Diagnosing Common Vitamin and Mineral Abnormalities in Dairy Cattle

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INTRODUCTION

Many minerals and vitamins have been proven to be essential for optimal growth, physiologic function, and productivity in animals. Data from the Utah Veterinary Diagnostic Laboratory indicated a significant increase in incidence of vitamin and mineral deficiencies and excesses. Much of the deficiencies appear to be associated with producers decreasing or completely stopping the practice of vitamin-mineral supplementation of replacements due to the economy and costs. A common finding with many of the diagnosed deficiencies is a lack of vitamin-mineral supplementation to replacement heifers, resulting in them coming into their first lactation deficient. Mineral excesses are commonly associated with heavy use of chelated minerals in lactation total mixed rations (**TMR**).

Increasing incidence of adverse neonatal health effects, due to vitamin or mineral deficiencies are commonly encountered at the Veterinary Diagnostic Laboratory. A lack of or inadequate supplementation of replacement heifers results in maternal depletion of body reserves of minerals and, subsequently, in poor calf health.

Interestingly, the same deficiencies commonly identified in first lactation cows are only rarely observed in multiparous cows. In fact, excessive supplementation in the lactation rations tends to be more common and results in excessive mineral concentrations, especially for copper (**Cu**) and selenium (**Se**).

DEFICIENCY 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 forms, which can dramatically alter their bioavailability and utilization. This is especially important with the increasing use of *chelated* minerals, as they can have significantly greater overall bioavailability than inorganic minerals.

Although not possible for some of the minerals, the most specific means of diagnosing a mineral deficiency is by testing animals for unique functional deficits or deficiencies of specific mineral-containing proteins or enzymes. This type of testing is often impractical from a field perspective, due to individual test costs or rigorous sample handling requirements. But, when possible, this type of testing eliminates the need to know the specific molecular characteristics of a dietary mineral and the potential for competitive interactions of antagonists of absorption/utilization. For minerals that do not have identified physiologic indices for testing, direct quantification from animal tissues or serum may provide a reliable indication of the overall mineral status of the animal or group.

Vitamin and mineral deficiencies can be suggestively diagnosed by the development of clinical disease or by post-mortem identification of tissue lesions. But, proof of deficiencies often requires analytical verification since most do not have very unique clinical signs or lesions. In some instances, circumstantial proof of a deficiency can be provided by positive response to supplementation of suspected deficient vitamins or minerals. But, positive response may have nothing to do with the supplementation, being just a correction of some other clinical condition.

An individual vitamin or mineral may have multiple means of measurement for identification of deficiencies, but most have one that is more specific than the others. For example, dietary concentrations may or may not be reflective of the amount that is bioavailable. Or, an individual tissue concentration may or may not reflect functional availability at the target or functional site.

The age of the animal being tested also is important for proper interpretation of status. For example, feti accumulate some minerals at different rates during gestation, necessitating adequate aging of the fetus for interpretation. In addition, some

minerals, for which little is provided in milk, accumulate at higher concentrations during gestation in order to provide neonates with adequate body reserves for survival until they begin foraging. This is especially prevalent with Cu, iron (**Fe**), Se, and zinc (**Zn**). Thus, the *normal ranges* for these minerals in body storage tissues would be higher in early neonates than in an adult animal. One must make sure that the testing laboratory is interpreting the results based on the age of the animals tested, as some interpret all as adults.

When individual animals are tested, the prior health status must be considered in interpreting vitamin and mineral concentration of tissues. Disease states can shift mineral from tissues to serum or serum to tissues. For example, diarrhea can result in significant loss of sodium (**Na**), potassium (**K**), and calcium (**Ca**) from the body. Or, acidosis will cause electrolyte shifts between tissues and circulating blood. It is known that infectious disease, stress, fever, endocrine dysfunction, and trauma can alter both tissue and circulating serum/blood concentrations of certain minerals and electrolytes. Thus, 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 are available from live animals that can be analyzed for vitamin-mineral content. The most common samples from live animals are serum and whole blood. These samples are adequate for measurement of several minerals, but it must be recognized that some disease states, as well as feeding times, can result in altered or fluctuating concentrations. Other samples from live animals that are occasionally used include liver biopsies, urine, and milk. But, since milk mineral content can vary through lactation, across lactations, and be affected by disease it is not typically used to evaluate mineral status. Hydration status affects urinary mineral concentrations, rendering it a poor sample for status evaluation. For vitamin A and E, serum is the best sample from live animals.

