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NUTRITION: *Invited Review*

Invited Review: Assessing trace mineral status in ruminants, and factors that affect measurements of trace mineral status

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ABSTRACT

Purpose: The purpose of this article is to review criteria for assessing copper, zinc, manganese, and selenium status in ruminants. Factors that affect measurements of trace mineral status also will be discussed.

Sources: Published scientific literature was the primary source of information reviewed.

Synthesis: When assessing mineral status, it is always good to analyze the diet or forages being consumed for the mineral of interest as well as other minerals that may affect its requirement. Liver is the best indicator of both low and excess Cu status. Plasma Cu concentrations do not decrease below normal values until liver Cu stores are mostly depleted, but a plasma Cu concentration less than 0.4 mg/L suggests Cu deficiency. Severe Zn deficiency can be diagnosed based on extremely low plasma or serum Zn concentrations (less than 0.5 mg/L) or on clinical signs of Zn deficiency that respond to Zn supplementation. It is important to note that infections or acute stress may cause plasma Zn concentrations to temporarily decrease to levels consistent with Zn deficiency. There is currently no reliable indicator of marginal Zn deficiency. Several criteria have been measured in an attempt to assess Mn status. However, no criteria have been demonstrated to accurately predict Mn deprivation. Whole blood or liver Se concentrations are useful in assessing Se status. When interpreting whole blood or liver Se concentrations, it is important to consider whether dietary Se is being derived from organic or inorganic sources.

Conclusions and Applications: The most appropriate measurement criteria to assess trace mineral status in ruminants depend on the trace mineral being considered. Liver copper is generally a good measure of low as well as excess Cu status. Liver or whole blood Se concentrations are reliable measures of Se status if one takes into account the source of dietary Se. Unless clinical deficiency signs are apparent, it is more difficult to assess Zn and Mn status. In the absence of disease or stress, plasma or serum Zn concentrations below 0.5 mg/L suggest possible severe deficiency. No reliable predictor of marginal Zn deficiency has been determined. Currently, no reliable indicator of Mn status has been identified.

Key words: trace mineral status, ruminants, copper, zinc, manganese, selenium

INTRODUCTION

Analyzing the diet or forages being grazed for the trace mineral in question, as well as other minerals that can affect the requirement of the mineral, is always a good place to start when assessing mineral status. The trace mineral content of forages (Mortimer et al., 1999) and by-product feeds (DePeters et al., 2000) varies considerably. In some areas mineral analysis of water can also be useful in assessing trace mineral status. The most appropriate measurement criteria in the animal vary depending on the trace mineral. Liver and plasma or serum are the most common criteria used to predict trace mineral status. Red blood cell concentrations of iron (Fe), zinc (Zn), manganese (Mn), and selenium (Se) are considerably greater than concentrations in plasma (Underwood, 1977). Therefore, hemolysis of red blood cells will result in falsely elevated levels of these trace minerals in plasma or serum. In the literature liver trace mineral concentrations are expressed either on a DM basis or wet weight basis. In our opinion liver trace mineral concentrations should be expressed on a DM basis because of dehydration and other factors that may affect liver moisture content. For example, liver biopsy samples obtained from cull dairy cows before being transported to a commercial slaughter plant analyzed 22.6% DM, whereas liver samples collected from these cows following slaughter averaged 31.6% DM (T. E. Engle, Colorado State University, Fort Collins, personal communication). For the purpose of this article, liver concentrations in ruminants (except fetal liver) expressed

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in publications on a wet tissue basis have been converted to a DM basis assuming a liver DM content of 28% (Herdt and Hoff, 2011). The DM content of fetal liver will vary depending on the stage of fetal development. Therefore, fetal liver trace mineral concentrations are reported as they were expressed in the original publication.

Concentrations of certain trace minerals reported in older literature are likely less accurate than those measured using newer and more sensitive analytical methods. This is especially true for reported serum or plasma Mn concentrations. Low concentrations of Mn can be accurately measured today using flameless (graphite furnace) atomic absorption spectrophotometry or inductively coupled plasma mass spectrometry.

Reference values indicative of deficiency or marginal trace mineral status vary in textbooks and scientific literature (Kincaid, 2000; Suttle, 2010) and among diagnostic laboratories. Diagnostic ranges reported in *Mineral Levels in Animal Health* by R. Puls (1994) are frequently used in diagnostic laboratories. In this book Puls defined deficient as "levels at which subclinical or pathological signs of deficiency should be apparent," marginal as "levels at which subclinical effects may prevail, such as reduced immune response or growth rate," and adequate as "levels sufficient for optimum functioning of all body mechanisms with a small margin of reserve to counteract commonly encountered antagonistic condition." The objective of this article is to review criteria for assessing copper (Cu), Zn, Mn, and Se status in ruminants. Factors that affect measurements of trace mineral status will also be discussed.

COPPER

Copper deficiency is considered one of the most widespread trace mineral deficiencies, especially in grazing cattle. In a study involving 2,007 beef cows and heifers from 256 herds in 18 states, 38.9% were classified as marginally deficient in Cu, based on a serum Cu concentration equal to or less than 0.65 mg/L (Dargatz et al., 1999). Bioavailability of Cu is greatly affected by sulfur (S), molybdenum (Mo), and Fe. Therefore, when deciding whether and how much supplemental Cu is needed, it is important to not only consider Cu but also dietary S, Mo, and Fe concentrations in forages and other feed ingredients. Mortimer et al. (1999) reported that 66.7% of forage samples (n = 709) from 23 states analyzed below the beef cattle recommendation of 10 mg of Cu/kg of DM (NASEM, 2016). Over 40% of forage samples in this study had a Cu:Mo ratio that suggested at least marginal Cu deficiency. Liver Cu concentrations are generally considered the best indicator of Cu status in ruminants. Plasma, serum, and hair Cu concentrations, and activities of superoxide dismutase (**SOD**) in erythrocytes and diamine oxidase in plasma, have also been used to evaluate Cu status. Markers that have been used to assess Cu status will be discussed in more detail below.

Liver Cu (Adults and Growing Ruminants with Developed Rumens)

Liver is the major storage organ for Cu, and Cu can readily be mobilized from the liver into the blood to supply Cu for biochemical functions. Depending on liver Cu concentrations and level of dietary Cu antagonist, liver Cu can maintain normal plasma Cu concentrations for months even in ruminants fed low-Cu diets. In nonlactating Holstein cows with low initial liver Cu status, liver Cu decreased from approximately 61 to 22 mg/kg of DM after 161 d without Cu supplementation (Grace et al., 2012). Liver Cu concentrations in cows with moderate initial liver Cu decreased from approximately 123 to 92 mg/kg during the 161-d study. Cows in this study were fed silage, pasture, and hay that analyzed 6.5 to 9 mg of Cu/kg of DM, 0.5 to 0.6 mg of Mo/kg, and 0.17 to 0.28% S (Grace et al., 2012). In beef cows, plasma Cu concentrations did not decrease below normal concentrations until liver Cu concentrations were less than 40 mg/kg of DM (Claypool et al., 1975). Studies in growing cattle indicate that plasma Cu concentrations do not decrease substantially until liver Cu is less than 20 mg/kg of DM (Phillippo et al., 1987; Hansen et al., 2008).

