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This review accompanies the relevant episode of the Cutting Edge veterinary podcast. In each episode of this podcast, 3rd year students in the University of Calgary's veterinary medicine program fill you in on the most up-to-date literature and evidence-based practices on topics that matter to you, the practising veterinarian.

Micronutrients in Western Canadian Beef Herds

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Introduction

Micronutrient status is an important determinant of health in cattle. There have been numerous minerals identified as nutritionally essential[1,2]. Many of these have been identified as important for immune function, reproductive success, and productivity[3]. The focus of this paper will be on micronutrient challenges and imbalances in western Canadian beef herds. As not all micronutrients pose the same challenges, emphasis will be on copper, selenium, molybdenum, sulfur, and vitamin A. Effective management of micronutrient uptake can be difficult due to two main reasons: 1) difficulty of assessing micronutrient levels and 2) difficulty of accurate supplementation which at times can be complicated by antagonistic actions of certain minerals. In this paper we will discuss the physiological role of the identified micronutrients, the current predicted levels in western Canadian beef herds, the effects of imbalances, how to evaluate micronutrient status, and ways to supplement herds.

Physiological Role

To understand the importance of balanced micronutrients, it is imperative to understand the role these factors play in bovine physiology. Copper is essential for innate immune function due to its role in a variety of metalloenzymes. The most notable copper-dependent enzyme is ceruloplasmin, which is a major bovine acute phase protein. Ceruloplasmin is responsible for lipid peroxidation, oxidation of ferrous iron to its nontoxic form, and tissue repair. It also plays a role in increasing immune function by its actions on various enzymes and the regulation of phagocytosis and antimicrobial activities [4]. During inflammatory events in cattle with adequate copper, ceruloplasmin concentrations increase sharply, likely due to its immune and regulatory roles. This response is suppressed in copper deficient cattle[2,5]. Copper is also essential for normal reproductive function and deficiency is associated with low fertility due to delayed or suppressed estrus, longer postpartum interval, anestrus, abortion and early embryonic death[6]. Copper status can be complicated by the presence of antagonists such as sulfur and molybdenum. In the rumen, molybdenum reacts with sulfur to form thiomolybdates that have high affinity to bind to copper[7]. Deficiency may occur when the diet contains Copper:Molybdenum ratios below 3:1[8]. High dietary concentrations of sulfates can also reduce copper bioavailability independent of dietary molybdenum. This occurs through

the formation of insoluble copper sulfide in the rumen[9]. Furthermore, iron, zinc, and calcium may also interfere with copper to decrease its bioavailability[1]. These mineral interactions have been implicated as responsible for reduced conception rates, anovulation, anestrus, decreased basal LH levels, reduced peak surge of LH and reduction of number and frequency of LH pulses[10]. These reproductive consequences were observed to be independent of copper status, suggesting that copper should not be considered independently from the other minerals that interact with it.

Selenium plays an important role in the antioxidant glutathione peroxidase, which is a powerful mediator of oxidative stress[2,11]. As a result, deficiency manifests as oxidative stress and tissue degeneration, as is seen with white muscle disease (nutritional myodegeneration). Moderate cases often involve myodegeneration causing muscle weakness, difficulty standing, dysphagia, and pain; more severe cases often involve cardiomyopathies, respiratory distress, and even death within 24 hours[12,13]. Selenium toxicity is possible with improper supplementation but much less common, and may manifest as alkali disease (chronic selenosis), or blind staggers (acute selenosis)[14].

Vitamin E and selenium act synergistically to decrease free radicals produced by the body during normal metabolism[15]. Vitamin E also has antioxidant activities that enhance neutrophil function by preventing oxidative damage[15].

Even though sulfur has the potential to be detrimental in ruminant health due to its interactions with other micronutrients, certain levels of the element are crucial for cattle health. Sulfur has a multitude of physiological roles. These include being a component of glutathione, vitamin B, amino acids, and coenzyme-A[16]. Due to these roles sulfur is important for hoof and hair health, milk and tissue protein production, blood clotting, and endocrine function[16].

Vitamin A plays an important role in vision, growth, immunity, cellular metabolism, bone development, and reproduction[17]. The many subforms of vitamin A all have roles specific to their form. For example, vitamin A exists as retinol, retinyl esters, retinoic acid, and retinaldehyde. In cattle, vitamin A is absorbed in the small intestine and transported to the liver where it can be stored or further metabolized for use in cells throughout the body. The various functions of vitamin A make it a necessary element for cattle health and homeostasis[17].

