a herd of 200 cows, with a 95% confidence and within .5 SD, requires an n of 15. Because the SD of plasma Cu concentrations is large, an estimate within .3 SD of the mean may be desired, which would require an n of 36. If the goal is to determine the proportion of a population that might be Cu-deficient, and if the proportion is suspected to be less than 25%, for a 95% confidence and an N of 200, n is 119. Finally, if the goal is to determine whether any animals are Cu-deficient in a herd of 200, to be 95% certain of detecting a 1% prevalence, n = 155. However, to have 95% confidence of detecting a deficient animal when the prevalence is 5%, then n = 51. Thus, n is a function of the confidence limits, prevalence, N, and SD. Sometimes preliminary testing is needed to estimate the SD.

Copper

Suggested values for classifying the Cu status of cattle are given in Table 1. The concentration of Cu in the liver of ruminants is correlated to the bioavailable Cu in the diet in ruminants (McDowell, 1992). In sheep, liver contains approximately half of the total Cu in the carcass (Langlands et al., 1984). In addition to bioavailable dietary Cu, the concentration of Cu in the liver is affected by physiological needs (e.g., fetal growth). In pregnant cows fed 5.5 ppm Cu, liver Cu declined continuously until parturition during the 8-wk nonlactating period. However, dietary supplementation of 10 ppm Cu prevented the decline in liver Cu (Xin et al., 1993).

Liver of newborn ruminants normally contains high concentrations of Cu (> 200 mg of Cu/kg liver DM; Hidiroglou and Williams, 1982; Branum et al., 1998) that are affected by maternal Cu status. For example, the concentration of Cu in liver of fetuses from cows with > 25 mg of Cu/kg of liver DM was higher than that in liver of fetuses from dams with < 25 mg of Cu/kg of liver DM (Gooneratne and Christensen, 1989). Lambs with swayback had 17 mg Cu/kg liver, compared to normal lambs with 109 mg Cu/kg liver (McC. Howell and Davison, 1959).

When Cu intakes of animals are less than physiological needs, concentrations of Cu and activities of ceruloplasmin (**Cp**) in plasma are not consistently reduced until liver Cu is < 40 mg/kg (Claypool et al., 1975; Mills, 1987). Engle et al. (1964) found a significant correlation (r = .57, $P < .01$) between concentrations of Cu in liver and plasma when there was < 33 mg of Cu/kg of liver. Claypool et al. (1975) suggested that plasma Cu values of .5 μg/mL or less are indicative of low stores of Cu in liver.

Factors other than Cu intake affect the concentrations of Cu in plasma. Copper in serum was higher at estrus than at d 21 in nulliparous heifers (Small et al., 1997) and depressed in beef cows on the day of calving (Small, 1997). Xin et al. (1993) found plasma Cu was lowest at 5 wk prior to parturition. Serum Cu values are increased by infection (Etzel et al., 1982). Finally, Cu metabolism is influenced by genetics, and significant differences exist among breeds of sheep in concentrations of Cu in plasma and liver (Woolliams et al., 1985).

Intakes of Zn, Fe, Mo, and S affect Cu utilization (McDowell, 1992). Large intakes of Zn reduce concentrations of Cu in plasma and liver of cattle and sheep (Ott et al., 1966a; Kincaid et al., 1976; Kellogg et al., 1989). Dietary Mo can inhibit uptake and utilization of Cu. In the rumen, Mo combines with reduced sulfur to form tetrathiomolybdate that binds Cu and prevents its absorption. Other thiomolybdates and molydate are absorbed into blood and bind endogenous Cu to render it unavailable for metabolic purposes (Mason, 1982). In plasma, Mo is bound to protein, removes Cu from liver, and exacerbates urinary Cu loss, although some of the Cu-Mo complex accumulates in the kidney (Kincaid and White, 1988). The presence of a Cu-Mo complex in plasma explains why plasma Cu has been reported to increase in ruminants consuming Mo (Suttle and Field, 1968) even though liver Cu is being depleted and Cp activity is reduced (Kincaid, 1980). The Cu-Mo complex in plasma is precipitated by trichloroacetic acid (**TCA**; Paynter, 1982;] thus, plasma samples should be treated with TCA prior to Cu determination to prevent an overestimate of the Cu status.

In plasma, 70 to 90% of the Cu is associated with Cp. Accordingly, the activity of Cp is closely correlated with serum Cu in cattle (r = .83) and sheep (r = .92; Blakely and Hamilton, 1985). Ceruloplasmin is very stable and retains activity in samples during shipment and handling. Activities of Cp increase sharply around parturition (Kincaid and White, 1988) and, as an acute phase protein, Cp increases during infection (Etzel et al., 1982) unless the cows have a marginal or low Cu status (Erskine and Barlett, 1993). Activities of Cp are 18 to 35% lower in serum than in plasma, and concentrations of Cu are approximately 14% lower in serum than in plasma (Kincaid et al., 1986).

Erythrocytes have a labile fraction of Cu that is loosely bound to protein and a more stable Cu fraction that includes superoxide dismutase (**SOD**). Approximately 60% of the Cu in erythrocytes is associated with SOD. Activity of SOD is not a sensitive measure of Cu status because SOD activity does not fall with deficient intakes of Cu until after plasma Cu and Cp are reduced (Andrewartha and Caple, 1980; Ward and Spears, 1997; Gengelbach and Spears, 1998).