Liver Cu is affected by dietary Cu, age, species, and breed and possibly by sex and gestation. Increasing dietary Cu increases liver Cu even in ruminants already receiving adequate Cu. Newborn bovines generally have much greater liver Cu concentrations than older cattle (Underwood, 1977). This is not true for sheep as liver Cu generally increases with age (Underwood, 1977). Most sheep breeds retain more Cu in their livers than cattle when fed diets adequate in Cu. Large differences exist among sheep breeds in their ability to accumulate Cu in their livers (Littledike and Young, 1993; Suttle et al., 2002). Liver Cu concentrations also differ among cattle breeds, especially when diets are deficient or marginally deficient in Cu (Mullis et al., 2003; Dermauw et al., 2014). Sex also appears to affect liver Cu in cattle. Male fetuses (Fry et al., 2013) and fattening males (Miranda et al., 2006) had greater liver Cu concentrations than females. Liver Cu decreases in late pregnancy in beef (Fry et al., 2013) and dairy cows (Xin et al., 1993) fed diets marginal (5.5 to 6.6 mg of Cu/kg) in Cu but not in those supplemented with 10 mg of Cu/kg of DM.

Suttle (2010) has defined minimal liver Cu concentrations in ruminants, with developed rumens, based on the likelihood of a Cu-responsive disorder. Using this definition liver Cu less than 7 mg/kg of DM indicates a high risk, and concentrations of 7 to 20 mg/kg of DM suggest a possible risk for disorders that respond to Cu supplementation. In growing cattle liver Cu concentrations of approximately 10 mg/kg of DM or lower have been associated with reduced growth rate, compared with Cu-supplemented animals (Phillippo et al., 1987; Spears et al., 2004; Hansen et al., 2008). The growth response to Cu

supplementation is most evident in cattle fed diets high in Mo and S (Phillippo et al., 1987).

Diagnostic laboratories generally use considerably greater liver Cu concentrations as indicators of deficiency or marginal Cu status. Puls (1994) suggests liver Cu concentrations of 2 to 36 mg/kg as deficient, 18 to 90 mg/kg as marginal, and 90 to 360 mg/kg as adequate.

Liver Cu (Newborn Ruminants)

Liver Cu is generally greater in newborn calves than in older cattle. Copper stored in the liver serves to provide the young animal with Cu when receiving primarily milk, which is low in Cu. Elevated liver Cu at birth also is due to the inability of the fetal liver to synthesize ceruloplasmin (**Cp**; Underwood, 1977). A high percentage (70–90%) of Cu in plasma leaving the liver is present in Cp. Plasma Cu concentrations are extremely low at birth but increase within 1 to 2 d of age as the liver starts synthesizing Cp.

The critical concentration of Cu in the newborn calf liver needed to prevent deficiency, during the nursing period, has been suggested at 300 mg/kg of DM (Gooneratne et al., 1989). Fetal liver Cu concentrations are not affected by stage of gestation (Abdelrahman and Kincaid, 1993). However, the total amount of Cu deposited in fetal liver increases throughout fetal development. Transfer of Cu to fetal liver is especially high in the last trimester when most of the fetal growth occurs. Liver Cu is lower than normal in fetuses from cows with low Cu status (Gooneratne et al., 1989; Fry et al., 2013). As indicated earlier, fetal liver Cu is greater in male than female fetuses (Fry et al., 2013).

Young ruminants may be more susceptible to Cu toxicosis than older animals. Calves are born with high liver Cu concentrations, if their dams were adequate in Cu. Absorption of Cu in lambs is considerably greater before rumen development compared with after rumen development (Suttle, 1973). Based on a study of calves (from birth to 1 yr of age) necropsied at the California Animal Health and Food Safety Laboratory, dairy calves had liver Cu concentrations 175 to 215 mg/kg of DM greater than beef calves (Puschner et al., 2004). In this study the estimated liver Cu concentration at birth was approximately 130 mg/kg of DM greater for dairy than beef calves. The greater liver Cu in dairy calves at birth likely relates to greater Cu supplementation to maternal diets and perhaps greater bioavailability of Cu from dairy versus beef cow diets. The high liver Cu concentrations generally observed in dairy calves raises questions regarding Cu supplementation to milk replacers used for heifer development and veal calf production.

A study with Holstein and Ayrshire-Holstein crossbred male calves indicated that they could tolerate up to 500 mg of Cu/kg of DM (from $CuSO_4$) in milk replacers for up to 6 wk (Jenkins and Hidiroglou, 1989). However, gain was reduced and liver Cu concentrations were greatly increased in calves supplemented with 200 or 500 mg of Cu/kg of DM. Liver histology was not evaluated in this study. Copper toxicosis was diagnosed in 7 veal calves, 10 to 16 wk of age, based on liver damage and high liver and kidney Cu concentrations (Sullivan et al., 1991). Liver Cu concentrations in the calves ranged from approximately 950 to 2,400 mg/kg of DM, and the severity and extent of liver lesions appeared to correlate with liver Cu concentrations. The Cu content of the diet was not indicated in the case report, but it did indicate that the calves had been supplemented with various Cu-containing hematinics. Robinson et al. (1999) reported that 3 to 5% of livers from veal calves in Indiana were condemned due to abnormalities in color and texture. To investigate this problem, 258 Holstein calves were purchased from Wisconsin dairy farms at 1 to 3 d of age and transported to a veal facility in Indiana. Calves were given milk replacer that analyzed 12.8 mg of Cu/kg of DM. Some of the calves in this study underwent a clinical hemolytic crisis before 8 wk of age that resulted in generalized icterus and decreased red blood cell counts. These calves either died or recovered following treatment (treatment not described). Livers from slaughtered calves and calves that died were divided into 4 categories based on severity of histopathologic alterations. Concentrations of hepatic Cu tended to decrease and kidney Cu concentrations increased as the severity of liver lesions increased, especially in calves with severe liver damage. They postulated that elevated liver Cu resulted in hepatic damage that reduced the hepatocellular mass capable of storing Cu (Robinson et al., 1999).

High winter mortality in female Jersey calves characterized by mild hepatopathy and enteropathy was reported in Scotland (Hunter et al., 2013). Calves that died had liver Cu concentrations of approximately 1,200 mg/kg of DM and also high kidney Cu concentrations. Newborn male calves on this farm were found to have liver Cu concentrations of approximately 800 mg/kg of DM. The high liver Cu concentrations at birth were due to their dams being supplemented at high (41–60 mg of Cu/kg of DM) Cu levels. In addition to already high liver Cu concentrations at birth, female calves were fed milk replacer supplemented with 10 mg of Cu/kg of DM and creep feed supplemented with 35 mg of Cu/kg of DM.

Plasma and Serum Cu

Absorbed Cu is transported to the liver bound to albumin. Copper not excreted in bile, stored, or used for Cu-dependent enzymes in the liver leaves the liver largely bound to Cp. Ceruloplasmin is a multifunctional protein (Healy and Tipton, 2007). It has ferroxidase activity and is important in the oxidation of ferrous Fe (Fe^{+2}), stored in the liver in ferritin, to ferric Fe (Fe^{+3}). This conversion is necessary for the release of stored Fe^{+2} because transferrin, the Fe transport protein in blood, only binds Fe^{+3}. Ceruloplasmin is also an acute phase protein that increases during an infection or during acute stress, if liver Cu is adequate. In Cu-deficient calves, Cp increased only

slightly following a viral and bacterial respiratory disease challenge (Stabel et al., 1993). Ceruloplasmin may also act as an antioxidant by scavenging oxygen radicals, and it has been proposed to transport Cu to certain cellular sites.

Activity of Cp is highly correlated with plasma Cu concentrations (Legleiter and Spears, 2007). Serum Cu concentrations are widely reported in the literature. However, plasma Cu represents a more accurate measure of circulating Cu concentration. Copper should be measured in plasma rather than serum because in the clotting process, some of the Cu present in Cp is sequestrated into the clot (Laven et al., 2008). Ceruloplasmin activity is 18 to 35% lower in serum than in plasma, and Cu concentrations are 14% lower in serum compared with plasma (Kincaid et al., 1986).

