Diagnosing Trace Mineral Deficiencies and Excesses in Transition Dairy Cows

Brent Hoff

Animal Health Laboratory, P.O. Box 3612, University of Guelph, Guelph, ON N1H 6R8
Email: bhoff@uoguelph.ca

Take Home Messages

- Trace minerals are required for normal functioning of all biochemical processes in the body. If minerals are adequate in the diet, but animals are found to be deficient, antagonistic interactive effects of other minerals need to be investigated.
- Deficiencies of essential trace minerals, depending on severity, can result in clinical or subclinical deficiency signs. These clinical signs may be very subtle and difficult to identify.
- Historically, testing for deficiencies has been performed on diets and/or dietary components to ensure "adequate" concentrations. However, general mineral analysis does not identify the chemical form of these minerals, which can dramatically alter their bioavailability and utilization. There are also many trace mineral antagonists with element-to-element interactions.
- Appropriate diagnosis of mineral status involves thorough evaluation of groups of animals. The evaluation should include a detailed health history, feeding history, supplementation history, and analysis of the appropriate sample from several animals for their mineral status.

Introduction

Trace minerals are required for essentially all biochemical processes in the body. Many of these minerals are necessary for optimal growth, physiologic function, and productivity in animals. This paper focuses on 8 trace minerals: cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn). These trace minerals have been chosen because nutritional deficiencies or disturbances in their metabolism are relatively common, and substantial information is available about their

WCDS Advances in Dairy Technology (2016) Volume 28: 287-297

metabolism and the amount needed for optimal health and productivity in animals. Testing of blood, serum, or tissues for total mineral concentration is a popular and potentially valuable means of assessing trace mineral nutritional status, and is generally more practical than expensive functional approaches of measuring specific mineral-containing proteins or enzymes. Modern analytical techniques make blood and tissue trace mineral analysis practical and relatively inexpensive. Of particular importance is the recent application of inductively coupled plasma/mass spectroscopy (ICP/MS) analysis to the diagnostic evaluation of animal samples. This technique is fast, extremely sensitive, precise and accurate, and allows for the simultaneous measurement of a wide array of trace minerals (Herdt and Hoff, 2011).

Trace minerals play a key role in supporting immune function; therefore, maintaining adequate trace mineral status during the dry period is an important component in achieving good cow health around parturition, when the cow experiences significant metabolic and physiological changes and her immune system is stressed.

Direct measurement of trace mineral content in blood and tissue is subject to considerable limitations in evaluating nutritional status. Consider the assessment of trace mineral nutrient evaluation in animals as described by Suttle (2010) in Figure 1. This conceptual approach recognizes that during periods of inadequate dietary intake, depletion of storage pools and transport forms are well defined and accessible for measurement. This concept can be readily applied in clinical use. However, from a diagnostic standpoint, not all trace elements fit well into this scheme because for some there is no recognizable storage pool and for others the transport and functional pools overlap.

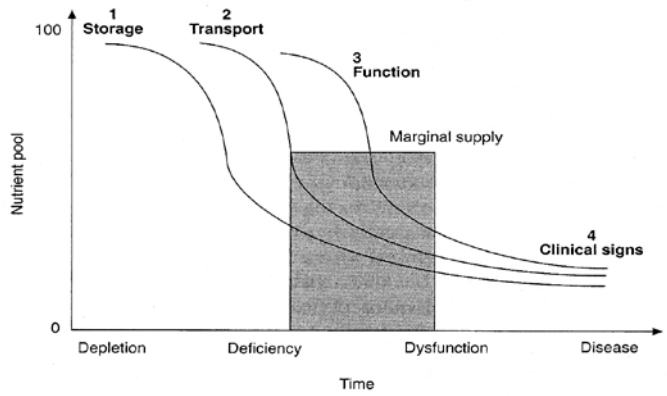

Figure 1: The sequence of pathophysiological changes that can occur in mineral-deprived livestock (Suttle, 2010)

Furthermore, factors other than nutrition are known to affect serum trace mineral concentrations. Most notably, homeostatic forces modulate the serum concentrations of most trace minerals within a range of homeostatic set points that vary in width among the different minerals. Other factors such as physiologic state (e.g., pregnancy, lactation, and gestation) may influence serum trace mineral concentrations. The presence of inflammation also has a large influence on serum concentrations of some minerals.

