Understanding copper nutrition in small ruminants

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Abstract

The objective of this presentation is to address pertinent aspects of copper metabolism, nutrition, and disease concerns relative to small ruminant feeding programs. Clinical manifestations of copper deficiency are associated with a critical decline in activity of a specific copper-dependent metalloenzyme. Though any disease process may occur in all small ruminant species, there seems to be some species differences in presentation of copper diseases. Goats tend to have greater problems with copper deficiency due to their higher requirements compared to other small ruminants. Copper toxicosis results from the accumulation of copper from the diet culminating in uncontrolled copper ion release from storage and subsequent oxidative damage. Toxicosis is of concern for sheep, llamas, and alpacas and less so for goats, though clinical presentation is not equivalent across the species. All dietary ingredients need to be accounted for in addressing small ruminant dietary copper management in an effort to prevent copper-related disease risks.

Résumé

Cette présentation examinera quelques aspects pertinents du cuivre ayant trait aux programmes d'alimentation des petits ruminants : métabolisme, qualité nutritive et incidence sur la santé. La carence en cuivre se manifeste cliniquement par un déclin critique de l'activité d'un métallo-enzyme qui dépend spécifiquement de ce métal. Bien que les maladies se manifestent de manières diverses chez tous les petits ruminants, les maladies reliées au cuivre semblent se manifester différemment entre certaines espèces. Par exemple, les chèvres semblent souffrir davantage d'une carence en cuivre que les autres petits ruminants, en raison de ses besoins plus élevés de ce minéral. D'autre part, la toxicose de cuivre, qui résulte de l'accumulation de ce minéral provenant de la ration alimentaire, cause une libération incontrôlée d'ions de cuivre à partir des tissus de réserves et entraîne des dommages dus à l'oxydation qui s'en suit. La toxicose de cuivre est un problème chez les ovins, les lamas et les alpagas, mais à un moindre degré chez les caprins, bien que la manifestation clinique diffère entre les espèces. Chez les petits ruminants, la gestion du cuivre alimentaire doit donc tenir compte de tous les ingrédients de la ration pour prévenir les risques de maladies reliées à ce minéral.

Introduction

Though most microminerals account for a small portion of the total consumed diet, they play many important biochemical roles in the body. Copper (Cu) is an essential micromineral required by all small ruminant species to maintain normal body functions. However, Cu nutrition is a double-edged sword being associated with both deficiency and toxicity disease concerns, with much variation among the small ruminant species. Although all microminerals are of importance, Cu is receiving more attention as a result of the potential toxicity concerns with sheep, llamas, and alpacas and for deficiency issues in goats.[8] There is evidence of increasing Cu content in forages, and feeding with other copper-containing supplements (pellets and mineral) may predispose animals to a greater risk for toxicity problems. The objective of this presentation is to provide an overview of Cu nutrition by describing biologic functions and associated deficiency and toxicity disease conditions in small ruminants. Additionally, Cu requirements, assessment of Cu status, and dietary supplementation practices, especially concerns relative to toxicity risks, will be addressed.

Biological Roles

Essentiality of Cu in animals was not discovered until 1928, though it had been discovered in animal and plant tissues much earlier. Copper performs a number of essential roles in the body as a component of various proteins (e.g., metalloenzymes) that require Cu to sustain their biologic functions.[4,13] Some examples of Cu metalloenzymes include ceruloplasmin, tyrosinase, lysl oxidase, cytochrome C oxidase, and superoxide dismutase. Through the action of these metalloenzymes, Cu has been associated with iron regulation and red blood cell function, cellular respiration, bone and connective tissue formation, hair pigmentation, nerve tissue and cardiac development, and immune function.

Ceruloplasmin is a blood protein with multiple functions and contains seven copper atoms, thus accounting for a majority of copper in blood. Ceruloplasmin converts stored iron (+2) into the ferric (+3) state to be incorporated into hemoglobin or myoglobin for oxygen transport. Ceruloplasmin is a liver generated acute phase protein with an important role in the non-specific immune response, and its blood concentration will be elevated in response to an infectious agent. Functional

activity of most cell types responsible for the various immune system responses are influenced by Cu through superoxide dismutase activity.

Of interest to fiber producing small ruminants, Cu plays an important role in fleece coloration and quality. Tyrosinase (polyphenyl oxidase) is the enzyme responsible for the formation of melanin, a pigment responsible for hair and skin coloration. Hair or fleece would appear lighter in color or "bleached out" without the presence of melanin. Cross linkages of disulfide groups within the keratin structure of hair provide the physical properties of fleece and are dependent upon Cu. Sheep with copper deficiency are described as having "steely wool", where the crimp has been lost and the fiber is straight.