Although some laboratories suggest hair sampling to evaluate mineral status or exposure, hair is a relatively poor matrix to give reliable data on mineral status, except for Se. Since body hair in livestock is notoriously contaminated with environmental contamination (dirt, manure, etc.), the hair MUST be cleaned thoroughly to remove contamination prior to testing. But, thorough cleaning can also leach out some of the true minerals in the hair, which results in measuring less than what was truly present.

Serum should be separated from the red/white blood cell clot within 1 to 2 hr of collection. If the serum sets on the clot for longer periods of time, minerals that have higher intracellular content than serum can leach into the serum and falsely increase the serum content. Minerals for which this commonly occurs include K and Zn. In addition, hemolysis from both natural disease and due to collection technique can result in increased serum concentrations of Fe, magnesium (**Mg**), manganese (**Mn**), phosphorous (**P**), K, Se, and Zn. Vitamin A and E can begin breaking down in serum if not separated from the red blood cells and frozen within 1 - 2 hr of collection. Serum for vitamin A and E analysis should be stored to prevent breakdown from sunlight exposure.

The best type of collection tube for serum or whole blood is royal blue-top vacutainer tubes, as they are certified trace-metal free. Typical red-top clot tubes can give abnormally increased results for Zn content as a Zn containing lubricant is commonly used on the rubber stoppers. For minerals other than Zn or vitamins A and E, serum samples from the typical red-top clot tubes or separator tubes are adequate.

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 or coagulation of solids, respectively, will result in loss of the overall integrity of the sample.

Liver biopsies, because of their small size, are susceptible to desiccation unless properly stored. Small biopsies should be placed into SMALL tubes, with the sample pushed all the way to the bottom. Small 1 to 2 ml micro-centrifuge tubes work well for this. By placing the sample at the very bottom of the tube, one minimizes the air to sample interface area and minimizes potential for desiccation. The samples can then be frozen for transport.

POST-MORTEM ANIMAL SAMPLING

A variety of post-mortem animal samples are available that can be analyzed for vitamin-mineral content. The most common tissue analyzed for mineral content is liver, as it is the primary storage

organ for many of the essential minerals. In addition, bone is used as the primary storage organ for Ca, P, and Mg. For vitamin A and E, liver is the tissue of choice for analysis, but it needs to be relatively fresh. Tissue degradation will correspondingly decrease the vitamin A and E present.

Post-mortem samples should be stored frozen until analyzed to prevent tissue degradation of the vitamins. If samples are to be analyzed within 1 – 2 d, they can be stored under refrigerated conditions.

COPPER

Copper deficiency is one of the most commonly encountered nutritional problems in ruminants, but Cu excess is also commonly encountered, especially in sheep or in dairy cattle. Excessive Cu is a relatively common finding in multiparous dairy cattle, while most deficiencies are identified in calves or first lactation cows. In contrast, Cu deficiency is rare in non-ruminants. Clinical signs of deficiency can present as a large array of adverse effects, including reduced growth rates, decreased feed conversion, abomasal ulcers, lameness, poor immune function, sudden death, achromotrichia, poor lactation, and impaired reproductive performance.

Cows will do all they can to ensure adequate Cu is in calves when they are born. They can actually deplete their own body reserves to ensure neonatal adequacy. As such, neonates diagnosed with Cu deficiencies are proof of maternal deficiencies. With Cu being an essential component of the immune function, this maternal deficiency likely results in poor colostrum quality and inadequate neonatal protection even with adequate intake.

The best method for diagnosing Cu status is via analysis of liver tissue, although much testing is performed on serum. Deficiency within a herd will result in some animals that have low serum Cu concentrations, but serum content does not fall until liver Cu is significantly depleted. In herds that have had livers tested and found a high incidence of deficiency, it is not uncommon for a high percentage of the animals to have *normal* serum concentrations. At the Utah Veterinary Diagnostic Laboratory, it is commonly recommended that a minimum of 10 animals be tested in order to have a higher probability of diagnosing a Cu deficiency via serum quantification. Even with herd deficiency, low serum Cu concentrations may only be seen in less than 10% of the individuals. Herds that may be classified as marginally deficient based on liver testing may have predominantly *normal* serum Cu concentrations. Thus, serum Cu analysis should be viewed as a screening method only. Another factor that can influence diagnosis of Cu deficiency in serum is the presence of high serum (**Mo**). As the Cu-sulfur (**S**)-Mo complex that forms is not physiologically available for tissue use, *normal* serum Cu content in the presence of high serum Mo should always be considered suspect. In addition, the form of Se supplementation can alter the normal range for interpretation of serum Cu status, with selenite supplemented cows having a lowered normal range for serum Cu.