▪ Deficiency and Toxicity Diagnoses

Historically, testing for deficiencies has been performed on diets and/or dietary components to ensure “adequate” concentrations in the diet. However, general mineral analysis does not identify the chemical form of these trace minerals, which can dramatically alter their bioavailability and utilization. This is especially important when considering the increasing use of “chelated” minerals, as they can have significantly greater overall bioavailability than the inorganic minerals. Mineral deficiencies can be presumptively diagnosed by development of clinical disease or by post-mortem identification of tissue lesions. Proof of deficiency requires analytical verification because most deficiencies do not have unique clinical signs or lesions. Circumstantial proof of a deficiency may be provided by positive response to supplementation of a suspected deficient mineral. The problem is that a positive response may have nothing to do with the supplementation and may be just a time-responsive correction of some other clinical condition (Hall, 2015).

The action of trace minerals is dose dependent, and even essential trace minerals can produce toxic effects when consumed at high concentrations. The toxic effects of trace minerals can be subtle, with no clinical signs. For example, Lyman (2013) reported copper toxicity in all age groups of Wisconsin Holsteins, causing subclinical liver damage. A review of 225 WVDL submissions showed a mean copper level of 143 ppm (25-100 ppm is the recommended copper level for adult dairy cows).

Most of the trace minerals have several means of measurement for identification of deficiencies, but most have one that is more specific than the others. A good example is serum copper concentrations. Unless serum copper is at a critically low value, it has no significant predictive value in assessing potential for copper deficiency disease. Another example is the debate between serum and whole blood selenium values. Serum selenium represents the transport pool and is very sensitive to dietary changes and liver mobilization. On the other hand, whole blood selenium values represent both transport and a portion of the biochemical function pools. This measure is somewhat less sensitive to changes as a result of a greater proportion of whole blood selenium being present as the erythrocyte enzyme, glutathione peroxidase. If we were to assess a potential response to dietary change,

serum selenium values would respond within a day or so while whole blood, like liver values, may take a month or more to show a significant change. This could dramatically impact interpretation of the dietary response.

Liver mineral concentrations are good markers for the storage pool; however, they are not always highly associated with the presence of disease. Liver mineral concentrations may give us some insight into the adequacy of the mineral supplementation program and potential for disease. The assessment of mineral status in fetal and neonatal animals is quite different than adult animals. The fetus can concentrate trace minerals in its liver from the dam, and therefore the comparison to adult values is inappropriate. This is especially relevant for copper, iron, selenium, and zinc. We are currently developing databases determining normal trace mineral concentrations in the fetal and neonatal liver. Also, a few more predictive markers for specific nutrient pools need to be identified.

When individual animals are tested, their prior health status must be considered in interpreting the mineral concentrations in tissues. Infectious disease, fever, stress, endocrine dysfunction, and trauma can alter both tissue and circulating serum/blood concentrations of many minerals. Therefore, evaluation of multiple animals is much more reflective of mineral status within a group than testing individual animals that are ill or have died from other disease states.

▪ Live Animal Sampling

A variety of samples from live animals can be analyzed for trace minerals. Testing of blood, serum, or liver samples for total mineral concentrations is a popular and potentially valuable means of assessing mineral nutritional status that is generally more practical than the more functional approaches mentioned earlier. Other samples from live animals occasionally used for analysis include urine and milk. Hydration status significantly affects urinary mineral concentrations and the mineral content in milk varies through lactation and can be greatly affected by disease. Hydration status is not typically considered when evaluating whole animal mineral status.

Serum samples should be separated from the red/white blood cell clot within 1 to 2 hours of collection. If the serum sits on the clot for a longer period of time, minerals that are present in high levels in cells within the clot can leach into the serum and falsely increase the serum content. Minerals for which this occurs include iron, zinc, and potassium. In addition, hemolysis from natural disease or due to collection technique can result in falsely increased levels of manganese, selenium, and zinc.

The best type of tube for serum or whole blood mineral analysis is the royal-blue top vacutainer tube, as it is certified trace-metal free. The red-top vacutainer tubes can give abnormally increased results for zinc content as a zinc-containing lubricant is commonly used on the rubber stoppers.

Samples should be appropriately stored for adequate sample preservation. Liver biopsies, urine, and serum can be stored frozen long-term or refrigerated if mineral analysis is to be completed within a few days. Whole blood and milk should be refrigerated but not frozen, as cell lysis will compromise the integrity of the sample.

