

**COPPER DEFICIENCY IN THE PACIFIC NORTHWEST:  
FIELD EXPERIENCES, DIAGNOSIS AND CORRECTION**

Clive C. Gay  
Field Disease Investigation Unit  
Department of Veterinary Medicine & Surgery  
Washington State University  
Pullman, Washington

The clinically overt disease syndromes associated with severe deficiencies of trace elements have been recognized for many years. The diagnosis of these conditions can be made primarily on clinical or pathological grounds with laboratory analysis to confirm the diagnosis. In recent years, however, it has been recognized that trace element deficiencies can be associated with syndromes that are not clinically distinct and that manifest primarily with suboptimal production or reproduction and increased disease susceptibility. In many instances, the occurrence of these syndromes has been recognized as the result of controlled response trials conducted in deficient herds to determine the effect of the deficiency state.

Copper deficiency may occur as a simple deficiency where the concentrations of copper in the diet are markedly deficient. Copper deficiency can also occur as a conditioned deficiency where copper concentrations in the diet may be marginal to normal but absorption and utilization of the ingested copper are interfered with by other constituents of the diet.<sup>(1)</sup> Prominent amongst these are molybdenum, sulphur and iron.<sup>(2)</sup> Copper deficiency most commonly occurs as a conditioned deficiency in Washington state and this is probably true for other areas of the western United States.<sup>(3,4,5)</sup> The clinical syndromes associated with copper deficiency vary from area to area and this variation probably reflects differences in the type of deficiency and the influence of the conditioning factors, the severity and rapidity of the depletion, and differences in the time during the growth or production cycle when it is operative. Conditions which lead to copper deficiency can be widespread throughout an area or localized to certain fields.

#### **Soil Associations**

Copper exists in soil in many chemical forms, some of which are available to the plant and others not. Total copper in the soil is of little value as a primary diagnostic guide to animal disorders and the usefulness of available copper is also limited.<sup>(6,7)</sup>

Severely leached soils, sandy soils, calcareous or alkaline soils, soils with high water tables for a significant portion of the year, and peat and muck soils are soils that are commonly

associated with either primary or secondary (conditioned) copper deficiency and should be suspect.

In the Pacific Northwest the main soil associations have been with alkaline silt loams, soils with high water tables and with peat and muck soils.

Although soil testing is of limited value, knowledge of soil type does have important value. We have found that when copper deficiency has been confirmed on a given farm its occurrence on other farms can be predicted (and subsequently confirmed) by the use of soil maps. This has resulted in the detection and correction of copper deficiency problems on farms that were unaware that they had a problem.

### Plant Associations

Plant associations with copper deficiency are complex because of the interaction between copper and other conditioning substances on the availability and absorption of copper by the ruminant.<sup>(2,8)</sup> Of the conditioning factors molybdenum and sulfate are most important. These interact within the gut to form thiomolybdates which complex copper and make it unavailable for utilization.

**Copper** In general the copper content in grasses ranges from 4-9 parts per million DM.<sup>(10)</sup> Legumes generally have higher concentrations of copper than grasses under similar growing conditions. Pastures with less than 5 parts per million of copper may be associated with copper deficiency especially if there are significant levels of molybdenum or sulphur.<sup>(6)</sup> Pastures with less than 2 parts per million copper may be associated with primary copper deficiency. The uptake of copper by grasses is decreased with decreasing soil pH and tends to be higher early in the growing season. The availability of copper to the animal varies with the type of plant and the stage of maturity. Availability is highest in the mature pastures and in hay and lowest in rapidly growing young pastures.<sup>(2)</sup> Silages are intermediate.

**Molybdenum** High molybdenum intakes in the diet can induce copper deficiency even when the copper content of the pasture is quite high. Pasture molybdenum concentrations of greater than 5 parts per million are commonly associated with secondary copper deficiency. Lower concentrations (1-4 parts per million) may be associated if copper concentrations are low and there is also a concurrent intake of sulphur. Molybdenum uptake by plants is higher under high moisture conditions and on soils with high water tables for significant portions of the year.<sup>(9)</sup> Molybdenum uptake by plants increases with increasing soil pH and can be significantly influenced by subsoiling and fertilization practices such as liming. Legumes take up more molybdenum than do grasses.