Cytochrome oxidase activity in neutrophils and other tissues has been suggested as a potentially useful marker of Cu status. However, cytochrome oxidase activity in circulating leukocytes of sheep and cattle declined more slowly than plasma Cu or Cp and therefore was less sensitive to Cu status (Boyne, 1978). Neutrophils isolated from heifers fed approximately 7 ppm Cu were less effective at killing ingested bacteria than neutrophils of heifers supplemented with 20 ppm Cu, although phagocytosis and superoxide production were not affected (Torre et al., 1996). Thus, the killing ability of neutrophils may have a useful role in Cu assessment.

In beef calves, low Cu status has been associated with the development of abomasal ulcers. Calves with abomasal ulcers had 45 to 48 μg of Cu/g of liver, compared to control calves with 245 μg of Cu/g (Lilley et al., 1985). These investigators suggested the effect could be mediated via impaired immunity in Cu-deficient calves or structural weakness in abomasum of Cu-deficient calves. Lysyl oxidase **(LO)** is a Cu-dependent enzyme involved in the formation of cross-linkages in collagen and elastin that gives structural strength to these tissues (Gallop et al., 1972). Unless an assay for LO is developed for an easily sampled tissue, use of LO in assessment would be difficult because of the invasiveness required to obtain aorta, abomasums, or other suitable tissue for assay.

Zinc

Suggested values for classifying the Zn status of cattle are given in Table 2. In ruminants, concentrations of Zn in plasma are reduced during a Zn deficiency. Cattle consuming diets severely deficient in Zn (1.2 ppm dietary Zn) have depressed concentrations of Zn in plasma within 36 h (Mills et al., 1967). Neathery et al. (1973a) found plasma Zn was reduced (.79 vs .96 ppm Zn) after 6 wk in cows fed 17 vs 40 ppm Zn. Sheep deficient in Zn had serum Zn values of .44 μg/mL, and serum Zn increased to .78 μg/mL when they were given a ZnO supplement (Suliman et al., 1988). The Zn-deficient sheep also had reduced alkaline phosphatase activity and increased lactate dehydrogenase and displayed wool biting. McDowell et al. (1991) surveyed 11 dairy goat herds in Florida and found plasma Zn was lower in goats with seasonal dermatosis (.54 vs .83 μg/mL). Previously, Neathery et al. (1973b) reported that goats fed 4 ppm Zn had .62 μg of Zn/mL of plasma and showed signs of Zn deficiency.

Dietary concentrations of 600 ppm Zn nearly doubled the concentration of Zn in plasma of calves (Ott et al., 1966b; Stake et al., 1975). Heifers responded with higher plasma Zn than steers (Ott et al., 1966b). Lambs fed diets with 500 ppm supplemental Zn had 1.22 μg of Zn/mL of plasma, compared to .95 μg of Zn/mL of plasma in control lambs (Ott et al., 1966a).

Concentrations of Zn in plasma fluctuate with age, stress, infections, and feed restriction. Plasma Zn is very high (2.3 μg/mL) in newborn calves and drops to 1.2 μg/mL by 12 wk of age (Kincaid and Hodgson, 1989). Plasma Zn, as part of an acute phase response, is initially reduced by infection (Wellinghausen and Rink, 1998), only to become elevated within a few days. Serum Zn also is decreased by hyperthermal stress and ketosis in cows and is increased in cows with mastitis and in older cows (Wegner et al., 1973).

The relationship between Zn intake and concentrations of Zn in liver is affected by age of the ruminant (Kincaid et al., 1976). Calves readily absorb and bind large amounts of Zn as metallothionein (**MT**) in liver in response to elevated Zn intakes (Kincaid et al., 1976). For example, diets supplemented with 600 ppm Zn fed to young calves caused Zn in liver to increase by 600% but did not affect Zn in liver of mature cows (Kincaid et al., 1976). Once the added Zn is removed from the diets of calves, concentrations of Zn in liver return to normal within a few weeks (Kincaid and Cronrath, 1979). There are four isoforms of MT, and increased dietary Zn increases induction of both MT-Ia and MT-II mRNA in liver and kidney tissue but not in the duodenum, muscle, or skin. Increased concentrations of MT-IA protein account for most of the increased Zn in liver (Lee et al., 1994).

Concentrations of MT in serum and erythrocytes may be useful as indicators of Zn status that are less affected by infection. Another potential measure of Zn status includes the unused capacity of plasma to bind Zn. In calves fed 20 or 70 ppm Zn, there was no difference in plasma Zn concentrations, whereas the percentage of unsaturated plasma Zn binding capacity reflected Zn intakes (Kincaid and Cronrath, 1979). Other potential measures in blood for determining Zn status include Zn concentrations in lymphocytes, granulocytes, and platelets. In human, these measures were shown to be more responsive than plasma Zn to Zn status (Prasad, 1998).

Among the various roles for Zn in immunity are gene expression, mitosis, and apoptosis of lymphoid cells. Because DNA polymerase, the major enzyme regulating DNA replication, is Zn-dependent (Shankar and Prasad, 1998), proliferative responses of macrophages, T cells, or B cells may have use as early indicators of Zn status. Engle et al. (1997) found the cell-mediated immune response to phytohemagglutinin was reduced in calves fed 17 ppm Zn compared to calves fed 40 ppm Zn, even though Zn in plasma and liver were not affected.

Selenium

Suggested values for classifying the Se status of cattle are given in Table 3. Measures for estimating the Se status of livestock include concentrations of Se in liver, serum, and whole blood; glutathione peroxidase (**GPx**) activities in erythrocytes and liver; and mRNA levels for GPx or hydroperoxide glutathione peroxidase (Kincaid, 1995). In serum of cows, Se is associated with albumin, GPx, and selenoprotein P (Awadeh et al., 1998). These various measures can lead to different interpretations unless the level and chemical form of the dietary Se are considered. Whole blood has a Se concentration that is approximately three

times higher than that in serum (Scholz and Hutchinson, 1979) and often is better for Se determination because any hemolysis of the erythrocytes will cause serum to have a false high value for Se (Maas et al., 1992).