The normal range for plasma Cu concentration is 0.6 to 1.5 mg/L, with values usually between 0.8 and 1.2 mg/L (Underwood, 1981). Plasma Cu concentrations less than 0.6 mg/L may indicate marginal Cu status, and values less than 0.4 mg/L suggest Cu deficiency. As indicated earlier, plasma Cu concentrations do not decrease until liver Cu concentrations decrease below 40 mg/kg of DM (Claypool et al., 1975).

Plasma Cu concentrations can be affected by dietary Cu, gestation, disease, and possibly the occurrence of estrus. Copper supplementation to Cu-deficient animals greatly increases plasma Cu, whereas Cu supplementation to diets adequate in Cu results in little or no increase in concentration. Plasma Cu decreases slightly in late pregnancy and then increases during lactation in dairy cows (Xin et al., 1993). The increase in plasma Cu during lactation, relative to plasma concentrations in gestation, is even observed in beef heifers fed Cu-deficient diets (Gengelbach et al., 1994). Induction of Cp synthesis in the liver by disease or acute stress results in increased plasma Cu concentrations in cattle adequate in Cu (Stabel et al., 1993). Serum Cu concentrations were greater when beef heifers and cows were bred at estrus than at 21 d after breeding (Small et al., 1997).

Interaction of Molybdenum and Sulfur with Plasma Cu

It is well documented that high concentrations of Mo and S reduce Cu bioavailability. Sulfide can react with molybdate in the rumen to form various thiomolybdates. Thiomolybdates (especially tri- and tetrathiomolybdates) reduce Cu absorption by forming insoluble complexes with Cu that do not release Cu even under acidic conditions (Suttle, 2010). This can lead to reduced liver Cu and eventually reduced plasma Cu concentrations. Certain thiomolybdates also can be absorbed and affect systemic metabolism of Cu (Gooneratne et al., 1989). Thiomolybdate absorption is most likely when the production of thiomolybdates exceeds the amount that can react with Cu to form insoluble complexes in the rumen. One of the systemic effects is thiomolybdates binding strongly to plasma Cu bound to albumin, which results in reduced transport of available Cu for biochemical processes (Gooneratne et al., 1989). This can result in high plasma Cu concentrations even in ruminants deficient in Cu. Although plasma Cu concentration is above normal, Cp activity remains low. Increased plasma Cu due to absorption of thiomolybdates is rare. Reduced plasma Cu in ruminants receiving diets high in Mo and S is much more common.

Copper Metalloenzymes

Erythrocyte (Gengelbach and Spears, 1998; Ward and Spears, 1997) SOD activity has been measured as an indicator of Cu status. Plasma Cu and Cp activity were reduced much earlier in Cu deficiency than erythrocyte SOD activity (Ward and Spears, 1997). Reduced SOD activity appears to indicate a prolonged Cu deficiency.

Plasma diamine oxidase is another Cu-containing enzyme that has been used to assess Cu deficiency. Activity of diamine oxidase was reduced in Cu-deficient calves and was highly correlated with liver and plasma Cu concentration, and plasma Cp activity (Legleiter and Spears, 2007).

Copper-dependent enzymes can be assayed in a research setting. However, they are much more involved to measure than measuring plasma or liver Cu concentrations, and most diagnostic laboratories do not offer these services. It is also difficult to standardize enzyme assays. Measuring SOD involves repeated washing and centrifugation of red blood cells followed by lysing cells and further processing. The diamine oxidase assay needs to be run with fresh plasma that has not been frozen (Legleiter and Spears, 2007).

Hair and Milk Cu Concentrations

Hair and milk Cu concentrations are not reliable indicators of Cu status. Copper concentrations in hair decrease slowly in Cu deficiency, and low values indicate prolonged deficiency (Suttle, 2010). Variation among individual animals is also high. Copper deficiency has been reported to decrease milk Cu concentrations (Underwood, 1977). However, milk Cu concentrations are affected by stage of lactation and are generally quite low.

Assessing Excess Liver Cu Concentrations

Copper toxicosis is a major problem in sheep and is of increasing concern in dairy cattle. Problems involved in providing adequate dietary Cu but not supplying concentrations that may lead to Cu toxicosis were recently reviewed (López-Alonso and Miranda, 2020). Chronic Cu toxicosis is characterized by gradual accumulation of Cu in the liver over a period of time. As Cu accumulates in the liver, no clinical signs are generally evident until shortly before the hemolytic phase occurs. During the hemolytic phase in sheep, Cu is rapidly released from the liver into the blood, resulting in hemolysis, hemoglobinemia, hemoglobinuria, and elevated kidney Cu concentrations. Elevated kidney Cu concentrations are consistently observed in

cows that die from Cu toxicosis (Bradley, 1993; Bidewell et al., 2012). Clinical signs that may be observed following the hemolytic phase include anorexia, dullness, jaundice of mucous membranes, excessive thirst, dark-colored urine, and frequently death (Howell and Gooneratne, 1987). Liver Cu concentrations may decrease slightly following the hemolytic crisis (Howell and Gooneratne, 1987). Before the hemolytic phase or crisis, liver damage occurs and enzymes concentrations indicative of liver damage may become high, but plasma or serum Cu is normal. In sheep histological and biochemical evidence of liver damage may be seen with liver Cu as low as 350 mg/kg of DM (Suttle, 2010). Clinical signs of Cu toxicosis usually do not occur until liver Cu concentrations reach 1,000 mg/kg of DM or greater. Samples of liver obtained by biopsy or from cull cows at slaughter in the United States (Strickland et al., 2019a) and the United Kingdom (Kendall et al., 2015) have indicated that approximately 40% of dairy cows have liver Cu concentrations above 500 mg/kg of DM. Studies have indicated that high liver Cu concentrations may be associated with increased hepatic oxidative stress, even in dairy cows not showing clinical signs of toxicosis (Lyman et al., 2015; Strickland et al., 2019b). In animals not showing clinical signs of toxicosis, liver Cu concentrations considered high vary from greater than 450 mg/kg of DM in cattle (Grace et al., 2010) to 700 to 2,000 mg of Cu/kg of DM in cattle and sheep (Puls, 1994). Suttle (2010) suggested a marginal toxic concentration for liver Cu of 350 to 1,500 mg/kg of DM in both cattle and sheep. The concentration of Cu in the liver likely to result in toxicosis varies with breed (especially in sheep), feed toxins that cause hepatic damage, and stressors (NASEM, 2005). Liver Cu concentrations in Holstein cows that die from Cu toxicosis generally exceed 1,000 mg/kg of DM (Perrin et al., 1990; Bradley, 1993). Jersey cows are more susceptible to Cu toxicosis than Holsteins. Recent case reports from New Zealand have indicated Cu toxicosis in Jersey cows with liver Cu concentrations of 525 to 885 mg of Cu/kg of DM (Johnston et al., 2014; Morgan et al., 2014). In a herd of 139 Jersey and 249 Holstein cows, 8 died from Cu toxicosis over a 17-mo period (Bidewell et al., 2012). Seven of the 8 that died were Jersey cows.

Liver Cu concentrations can be assessed by obtaining liver biopsies or obtaining liver samples from cull cows at slaughter to determine whether cows are at risk for chronic Cu toxicosis. Liver damage that occurs before clinical signs of Cu toxicosis often results in increased serum activities of liver enzymes. Glutamate dehydrogenase has been shown to be high in serum of dairy calves (Hunter et al., 2013) and dairy cows (Laven et al., 2004; Johnston et al., 2014) with high liver Cu concentrations. Laven et al. (2004) reported that high glutamate dehydrogenase activities observed in cows fed high dietary Cu decreased by 6 wk following removal of supplemental Cu. Serum aspartate aminotransferase activity has also been positivity correlated with high liver Cu concentrations in lambs (Woolliams et al., 1982) and calves (López-Alonso et al., 2006). Serum or plasma Cu is not high until clinical signs of Cu toxicosis are apparent. However, whole blood Cu concentrations were positively correlated with liver Cu concentrations in cattle (López-Alonso et al., 2006). Erythrocyte Cu concentrations were increased in calves supplemented with high Cu in milk replacer (Jenkins and Hidiroglou, 1989).