Current Predicted Levels in Western Canadian Beef Herds

Micronutrient deficiency is suspected to be an underlying issue in most beef herds in western Canada[3]. The most common mineral deficiencies in cattle sampled between 2003-2012 include copper, iron, manganese, and magnesium[3,18]. Deficiencies in selenium, vitamin E, and vitamin A have also been reported in North American herds[3,19]. These deficiencies may have significant impacts on cattle due to the important roles of micronutrients on health and physiology. A 2023 study that evaluated cattle from British Columbia, Alberta, Saskatchewan, and Manitoba found that in 82% of herds, at least one cow per herd was copper deficient[3]. A previous study also found that 66% of individual cattle had less than adequate serum concentration of copper[19].

Selenium deficiency is less common than copper deficiency[3]. Of those sampled in a study in 2019, only 2% of all animals sampled were considered deficient, and 1/3 of cattle were considered below adequate levels[3]. In this study, cattle were considered

deficient if serum concentrations were below 0.025 ppm, and considered below adequate if serum concentrations were below 0.08 ppm[3]. These levels were based on commonly used reference values in North American labs[3]. The proportion of cattle that were deficient is less than another study from 2001, where 11% were considered deficient, and 87% had less than adequate levels[20].

Sulfur deficiency is much less common, as it has high bioavailability and is found in most feed sources[2]. In fact, excess sulfur is seen more frequently due to the formation of thiomolybdates and insoluble sulfide complexes in the rumen, resulting in secondary copper deficiency[9].

A study which reported vitamin status in beef calves from herds in western Canada found that serum vitamin A concentrations were considered less than adequate in 58.3% of calves sampled[15]. Calves receive most of their vitamin A through milk and colostrum therefore measuring their serum levels is considered representative of the herd's vitamin A status[21]. This study also corrected for failure of transfer of passive immunity by measuring serum IgG concentrations[15]. One potential confounder in this study was that it was conducted during drought years of 2001-2002 which may have impacted the results by increasing the levels of deficiencies. However, similar conditions have prevailed throughout western Canada in recent years, making vitamin A levels a valid concern for today's beef production systems.

Subsequent Effects of Micronutrient Imbalance

Copper deficiency may be primary or secondary in origin[1]. Primary deficiencies are found in individuals that do not consume enough dietary copper, secondary deficiencies are found in individuals that consume sufficient copper but the mineral is not biologically available[19]. An example of secondary deficiency is when molybdenum reacts with sulfur to form thiomolybdates that have high affinity for binding to copper[7]. Copper levels are maintained by liver stores so cattle can be clinically normal despite their plasma concentrations reflecting a deficient state[5]. This is because clinical signs often do not appear until the liver stores are spent and the animal is severely deficient[5]. Signs of copper deficiency can vary and range from apparent to non-specific. Severe deficiency results in decreased growth, anemia, colour changes in hair coat, and compromised immune function[22]. Copper is often a risk factor for reproductive failure in beef herds, and decreased serum copper concentrations have been associated with increased odds of cows less than 10 years of age not being pregnant compared to cows less than 10 years of age with adequate serum copper levels[9]. This effect is even more substantial when the cows are copper deficient prior to breeding. Copper supplementation has also been reported to increase growth and average daily gain in grazing steers compared to individuals whose diets were not supplemented with copper[22].

While over-supplementation of copper is rare, toxicity is possible yet can be hard to detect due to a subclinical hypercupremia[23]. Buildup of copper in the liver may transiently elevate hepatic enzymes, but this is not a reliable indicator without weekly blood tests[23]. Copper toxicity tends to cause clinical signs after a stressful event, where a spike in blood copper levels causes intravascular hemolysis. This can lead to anemia, icterus, depression, increased thirst, anorexia, hemoglobinuria, and death within 24 hours[24].

Deficiencies in selenium levels also have considerable impacts on cow health, including reduced weight gain, tissue degeneration, myocardial and skeletal muscle necrosis, retained placenta, reproductive inefficiency, mastitis, increased calf morbidity, and reduced immune competence due to decreased function of phagocytic cells[2,25]. Selenium has an important interaction with vitamin E and a shared role in the development of white muscle disease and weak calf syndrome when deficient[2]. Selenium deficiency has also been associated with an increased risk of retained placenta, and reduced function of phagocytic cells leading to decreased immune competence[2].

Sulfur plays an important role in the synthesis of ruminal microbial protein. However, levels above requirement have a negative effect on animal performance by decreasing copper concentrations in plasma [8,9]. Excess sulfur can also decrease average daily gain and gain-to-feed ratio in growing heifers[8]. Depending on dose, duration and bioavailability of sulfur ingested, it may interact with thiamine metabolism resulting in polioencephalomalacia. Sulfur toxicity is a major cause of polioencephalomalacia, a neurological disease that causes anorexia, blindness, and ataxia progressing to recumbency and death[26].