Concentrations of Se in whole blood are responsive to Se intake (Levander, 1986). Clinical deficiencies in ruminants are associated with values of < 30 ng of Se/mL of whole blood (Sheppard et al., 1984; Pehrson et al., 1986). Based on mastitis resistance, Smith et al. (1988) recommend that whole blood contain at least 200 ng of Se/mL. For plasma, most researchers (Pehrson et al., 1986; Smith et al., 1988; Gerloff, 1992) recommend that Se exceed 70 ng/mL.

Concentrations of Se in whole blood of newborn calves and their dams are highly correlated ($r = .74$, $P < .05$; Kincaid and Hodgson, 1989). Cows in late pregnancy need 3 to 5 mg of Se/d to ensure adequate Se reserves in tissues of newborns (Abdelrahman and Kincaid, 1995). Relatively large amounts of Se are transferred from the dam to the fetus during the last trimester of pregnancy; therefore, Se levels in maternal blood are reduced unless Se intakes of cows exceed 3 mg/d. The efficiency of maternal transfer of Se to the fetus is affected by the chemical form of the Se in the diet. Compared to selenite, more Se from selenomethionine is transferred from the dam to the fetus and into milk (Kincaid and Rock, 1999; Knowles et al., 1999).

Concentrations of Se and activities of GPx are highly correlated ($r = .92$, $P < .001$) in blood of sheep and cows ($r = .59$, $P < .001$; Thompson et al., 1976), but the correlation coefficients are reduced by Se supplementation. Activities of GPx reported between laboratories are variable because the assay is difficult to standardize and the enzyme is subject to deterioration with shipment (Stowe and Herdt, 1992). There also is considerable interlaboratory variation in Se determinations; reported ratios of Se in whole blood to serum range from 3.3:1 to 1.8:1 in replicate samples (Waldner et al., 1998).

Between 4% and 9% of total body Se is in the liver of sheep (Langlands et al., 1984), and liver tends to have the highest concentration of Se among tissues. Hence, liver Se concentrations are used as a measure of Se status (Table 3). However, the largest body burden of Se is found in muscle.

Manganese

Suggested values for classifying the Mn status of cattle are given in Table 4. Typical concentrations of Mn are 5 to 10 ng/mL of plasma in cows (Gibbons et al., 1976) and 1.8 to 4.0 ng/mL in sheep (Masters et al., 1988). Unfortunately, concentrations of Mn in plasma are not good indicators of Mn intake. Concentrations of Mn in the red blood cells are higher than in plasma and have been used to assess status. For example, cows fed diets with 8 ppm Mn had 130 ng of Mn/mL of whole blood, compared to 210 ng of Mn/mL of whole blood in cows supplemented with 60 ppm Mn (Hidiroglou et al., 1978).

The liver efficiently removes Mn from plasma, whether the Mn is as Mn^{2+} or bound to α_2 macroglobulin, but not Mn^{3+} bound to the transferrin complex (Gibbons et al., 1976). The Mn that is taken up by the liver is excreted endogenously via the bile, and accumulations of Mn in the liver often do not reflect dietary intakes of Mn. Although a Mn-dependent SOD exists within cells, only the activity of MnSOD in the heart has been significantly correlated with intake of Mn (Masters et al., 1988).

Dietary Mn affects the concentration of Mn in bones and other tissues. Calves of cows fed diets containing 16 ppm Mn had deformed bones and lower Mn concentrations in bones, liver, kidney, blood, and gonads (Bentley and Phillips, 1951). Calves fed milk containing .5 ppm Mn had 12 μg Mn/g of liver, compared to 22 μg Mn/g of liver in calves supplemented with the equivalent of 15 ppm dietary Mn. Supplementation also increased Mn in the rib shaft, but not in the tibia (Carter et al., 1974).

Cobalt

Suggested values for classifying the Co status of cattle are given in Table 5. Because the metabolic role of Co is as a component of vitamin B_{12} (Smith,1997), assessment of Co nutriture often centers on measures of B_{12} status, although concentrations of Co in liver and performance response of ruminants to Co supplementation also can be used in assessment (McDowell, 1992). Vitamin B_{12} is a cofactor for the enzyme methylmalonyl-CoA mutase that catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA (Smith, 1997). In a vitamin B_{12} deficiency, methylmalonic acid (**MMA**) accumulates, and the elevated concentrations of MMA in plasma (Rice et al., 1989; Kennedy et al., 1991) and urine (Quirk and Norton, 1988) may be used for differential diagnosis. Sheep are more sensitive to the effects of a Co deficiency than are cattle (Kennedy et al., 1995). The upper limit for normal MMA in plasma is 2.0 μmol/L in cattle (Paterson and MacPherson, 1990) and 5.0 μmol/L in sheep (Rice et al., 1989). Concentrations of MMA in plasma are elevated in the early stages of a Co deficiency in sheep, preceding the onset of loss of production and clinical signs of the disease (Rice et al., 1989). Although some MMA may arise from the rumen, increased MMA still indicates a functional B_{12} deficiency (Rice et al., 1989; Paterson and MacPherson, 1990).