**Sulphur** The sulphur content of plants is highest during the early growth stages and falls with maturity. Sulphur content of improved pastures is generally higher than that of native or unimproved pastures and copper deficiency can be induced by pasture improvement practices in copper marginal areas.<sup>(2,11)</sup> Sulphur concentrations greater than 0.2% may have a risk for copper deficiency.

#### **Copper, Molybdenum, Sulfate Interactions**

Experimental work has shown that an increment of 4 mg/kg in dietary molybdenum is sufficient to reduce the availability of dietary copper by 50%.<sup>(12)</sup> An increment of 1 g sulphur/kg diet has a similar effect on copper availability. Copper deficiency may occur as a primary deficiency when pasture copper levels are low or may occur as a secondary deficiency when molybdenum and/or sulphur levels are high. The copper to molybdenum ratio provides some indication of the potential of the pasture to induce a conditioned copper deficiency. If the Cu:Mo ratio is less than 2 there is a high probability for a conditioned copper deficiency. If it is between 2 and 5 there is a possibility of deficiency if the copper concentrations are also low or if sulfate is high. Molybdenum and sulphur intakes in surface and well water may need to be taken into consideration.

**Iron** An intake of 800 parts per million of iron in the diet has been shown to markedly reduce copper availability under experimental circumstances and diets with greater than 400 parts per million of iron may contribute to the limitation of copper availability.<sup>(2,21)</sup> Soil ingestion may account for 2-10% of the dry matter intake during certain periods of the year and can result in high iron intakes. Similarly soil contamination of silage can be associated with high iron intakes.

Reference values for pastures are given in the appendix tables.

#### **Syndromes Associated with Copper Deficiency**

Descriptions of the clinical syndrome may be found in standard texts and other articles.<sup>(13,14,15)</sup> It must be emphasized that the manifestations of copper deficiency vary between geographic areas and in association with the cause and type of deficiency. For example, reproductive inefficiency has been experimentally demonstrated in cattle with molybdenum induced copper deficiency but not with iron induced deficiency.<sup>(22)</sup>

Depressed growth rate is probably the most common manifestation of copper deficiency and occurs in both sucking and weaned calves, in lambs and kids, and in yearling cattle. In cattle the effects are most severe in cattle under one year of age

but also occur in yearlings. In severe deficiency areas in Washington state, we have measured growth depressions of 60 to 100 lbs for the grazing season. The syndrome is frequently accompanied by diarrhea in a variable proportion of the group. Diarrhea is common where high molybdenum pasture concentrations cause a conditioned copper deficiency.

Changes in the coat color of cattle are often cited as early signs of copper deficiency and certainly occur in some copper deficient cattle. In Washington state we have observed these changes in some areas but have not found them in others. The coat of Hereford cattle develops a yellow dun color and Angus cattle show grey discoloration particularly around the eyes and at the peripheral hairs of the ears. Changes in wool character can be one of the earliest signs of deficiency in sheep. The wool lacks character, develops crimps 3 to 4 times normal width and has reduced tensile strength. These characteristics are more easily seen in fine wool breeds than Down breeds.

Adult cattle may show no signs of copper deficiency except in severe deficiency states. A proportion may show diarrhea and some in the group may have lowered body condition. Abortion and infertility have been associated with copper deficiency in some areas of the world but we have not seen these associations.

In young calves there may be an enlargement of the medial and lateral aspects of the distal metacarpal and metatarsal physal regions associated with abnormal bone development at the distal growth plates. The presenting history in some copper deficient cattle herds has been an increased fracture rate particularly involving the scapula, humerus and femur of yearling cattle. Young lambs with copper deficiency are frequently osteoporotic.