Removing supplemental Cu and supplementing with Mo (Bradley, 1993; Morgan et al., 2014) or Mo and S (Johnston et al., 2014) has been very effective in alleviating Cu toxicosis and reducing liver Cu concentrations in dairy cows. Supplementing 200 mg of Mo/cow per day for 26 d reduced liver Cu by 61% in Jersey cows in a herd experiencing chronic Cu toxicosis (Morgan et al., 2014). When a Cu antagonist is not supplemented, the rate of decrease in liver Cu, following the removal of supplemental Cu, is slow in dairy cows with high liver Cu concentrations (Grace et al., 2012; Hittmann et al., 2012). Nonlactating Holstein cows lost liver Cu at a rate of 0.57%/d over a 161-d period when fed a non-Cu-supplemented diet containing 6 to 7 mg of Cu/kg of DM (Grace et al., 2012). In Holstein calves fed milk replacer low in Cu followed by a semi-purified diet containing 1 to 2 mg of Cu/kg of DM, approximately 150 d has been required to reduce plasma Cu concentrations to levels consistent with low or marginal Cu status (Stabel et al., 1993; Gengelbach and Spears, 1998).

ZINC

Severe Zn deficiency has been observed in ruminants (Underwood, 1981) and can be diagnosed based on extremely low plasma or serum Zn concentrations (less than 0.5 mg/L) or presence of clinical Zn deficiency signs that respond to Zn supplementation. However, severe Zn deficiency in ruminants is rare. A marginal Zn deficiency is more likely under practical conditions. Unfortunately, there is no reliable indicator of marginal Zn deficiency, other than a positive production response (growth, reproduction, milk production, or health) of the animal to increased dietary Zn. Analyzing diets and forages for Zn is important in predicting the adequacy of dietary Zn. Of 709 forage samples collected from 23 states, 70% were below the beef cattle recommendation of 30 mg of Zn/kg of DM (NASEM, 2016). However, factors that affect bioavailability of Zn in ruminants are poorly defined (Spears, 2003).

Plasma and Serum Zn

Normal Zn concentrations in serum or plasma generally range between 0.6 and 1.2 mg/L (Suttle, 2010). Low plasma or serum Zn generally reflects either Zn deficiency or acute stress or infection. Plasma Zn concentrations below 0.60 mg/L may indicate Zn deficiency in the absence of acute stress or infection (Suttle, 2010). Under conditions of infection or acute stress, plasma Zn may decrease to concentrations (less than 0.40 mg/L) consistent with

Zn deficiency (Suttle, 2010). Plasma Zn decreases around parturition in beef (Dufty et al., 1977) and dairy cows (Goff and Stabel, 1990) and returns to baseline levels by d 3 of lactation (Goff and Stabel, 1990). Decreases in plasma Zn at parturition are greatest in cows exhibiting dystocia (Dufty et al., 1977) or milk fever (Goff and Stabel, 1990). The decrease in plasma Zn during stress or disease is a normal physiological response, and increasing plasma Zn under these conditions may actually be detrimental to animal health (Chesters and Will, 1981). The decrease in circulating Zn concentrations is due to stress hormones or cytokines inducing the Zn binding protein metallothionein, which temporarily binds Zn in the liver (Suttle, 2010).

In addition to acute stress or disease, plasma Zn concentrations are affected by dietary Zn and age. In ruminants fed diets severely deficient in Zn, plasma or serum Zn concentrations decrease below 0.5 mg/L before the development of clinical signs of deficiency. Serum Zn was decreased below 0.4 mg/L in growing lambs fed a diet containing 3.7 mg of Zn/kg of DM by 14 d (Droke and Spears, 1993). However, deficiency signs such as reduced feed intake and gain, and parakeratotic lesions, were not observed until after 28 d (Droke and Spears, 1993). In young calves fed a diet containing 3 mg of Zn/kg of DM, serum Zn concentrations decreased to 0.18 mg/L by 2 wk (Ott et al., 1965). Decreased weight gain was observed by 3 wk, and parakeratotic lesions started to develop by 4 wk (Ott et al., 1965). The decrease in plasma Zn is less rapid in older sheep fed a Zn-deficient diet, probably because their greater BW provides more mobilization of Zn from tissue turnover (Masters and Moir, 1983). Ewes fed a diet containing 4 mg of Zn/kg of DM throughout gestation had reduced feed intake and produced lambs with lighter birth weight (Masters and Moir, 1983). However, parakeratosis and other clinical signs of Zn deficiency were not observed, although plasma Zn concentrations decreased below 0.5 mg/L by 6 wk of pregnancy (Masters and Moir, 1983). Collectively, these studies indicate that plasma or serum Zn concentrations below 0.5 mg/L may indicate severe Zn deficiency, and that observed clinical signs of Zn deficiency such as parakeratosis are less likely to be apparent in older animals.

Moderate Zn supplementation (20–150 mg/kg) to diets marginal or adequate in Zn has little or no effect on plasma or serum Zn concentrations in calves (Kincaid et al., 1997; Wright and Spears, 2004) and pregnant ewes (Masters and Fels, 1985). However, supplementing Zn-adequate diets with high (300–500 mg/kg) Zn concentrations will increase plasma or serum Zn levels in calves (Kincaid et al., 1997; Wright and Spears, 2004). In sheep Zn concentrations are greater in neonatal than in maternal plasma regardless of Zn status (Masters and Moir, 1983; Apgar and Fitzgerald, 1987). Plasma Zn concentrations in calves shortly after birth are also greater than in their dams (Dufty et al., 1977). In Holstein calves, plasma Zn concentrations were high at birth and decreased with age up to 9 wk (Kincaid and Hodgson, 1989).

There are several possible sources of Zn contamination during the collection of blood. Contamination can occur from evacuated tubes, lubricants and anticoagulants added to tubes, and rubber stoppers (King et al., 2016). Evacuated tubes designated for trace mineral analysis with siliconized rather than rubber stoppers should be used. Some rubber stoppers contain Zn and can result in falsely high plasma or serum Zn concentrations. Red blood cells are much greater in Zn than plasma (King et al., 2016). Therefore, hemolysis of red blood cells can greatly increase Zn concentrations in serum or plasma. Collection of plasma rather than serum is preferred for Zn determination because the extent of hemolysis is usually less when collecting plasma.

Tissue Zn Concentrations

Zinc concentrations in pancreas and bone appear to decrease the most in severe Zn deficiency in calves (Miller and Miller, 1962; Ott et al., 1965). Liver Zn concentrations in weaned calves and cows generally range from 80 to 130 mg/kg of DM. Fetal liver Zn concentrations are considerably greater than dam liver when expressed a DM basis (Gooneratne and Christensen, 1989) or wet tissue basis (Graham et al., 1994). Liver Zn is not a reliable indicator of Zn status in cattle. Liver Zn concentrations were only slightly decreased (107 vs. 130 mg of Zn/kg of DM; Miller and Miller, 1962) or not decreased at all (23.9 vs. 24.4 mg of Zn/kg of wet tissue; Ott et al., 1965) in Zn-deficient calves that received a semi-purified diet containing 3 mg of Zn/kg of diet, compared with those receiving adequate Zn. Lactating dairy cows fed diets containing 39.5 or 16.6 mg of Zn/kg of DM for 6 wk had similar liver Zn concentrations (Neathery et al., 1973a). Supplementing high Zn levels (300–600 mg of Zn/kg of diet) will increase liver Zn concentrations in calves above normal values (Kincaid et al., 1976: Kincaid et al., 1997; Wright and Spears, 2004). In nonlactating cows, supplementation of a control diet, containing 41 mg of Zn/kg of DM, with 600 mg of Zn/kg for 23 d did not affect liver Zn concentrations, suggesting that homeostatic control mechanisms for regulating liver Zn levels are more effective in mature animals (Kincaid et al., 1976). Liver Zn concentrations were reduced in lambs fed a diet severely deficient in Zn (Ott et al., 1964) and in neonatal lambs born to ewes fed a diet containing 4 mg of Zn/kg during pregnancy (Masters and Moir, 1983).