Concentrations of vitamin B_{12} in liver, but not in serum, reflect the body's reserve. Serum B_{12} concentrations are not a good measure of B_{12} status because they change very rapidly with Co intake and they also are increased by liver disease, stress, and starvation (Paterson and MacPherson, 1990). Thus, the correlation between vitamin B_{12} in serum and liver is often low unless the sheep are severely deficient. Vitamin B_{12} concentrations in plasma are a passive marker of Co and vitamin B_{12} status, because B_{12} in plasma is bound to transcobalamins that have no active role. During the assay for B_{12} in plasma of cattle, a large proportion of total plasma B_{12} is not released from transcobalamin I, the prinicipal B_{12} carrier protein in bovine plasma (Price et al., 1993).

Another measure of vitamin B_{12} status arises from the enzyme methionine synthetase, which transfers methyl groups in the folic acid cycle (Smith, 1997). A deficiency of vitamin B_{12} subsequently impairs conversion of formiminoglutamic acid (**FIGLU**) to glutamic acid (Smith, 1997). Hence, FIGLU accumulates and the increased concentration of FIGLU in urine is an indicator of Co deficiency (Quirk and Norton, 1988). Finally, in a vitamin B_{12} deficiency there are increased concentrations of branched-chain fatty acids in the carcass (Kennedy et al., 1994). No suggestion has been made concerning how to incorporate this change in fat deposition into the assessment of Co status.

Iron

Suggested values for classifying the Fe status of cattle are given in Table 6. Iron deficiencies, except in young ruminants (Mollerberg, 1975) and milk-fed calves (Matrone et al., 1957), do not normally occur in ruminants. Concentrations of Fe in liver and spleen increase in cows until 6 yr of age (Blum and Zuber, 1975). Iron depletion is normally divided into three stages: stage 1 is depletion of tissue Fe reserves; stage 2 is characterized by reduced serum Fe and increased total Fe binding capacity; and stage 3 is characterized by anemia (Johnson, 1990). Stage 1 Fe deficiency is the most difficult to diagnose but can be assessed by determining concentrations of ferritin in serum (Johnson, 1990). Stage 2 Fe deficiency can be determined from the reduced concentrations of Fe in serum that precedes development of anemia.

Iodine

Suggested values for classifying the I status of cattle are given in Table 7. Iodine deficiencies can occur in ruminants in areas of endemic low I in soils or in ruminants fed uniodized salt and feeds containing goitrogens (McDowell, 1992). Assessment of I status is done by measurement of serum I, protein-bound I, thyroxine ($\mathbf{T_4}$), or the presence of goiter (McDowell, 1992). Concentrations of I in plasma are significantly affected by dietary I but also increase in the weeks prepartum and decline immediately postpartum (Aumont et al., 1989). Maternal plasma I can be more useful than T_4 for assessment in gestating ruminants, because low I intakes during pregnancy can result in goiter in newborn lambs even though serum T_4 of the dam may not be affected by the temporary low I intake (Azuolas and Caple, 1984). The concentration of I in milk reflects dietary intake of I; however, there also is a seasonal pattern of milk I concentrations not associated with I intake (Azuolas and Caple, 1984). An obvious limitation to milk I for I assessment is that the fetus is most sensitive to maternal I status during late gestation, a time when the dam is not lactating. Urinary I excretion is used in I assessment of human populations (Boyages, 1993) and could have use in assessment of livestock (Aumont et al., 1989) if standards were developed.

Molybdenum

Molybdenum is a trace element with known functions (i.e., activities of sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase; Johnson, 1997), but a nutritional deficiency of Mo has not been reported in livestock (McDowell, 1992). Assessment of Mo status is usually concerned with Mo toxicity or conditioned Cu deficiency (see the section on copper above). Molybenum-induced effects on health of livestock are more likely to occur in ruminants than in nonruminants because the Mo can combine with reduced S to form thiomolybdates in the rumen, thereby reducing absorption of Cu, leading to a conditioned Cu deficiency (McDowell, 1992). Because no nutritional deficiencies of Mo under practical conditions have been reported in ruminants, no estimates are available on minimum values for Mo in blood and tissues. Soluble forms of dietary Mo are readily absorbed by animals and cause Mo concentrations in serum, whole blood, milk, liver, and kidney to be increased severalfold (Kincaid and White, 1988; Wittenberg and Devlin, 1988).

Summary

Assessment begins with clear objectives. Next, the appropriate measurement criteria must be determined and the sampling size must be estimated. Possible sources of sample contamination should be identified and steps taken to avoid them. Accurate assessment of the trace mineral status also requires valid laboratory analyses and critical interpretations. Concentrations of trace elements in liver best represent endogenous stores; however, blood is most frequently analyzed because of the relative sampling ease. Concentrations of trace elements in blood are reduced in clinically deficient animals but may not reflect marginal status. Improved blood tests that are based on immunity or reactive pools of trace elements await development.

Implications

Deficiencies and imbalances of trace elements can affect productivity of ruminants. Whereas improvement in feed intake can occur quickly in response to zinc or cobalt supplementation, productivity responses to copper or selenium may be slower to become evident by improved health of newborns, greater resistance to mastitis, or increased weaning weights of calves. Assessment of trace element status identifies whether current mineral supplementation of livestock is adequate and whether improved productivity is likely to occur with changes in supplementation.

Literature Cited

Abdelrahman, M. M., and R. L. Kincaid. 1995. Effect of selenium supplementation of cows on maternal transfer of selenium to fetal and newborn calves. J. Dairy Sci. 78:625-630.

Andrewartha, K. A., and I. W. Caple. 1980. Effects of changes in nutritional copper on erythrocyte superoxide dismutase activity in sheep. Res. Vet. Sci. 28:101-104.

Aumont, G., M. Lamard, and J. C. Tressol. 1989. Iodine nutrition in ewes: effects of low to high iodine intake on iodine content of biological fluids in pregnant and lactating ewes. Reprod. Nutr. Dev. 29:113-125.