Excessive Cu in dairy cattle is a common finding at the Utah Veterinary Diagnostic Laboratory. Liver Cu concentrations greater than 200 ppm are routinely identified. But, in recent years, several cases of deficiencies also have been identified, due to poor mineral supplementation programs. These have most commonly been in first lactation cows that were not adequately supplemented in the growth, breeding, or pre-lactation period.

Excessive liver Cu has the potential of causing adverse health and production effects. Liver Cu concentrations greater than 150 - 200 ppm have been shown to cause increased liver enzyme leakage, indicating adverse effects on tissue health. Adverse liver health and function can adversely affect feed/energy utilization and overall productivity of an individual. As Cu concentrations get even higher, further liver damage can occur.

Over-supplementation of Cu, as indicated by excessive liver Cu, in dairy cows has been increasing over time. The nature of these findings tends to correspond to increasing use of chelated minerals in dairy rations. Due to the increased bioavailability of chelated minerals, adding them to rations at the same concentrations as inorganic Cu can result in over-supplementation. Excessive dietary Cu also plays a role in the adverse interaction with other minerals. For example, excessive dietary Cu adversely impacts the absorption of Zn.

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

MANGANESE

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 Mn deficient can be weak, small, and develop enlarged joints or limb deformities. Manganese deficiencies in beef cattle, although rare, would most commonly be seen in areas of highly alkaline soils, due to much poorer plant uptake, but this is not very commonly identified.

Manganese at sub-normal to deficient concentrations is identified routinely in dairy cows. Of interest is the fact that most testing of beef cattle (greater than 95 %) finds normal Mn concentrations in liver, blood, and serum; but in these same matrices many dairy cattle tested are below recommended normal concentrations (unpublished data, Utah Veterinary Diagnostic Laboratory). This may, in part, be due to high Ca and P content of dairy rations, which can be antagonistic to the bioavailability of Mn. Another potential factor that can play a role in Mn status differences in beef vs. dairy cattle can be the diet. Some dairy herds that are grazed on grass have a similar lack of deficiencies or sub-normal concentrations of Mn as those observed in beef cows. Grasses typically have higher Mn content than forbs and the chemical form of Mn may be more bio-available than inorganic Mn supplements.

Of the samples available, liver is the most indicative of whole body status, followed by whole blood and then serum. As red blood cells have higher Mn content than serum, hemolysis can result in increased serum content. Since the normal serum concentration of Mn is quite low, many laboratories do not offer this analysis because of inadequate sensitivity. Overall, response to supplementation has frequently been used as a means of verifying Mn deficiency, but it is critical that a bioavailable form be utilized.

Unlike Cu, Se, Fe, and Zn, late term feti and neonates have lower Mn content than adult animals. Calves generally will have similar normal ranges to adults by 5 - 6 mo of age. For wet weight normal liver Mn, adult normal range is 2.0 to 6.0 ppm, while a neonatal normal range is 0.9 to 4.5 ppm.

SELENIUM

As an essential mineral, Se is commonly identified as deficient in ruminants, but infrequently in dairy cattle. In dairy cattle, the most common finding of Se deficiency is in replacement and first lactation heifers. This type of deficiency has significantly increased over the past 5 yr. Selenium deficiency is associated with reduced growth rates, poor feed efficiency, poor immune function, impaired reproductive performance, and damage to muscle tissues. *White muscle disease*, a necrosis and scarring of cardiac and/or skeletal muscle, is linked to severe Se deficiency; although, it can be caused by vitamin E deficiency as well.

Cows will do all they can to ensure adequate Se is in calves when they are born. They will actually deplete their own body reserves to ensure neonatal adequacy. As such, neonates diagnosed with Se deficiencies are proof of maternal deficiencies. With Se being an essential component of the immune function, this maternal deficiency likely results in poor colostrum quality and inadequate neonatal protection even in calves that get adequate volumes of colostrum.