Deficiency in vitamin A and E have been suggested as one of the most common causes of death in calves born alive[15]. Since calves are dependent on colostrum for most of their vitamin A and E, this finding could be associated with inadequate colostrum intake and therefore decreased immunity[15,21]. Signs of vitamin A deficiency may include blindness in both newborn calves and growing animals, reduced performance, increased susceptibility to disease, and reproductive failure in cattle[17,21].

Evaluating Micronutrient Status

Evaluating micronutrient status in beef herds can be a challenging process for producers[3]. The signs of deficiency can be vague, other issues may be suspected more readily, or producers may not be aware of the potential risks associated with micro-nutrient imbalances[2,9]. To complicate matters further, when micronutrients are deficient, the consequences can act synergistically with other comorbidities to negatively impact cow health. One may assume that evaluating micronutrient levels in the animal's feed and supplements is sufficient for analyzing nutrient status. This is not the case, as most beef herds spend a majority of their year on pasture where mineral concentrations are variable, and intake of a supplement may vary between individuals[1]. To address these issues veterinarians may turn to lab work diagnostics to directly evaluate the animals' status.

When evaluating copper levels, the most diagnostic sample is a liver biopsy due to the liver's role as storage in the body[1]. Plasma levels are a less accurate measurement because blood levels of copper are maintained at a constant concentration through depletion of the liver stores, masking the deficiency[18]. An additional complication is that in serum samples, thiomolybdate-bound copper may be detected as free copper and falsely elevate detected levels[3]. When it comes to chronic copper toxicity, liver biopsies are the most reliable test for detection, whereas serum copper levels tend to remain stable regardless[7].

A liver biopsy can be obtained by using a Tru-Cut biopsy needle to penetrate muscle and peritoneum before reaching the liver[27]. On a herd level however, liver

biopsies can be impractical and therefore plasma sampling may be more realistic for producers and veterinarians. The information gathered when sampling for copper status must also be considered with seasonal and physiological fluctuations in mind. For example, maternal copper levels decrease towards the end of pregnancy, as levels in the growing fetus are increasing[28].

Fertilizer use, sulfur-rich feed sources such as molasses or distillers' grains, high-sulfate water sources, and acid rain deposition on forages all contribute to the sulfur intake of beef cattle[2]. Each of these sources must be taken into account when calculating the total sulfur intake of a herd. Sulfur intake can be estimated by testing feed sources, supplements, and water[2]. The total sulfur content of these three sources should be enough to accurately gauge sulfur intake. Cattle can tolerate a maximum of 30 g/day of sulfur before copper and selenium antagonism become an issue[2]. If this limit is exceeded, changes must be made to the diet or water, depending on which source is the issue. Alternatively, copper and selenium may be supplemented to avoid deficiencies. A common misconception is that the characteristic "rotten egg" smell of sulfur can be used to identify high-sulfate water. However, this odor comes from hydrogen sulfide volatilizing off the surface of the water, which humans can detect in parts per billion[2]. This is too little sulfur to cause a noticeable effect on cattle health and is not a reliable indicator of quantity.

Serum or whole blood samples provide adequate information to assess selenium and molybdenum levels. Selenium is absorbed from the gut based on dietary availability without homeostatic interference. If in excess in the blood, it is removed minimally through the bile or majorly via renal excretion[1]. As serum is the carrier vehicle for renal excretion, serum sampling is a fairly accurate way of measuring selenium status[1]. Whole blood sampling may be equally advantageous, as erythrocytes also carry selenium and may provide a more long-term evaluation of levels, as the half-life of erythrocytes may be longer than the time selenium stays in plasma before excretion[1]. Molybdenum is absorbed from the diet and can be evaluated from a variety of samples, these include serum, whole blood, and milk[1]. If there is a concern regarding excess molybdenum, or if a secondary copper deficiency is suspected, sampling the liver or serum may be used to detect a micronutrient excess[1].

Vitamin A and E levels may also be analyzed via a serum sample[15]. Feed samples may be analyzed for carotenoid content (vitamin A precursor) in mg/kg or mg/lb[29].

Furthermore, besides live animal testing, post-mortem sampling of affected or deceased animals may also be beneficial if micronutrient deficiencies are suspected. Sampling the liver and kidney to assess mineral and vitamin status would be necessary in addition to all other routinely sampled tissues[30,31]. Ideally a fresh sample on ice is submitted, however frozen, or formalin fixed samples would also be acceptable[31]. These directions may differ based on the diagnostic laboratory completing the testing, therefore labs should be contacted directly for specific directions.

How to Supplement

Micronutrient supplementation is complicated by many factors, which include: regional needs based on soil types, use of different forages, increasing herd sizes, and variable free-choice supplement intake by cattle. Understanding the micronutrient

concentrations in feed may be insufficient when intake is difficult to measure. Additionally, predicting interactions and biological availability adds further challenges.