Awadeh, F. T., M. M. Abdelrahman, R. L. Kincaid, and J. W. Finley. 1998. Effect of selenium supplements on the distribution of selenium among serum proteins in cattle. J. Dairy Sci. 81:1089-1094.

Azuolas, J. K., and I. W. Caple. 1984. The iodine status of grazing sheep as monitored by concentrations of iodine in milk. Aust. Vet. J. 61:223-227.

Bentley, O. G., and P. H. Phillips. 1951. The effect of low manganese rations upon dairy cattle. J. Dairy Sci. 34:396-403.

Blakely, B. R., and D. L. Hamilton. 1985. Ceruloplasmin as an indicator of copper status in cattle and sheep. Can. J. Comp. Med. 49:405-408.

Blum, J. W., and U. Zuber. 1975. Iron stores of liver, spleen and bone marrow, and serum iron concentrations in female dairy cattle in relationship to age. Res. Vet. Sci. 18:294-298.

Boyages, S. C. 1993. Clinical review 49 Iodine deficiency disorders. J. Clin. Endocrin. Metab. 77:587-591.

Boyne, R. 1978. Changes in leucocyte cytochrome oxidase activity associated with deficiency of copper in laboratory and farm animals. Res Vet. Sci. 24:134-138.

Branum, J. C., G. E. Carstens, E. H. McPhail, K. W. McBride, and A. B. Johnson. 1998. Effects of prenatal dietary copper level on immune function of calves at birth and 56 days of age. J. Anim. Sci. 76 (Suppl.1):43 (Abstr.).

Carter, J. C., W. J. Miller, M. W. Neathery, R. P. Gentry, P. E. Stake, and D. M. Blackmon. 1974. Manganese metabolism with oral and intravenous ^{54}Mn in young calves as influenced by supplemental manganese. J. Anim. Sci. 38:1284-1290.

Claypool, D. W., F. W. Adams, H. W. Pendell, N. A. Hartmann, Jr., and J. F. Bone. 1975. Relationship between the level of copper in the blood plasma and liver of cattle. J. Anim. Sci. 41:911-914.

Dargatz, D. A., and P. F. Ross. 1996. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. J. Anim. Sci. 74:2891-2895.

Engel, R. W., W. A. Hardison, R. F. Miller, N. O. Price, and J. T. Huber. 1964. Effect of copper intake on concentration in body tissue and on growth, reproduction and production in dairy cattle. J. Anim. Sci. 23:1160-1164.

Engle, T. E., C. F. Nockels, C. V. Kimberling, D. L. Weaber, and A. B. Johnson. 1997. Zinc repletion with organic and inorganic forms of zinc and protein turnover in marginally zinc-deficient calves. J. Anim. Sci. 75:3074-3081.

Erskine, R.J., and P. C. Barlett. 1993. Serum concentrations of copper, iron, and zinc during *Escherichia coli*-induced mastitis. J. Dairy Sci. 76:408-413.

Etzel, K. R., M. R. Swerdel, J. N. Swerdel, and R. J. Cousins. 1982. Endotoxin-induced changes in copper and zinc metabolism in the Syrian hamster. J. Nutr. 112:2363-2373.

Gallop, P. M., O. O. Blumenfeld, and S. Seifter. 1972. Structure and metabolism of connective tissue proteins. Annu. Rev. Biochem. 41:617-672.

Gengelbach, G. P., and J. W. Spears. 1998. Effects of dietary copper and molybdenum on copper status, cytokine production, and humoral immune response of calves. J. Dairy Sci. 81:3286-3292.

Gerloff, B. F. 1992. Effect of selenium supplementation on dairy cattle. J. Anim. Sci. 70:3934-3940.

Gibbons, R. A., S. N. Dixon, K. Hallis, A. M. Russell, B. F. Sansom, and H. W. Symonds. 1976. Manganese metabolism in cows and goats. 1976. Biochim. Biophys. Acta 444:1-10.

Gooneratne, S. R., and D. A. Christensen. 1989. A survey of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Can. J. Anim. Sci. 69:141-150.

Hancock, D. 1996. Strategic laboratory use. Bovine Proc. 28:118-125.

Hidiroglou, M., S. K. Ho, and J. F. Standish. 1978. Effects of dietary manganese levels on reproductive performance of ewes and on tissue mineral composition of ewes and day-old lambs. Can. J. Anim. Sci. 58:35-41.

Hidiroglou, M., and C. J. Williams. 1982. Trace elements status of fetuses from ewes fed a copper-deficient ration. Am. J. Vet. Res. 43:310-313.

Johnson, J. L. 1997. Molybdenum In: B. L. O'Dell and R. A. Sunde (Ed.) Handbook of Nutritionally Essential Mineral Elements. pp 413-438. Marcel Dekker, New York.

Johnson, M. A. 1990. Iron: Nutrition monitoring and nutrition status assessment. J. Nutr. 120:1486-1491.

Kellogg, D. W., J. M. Rakes, and D. W. Gliedt. 1989. Effect of zinc methionine supplementation on performance and selected blood parameters of lactating dairy cows. Nutr. Rep. Int. 40:1049-1057.

Kennedy, D.G., S. Kennedy, W. J. Blanchflower, J. M. Scott, D. G. Weir, A. M. Molloy, and P. B. Young. 1994. Cobalt-vitamin B_{12} deficiency causes accumulation of odd-numbered, branched-chain fatty acids in the tissues of sheep. Br. J. Nutr. 71:67-76.