Anemia has not been a feature of copper deficient cattle in our area although it is recorded as a feature in other area studies. Anemia is common in copper deficient sheep.

Enzootic ataxia (Swayback) may be congenital or may first appear at one or two months of age. Congenitally affected lambs are born dead or may be born weak and unable to rise. Lambs that are ambulatory or that develop the syndrome at a later age show incoordination, especially of the hind limbs. Lambs may become recumbent but there is no true paralysis. Swayback-like disease is recorded in calves in North America but is rare. It can be a common manifestation in goats.

Increased disease susceptibility may be a feature of copper deficiency. There is a large developing body of literature to show that copper deficiency can impair innate, humoral and cellular immune functions. The majority of these studies involve laboratory animals under very controlled dietary regimens and are demonstrated using in-vitro measures. It is not known to what extent these studies can be extrapolated to agricultural animals under farm conditions. However, neutrophils and pulmonary macrophages from

copper deficient cattle and sheep have been shown to have impairment of microbicidal activity.<sup>(16,17,23)</sup> This impairment occurs relatively early in the development of copper deficiency. Copper deficient sheep have been shown to have increased mortality and higher risk of death from infections with Escherichia coli and Pasteurella hemolytica than copper normal sheep in the same flock.<sup>24)</sup> Our own field studies and reports from other areas suggest a higher disease prevalence in copper deficient animals but the full importance of copper deficiency to increased disease susceptibility remains to be determined.

### Diagnosis of Copper Deficiency

The diagnosis of copper deficiency and the establishment of the copper status of the herd must be made by animal tissue analysis. Pasture analysis and knowledge of factors that may affect subsequent copper intake and availability are of value in predicting the possible course of this status.

The liver is the main store of copper in the body. Under conditions of copper deficiency, liver concentration of copper will start to decrease before blood concentrations fall. When liver copper concentrations fall to a level of approximately 40 parts per million, blood concentrations will start to fall. Cattle can tolerate a period of hypocupremia but if the deficiency persists then clinical hypocuprosis will occur.

### Blood Analysis

Blood is easy to collect and blood analysis is the cheapest in total cost. Blood concentrations only fall after there has been significant depletion of liver reserves.

The number of samples that are required to obtain an estimate of the herd mean will vary according to the stage of the deficiency. Where there is overt clinical deficiency blood concentrations of individuals within the group will all have fallen to extremely low values with little individual variation and a small sample size will provide a valid estimation of the mean. Where there is a developing deficiency there can be considerable variation in blood copper concentration between individuals and a larger sample size will be required. This sampling should also be coupled with pasture sampling so that the future trend in animal copper status can be examined. In our studies we have found that 7 samples taken from each group of animals under consideration will allow a satisfactory determination of the mean in most investigations. This number may not apply in other geographic areas with more slowly developing periods of copper depletion. The samples should be taken at random and a single sample taken from an obviously sick animal can be misleading. Blood copper can be elevated by inflammatory conditions and liver disease which is

one reason for random sampling rather than sampling obviously sick animals. The sample must be taken without external contamination and vacutainer tubes are best for this purpose.

Serum copper concentrations will be lower than plasma copper concentrations because some of the copper containing ceruloplasmin is trapped in the clot. Nevertheless, serum can be used and is the most convenient in terms of transport to diagnostic labs. If serum is decanted in your own laboratory care should be taken to avoid contamination. The reference values in serum are given in Table 1. If you wish to convert to plasma copper concentrations the relationship is:  $\text{plasma copper} = 1.2 * \text{serum copper} - 0.032$ .<sup>(18)</sup>

Ceruloplasmin can be used to determine copper status as there is a direct relationship between blood copper concentrations and ceruloplasmin ferro oxidase).<sup>(20)</sup> Ceruloplasmin contains 70-90% of the plasma copper. Reference values are given in Table 2.

Hair analysis poses problems because of the potential for contamination. Also hair growth takes place over several months. A value of less than 5 mg copper/kg dry matter in a clean recently grown hair sample may indicate an increased risk of hypocuprosis. Our experience has found hair analysis of limited value as a test for copper deficiency.