There is a relatively wide range for copper supplementation in cattle because requirements are highly dependent on availability and the presence of other dietary elements that can influence absorption and metabolism[11]. The National Research Council suggests that cattle require 10 mg Cu/kg DM daily, with additional copper being required if antagonists are present in the feed[2]. Copper supplementation can be done in a number of ways. Typically, it is given orally through a mineral lick block or an organic (carbon-containing) supplement in the feed[32]. However, it is difficult to ensure that each animal is taking in enough copper unless they are being force-fed as part of a total mixed ration. Despite the fact that most producers supply free-choice minerals to their herds, the majority of beef cattle in western Canada are still copper deficient. An experimental trial in Saskatchewan showed that cows fed free-choice mineral pre-calving were six times more likely to be below adequate levels of copper at calving and calved five days later than cows force-fed mineral in silage[3]. For producers who feed a total mixed ration, giving a pre-measured amount of mineral in the feed is one option to improve intake.

Other options for copper supplementation include boluses, drenches, pastes, and injectable products[33]. Multimin 90 is one injectable product that also contains selenium, manganese, and zinc. This product has been shown to have little effect on plasma copper, which is generally stable, but does increase liver copper for approximately a month[33]. Conversely, oral pastes or drenches were found to have little effect on liver copper levels and are likely insufficient for treating deficient cattle due to their tendency to bind to feed particles[33]. Long-acting oral boluses may be an option for a more gradual increase in liver mineral concentrations, as they require 120 days to take effect[2]. Current studies have not shown a significant increase in conception rates after injecting cattle with Multimin[34]. However, yearling bulls have been shown to exhibit improved semen motility and morphology after Multimin supplementation at 7 and 10 months[35]. While injectable copper is generally accepted as a method of improving liver copper, care must be taken not to inadvertently cause toxicity, especially since Multimin 90 contains other micronutrients. To avoid this, injectable products containing only copper may be compounded as well. However, this is considered extra-label drug use and is not regulated by Health Canada, so special attention must be paid to meat withdrawal[36]

For treatment of selenium deficiency, Multimin 90 was found to effectively increase both plasma and liver concentrations. Similar to copper, boluses were found to have little effect on liver selenium[33]. Injectable solutions containing selenium and vitamin E are also available but depending on the product, may not be sufficient to correct vitamin E deficiency on their own[15]. Selenium and vitamin E both play a vital role in scavenging peroxides and free radicals, but supplementing one nutrient does not reduce the need for the other[37]. It is advised that producers in selenium deficient areas give newborn calves an injection to prevent diseases such as white muscle disease[14]. Since much of the selenium provided to calves is supplied through the placenta, it is also suggested that late gestation cattle are monitored to ensure that they do not use up their own reserves on their calves[14]. After injection, selenium must be maintained through adequate dietary supplementation. For mature cattle, selenium may be

supplemented through selenium enriched cereals, mineral selenium (selenite), or organic selenium (yeast)[14].

Vitamin A is typically best consumed as beta carotene through lush green pasture grass[29]. Vitamin A consumed through grass is stored in the liver and may be drawn upon for 2-3 months[29]. Deficiencies are more common during the winter or periods of drought, when fresh grass is less available[38]. While hay may contain enough carotenoids to meet the needs of a herd, supplementation is often recommended when feed is poor, animals are stressed, or during gestation[29]. Where hay quality is marginal, it is recommended to supplement 50% of a cow's daily vitamin A requirements as an adjunct to the forage provided[38]. As with minerals, vitamin A can be supplemented through a water source or free-choice, but these methods both result in variable intake per cow[29]. Force-feeding vitamin A in a total mixed ration is often the most effective form of supplementation[29]. Alternatively, cattle may be drenched periodically or receive an injection of vitamin A every 28 days[38].

Conclusion

While interactions between micronutrients in beef cattle are generally well-understood, there is still significant work to be done in terms of improving micronutrient status of Canadian beef herds. Deficiencies in copper and selenium are commonly seen, and their levels paired with other micronutrients such as molybdenum play a crucial role in the health of beef cattle. Feed, plasma, and liver biopsy testing are all methods of monitoring beef herds to determine their mineral status. While liver samples are often the best choice, logistically they are challenging to perform on large numbers of cattle. Even in herds with free choice supplements available, there is no guarantee that cattle are within acceptable physiological micronutrient ranges. Where deficiencies are found, supplementation in feed, injections, and in some cases, boluses or drenches may be used to bring the cattle back into normal ranges. Being proactive about micronutrient testing of feed and other sources (e.g. water, pasture) will help improve immunity and fertility in Canadian beef herds, which ultimately has a positive impact on production, health, welfare, and the financial success of producers.

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