Liver biopsies, because of their small size, are susceptible to desiccation unless properly stored. These small biopsy samples should be placed into small tubes, with the sample pushed all the way to the bottom. Small 1-2 ml micro-centrifuge tubes work well for this (See AHL website, LabNote 19, http://www.guelphlabservices.com/files/AHL/AHL%20LabNotes/LabNote19.pdf). Placing the sample at the bottom of the tube minimizes the air-to-sample interface area and the potential for desiccation. The sample can then be frozen.

▪ Post-mortem Animal Sampling

A variety of post-mortem samples can be analyzed for trace minerals. Liver tissue is the most common tissue analyzed for mineral content, as it is the primary storage organ for many of the essential minerals. Post-mortem samples can be stored frozen until they are analyzed. Other samples, such as kidney, source material, feed, and water, may also be needed depending on the deficiency or excess suspected.

▪ Trace Mineral Functions and Bioavailability

Cobalt (Co)

Cobalt deficiencies have not been reported in Alberta, although cobalt levels in feed and livestock have not been widely studied in the province (www.agric.ab.ca). The only known function of cobalt is its role as a component of vitamin B-12. Ruminal microorganisms are able to synthesize vitamin B-12 from dietary cobalt. A lack of dietary cobalt for vitamin B-12 synthesis by rumen microorganisms can also alter ruminal fermentation. Deficiency is associated with decreased feed intake, lowered feed conversion, reduced growth, weight loss, hepatic lipidosis, anemia, immunosuppression, and impaired reproductive function (Herdt and Hoff, 2011).

Copper (Cu)

More than 90% of feed produced in Alberta is low in copper. Deficiencies occur through prolonged consumption of forages low in copper and/or the consumption of forages containing elevated levels of molybdenum or sulfur, which are natural antagonists of copper.

Copper deficiency is one of the most commonly encountered nutritional problems in ruminants, but copper excess is also commonly encountered in dairy cattle. Excessive copper is a relatively common finding in multiparous dairy cows, while most deficiencies are identified in calves and first lactation cows (Lyman, 2013; Hall, 2010). Copper is an essential trace element for livestock and has two functions. Copper is a component of a number of enzymes in which it serves a catalytic function. These enzymes are important for the structural integrity of collagen and elastin, detoxification of superoxide radicals, pigmentation, iron transport, and energy metabolism. Copper can also be a structural component in macromolecules, acting as a coordinating center.

Clinical signs of deficiency can appear as reduced growth rate, decreased feed conversion, poor immune function (failure to respond to vaccinations), impaired reproductive function, anemia, and rough, dull hair coat. Cows can deplete their own body reserves to ensure neonatal adequacy. Therefore, copper deficiency in calves would indicate that the dam is deficient and that she would likely also have poor colostrum quality, leading to inadequate neonatal protection even with adequate volume of colostrum.

The best method for copper evaluation is via analysis of liver tissue (storage pool) because depletion of hepatic copper is the earliest sign of inadequate copper consumption. Copper evaluation can be reliably determined on liver biopsy samples as small as 50 to 75 mg of fresh tissue. Such samples are easily obtained with Tru-Cut-style biopsy instruments (AHL website).

Deficiency in a herd will result in some animals that have low serum values, but serum content does not fall until liver copper is significantly depleted. In herds that have been sampled with liver biopsies and found to have a high prevalence of deficiency, it is not unusual to see a high percentage of “normal” serum copper levels (Hall, 2010). In Guelph, we have identified herds as “marginally deficient” from liver biopsies, and most of the cows have “normal” serum copper levels. Thus serum copper analysis should be viewed as a screening method only.

The recommended adequate wet weight liver copper concentration in adult cattle is 25 to 100 ppm. In comparison, a late-term fetal or early neonatal liver should have 65 to 150 ppm copper to be considered adequate.

Iodine (I)

The primary role of iodine is in the synthesis of hormones by the thyroid gland. Thyroid gland hormones regulate energy metabolism, reproduction, thermoregulation, growth and development, circulation, and muscle function. The levels of iodine in forages in Alberta are low and supplementation is necessary. Clinical signs, such as goiter, decreased milk yield, impaired fertility, and increased incidence of retained placenta, have been reported.

Evaluation of iodine is of interest because of the large potential for dietary deficiency, the possibility of toxicity, and the transfer of iodine to human food products, especially dairy products. Overt iodine deficiency is manifested as goiter, which is enlargement of the thyroid gland. Goiter may occur in utero and not be observed until birth. Congenital goiter may occur in the offspring of dams that are not themselves suffering from overt iodine deficiency. For post-mortem diagnosis of iodine deficiency, the tissue of choice is thyroid gland. Low iodine levels indicate iodine deficiency (Herdt and Hoff, 2011). At high iodine intakes, liver concentrations may increase more than normal, but hepatic concentrations are not useful in diagnosing iodine deficiency.