Diagnosis of a deficiency can be made by analysis of liver, whole blood, or serum for Se content or by analysis of whole blood for glutathione peroxidase, a Se-dependent enzyme. The most specific analysis is that of whole blood glutathione peroxidase, as it verifies true functional Se status. Liver is the optimal tissue to analyze for Se content as it is a primary storage tissue. With serum and whole blood, the former better reflects recent intake, while the latter better reflects longer term intake status. Since seleno-proteins are incorporated into the red blood cells when they are made and the cells have a long half-life, Se content of whole blood is a better reflection of intake over the previous months than serum.

In order to adequately diagnose Se deficiency, the dietary form of the Se intake by the animals is important. Natural Se, predominantly in the form of seleno-methionine is metabolized and incorporated into Se dependent proteins, but can also be incorporated into non-specific proteins in place of methionine. Inorganic Se is also metabolized and predominantly incorporated into Se-dependent proteins. Thus, *normal* concentrations in serum and whole blood differ depending on whether the dietary Se is a natural organic form or an inorganic supplement.

Selenium excess is commonly identified in multiparous dairy cows. If the Se excess is great enough, it can result in poorer reproductive performance, poor calf survival, and imbalances of other minerals. Excessive Se can also interfere with Zn absorption. The recommended adequate liver Se concentration range in adult cattle is 0.25 to 0.50 ppm, with late-term fetal or neonatal liver normal being 0.35 to 0.75 ppm.

ZINC

Zinc is an essential mineral that is required by all cells in animals. Zinc plays a role in numerous enzymatic reactions. Deficiencies of Zn are associated with reduced growth, poor immune function, diminished reproductive performance, and poor offspring viability, as well as skin lesions in severe cases. Tissue Zn concentrations do not reflect body status well. Of the common samples tested, liver and serum are the best indicators of Zn status. But, serum and liver Zn can be altered by age, infectious diseases, trauma, fever, and stress. Response to added Zn has shown that some animals with low-normal liver or serum Zn can show improvement in some clinical conditions.

Over the past few years, the number of dairy cows found to have sub-normal to deficient Zn status has been increasing. It is important to note that almost all of these cases are in multiparous cows that are also found to have excessive Cu and Se in the liver. Thus, knowing that dietary excesses of both Cu and Se can interfere with Zn absorption, one must conclude that the low Zn is likely a secondary effect. In fact, several cases have corrected the low Zn by nothing more than decreasing the excessive Cu and Se in the rations.

VITAMIN A

Vitamin A is an essential fat soluble vitamin in ruminants. It is essential for all cell replications and is especially important in epithelial integrity. It plays an important role in tight junctions between cells, as well as being an important antioxidant in the body and in mucosal secretions. Vitamin A deficiency is associated with poor growth rates, poor feed intake, poor immune function, poor reproductive performance, and high incidences of diarrhea in calves. Loss of efficient tight junctions in the epithelial cell lining of the digestive tract allows opportunistic pathogens to invade and cause disease.

Vitamin A is provided in the diet via green growing vegetation or supplementation. Dead, brown forages have relatively no Vitamin A content. Thus, for grazing livestock, they must accumulate enough body reserves to carry them through the winter and have enough left to provide adequate vitamin A to their offspring. Therefore, it is more common to see vitamin A deficiencies in the spring after significant drought years, due to decreased time for body reserve accumulation. Unlike minerals, much of the vitamin A provided to the neonate is via the colostrum and in milk fats. Also, early calving in beef herds has increased the incidence of neonatal vitamin A deficiencies due to lack of green forage for the cows at the time of parturition.

Vitamin A deficiency is not generally encountered in dairy cows, but is occasionally seen in dairy calves. In vitamin A deficiency cases this author has investigated, most occur in very intensely managed herds. As dairy rations typically have good vitamin A supplementation, one would not expect to see these deficiencies in calves. But the potential for vitamin A loss in the processing of colostrum must be considered. Vitamin A can be broken down by heat. So, although not yet proven, one should consider the possibility that the vitamin A could be lost either in thawing colostrum too fast or when colostrum is pasteurized to prevent spread of disease.

Vitamin A analysis can be efficiently performed on serum or liver tissue. It is important that samples be stored frozen and protected from light to prevent degradation of the vitamin A.