Normal development of bone and connective tissue is dependent upon the copper-dependent enzyme lysl oxidase. This enzyme is responsible for modifying specific amino acids within the collagen protein structure that facilitates cross linkages between collagen fibers within connective tissue. These cross linkages impart properties of rigidity or elasticity to the collagen structure. Rigid connective tissue provides the scaffolding for mineralization in the development of bone. Elastic properties of collagen are seen in the large blood vessels such as the aorta, which must withstand wide fluctuations in pressure in circulating blood.

The cell's ability to generate energy through the transfer of electrons to the final receptor oxygen to form water is one of the functions of cytochrome C oxidase. Integrity of the nervous system is dependent upon the specialized phospholipid coating (myelin) around nerve fibers facilitating transmission of nerve signals. Myelin formation in the brain and spinal cord is related to cytochrome C oxidase activity. Besides the structural effects on nervous tissue, Cu is linked to nervous and cardiac tissue communications through its effect on production of neurotransmitters and heart muscle fiber (myofibril) development. Both cytochrome C oxidase and dopamine- -monooxygenase are responsible for these biologic functions of Cu.

Deficiency Diseases

With the number of biological functions attributed to Cu, a spectrum of disease entities has been associated with a deficient nutrient status. A common Cu deficiency disease occurring in a wide range of animals is anemia. Anemia can be characterized by the size and pigment (hemoglobin) content of red blood cells. In older ruminants, Cu deficiency can induce a macrocytic, hypochromic anemia due to low ceruloplasmin activity, whereas in lambs the anemia is characterized as microcytic and hypochromic.[13] Anemia and poor condition attributed to Cu deficiency has been reported in two llamas (14 and 23 months of age).[1] Although anemia can result from a number of nutritional and non-nutritional insults, affected llamas in this report had low blood Cu concentrations (3.8 - 8.3 µg/dL) and responded to Cu supplementation with increased blood Cu concentrations (66.7 – 82.6 µg/dL). These Cu concentrations are consistent with recognized Cu deficiency in other species, and are below reported values (mean 35.6, range 16.5 – 55.3 µg/dL) for healthy llamas.[12] Similar copper-responsive anemia can be seen in goats.

Copper deficiency has been associated with neurologic degeneration in sheep and goats as a result of abnormal myelin formation, with resulting disease signs of muscular weakness of the legs and ataxia.[4,13] Three different reports of possible association between Cu deficiency and neurologic disease and ataxia in llamas and alpacas have been published.[5,9,11] Presenting signs in the affected animals included hind limb ataxia, paralysis or head tremors; similar to what is seen in sheep and goats. Blood Cu concentrations of the affected animals in these reports were not in the deficient range; however, blood Cu is not always a reliable measure of Cu status. Response to Cu supplementation in these cases was not well defined, thus questioning whether or not this truly was a Cu deficiency disease process. In one of the reports, a six-month-old llama was described with progressive paralysis, and liver and kidney Cu concentrations were found to be deficient. More evidence is needed to determine if there is a role for Cu deficiency in neurologic disease of camelids, similar to that described for sheep and goats.

Other potential Cu-deficiency diseases have not been reported in llamas and alpacas, and there is no indication that there is potential for such diseases. One might surmise there is the possibility for achromotrichia (loss of fleece pigmentation) and fleece structural changes in llamas and alpacas similar to what is seen in sheep and other species with Cu deficiency. Bone development abnormalities (similar to rickets), blood vessel ruptures, and heart degeneration are other recognized Cu deficiency diseases, but these are not seen in all species. Copper deficiency diseases will vary among animal species.

The disease conditions discussed thus far are classified as clinical disease syndromes. Clinical disease is characterized as the "classical" disease processes associated with a specific nutrient. In contrast to clinical disease is what is termed "subclinical" disease. Subclinical disease is described as non-specific consequences of a nutrient being marginally deficient or toxic. Subclinical Cu deficiency is associated with impaired immune response and greater susceptibility to disease, reduced reproductive fertility, and poor growth or lactation. Clinical disease is more readily identified, but is not nearly as prevalent as subclinical disease. With difficulties in assessing Cu status using blood concentrations, subclinical disease becomes a difficult process to diagnose.