Hair Zn Concentrations

Severe Zn deficiency reduces hair (Miller et al., 1965) and wool (Apgar and Travis, 1979) Zn concentrations under controlled conditions. Hair Zn can be affected by color of hair, age, and the procedure used to wash hair (Miller et al., 1965). Hair samples must be washed with detergents to remove Zn contamination from soil and manure. Hair Zn is also quite variable from one animal to another. Although some studies have observed a relationship between Zn intake and hair Zn, the variation in hair Zn concen-

trations among studies has been greater than differences due to dietary Zn (Perry et al., 1968; Beeson et al., 1977; Mayland et al., 1980).

Milk Zn Concentrations

Zinc concentrations in milk are affected by dietary Zn and stage of lactation, with Zn concentrations decreasing with advanced stage of lactation (Underwood, 1977). Milk Zn concentrations were 23% lower in Holstein cows fed 16.6 mg of Zn/kg compared with cows receiving 39.5 mg of Zn/kg of diet (Neathery et al., 1973b). In a more recent study, increasing dietary Zn from 41 to 63 mg/kg of diet did not alter milk Zn concentrations in dairy cows (Cope et al., 2009). The addition of 1,000 or 2,000 mg of Zn/kg to a control diet greatly increased milk Zn concentrations (Miller et al., 1989). Zinc concentrations in colostrum are 3 to 4 times greater than normal milk (Underwood, 1977). Colostrum Zn concentrations were markedly lower in ewes fed a diet extremely (<1.0 mg/kg) deficient in Zn throughout pregnancy (Apgar and Fitzgerald, 1987) but not in ewes fed a diet containing 4 mg of Zn/kg (Masters and Moir, 1983) compared with ewes receiving adequate Zn.

Marginal Zn Deficiency

There is no reliable indicator of marginal Zn deficiency. In some studies Zn supplementation has increased gains in growing cattle (Price and Humphries, 1980; Spears and Kegley, 2002) or nursing calves (Mayland et al., 1980) with normal plasma or serum Zn concentrations (0.7–1.2 mg/L). Price and Humphries (1980) conducted field trials on 21 farms in Scotland to determine whether supplemental Zn would affect growth of growing and fattening cattle fed typical winter rations. Cattle on each farm were divided into 2 groups, based on weight, breed, and sex, with one group receiving the standard (control) farm diet and the other group receiving 60 mg of supplemental Zn/kg of diet. Mean initial plasma Zn concentrations on the different farms ranged from 0.73 to 1.10 mg/L, and Zn concentrations of diets ranged from 13 to 32 mg/kg of DM. Body weight gain responses to Zn supplementation on the 21 farms ranged from −0.14 to +0.22 kg/d during the 100- to 140-d feeding period. Gain responses to Zn supplementation were not related to plasma Zn concentration or the Zn content of the basal diet (Price and Humphries, 1980).

MANGANESE

Several criteria including plasma or serum Mn; whole blood, liver, and hair Mn concentrations; and Mn-dependent SOD activity have been measured to assess Mn status in ruminants. However, measures of Mn status have been extremely variable among studies, and no criteria have been demonstrated to accurately predict Mn deprivation. Even in animals exhibiting classical signs of Mn deficiency, such as impaired reproduction or skeletal disorders, measures of Mn status have not always differed from controls. Furthermore, age, sex, genetics, and other factors may affect measures of Mn status. Analyzing feedstuffs for Mn also has not always been reliable in determining whether diets are adequate in Mn.

It is difficult to formulate a diet using practical feed ingredients that will guarantee that animals will become Mn deficient. In young lambs fed a semi-purified diet, containing 0.8 mg of Mn/kg of DM, clinical signs of deficiency including joint pain, with poor locomotion and balance, appeared by 12 wk (Lassiter and Morton, 1968). At the end of the 22-wk study, liver, heart, and wool Mn concentrations were reduced in lambs fed the low Mn diet compared with those receiving 29.9 mg of Mn/kg of DM. Ewes fed a semi-purified diet containing 8 mg of Mn/kg of DM from 5 mo before breeding and throughout gestation had reduced whole blood Mn concentrations and required more services per conception (2.5 vs. 1.5) than ewes supplemented with 60 mg of Mn/kg of DM (Hidiroglou et al., 1978a). Hidiroglou et al. (1978b) fed ewes a semi-purified diet containing either 5 or 60 mg of Mn/kg of DM after breeding until lambing. Whole blood Mn (22.1 vs. 12.0 μg/L) and liver Mn concentrations (9.5 vs. 6.8 mg of Mn/kg of DM) were greater in Mn-supplemented ewes at lambing. Newborn lambs from Mn-supplemented ewes also had greater liver Mn concentrations (15.1 vs. 12.2 mg/kg of DM). Considerable variation in liver Mn among ewes and lambs within a treatment was observed in this study (Hidiroglou et al., 1978b).

In a long-term study, Holstein heifers were assigned to a low-Mn diet (7–10 mg of Mn/kg of DM) or Mn-supplemented diet (30 mg of Mn/kg of DM; Bentley and Phillips, 1951). This study lasted almost 4 yr and covered 2 calf crops. Heifers fed the low-Mn diet exhibited delayed first estrus and increased number of services per conception. Four of 14 calves born to heifers fed low Mn had weak legs and pasterns. However, liver and whole blood Mn concentrations in cows and calves were not significantly affected by dietary Mn (Bentley and Phillips, 1951).

Diet Mn

Analyzing feedstuffs for Mn is generally considered the best indicator of dietary Mn adequacy (Underwood, 1981; Puls, 1994). It is also important to consider other minerals that may affect Mn bioavailability, especially Fe, which appears to share a common intestinal transporter with Mn (Hansen et al., 2010). High dietary Ca and P are well documented to affect Mn requirements in poultry and may affect requirements in ruminants.

Other factors also may affect Mn bioavailability in ruminants. A condition referred to as congenital joint laxity and dwarfism has been described in young calves in several countries (White and Windsor, 2012). This condition has been linked to low serum or liver Mn concentrations, and clinical signs have included superior brachygnathism, disproportionate dwarfism, and joint problems that affect

the ability of calves to stand and walk. Birth of calves with congenital joint laxity and dwarfism has been associated with prolonged drought conditions (White and Windsor, 2012) or feeding silage during gestation (Hidiroglou et al., 1990). Beef cows wintered on red clover or grass silage in Canada had lower serum Mn concentrations than cows fed grass hay (Hidiroglou et al., 1990). Incidence of congenital joint laxity and dwarfism in calves born to cows fed red clover or grass silage was 38 and 28%, respectively, whereas all calves born to cows fed hay were normal. Manganese concentration in the hay (51 mg/kg of DM) was actually lower than in the silages (64 or 63 mg of Mn/kg of DM).

Blood Mn

Manganese concentrations are considerably greater in red blood cells than in plasma (Underwood, 1977). Concentrations of Mn in blood are much lower than Cu and Zn. Whereas Cu and Zn concentrations in plasma or serum are expressed as milligrams per liter, Mn is only present in microgram-per-liter concentrations. The low concentrations of Mn in blood results in greater likelihood of errors in analysis, and this is evident in the wide variation of values reported in the literature. Accurately measuring Mn in whole blood, plasma, or serum requires the use of flameless atomic absorption spectrophotometry, neutron activation, or inductively coupled plasma mass spectrometry.