### Liver Copper

The liver copper concentration is the best indicator of copper adequacy as it provides a direct measure of the main copper storage area of the body. The disadvantage of this method of assessment is that it requires surgical biopsy and is more easily subject to contamination. Although of value in experimental studies, liver biopsy does not appear to be a practical field procedure under range conditions. In sheep it may be accompanied by a mortality of 1-2% and an increased risk of black disease in unvaccinated sheep. Furthermore, not all biopsies give a useful sample. A further disadvantage is the within group variation in liver copper concentrations which may require a large sample size for a valid estimation of the group mean.

Liver samples have proved of value in monitoring situations where the owner is requested to collect a liver sample from cattle or sheep that have died from any cause. These are stored in the freezer until collected for analysis. Reference values for liver copper are given in Table 3.

### **Copper Enzymes**

Copper is a component of several enzyme systems and the measurement of these could provide a more accurate measure of rate limiting factors associated with copper deficiency, i.e. growth rate, than current assays of blood or liver copper concentrations. The limitation of blood copper analyses is that animals need to be hypocupremic for several weeks before clinical signs of copper deficiency become apparent. The measurement of an actual functional copper enzyme might provide a better indication of the impending or clinical significance of the deficiency and this area is the subject of much research at present. However, none of these enzyme analyses currently have accepted values for this purpose nor are these analyses routinely available at diagnostic laboratories.

There is evidence from experimental animal studies that blood copper values may be within the normal range in cattle which have clinical copper deficiency in association with high molybdenum intakes. We have not found this to be true in copper deficient cattle in high molybdenum levels in the Pacific Northwest.

### **Feed Analysis for Diagnosis**

In our experience, pasture sampling, by itself, cannot be used to diagnose the occurrence of copper deficiency in cattle. Pasture analysis can be used to determine the nature of the copper deficiency, for example, whether it is a primary deficiency or secondary due to high molybdenum and/or sulfate intakes. This information is of considerable value in determining the level and frequency of corrective copper supplementation or treatment that is required to alleviate the syndrome. Composite grass and legume samples should be taken from several areas in the field.

Pasture analysis is also of great value in predicting the probable trend in herd blood copper concentration when samples are taken at the same time. Pasture analysis should cover copper, molybdenum, iron and sulfur and the relative concentrations of each are as important as the individual values. Guidelines for interpretation are in the appendix.

### **Response Trials**

In general there needs to be a prolonged period of copper depletion before there are pathological effects and seasonal hypocupremia can occur without production or economic significance. Similarly, a low herd blood copper concentration, by itself, is not necessarily predictive of a production or clinical deficiency problem. The ultimate determination of whether the presence of hypocupremia has clinical or production significance rests with a treatment response trial. In such a trial a treatment group of animals and a nontreated group (control group) are established and the response to treatment or difference between the groups is

measured according to the production criteria that are being examined. The length of the trial will vary according to the production parameter that is of interest. In the case of illthrift or poor growth rate a significant difference may be observed within a trial lasting 1 or 2 months. In this case it would involve weighing the animals, dividing them into 2 equal groups based on weight and sex, treating one of the groups and taking subsequent weights to determine the response.

Treatment response trials should also include sampling to determine that the level and/or frequency of treatment or supplementation has been sufficient to correct the problem. This is very important. For example in the Pacific Northwest we have deficiency problems that in certain areas require a copper injection once every 6 months and in other areas require an injection every 4 to 6 weeks. Similarly, levels of copper inclusion in the salt/mineral mix that are required to alleviate the problem can vary from 0.2% to 0.6% copper. Treatment response trials and continual monitoring give this information.

Treatment response trials are the only real method of determining whether the presence of copper deficiency has clinical or economic significance. However, for any one area or for any given farm it is most unlikely that this type of trial will be conducted by a university or research institution. These trials are the province of the practitioner in cooperation with the producer client and nutritionist using the county agent or local university for consultative advice.