Toxicity Disease

Sheep are well known to be extremely sensitive to excess dietary Cu (>4.54 mg/lb or 10 mg/kg);[13] however, camelids do not seem to be as keenly sensitive but are prone to toxicity.[14] At least four published case studies have reported Cu toxicity or suspected toxicity in llamas and alpacas.[2,3,6,16] A number of clinical situations have occurred throughout the US where Cu toxicity was highly suspected. It would seem that Cu toxicity is a disease of greater concern for llamas and alpacas. The challenge is being able to recognize disease risk early and initiate appropriate dietary changes, as there is no treatment once the disease process has initiated.

Copper toxicity is the result of the highly reactive nature of the Cu ion when not protectively bound in tissues or blood.[7] Like most minerals, copper is a strong oxidizing agent that can damage cellular membranes and proteins. In most situations, Cu toxicity is a progressive disease process where excess dietary Cu accumulates in the liver until it becomes saturated. The liver is the primary mineral storage organ of the body, and contains special protein molecules capable of binding minerals and keeping them isolated from doing any damage to the surrounding tissues. Stored Cu is inefficiently excreted through bile produced in the liver and transported to the intestinal tract to be lost in fecal matter. The ability to excrete Cu is species-dependent and accounts for the observed differences in sensitivity to Cu toxicity. Once the liver has become saturated with stored Cu, excess will spill out into the liver and blood, wreaking oxidative havoc.

Clinical Cu toxicity in sheep is characterized by hemolysis due to oxidative damage from the Cu ion released into the bloodstream. With hemolysis, there is leakage of free hemoglobin into the blood (hemoglobinemia) and urine (hemoglobinuria). Hemoglobin in urine will induce damage to the kidneys. The hemolytic crisis stage of the disease is nearly always fatal. The disease process is different in llamas and alpacas, as the "hemolytic crisis" has not been reported or seen in field cases. The common theme in llama and alpaca Cu toxicity cases is the documentation of severe and widespread degenerative changes (necrosis) in the liver. This is a different process than hepatic lipidosis (fatty liver). Liver necrosis may or may not be associated with elevated blood enzyme activities assessing liver function. Affected llamas and alpacas will have highly elevated blood (>200 µg/dL) and liver (>600 µg/g dry weight) Cu concentrations, as well as elevated kidney (>10 µg/g dry weight) Cu concentrations.[14]

Typically, blood and kidney Cu concentrations are not highly elevated until the final initiation of the disease process; therefore, they may not be useful in diagnosing potential risk. Additionally, kidney Cu concentrations are only determined in animals that have died. The difficulty in dealing with this disease is that animals may only show minimal signs of poor-doing prior to the final demise. Stress factors or previous liver disease may precipitate the disease.

Key to understanding and preventing Cu toxicosis is nutritional management practices. Based on these published reports and the documentation of dietary factors, llamas and alpacas are seemingly prone to Cu toxicity when fed diets with greater than > 9.1 mg/lb (20 mg/kg or ppm) total Cu and a high ratio (16:1) of Cu to molybdenum (Mo) in comparison to sheep, where diets > 4.54 mg/lb (10 mg/kg) can potentially be toxic.[7] Molybdenum is a trace mineral that plays an important role in dietary Cu availability.

Requirements

Defining a "true" requirement, meaning how many milligrams (mg) per day to support a given physiologic state, for a trace mineral is difficult at best. Often a trace mineral requirement is described in terms of dietary concentration, namely parts per million (ppm). Ideally, a trace mineral requirement would be defined in terms of how many mg of mineral were needed to support specific physiologic states such as maintenance, pregnancy, lactation, growth, and work/activity. Obviously to determine such needs, specific feeding trials must be completed. The recent National Research Council (NRC) publication for small ruminants has defined specific mineral requirements for sheep based on factorial models and for goats based on dietary concentration.[8] There were no mineral recommendations for llamas and alpacas, with the exception of selenium, as there are no published studies defining feeding protocols specific to llamas and alpacas.[8] For sheep, the suggested dietary Cu concentration ranged from 2.27-3.63 mg/lb (5-8 mg/kg), while for goats the recommendation was 6.8-9.1 mg/lb (15-20 mg/kg). Clearly, mineral products cannot be formulated to meet the Cu requirements of both species appropriately.

Based on clinical reports of Cu-associated disease conditions, it appears camelids are not significantly different from other species relative to their Cu requirement; other than a concern for sensitivity to Cu toxicity similar to sheep. Assuming no inherent differences among species, mineral requirements for beef cattle, sheep, and goats can be used to generate camelid requirements. The small ruminant NRC recommends mineral requirements defined for sheep as appropriate for llamas and alpacas. Using the requirements from beef cattle, sheep, and goats, an averaged requirement of 0/068 mg/lb (0.15 mg/kg) of body weight was derived.[15] This would calculate to a daily Cu requirement between 9 and 24 mg/day for llamas or alpacas varying in body

weight from 130 to 350 lb (59-159 kg). Assuming a dietary intake of 1.25 to 1.5% of body weight, suggested dietary Cu content should be between 9 and 12 ppm (dry matter basis). This determination is consistent with Cu requirements for other species and accounts for a slightly lower intake capacity, which increases dietary concentration slightly.