VITAMIN E

Vitamin E is an essential fat soluble vitamin in ruminants. It is essential for all cells as an important antioxidant in the body in conjunction with Se. Vitamin E deficiency is associated with poor growth rates, poor immune function, poor reproductive performance, poor muscle function, poor cardiovascular function, and *white muscle disease*.

Vitamin E is provided in the diet via green growing vegetation or supplementation. Dead, brown forages have relatively no Vitamin E content. Thus, for grazing livestock, they must accumulate enough vitamin E to carry them through the winter and have enough left to provide adequate vitamin E to their offspring. Therefore, it is more common to see vitamin E deficiencies in the spring after significant drought years, due to decreased time for body reserve accumulation. Much of the vitamin E provided to the neonate is via the colostrum and in milk fats, although it is also transferred, a small amount, across the placenta. Also, early calving in

beef herds has increased the incidence of neonatal vitamin E deficiencies due to lack of green forage for the cows at the time of parturition.

Vitamin E deficiency is not generally encountered in dairy cows, but is occasionally seen in dairy calves. In vitamin E deficiency cases this author has investigated, most occur in very intensely managed herds. As dairy rations typically have good vitamin E supplementation, one would not expect to see these deficiencies in calves. But the potential for vitamin E loss in the processing of colostrum must be considered. Vitamin E can be broken down by heat. So, although not yet proven, one should consider the possibility that the vitamin E could be lost either in thawing colostrum too fast or when colostrum is pasteurized to prevent the spread of disease.

Vitamin E analysis can be efficiently performed on serum or liver tissue. It is important that samples be stored frozen and protected from light to prevent degradation of the vitamin E.

EFFECTS ON IMMUNE STATUS

Deficiencies in vitamins and minerals have a 2-part impact on immune function in neonates. Firstly, since neonates are still developing their immune capabilities, these deficiencies have a direct negative impact on that development. And, indirect immune compromise is via the mother's poor immune function. At the time in which it is essential that mothers be immune competent in order to produce antibodies for the colostrum, when inadequately supplemented they are often deficient due to depletion from the movement of minerals to the fetus. Additionally, poor immune function at the time of vaccination can result in very poor vaccine response, which in turn results in poor immune memory and antibody production necessary for good quality colostrum. Thus, herd deficiencies would be expected to result in poor colostrum quality. This poor quality equates to a higher incidence of disease in the offspring due to poor maternal protection. Often this is seen as high incidence of neonatal diarrheas and/or high incidence of neonatal/juvenile pneumonias.

SUMMARY

A variety of samples can be tested for vitamin-mineral content, but may not provide any indication of the overall mineral status of the animal. Appropriate diagnosis of mineral status involves thorough evaluation of groups of animals. The evaluation should include a thorough health history, animal ages, feeding history, supplementation history, and analysis of several animals (at least 6-10 recommended per similarly fed group, depending on the group size) 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 supplements need to be investigated. As an example, high S or Fe can cause deficiencies in Cu and Se even when there are adequate concentrations in the diet. Or excessive Cu and Se can adversely impact the Zn status. If a free choice supplement is used instead of the supplement being incorporated into a TMR, true intake should be measured.

Overall, common vitamin-mineral deficiencies/excesses are significant hindrances to profitability in the livestock industry. Poor reproductive performance results in increased incidence of culling open cows. Poorer than optimal feed efficiency and productivity impact the bottom line in terms of pounds of milk sales. And, poor calf health results in deaths and disease. The resultant increased disease incidence results in lost income in terms of treatment costs and poorer overall growth rates and productivity in affected animals. Beef herds have been followed where deficiencies were observed, then corrected in which breed-back efficiency has improved by 10 % or more and weaning weight averages have improved by as much as 30 to 70 lb/calf or more. These changes amount to significant improvements in profitability in cattle operations.

In dairy operations, one must correctly identify cause of mineral status abnormalities. For example, if calves are identified to be Cu and Se deficient (indicating deficiencies in the cows) the lactation ration should not immediately have increased supplementation. In several cases the author has investigated, these deficiencies were in herds that were actually OVER-supplementing Cu and Se in the lactation ration. But, the deficiencies were all in calves from first calf heifers which were not being adequately supplemented by the heifer raising facility. Similarly finding excessive Cu and/or Se in multiparous cows does not indicate that mineral supplementation should be cut back for all groups of cows (replacement heifers on dairies are often fed quite different rations than those fed to the lactating herd).