For the antemortem diagnosis of iodine deficiency, evaluation of thyroid function is the most suitable means of evaluation. This evaluation involves, at a minimum, the determination of serum thyroxine concentrations (T4) and ideally should include measurement of thyrotropin-releasing hormone and thyroid-stimulating hormone. Direct measurement of serum iodine is less sensitive and specific for the diagnosis of iodine deficiency.

Iron (Fe)

Iron is an essential nutrient that is required in a variety of metabolic processes and is found in all body cells. The largest portion is found as a necessary component of the protein molecules hemoglobin and myoglobin. Iron plays a vital role in the transport of oxygen by hemoglobin and in oxygen storage and transport in muscle by myoglobin. Iron is essential for normal cellular function of all cell types and is found in plasma (transferrin), milk (lactoferrin), and liver (ferritin and hemosiderin).

Deficiency of iron is of limited practical significance in farm livestock. Confinement increases the possibility of iron deficiency in young suckling animals, or animals reared on a diet of milk. Severe blood loss from parasites or other causes also produces secondary iron deficiency. A variety of factors in feeds can have enhancing or inhibiting effects on iron bioavailability. Trace mineral interaction may also alter bioavailability; for example, excessive dietary cobalt or manganese may interfere with iron availability.

Both liver and serum concentrations are commonly used to diagnose iron deficiency and toxicosis. When using serum to measure iron deficiency, samples that are hemolyzed should not be used. Interpretation of the iron status should be made with consideration of the overall health of the animal, as inflammation and infection can alter serum and liver iron concentrations.

Manganese (Mn)

Manganese is involved in a broad array of enzyme systems in the body and affects a wide variety of biochemical processes including carbohydrate, fat and protein use. Manganese is also involved in proper bone development and maintenance. Pasture grasses and legumes are typically good sources of manganese, whereas corn silage and cereal grains are poor sources (Herdt and Hoff, 2011).

Manganese deficiency in ruminants is associated with impaired reproductive function, skeletal abnormalities, and less than optimal productivity. Cystic ovaries, silent heat, reduced conception rates, and abortions are reported reproductive effects. Neonates that are manganese deficient can be weak, small, and develop enlarged joints or limb deformities.

Manganese at sub-normal to deficient concentrations is identified routinely in dairy cattle. The lower levels in dairy cattle may in part be the result of high levels of calcium and phosphorous in dairy rations, which can be antagonistic to the bioavailability of manganese. This is not seen in beef cattle (Hall, 2010).

Of the samples available, liver is the most indicative of whole body status, followed by whole blood, and then serum. Hemolysis can result in a false increase in serum content. Response to supplementation has frequently been used as a means of verifying manganese deficiency, but it is critical that a bioavailable form be utilized. For example, manganese oxide has very poor bioavailability.

Unlike for copper, selenium, iron, and zinc, late-term fetuses and neonates have lower manganese content than adult animals. Calves will generally have similar normal ranges to adults by 5 to 6 months of age. For wet weight liver manganese, normal adult range is 2.0 to 6.0 ppm whereas the neonatal normal range is 0.9 to 4.5 ppm.

Molybdenum (Mo)

Early nutritional interest in molybdenum was centered on its impact on copper availability in ruminants. An essential role for molybdenum came from the discovery that the flavoprotein, xanthine oxidase, contains molybdenum, and

that its activity depends on the metal (Suttle 2010). Although molybdenum is an essential trace mineral, the requirements are very low and clear signs of deficiency have not been seen in cattle. The tolerance of livestock to high molybdenum intakes varies with the species, the amount and the chemical form of the ingested molybdenum, the copper status of the animal, and the diet and the forms and concentration of sulfur and iron in the diet. Cattle are the least tolerant species. Growth retardation, weight loss, and anorexia are common, and diarrhea is typical only in cattle. Cattle have clinical signs that mimic copper deficiency if they have less severe exposure, as a result of formation of thiomolybdates in the rumen, which can diminish copper absorption and bind systemic copper and render it non-functional.

The assessment of molybdenum status is usually undertaken when there are concerns about molybdenum toxicity or conditioned copper deficiency. Molybdenum in soluble dietary forms is readily absorbed, and serum, whole-blood, liver, and kidney values reflect dietary intake (Herdt and Hoff 2011). Assessment of serum and hepatic molybdenum concentrations is useful as a reflection of potentially excessive intake with concomitant secondary copper deficiency. High serum molybdenum concentrations should cause concern for the presence of thiomolybdates, which could affect the interpretation of serum or plasma copper concentrations.