There is little evidence that plasma or serum Mn is a reliable indicator of Mn status. The concentration of Mn in plasma leaving the liver is tightly regulated via biliary excretion. Controlling the amount of Mn in plasma leaving the liver is critical in preventing excess Mn from entering the brain, the major organ responsible for Mn toxicosis (Roth, 2006). Plasma or serum Mn was measured by flameless atomic absorption spectrophotometry in all of the studies cited in the following. Reported plasma or serum Mn concentrations in lambs have varied from approximately 2.0 (Masters et al., 1988) to 44 μg/L (Black et al., 1985). In growing heifers and steers plasma Mn concentrations have varied from approximately 10 to 20 μg/L (Legleiter et al., 2005; Hansen et al., 2006a). Increasing dietary Mn from approximately 16 to 68 mg/kg of DM did not affect plasma Mn in growing heifers (Hansen et al., 2006a). However, plasma Mn concentrations were affected by sampling day in this 196-d study. The addition of graded levels of Mn up to 240 mg/kg to a growing diet (analyzed 29 mg of Mn/kg of DM) or finishing diet (analyzed 8 mg of Mn/kg of DM) did not affect plasma Mn concentrations in steers (Legleiter et al., 2005). Increasing dietary Mn from 13 to 45 mg/kg of DM increased plasma Mn on d 52 but not on d 28 or 84 in a study with ram lambs (Masters et al., 1988). Plasma Mn is affected by age in lambs, with young lambs having much lower concentrations than 16-wk-old lambs (Paynter and Caple, 1984).

Whole blood Mn may better reflect Mn status than plasma or serum. As mentioned previously, ewes fed a semi-purified diet containing 5 (Hidiroglou et al., 1978b) or 8 mg of Mn/kg of DM (Hidiroglou et al., 1978a) had lower whole blood Mn concentrations than ewes supplemented with 60 mg of Mn/kg of DM. Calves born to heifers fed a diet containing 15.8 mg of Mn/kg of DM throughout gestation, and exhibiting signs of Mn deficiency at birth, had lower whole blood Mn concentrations than calves from heifers supplemented with 50 mg of Mn/kg of DM (Hansen et al., 2006b). However, at the end of the study, when calves averaged 67 d of age, whole blood Mn was slightly greater in calves from heifers fed the low-Mn diet. Increasing dietary Mn from approximately 43 to 60 mg/kg of DM in late gestation did not affect whole blood Mn concentrations in Holstein cows or their calves at birth (Weiss and Socha, 2005). Whole blood Mn concentrations were greater in newborn calves than in cows in this study.

Liver Mn

As mentioned earlier, lambs (Lassiter and Morton, 1968) and ewes (Hidiroglou et al., 1978b) fed semi-purified diets low in Mn had reduced liver Mn concentrations compared with Mn-supplemented animals. Beef calves born to dams receiving 13 mg of Mn/kg of DM and showing skeletal abnormalities also had lower liver Mn concentrations at birth than those born to cows fed 21 mg of Mn/kg of DM (Howes and Dyer, 1971). However, liver Mn was not reduced in calves born to dairy cows receiving a diet containing 7 to 10 mg of Mn/kg of DM relative to those born to cows supplemented with Mn (Bentley and Phillips, 1951).

Supplementation of graded levels of Mn to increase dietary Mn from 13 to 45 mg/kg of DM did not affect liver Mn concentrations in ram lambs (Masters et al., 1988). In growing heifers, supplementation of a control diet (15.8 mg of Mn/kg of DM) with 30 or 50 mg of Mn/kg of DM slightly increased liver Mn concentrations (Hansen et al., 2006a). However, differences among sampling days (d 98 and 196 of the study) were greater than treatment differences. Age has been shown to greatly affect liver Mn concentrations in sheep (Paynter and Caple, 1984). The addition of graded concentrations of Mn (ranging from 0 to 240 mg/kg of DM) to growing and finishing steer diets resulted in a linear increase in liver Mn at slaughter (Legleiter et al., 2005). Although a linear response was observed, the increase in liver Mn was small ranging from 12.1 mg/kg of DM in controls up to only 15.1 mg/kg of DM in steers supplemented with 240 mg of Mn/kg of DM. Liver Mn is not affected by stage of gestation in cows (Gooneratne and Christensen, 1989; Graham et al., 1994).

Fetal liver Mn concentrations in the third trimester were reported to be 77% (DM basis; Gooneratne and Christensen, 1989) and 67% (wet weight basis; Graham et al., 1994) of those found in maternal liver (Gooneratne and Christensen, 1989; Graham et al., 1994). Maternal and fetal liver Mn concentrations are positively correlated (Gooneratne and Christensen, 1989; Graham et al., 1994). Based on fetuses and stillborn calves submitted to the

Minnesota Veterinary Diagnostic Laboratory in 2010, fetuses with skeletal abnormalities had lower liver Mn concentrations than fetuses with normal skeletal features (Schefers, 2011).

Sheep appear to differ from cattle with regard to liver Mn concentrations. Newborn lambs had greater liver Mn concentrations than their dams, regardless of dietary Mn (Hidiroglou et al., 1978b). This is consistent with a study indicating that day-old and week-old lambs had greater liver Mn concentrations than adults (Paynter and Caple, 1984).

Hair Mn and Mn-Dependent SOD

Hair Mn concentrations are not a useful measure of Mn status (Underwood, 1981; Puls, 1994). Manganese-dependent SOD activity was increased in heart when dietary Mn was increased from 13 to 45 mg/kg of DM (Masters et al., 1988). However, Mn-dependent SOD was not affected in liver, kidney, testes, or muscle in this study. Furthermore, liver, heart, and lung Mn-dependent SOD activity is affected by age in lambs (Paynter and Caple, 1984).

SELENIUM

Forages and other feedstuffs produced in many areas contain Se concentrations that are deficient or marginal in terms of meeting requirements of ruminants. In the United States, the US FDA restricts the amount of Se that can be supplemented to diets to 0.3 mg of Se/kg of DM. Ruminants can ingest Se in organic and inorganic forms. Selenium occurring naturally in feedstuffs is present primarily as selenomethionine (SeMet). Most supplemental forms of organic Se, including selenized yeast, also contain largely SeMet. Inorganic Se is generally supplemented as sodium selenite (SeO_3). Organic and inorganic Se are metabolized differently in the body, and measures of Se status are affected by Se source. Before discussing measures of Se status, it is important to understand the metabolism of inorganic and organic Se.

Rumen Metabolism

Selenium absorption is much lower in ruminants than in nonruminants. Low absorption of Se in ruminants is believed to result from reduction of dietary Se to insoluble forms such as elemental Se or selenide by ruminal microorganisms (Spears, 2003). Organic Se is taken up by ruminal microorganisms to a greater extent than inorganic Se (Van Ryssen et al., 1989; Mainville et al., 2009; Galbraith et al., 2016). Following incubation with SeO_3, 42% of the Se in ruminal microorganisms was present as selenocysteine (SeCys) and only 20% as SeMet (Van Ryssen et al., 1989). In contrast, 79% of the Se present in ruminal microorganisms incubated with SeMet was present as SeMet. The greater uptake of organic Se by ruminal microorganisms may increase bioavailability of Se by reducing the amount of dietary Se reduced to insoluble forms in the rumen (Galbraith et al., 2016). Selenomethionine and Se yeast were approximately twice as bioavailable, based on erythrocyte glutathione peroxidase activity (**GSHpx**), as SeO_3 when supplemented to Se-deficient heifers (Pehrson et al., 1989). Glutathione peroxidase activity is generally not affected by source when organic and inorganic Se are supplemented to diets adequate in Se (Ortman and Pehrson, 1999; Juniper et al., 2006; Guyot et al., 2007). However, supplementation of organic Se results in greater concentrations of Se in blood, milk, liver, and muscle than inorganic Se, especially when supplemented to diets in quantities greater than requirements (Juniper et al., 2006, 2008).