Copper Availability and Metabolism

In the more recent NRC publications, mineral requirements have been adjusted for variable availability from dietary ingredients. It has been shown that minerals within forages are not as available for absorption as from mineral sources. Compounds such as oxalates and phytates in forages can bind minerals, reducing their availability. Copper availability in fresh pasture is lower than from hay.[13] When the plant is harvested some breakdown of compounds facilitates the release of Cu, making it more available. As with many other minerals, there are many documented interactions between minerals that can alter availability. Relative to Cu, high dietary iron (Fe), zinc (Zn), and calcium (Ca) can reduce Cu availability.[13] Iron is high in soil, and soil consumed by grazing animals may contribute to the observed lower Cu availability from pasture.

Interactions affecting Cu availability have been well studied as a result of a unique situation in ruminant animals. Bacteria in the fermentation vat (rumen or camelid C-1) can combine dietary molybdenum (Mo) and sulfur (S) to produce thiomolybdate compounds. These thiomolybdates chelate or bind Cu in the fermentation vat and prevent Cu from being absorbed in the intestine. Even if absorbed, the chelated Cu is not available for use by tissues. For any ruminant animal, including llamas and alpacas, availability of dietary Cu will be significantly influenced by dietary Mo and S content. In this regard, often the Cu requirement is defined relative to dietary Mo as a Cu-to-Mo ratio. For sheep and camelids that are more sensitive to Cu, a suggested dietary Cu:Mo ratio of 6 to 10:1 is recommended. A Cu:Mo ratio of 16:1 or greater is often associated with Cu toxicity problems.[10]

Feeding Recommendations

With the requirement numbers presented, one needs to provide sufficient amounts of Cu from the diet without greatly exceeding this requirement and potentially inducing toxicity. The challenge here is remembering dietary Cu is contributed by every ingredient fed to some extent. This is where many people become confused with the daily Cu requirement on a dietary concentration basis ranging from 5 to 20 ppm. However, many feed ingredients can contain much higher Cu content, for example mineral supplements might contain between 30 and 600 ppm Cu. Does this mean these feed ingredients are toxic? Possibly, but only if they were fed as a sole feed source (not practical or realistic), or in combination with other feed ingredients with high Cu content. Each feed ingredient will contribute to the overall total dietary Cu content, but only to the proportion of the total diet the individual feed represents.

In Table 1, a number of examples are provided to demonstrate the concept of ingredient contribution to dietary Cu content. For these examples, three feed ingredients (hay, pellet, and mineral) comprise the total diet. The same amount of hay (3.25 lb (1.50)/day), pellet (0.5 lb (0.2 kg)/day), and mineral supplement (0.015 lb/day or 0.25 oz/day) are provided in each example for simplicity, and only Cu content is varied. In these examples it can be seen that hay provides the largest amount of dietary Cu, even though it has the lowest Cu content. This is a direct result of hay being the largest proportion of the total diet. Example 1 shows Cu intake (21.2 mg/day) and dietary content (12.4 ppm) are in line with estimated requirements (20.4 mg/day; 12 ppm) for the defined animal (see table legend). In example 2, the pellet Cu content is increased from 26 to 46 ppm, yet dietary Cu intake and content are not greatly increased. Some are concerned about the Cu content of the mineral supplement, yet example 3 shows the mineral Cu reduced from 300 to 30 ppm, but Cu intake is reduced only by 2 mg/day. Of greatest concern is the situation in example 4 where hay Cu content increases from 9 to 25 ppm. In this situation, daily Cu intake and dietary content is greatly increased and, depending upon dietary Mo status, could potentially lead to Cu toxicity problems. Hay Cu content typically is between 4 and 14 ppm, though much higher Cu concentrations are being observed more frequently in many regions of the US. High forage Cu content may be the result of inappropriate fertilization practices, especially if poultry or pig manure are used. Dietary Cu is very high in poultry and pig diets, which accounts for the higher manure Cu content. Another concern is the use of copper sulfate footbaths on dairy cattle farms and the spread of this material on croplands. Given these situations, it is important to know just how purchased forages are raised, or testing forages to assess Cu status is another option.