Kennedy, D. G., F. P. M. O'Harte, W. J. Blanchflower, and D. A. Rice. 1991. Sequential changes in propionate metabolism during the development of cobalt/vitamin B12 deficiency in sheep. Biol. Trace Elem. Res. 28:233-241.

Kennedy, D. G., P. B. Young, S. Kennedy, J. M. Scott, A. M. Molloy, D. G. Weir, and J. Price. 1995. Cobalt-vitamin B_{12} deficiency and the activity of methylmalonyl CoA mutase and methionine synthase in cattle. Int. J. Vit. Nutr. Res. 65:241-247.

Kincaid, R. L. 1980. Toxicity of ammonium molybdate added to drinking water of calves. J. Dairy Sci. 63:608-610.

Kincaid, R. L. 1995. The biological basis for selenium requirements of animals. Prof. Anim. Sci. 11:26-29.

Kincaid, R. L., and J. D. Cronrath. 1979. Effects of dietary zinc upon tissue zinc and percent unsaturated plasma-zinc binding capacity. J. Dairy Sci. 62:572-576.

Kincaid, R. L., C. C. Gay, and R. I. Krieger. 1986. Relationship of serum and plasma copper and ceruloplasmin concentrations of cattle and the effects of whole blood sample storage. Am. J. Vet. Res. 47:1157-1159.

Kincaid, R. L., and A. S. Hodgson. 1989. Relationship of selenium concentrations in blood of calves to blood selenium of the dam and supplemental selenium. J. Dairy Sci. 72:259-263.

Kincaid, R. L., W. J. Miller, P. R. Fowler, R. P Gentry, D. L. Hampton, and M. W. Neathery. 1976. Effect of high dietary zinc metabolism and intracellular distribution in cows and calves. J. Dairy Sci. 59:1580-1584.

Kincaid, R. L., and M. J. Rock. 1999. Selenium intakes during late gestation on immunoglobulins and thyroid hormones in sheep. FASEB J. 13(4):A249.

Kincaid, R. L., and C. L. White. 1988. The effects of ammonium tetrathiomolybdate intake on tissue copper and molybdenum in pregnant ewes and lambs. J. Anim. Sci. 66:3252-3258.

Knowles, S. O., N. D. Grace, K. Wurms, and J. Lee. 1999. Significance of amount and form of dietary selenium on blood, milk, and casein selenium concentrations in grazing cows. J. Dairy Sci. 82:429-437.

Langlands, J. P., J. E. Bowles, G. E. Donald, and A. J. Smith. 1984. Deposition of copper, manganese, selenium and zinc in Merino sheep. Aust. J. Agric. Res. 35:701-707.

Lee, J., B. P. Treloar, and N. D. Grace. 1994. Metallothionein and trace element metabolism in sheep tissues in response to high and sustained zinc dosages. II. Expression of metallothionein m-RNA. Aust. J. Agric. Res. 45:321-332.

Levander, O. A. 1986. Selenium. In: W. Mertz (Ed.) Trace Elements in Human and Animal Nutrition vol. 2. pp 209-279. Academic Press, New York.

Lilley, C. W., D. W. Hamar, M. Gerlach, and J. L. Johnson. 1985. Linking copper and bacteria with abomasal ulcers in beef calves. Vet. Med. 80:85-88.

Maas, J., D. Galey, J. R. Peauroi, J. T. Case, E. S. Littlefield, C. C. Gay, L. D. Koller, R. O. Crisman, D. W. Weber, D. W. Warner, and M. L. Tracy. 1992. The correlation between serum selenium and blood selenium in cattle. J. Vet. Diag. Invest. 4:48-52.

Matrone, G., C. Conley, G. H. Wise, and R. K. Waugh. 1957. A study of iron and copper requirements of dairy calves. J. Dairy Sci. 40:1437-1447.

Mason, J. 1982. The putative role of thiomolybdates in the pathogenesis of Mo-induced hypocupraemia and molybdenosis: some recent developments. Irish Vet. J. 36:164-168.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1988. Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and the activity of manganese enzymes in tissues. Aust. J. Agric. Res. 39:517-524.

McC. Howell, J., and A. N. Davison. 1959. The copper content and cytochrome oxidase activity of tissues from normal and swayback lambs. Biochem. J. 72:365-368.

McDowell, L. R. 1992. Minerals in Animal and Human Nutrition. Academic Press, New York.

McDowell, L. R., B. J. Gordon, R. C. Merkel, V. Fadok, N. S. Wilkinson, and G. A. Kunkle. 1991. Mineral status comparisons in goats of Florida with emphasis on zinc deficiency. Small Ruminant Res. 5:327-335.

Miller, J. K., E. W. Swanson, and G. E. Spalding. 1975. Iodine absorption, excretion, recycling, and tissue distribution in the dairy cow. J. Dairy Sci. 58:1578-1593.

Miller, W. J. 1975. New concepts and developments in metabolism and homeostasis of inorganic elements in dairy cattle. A review. J. Dairy Sci. 58:1549-1560.

Mills, C. F. 1987. Biochemical and physiological indicators of mineral status in animals: copper, cobalt and zinc. J. Anim. Sci. 65:1702-1711.

Mills, C. F., A. C. Dalgarno, R. B. Williams, and J. Quarterman. 1967. Zinc deficiency and the zinc requirements of calves and lambs. Br. J. Nutr. 21:751-771.

Mollerberg, L. 1975. A hematologic and blood chemical study of Swedish purchased calves. Acta Vet. Scand. 16:1-8.

Neathery, M. W., W. J. Miller, D. M. Blackmon, R. P. Gentry, and J. B. Jones. 1973a. Absorption and tissue zinc content in lactating dairy cows as affected by low dietary zinc. J. Anim. Sci. 37:848-852.