Mammalian Metabolism

In the body SeO_3 is reduced to selenide, which then undergoes a series of reactions to form SeCys. Selenocysteine is the form of Se present in the active site of selenoenzymes, such as GSHpx (Sunde, 1997). Inorganic Se not used for synthesis of SeCys is largely excreted in the urine. There are 2 possible pathways for SeMet. Following intestinal absorption, SeMet enters the methionine pool, where it can be incorporated into nonspecific proteins in place of methionine or be further metabolized to selenide for synthesis of SeCys (Sunde, 1997). The amount of SeMet incorporated into nonspecific proteins is affected by dietary methionine (Butler et al., 1989). The greater Se concentrations in blood, milk, liver, and skeletal muscle in ruminants receiving organic Se versus inorganic Se is largely due to SeMet being incorporated into general proteins, when Se is supplemented to diets adequate or marginal in Se (Juniper et al., 2006, 2008).

Blood Se

Whole blood Se is considered a more desirable measure of Se status than serum or plasma Se concentrations (Maas et al., 1992; Herdt and Hoff, 2011). Plasma or serum Se concentrations are sensitive to short-term changes in Se intake. In cattle fed free-choice minerals, intake can vary greatly from day to day, and this can cause plasma or serum Se concentrations to not accurately reflect Se status. Hemolysis of red blood cells during processing will also falsely increase serum Se concentrations because 60 to 70% of the total Se in blood is present in erythrocytes (Maas et al., 1992; Herdt and Hoff, 2011). Because of the slow turnover of erythrocytes, whole blood Se gives a better overall measure of long-term Se status. The majority of Se in erythrocytes is present in GSHpx. Whole blood Se is positively correlated ($r = 0.85$) with whole blood GSHpx activity (Thompson et al., 1981; Koller et al., 1984).

Fetal whole blood Se concentrations are similar to maternal values, whereas serum Se concentrations are considerably lower in fetal compared with maternal serum (Van Saun et al., 1989). Young beef calves have lower whole blood and plasma Se concentrations than their dams (Pehrson et al., 1999; Guyot et al., 2007).

There is no agreement in the literature regarding Se concentrations in whole blood and plasma or serum that should be considered deficient, marginal, or adequate. Whole blood Se concentrations below 50 μg/L are generally considered deficient (Dargatz and Ross, 1996; Pehrson et al., 1999; Kincaid, 2000). Clinical signs of nutritional muscular dystrophy (**NMD**) have been observed in cattle with whole blood Se levels up to 30 μg/L (Pehrson et al., 1999). Unthriftiness with high mortality rates (25–45%) were observed in lambs with blood Se concentrations less than 5 μg/L, and milder cases of unthriftiness were seen in lambs with whole blood Se concentrations of 5 to 10 μg/L (Sheppard et al., 1984). Whole blood Se concentrations less than 10 μg/L were associated with increased fetal death losses in ewes (Sheppard et al., 1984). Selenium concentrations in whole blood considered marginal vary from 50 to over 100 μg/L (Puls, 1994; Dargatz and Ross, 1996). Concentrations of Se in whole blood considered to be adequate vary from 81 to 200 μg/L (Puls, 1994; Dargatz and Ross, 1996).

Greater whole blood Se concentrations may be needed to maximize immunity rather than to prevent clinical signs of Se deficiency. A blood Se concentration of at least 100 μg/L appeared to be necessary in calves for optimal antibody production following injection of a foreign protein (egg lysozyme; Swecker et al., 1989). Dairy cows with whole blood Se levels of 33 μg/L were more susceptible to experimentally induced *Escherichia coli* mastitis than those with average blood Se concentrations of 132 μg/L (Erskine et al., 1989). Jukola et al. (1996) reported that a whole blood Se concentration of 180 μg/L appeared to be critical for prevention of coagulase-negative staphylococci and *Staphylococcus aureus* mastitis in dairy cows.

Plasma or serum Se concentrations less than 25 μg/L are considered deficient, and concentrations between 30 and 60 μg/L are considered marginal by Puls (1994). Calves born to cows with plasma Se concentrations of approximately 15 μg/L exhibited an 8 to 11% incidence of NMD (Hidiroglou et al., 1985). Increased death loss due to diarrhea and unthriftiness was observed in calves born to cows with plasma Se concentrations of 40 μg/L before calving (Spears et al., 1986).

Source of Se should be considered when evaluating whole blood, and plasma or serum Se concentrations. When supplemented to diets marginal in Se (0.10–0.16 mg/kg) at supplemental levels of 0.10 to 0.20 mg of Se/kg of DM, whole blood Se concentrations have been 11 to 33% greater and plasma Se concentrations 22% greater in cattle supplemented with Se yeast compared with SeO_3 (Ortman and Pehrson, 1999; Juniper et al., 2006, 2008). The magnitude of differences in whole blood and plasma or serum concentrations between organic and inorganic sources becomes greater with greater supplemental levels. Ewes receiving a low-Se diet and supplemented with 0.7 mg of Se/d for 12 mo from Se yeast had a 35% greater whole blood and 24% greater serum Se concentration than ewes given the same amount of Se from SeO_3 (Hall et al., 2012). In ewes supplemented with 2.1 mg of Se/d for 12 mo, whole blood and serum Se concentrations were 61 and 40% greater, respectively, in ewes receiving Se yeast compared with those supplemented with SeO_3 (Hall et al., 2012).

Liver Se

Liver Se concentrations also reflect Se status. Selenium liver concentrations of fetuses and young calves are greater than in adults (Herdt and Hoff, 2011). Fetal liver Se concentrations are at least twice as high as maternal levels and are positively correlated with maternal liver Se concentrations (Gooneratne and Christensen, 1989; Van Saun et al., 1989). Concentrations of liver Se suggestive of deficiency in adult cattle and sheep vary greatly in the literature. Puls (1994) reported that liver Se concentrations between 0.07 and 0.61 mg/kg of DM are deficient, and values between 0.43 and 0.89 mg/kg of DM are marginal. In New Zealand, Thompson et al. (1998) reported a reference range for liver Se in cattle of 0.17 mg/kg of DM as deficient (defined as responsive to Se supplementation) and 0.17 to 0.24 mg/kg of DM as marginal. A liver Se concentration of 2.2 mg/kg of DM has been suggested as adequate in the bovine fetus (Van Saun et al., 1989). Calves born to cows with a liver Se concentration of 0.47 mg/kg of DM had an 8 to 11% incidence of NMD (Hidiroglou et al., 1985). In New Zealand liver Se concentrations in lambs with NMD averaged 0.19 mg/kg of DM compared with 0.47 mg/kg in normal lambs (Gabbedy et al., 1977).

Increasing dietary Se from inorganic or organic sources from deficient to adequate concentrations increases liver Se in lambs (Oh et al., 1976; Ullrey et al., 1977) and cattle (Knowles et al., 1999; Juniper et al., 2008). Supplementing dairy cows grazing forage containing 0.035 mg of Se/kg of DM with 2 or 4 mg of Se/d, from SeO_3, increased liver Se from 0.26 to 0.54, and 0.76 mg/kg of DM, respectively (Knowles et al., 1999). Increasing dietary Se from 0.02 to 0.12 mg/kg of DM, from SeO_3, increased liver Se concentrations in lambs from 0.18 to 0.65 mg/kg of DM (Oh et al., 1976). Lambs and steers receiving Se from natural feedstuffs have greater liver Se concentrations than those supplemented with a similar amount of Se from SeO_3 (Ullrey et al., 1977). Supplementation of Se yeast also results in greater liver Se concentrations than a similar quantity of Se from SeO_3 (Knowles et al., 1999; Juniper et al., 2008).