Given these dietary examples, it is imperative that all potential sources of Cu be accounted for in the diet to ensure adequate, but not excessive, Cu is consumed. As previously described, dietary Mo is an important factor to address in assessing dietary Cu status. From these examples both dietary ingredient Cu content and intake amount need to be considered. If testing feed ingredients for Cu content, one should also have Mo and S content determined. In feeding appropriately for Cu, one should first evaluate forage Cu content, then match pellet and mineral supplement accordingly. If pellet product contains more than 50 ppm Cu, then a mineral

Table 1. Contribution of individual feed ingredients (hay, pellet, mineral supplement) to total dietary copper (Cu) content. For comparison in the following examples daily Cu requirement for a 300 lb adult llama is 20.41 mg/day. Dietary Cu content can vary from 9 to 12 ppm assuming a total intake of 1.5 and 1.25% of body weight, respectively.

Example 1	**Forage**	**Pellet**	**Mineral**	**Total diet**
Intake, lb/day	3.25	0.5	0.015	3.77
Cu, ppm	9.0	26	300	12.4
Cu, mg/day	13.27	5.90	2.04	21.21
Example 2	**Higher pellet Cu content**			
Intake, lb/day	3.25	0.5	0.015	3.77
Cu, ppm	9.0	46	300	15.1
Cu, mg/day	13.27	10.43	2.04	25.74
Example 3	**Lower mineral Cu content**			
Intake, lb/day	3.25	0.5	0.015	3.77
Cu, ppm	9.0	26	30	11.34
Cu, mg/day	13.27	5.90	0.20	19.37
Example 4	**Higher forage Cu content**			
Intake, lb/day	3.25	0.5	0.015	3.77
Cu, ppm	25	26	300	26.23
Cu, mg/day	36.85	5.90	2.04	44.79

supplement with low (<100 ppm) Cu can be used. If the hay has a Cu content greater than 15 ppm, it may be necessary to feed a pellet with lower Cu content and a low Cu mineral. It must be remembered that high dietary Cu intake does not guarantee that a toxicity event will occur. Most reported toxicity cases are associated with dietary Cu content exceeding 25 to 30 ppm and a high (>16:1) Cu to Mo ratio.

Monitoring Cu Status

With concerns for disease related to either Cu deficiency or toxicity, methods to assess Cu status are of interest. Copper can be directly determined in serum, plasma, or liver samples. Serum or plasma Cu concentrations are most easily obtained and determined, though interpretation relative to dietary status is confounded. Only very low (<0.1 µg/ml) or very high (>5 µg/ml) blood Cu concentrations are diagnostic. Values within the normal reference range (0.3 to 0.8 µg/ml) could also be associated with marginally deficient or excessive dietary Cu intake. Liver Cu concentration is considered the best measure of dietary Cu status, but requires a liver biopsy to obtain a sample. If an animal dies from unknown causes, a sample of liver and kidney should be obtained for Cu concentration determination. Liver Cu concentrations below 25 ppm (dry weight basis) or above 500 ppm (dry weight basis) are suggestive of deficiency or toxicity, respectively.

Beyond Cu concentration determinations, Cu status can be assessed by measuring activities of Cu-specific enzymes. Ceruloplasmin activity in blood is a measure of Cu status, but it is influenced by infectious conditions, thus confounding their interpretation. Whole blood super oxide dismutase enzyme activity has also been associated with Cu status, though this enzyme is also influenced by zinc status. Enzyme activities are not very sensitive to dietary changes, as their activities are highly conserved by the body in the face of deficiency. They also do not reflect toxicity situations. Availability of laboratories capable of measuring these enzymes and having reference values for llamas and alpacas are limited. At this point, serum Cu concentration should be used as a screening tool to assess Cu status. This measure should be evaluated in conjunction with dietary Cu and Mo content.

Conclusions

Copper is an essential micromineral for all small ruminant species, though differences in the species Cu requirement results in differential risks for deficiency or toxicity disease. Sheep and camelids are more prone to copper accumulation from an over-supplemented diet, thus predisposing them to greater risk for toxicity problems. Goats have a much higher tolerance for Cu and have higher requirements compared to other small ruminants, and are more prone to present with deficiency

disease. A common problem in the feed industry is the lack of recognition for the difference in copper requirement between sheep and goats, with many products labeled for their use being formulated for low Cu content relative to sheep requirements. Proper Cu dietary supplementation requires that all feed ingredients be analyzed not only for their Cu content, but the content of important interfering substances to Cu availability such as iron, molybdenum, and sulfur. Ongoing monitoring of animal Cu status is a necessary component of a small ruminant health program.

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