Neathery, M. W., W. J. Miller, D. M. Blackmon, F. M. Pate, and R. P. Gentry. 1973b. Effects of long term zinc deficiency on feed utilization, reproductive characteristics, and hair growth in the sexually mature male goat. J. Dairy Sci. 56:98-105.

Ott, E. A., W. H. Smith, R. B. Harrington, and W. M. Beeson. 1966a. Zinc toxicity in ruminants. III. Physiological changes in tissues and alterations in rumen metabolism in lambs. J. Anim. Sci. 25:424-431.

Ott, E. A., W. H. Smith, R. B. Harrington, H. E. Parker, and W. M. Beeson. 1966b. Zinc toxicity in ruminants. IV. Physiological changes in tissues of beef cattle. J. Anim. Sci. 25:432-438.

Paterson, J. E., and A. MacPherson. 1990. A comparison of serum vitamin B_{12} and serum methylmalonic acid as diagnostic measures of cobalt status in cattle. Vet. Rec. 126:329-332.

Paynter, D. I. 1982. Differences between serum and plasma ceruloplasmin activities and copper concentrations: Investigation of possible contributing factors. Aust. J. Biol. Sci. 35:353-361.

Pehrson, B., J. Hakkarainen, and J. Tyopponen. 1986. Nutritional muscular degeneration in young heifers. Nord. Vet. Med. 38:26-30.

Prasad, A. S. 1998. Zinc and immunity. Mol. Cell. Biochem. 188:63-69.

Price, J., S. Ueno, and S. G. Wood. 1993. Recent developments in the assay of plasma vitamin B_{12} in cattle. In: M. Anke, D. Meissner, and C. F. Mills (Ed) Proc. Eighth International Symp. on Trace Elements in Man and Animals. pp 317-318. Verlag Media Touristik, Gersdorf, Germany.

Puls, R. 1988. Mineral Levels in Animal Health. Sherpa International, Clearbrook, B.C., Canada.

Quirk, M. F., and B. W. Norton. 1988. Detection of cobalt deficiency in lactating heifers and their calves. J. Agric. Sci. 110:465-470.

Rice, D. A., M. McLoughlin, W. J. Blanchflower, C. H. McMurray, and E. A. Goodall. 1989. Sequential changes in plasma methylmalonic acid and vitamin B_{12} in sheep eating cobalt-deficient grass. Biol. Trace Element Res. 22:153-164.

Rojas, M. A., I. A. Dyer, and W. A. Cassatt. 1965. Manganese deficiency in the bovine. J. Anim. Sci. 24:664-667.

Schalm, O. W. 1980. Blood and blood-forming organs In: H. E. Amstutz (Ed.) Bovine Medicine and Surgery II. pp 791. American Veterinary Publication, Santa Barbara, CA.

Scholz, R. W., and L. J. Hutchinson. 1979. Distribution of glutathione peroxidase activity and selenium in the blood of dairy cows. Am. J. Vet. Res. 40:245-248.

Shankar, A. H., and A. S. Prasad. 1998. Zinc and immune function: the biological basis of altered resistance to infection. Am. J. Clin. Nutr. 68(Suppl.):447S-463S.

Sheppard, A. D., L. Blom, and A. B. Grant. 1984. Levels of selenium in blood and tissues associated with some selenium deficiency diseases in New Zealand sheep. N. Z. Vet. Res. 32:91-95.

Small, J. A. 1997. Serum mineral concentrations in relation to parturition in beef heifers and cows fed conserved forage. Can. J. Anim. Sci. 77:63-68.

Small, J. A., E. Charmley, A.V. Rodd, and A. H. Fredeen. 1997. Serum mineral concentrations in relation to estrus and conception in beef heifers and cows fed conserved forage. Can. J. Anim. Sci. 77:55-62.

Smith, K. L., J. S. Hogan, and H. R. Conrad. 1988. Selenium in dairy cattle: Its role in disease resistance. Vet. Med. 83:72-78.

Smith, R. M. 1997. Cobalt. In: B. L. O'Dell and R. A. Sunde (Ed.) Handbook of Nutritionally Essential Mineral Elements. pp 357-387. Marcel Dekker, New York.

Stake, P. E., W. J. Miller, R. P. Gentry, and M. W. Neathery. 1975. Zinc metabolic adaptations in calves fed a high but nontoxic zinc level for varying time periods. J. Anim. Sci. 40:132-137.

Stowe, H. D., and T. H. Herdt. 1992. Clinical assessment of selenium status of livestock. J. Anim. Sci. 70:3928-3933.

Suliman, H. B., A. I. Abdelrahim, A. M. Zakia, and A. M. Shommein. 1988. Zinc deficiency in sheep: Field cases. Trop. Anim. Health Prod. 20:47-51.

Suttle, N. F., and A. C. Field. 1968. Effect of intake of copper, molybdenum and sulphate on copper metabolism in sheep. I. Clinical condition and distribution of copper in blood of the pregnant ewe. J. Comp. Pathol. 78:351-362.

Thompson, R. H., C. H. McMurray, and W. J. Blanchflower. 1976. The levels of selenium and glutathione peroxidase activity in blood of sheep, cows and pigs. Res. Vet. Sci. 20:229-231.

Torre, P. M., R. J. Harmon, R. W. Hemken, T. W. Clark, D. S. Trammell, and B. A. Smith. 1996. Mild dietary copper insufficiency depresses blood neutrophil function in dairy cattle. J. Nutr. Immunol. 4:3-24.