Milk Se

Selenium concentrations in colostrum are approximately 4 times greater than in milk (Weiss and Hogan, 2005; Guyot et al., 2007). Colostrum Se concentrations are reduced by Se deficiency in cows (Juniper et al., 2019) and ewes (Hidiroglou et al., 1971). Cows supplemented with Se yeast (Weiss and Hogan, 2005; Guyot et al., 2007) and hydroxy-SeMet (Juniper et al., 2019) have greater Se concentrations in colostrum than those receiving SeO_3.

Milk Se concentrations are also affected by dietary Se level and source. Beef (Ammerman et al., 1980; Hidiroglou et al., 1985) and dairy cows (Malbe et al., 1995: Knowles et al., 1999) receiving diets containing less than 0.05 mg of Se/kg of DM have milk Se concentrations less than 10 μg/L. Milk Se concentrations are generally greater than 20 μg/L in cows receiving adequate Se. Increasing dietary Se, from inorganic Se, in cows fed adequate Se has little or no effect on milk Se concentrations. In Holstein cows increasing dietary Se (SeO_3) from 0.2 to 0.3 mg/kg of DM for 13 wk slightly increased milk Se (Maus et al., 1980). However, increasing dietary Se from 0.3 mg/kg of DM up to levels as high as 0.7 mg/kg using SeO_3 did not further increase milk Se levels after 13 wk of supplementation (Maus et al., 1980). Supplementing graded levels of SeO_3 up to 53 mg of Se/d did not greatly affect milk Se concentrations in cows fed adequate Se (Fisher et al., 1980). Increasing dietary Se from 1 to 2 or 3 mg/d, from SeO_3, also did not affect milk Se in Hereford cows (Perry et al., 1977). Supplementing 2 mg of Se/d, from SeO_3, to dairy cows grazing forage containing 0.035 mg of Se/kg of DM increased milk Se concentrations (Knowles et al., 1999). Increasing Se from 2 to 4 mg of Se/d from SeO_3 did not result in further increases in milk Se (Knowles et al., 1999).

Cows supplemented with Se yeast have greater milk Se concentrations than cows given a similar concentration of Se from SeO_3 (Knowles et al., 1999; Juniper et al., 2006; Guyot et al., 2007). Increasing dietary Se above requirements with Se yeast further increased milk Se concentrations above those observed in cows supplemented with SeO_3 (Knowles et al., 1999; Givens et al., 2004). Greater milk Se concentrations in cows supplemented with Se yeast is due to greater Se concentrations in the casein fraction (Knowles et al., 1999). Juniper et al. (2006) showed that the amount and percentage of total Se in milk present as SeMet was greater in cows supplemented with Se yeast than in those receiving SeO_3. This is consistent with SeMet being incorporated into milk protein in place of methionine.

Glutathione Peroxidase

Glutathione peroxidase is a Se metalloenzyme that has been measured in erythrocytes (Thompson et al., 1981; Pehrson et al., 1999), whole blood (Koller et al., 1984; Spears et al., 1986), and plasma (Thompson et al., 1981) in research studies to assess Se status. Sex, age, and factors other than Se status that may affect GSHpx are not well defined. Temperatures and pH values used in GSHpx assays can vary and greatly affect reported enzyme activities (Suttle, 2010). Because of the difficulty involved in the GSHpx assay, most diagnostic laboratories do not offer this service.

APPLICATIONS

Providing ruminants with adequate amounts of trace minerals, without supplying excessive concentrations that may negatively affect productivity, can be challenging. Supplementation practices in the United States include (1) no supplemental trace minerals, (2) free-choice mineral supplements (containing trace minerals), (3) trace mineral additions to the TMR, and (4) injectable trace minerals. Trace mineral requirements are not static but can be affected by such factors as bioavailability of trace minerals from feedstuffs or supplemental sources, genetics, physiological status, and antagonists. Factors that may affect trace mineral requirements are discussed in detail in nutrient requirement publications.

Severe trace mineral deficiencies are often associated with clinical signs of deficiency. However, marginal trace mineral deficiencies can affect animal performance and health in the absence of obvious clinical signs. Trace mineral status is often assessed to address problems or at least perceived problems in health or production. Based on this review, recommended criteria for assessing Cu, Zn, Mn, and Se status in ruminants is presented in Table 1.

It is recommended that blood samples for trace minerals be collected in evacuated tubes designated for trace mineral analysis. Collecting plasma instead of serum will minimize hemolysis and should provide more accurate measures of circulating Cu concentrations. Liver trace mineral concentrations should be expressed on a DM basis because of factors that affect liver moisture content. Plasma Cu concentrations less than 0.6 mg/L or liver concentration less than 50 mg/kg of DM suggest marginal Cu status. Liver Cu concentrations less than 20 mg/kg of DM or plasma concentrations less than 0.4 mg/L are

Table 1. Recommended criteria for assessing Cu, Zn, Mn, and Se status in ruminants[1]

Trace mineral	Animal criteria	Dietary considerations
Copper	Liver Cu, plasma Cu	Cu, Mo, S, Fe
Zinc	Plasma Zn	Zn, Ca?
Manganese	?	Mn, Fe, Ca?, P?
Selenium	Whole blood Se, liver Se	Se, source of Se

[1]The question marks indicate some uncertainty with regard to whether these minerals affect bioavailability.

consistent with Cu deficiency. In the absence of disease or acute stress, plasma Zn concentrations less than 0.5 mg/L suggest possible severe Zn deficiency. No reliable indicator of marginal Zn status is currently known. Selenium concentrations in whole blood less than 50 μg/L or liver concentrations less than 0.50 mg/kg of DM are indictive of Se deficiency. Currently, there is no reliable indicator of Mn status. Research is needed to better define markers of Mn status, using improved analytical instrumentations for more accurately measuring Mn concentrations.

When evaluating dietary Cu concentrations, it is important to also consider the potent Cu antagonists Mo and S, and perhaps dietary Fe. High dietary Fe, when present in an available form, reduces bioavailability of Cu (Spears, 2003) and Mn (Hansen et al., 2010). However, analyzed dietary Fe concentrations are difficult to interpret because of unknown bioavailability from feed ingredients and possible soil contamination that can greatly increase analyzed values. Iron in most soil types is considered to be of low bioavailability to ruminants, but soil Fe appears to become more available when exposed to the acid environment during silage fermentation (Hansen and Spears, 2009). Calcium and P are known to be Mn antagonists in poultry. Research is needed to determine whether dietary Ca and P affect Mn bioavailability in ruminants. Factors that affect Zn bioavailability in ruminants are also not clearly defined. Earlier research examining the interaction of Ca and Zn in ruminants has been inconsistent (Spears, 2003). Studies are needed to determine whether high soluble Ca concentrations in the rumen affect Zn bioavailability and also degradation of phytate by microbial phytase. The interaction between Ca and phytate with Zn may be more important than previously thought if high soluble Ca concentrations reduce phytate degradation in the rumen.

Studies have indicated that approximately 40% of dairy cows in the United States have liver Cu concentrations in excess of 500 mg/kg of DM. Research is needed to determine whether high liver Cu concentrations in dairy cows, not showing clinical signs of toxicosis, affect longevity in the herd, milk production, or reproduction. If longevity or production is affected, liver Cu concentrations necessary to cause impairment need to be better defined. Studies are also needed to determine whether high liver Cu concentrations in dairy cows are due to Cu over supplementation of cows, excess Cu supplementation in milk replacers and heifer development diets, or a combination of the 2.

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