Selenium (Se)

Some of the physiological functions of selenium are still not clear, but much has been elucidated since the discovery of selenium as an integral part of cellular glutathione peroxidase enzymes (GPx). These enzymes prevent cellular damage by destroying hydrogen peroxide and lipid hydroperoxides. Selenium is also involved in the deiodination of thyroxine (T4) to the more metabolically active triiodothyronine (T3) in tissues. The immune system is adversely affected by selenium deficiency, and selenium deficiency increases the incidence of mastitis and retained placenta in dairy cows.

As an essential mineral, selenium is commonly identified as deficient in ruminants, but infrequently in dairy cattle. In dairy cattle, we see deficiency in dairy heifers and calves. Selenium deficiency is associated with reduced growth rates, poor feed efficiency, poor immune function, impaired reproductive performance, and damage to muscle tissue. “White muscle disease”, or nutritional myopathy, is linked to severe selenium and/or vitamin E deficiency.

Cows will do all they can to ensure adequate selenium levels in calves when they are born. They will deplete their own body reserves to ensure neonatal adequacy. Therefore, a calf born with selenium deficiency confirms maternal

deficiency. This means that the dam will most likely have poor colostrum quality and inadequate immune protection for her calf.

Diagnosis of deficiency can be made by analysis of liver, whole blood, or serum for selenium, or by analysis of whole blood for glutathione peroxidase. Serum reflects the recent intake of selenium. Whole blood better reflects the longer term intake of selenium. In order to adequately diagnose selenium deficiency, the dietary form of selenium intake is important. The “adequate” concentrations of serum and whole blood selenium differ depending on whether the dietary selenium is in a natural organic form or an inorganic form.

Selenium excess is commonly identified in multiparous dairy cows. If the selenium excess is great enough, it can result in poor reproductive performance, poor calf survival, and imbalances of other minerals. Excessive selenium can also interfere with zinc absorption. The recommended adequate liver selenium concentration in adult cattle is 0.25 to 0.50 ppm. In comparison, late-term fetal or neonatal liver values should be higher, at 0.35 to 0.75 ppm.

Zinc (Zn)

Zinc is an essential component of over 70 enzymes found in mammalian tissues. Enzymes that require zinc are involved in protein, nucleic acid, carbohydrate, and lipid metabolism. Zinc is also important for normal development and functioning of the immune system, in cell membrane stability, and gene expression.

Responses of dairy cattle to zinc supplementation of practical diets have been highly variable, suggesting that dietary factors affect zinc bioavailability; however, these are not well defined. Some studies would suggest that high dietary calcium reduces zinc status in cattle (Spears, 2003).

Deficiencies of zinc are associated with reduced growth, poor immune function, diminished reproductive performance, and poor offspring viability, as well as skin lesions in severe cases. Liver and serum are the best indicators of zinc status. Response to zinc supplementation has shown that some animals with borderline zinc levels can still show improvement in some clinical conditions (Hall, 2010).

A number of laboratories have found decreasing zinc levels in multiparous cows over the last few years. They have also found excessive copper and selenium in the livers of these cows. The dietary excess of copper and selenium can interfere with zinc absorption; therefore, the low zinc levels are likely a secondary effect. It should be possible to decrease the copper and selenium levels in the rations to increase the absorption of zinc.

▪ Conclusions

A variety of sample types can be tested for trace mineral content, but may not provide an indication of overall mineral status of the animal. Diagnosis of trace mineral status involves evaluation of appropriate samples from groups of animals, rather than individuals. The evaluation should include a thorough health history, feeding history, supplementation history, and analysis of several animals for their mineral status.

Dietary mineral evaluation should only be used to augment the mineral evaluation of animal groups. If minerals are deemed to be adequate in the diet, but the animals are found to be deficient, antagonistic interactive effects of other minerals and true average daily per animal intake of the supplement need to be investigated. For example, high sulfur or iron causes deficiencies in copper and selenium, and excessive copper and selenium can adversely impact zinc status.

Common trace mineral deficiencies or excesses are significant hindrances to profitability in dairy cattle. They may impact reproductive performance, milk production, and animal health. In dairy operations, one must correctly identify the cause of the mineral imbalance, and any abnormal supplementation. We have seen cases with excessive supplementation in multiparous cows, but the replacement heifers were on a different ration and were deficient.

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