Van Saun, R. J., T. H. Herdt, and H. D. Stowe. 1989. Maternal and fetal selenium concentrations and their interrelationships in dairy cattle. J. Nutr. 119:1128-1137.

Waldner, C., J. Campbell, G. K. Jim, P. T. Gulchon, and C. Booker. 1998. Comparison of 3 methods of selenium assessment in cattle. Can. Vet. J. 39:225-231.

Ward, J. D., and J. W. Spears. 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. J. Anim. Sci. 75:3057-3065.

Wegner, T. N., D. E. Ray, C. D. Lox, and G. H. Stott. 1973. Effect of stress on serum zinc and plasma corticoids in dairy cattle. J. Dairy Sci. 56:748-752.

Wellinghausen, N., and L. Rink. 1998. The significance of zinc for leukocyte biology. J. Leukoc. Biol. 64:571-577.

Wikse, S. E., D. Herd, R. Field, and P. Holland. 1992. Diagnosis of copper deficiency in cattle. J. Am. Vet. Med. Assoc. 200:1625-1629.

Wittenberg, K. M., and T. J. Devlin. 1988. Effects of dietary molybdenum on productivity and metabolic parameters of lactating ewes and their offspring. Can J. Anim. Sci. 68:769-778.

Woolliams, J. A., G. Wiener, C. Woolliams, and N. F. Suttle. 1985. Retention of copper in the liver of sheep genetically selected for high and low concentrations of copper in plasma. Anim. Prod. 41:219-226.

Xin, Z., D. F. Waterman, R. W. Hemken, and R. J. Harmon. 1993. Copper status and requirement during the dry period and early lactation in multiparous Holstein cows. J. Dairy Sci. 76:2711-2716.

Notes

1. Correspondence: phone: 509-335-2457; fax: 509-335-1082.

Table 1. Criteria for classification of cattle on copper in liver and plasma[a]

Diet	Cu in liver, µg/g DM	Cu in plasma, µg/mL
Clinically deficient	< 20	< .2
Deficient	< 33	.2 to .5
Marginal	33 to 125	.5 to .7
Adequate	125 to 600	.7 to .9
High	600 to 1,250	.9 to 1.1
Toxic	> 1,250[b]	> 1.2

[a]Adapted from Puls (1988) and Wikse et al. (1992).

[b]Ranges for liver Cu associated with toxicosis seem variable and have not been definitively established.

Table 2. Criteria for classification of cattle on zinc in plasma and liver[a]

Status	Zn in liver, µg/g DM	Zn in plasma, µg/mL
Deficient	< 20 to 40	.2 to .4
Marginal	25 to 40	.5 to .8
Adequate	25 to 200	.8 to 1.4
High	300 to 600	2 to 5
Toxic	> 1,000	3 to 15

[a]Adapted from Puls (1988).

Table 3. Criteria for classification of cattle on selenium in blood and liver[a]

Classification	Se in whole blood, ng/mL	Se in liver of adults, µg/g DM	Se in liver of newborns, µg/g DM
Severely deficient	< 60	.1 to .5	< 1.1
Marginally deficient	60 to 200	.6 to 1.25	1.1 to 2.2
Adequate	210 to 1200	1.25 to 2.5	2.3 to 8.0
High adequate	> 1,200	> 2.5	

[a]Adapted from Sheppard et al., 1984; Pehrson et al., 1986; Puls, 1988; Van Saun et al., 1989; Gerloff, 1992; Dargatz and Ross, 1996; and Knowles et al., 1999.

Table 4. Criteria for classification of cattle on manganese in blood, serum, and liver[a]

Status	Mn in whole blood ng/mL	Mn in serum ng/mL	Mn in liver µg/g, DM
Deficient	< 20	< 5	< 7
Marginal	20 to 60	5 to 6	7 to 15
Adequate	70 to 200	6 to 70	> 13

[a]Adapted from Rojas et al. (1965) and Puls (1988).

Table 5. Criteria for classification of cattle on metabolites in serum, liver, and urine[a]

	Serum		Liver		Urine	
Status	$B_{12,}$ ng/L	MMA[b], µmol/L	FIGLU, µmol/L[c]	$B_{12,}$ µg/g, wet wt	MMA, mg/L	FIGLU, µmol/L
Deficient	100	> 4	100 to 200	.04 to .15	30 to 150	50 to 2,000
Marginal	100 to 200	2 to 4		.15 to .30		
Adequate	200	< 2	0	.30 to 2.24	< 25	0 to 10

[a]Adapted from Puls (1988), Quirk and Norton (1988), and Paterson and MacPherson (1990).

[b] MMA = methylmalonic acid.

[c] FIGLU = formimino-glutamic acid.

Table 6. Criteria for classification of cattle on iron and ferritin in tissues[a]

Status	Fe in liver, µg/g wet wt	Fe in Kidney, µg/g wet wt	Fe in Serum, µg/100 mL	Serum ferritin, ng/mL
Deficient	< 40	< 20	15–120	2–10
Adequate	45–300	30–150	130–250	30–50
High	53–700	49–300	400–600	> 80

[a]Adapted from Puls (1988).

Table 7. Criteria for classification of cattle on iodine in tissues[a]

Status	Total I in serum, µg/100 mL	Protein bound I, µg/100 mL	Milk I, µg/L	Urinary I, µg/100 mL	Serum T_4, ng/mL
Deficient	1–5	3–5.3	8–25	NA[b]	< 7–30
Adequate	10–40	4.6–12.8	30–300	10–25	20–100
Excessive	70–300	20–100	500–3,500	> 50	34–120

[a]Adapted from Puls (1988).
[b]Not available.