### **Correction of Copper Deficiency**

Copper deficiency can be corrected by the administration of copper by injection, by incorporation of copper supplements in the salt/mineral mix, by the oral administration of copper oxide "needles" or by the addition of copper to the total ration. Extreme caution must be exercised with any copper supplementation program in sheep.

Copper glycinate may be given by injection. Four hundred mg of copper glycinate is equivalent to 120 mg of copper. It is given by subcutaneous injection. A significant proportion of the dose is sequestered at the site of injection due to the local reaction and is released over a period of time to the body. This sequestration is of advantage as it markedly reduces the possibilities of copper toxicity; it has a disadvantage in that local reactions are common and occasionally necrosis and abscessation occur. The duration of control achieved by a single injection varies substantially depending upon the nature of the deficiency. In simple deficiency situations a single injection may provide protection for up to six months. In severe molybdenum conditioned copper deficiency repeat injections may need to be given every 4-6 weeks. The frequency of treatment required can only be determined by a treatment response trial with continual monitoring. In general, the results obtained

on one soil type are applicable to herds on the equivalent soil type elsewhere.

Copper supplementation may also be achieved by the addition of copper, (usually copper sulfate or copper oxide), to the salt/mineral mix. Most trace mineralized salts contain only 0.02% copper which is far too little to be of nutritional consequence in conditioned deficiencies. Copper sulfate, for example, may need to be added to a level of 2% or greater depending upon the severity of the deficiency and the severity of the conditioning influences. The response of cattle to copper supplementation in the salt/mineral mix is variable within a group due to variation in salt intake. The level required for any one particular circumstance will need to be determined by response trial.

Copper glycinate will on occasion produce mortality when injected into sheep and should be used with extreme caution. Copper supplementation of salt/mineral mixtures in sheep should only be used where there is a confirmed copper deficiency. The lower level of inclusions should be used and should be monitored.

Adult cattle are relatively resistant to copper toxicity and overseas studies suggest that copper can be compounded into the total ration of cattle at a level of 25-35 mg/kg with comparative safety.

Copper is transferred to the fetus in utero and good fetal reserves of copper can be built up by treatment of the dam during pregnancy. This is more true for the calf than the lamb. In most situations the reserves will be sufficient to sustain the calf until the time of significant pasture intake at which time interfering substances in the pasture may rapidly induce a copper deficiency and direct supplementation to the calf will be necessary.

Because of the managemental problems associated with repeat injections in pastured cattle and calves and the variable intake problems associated with salt, there can be a considerable advantage to the use of a sustained release preparation, such as copper oxide "needles", that can be given orally. Field trials in Washington State have established that copper oxide "needles" administered via a bolus can provide sustained protection against the deficiency. The needles stick to the mucus on the wall of the rumen and abomasum and slowly release copper over a number of months. A single bolus of 25 grams given at the beginning of the grazing season provided protection against the deficiency throughout the season in trials in two separate deficiency areas of the state. Growth rates of treated calves were equivalent or superior to calves treated with copper glycinate or copper added to the salt.

**Table 1. Serum Copper Concentrations**

Calves and Yearlings-Suggested Threshold Levels

Serum Copper mg/l ppm)

>1.2	Copper toxicity; inflammatory disease, liver disease
0.7-1.1	Normal
0.4-0.7	Low normal. Frequently called marginal. We have observed no effects or response to supplementation at these levels in beef cattle.
0.2-0.4	Deficient. A growth response to supplementation is likely if these levels are sustained for any length of time.
<0.2	Clinical signs of overt clinical copper deficiency usually present.

**Table 2. Reference Values in Plasma Ceruloplasmin**

10-20 mg/dl	Normal
5-10 mg/dl	Deficient
<5 mg/dl	Severely deficient

Serum ceruloplasmin concentrations will be less than plasma. Reference values may vary slightly from laboratory to laboratory. Follow your laboratory's stated values.

**Table 3. Reference Values liver copper**

- normal liver copper concentrations often 60-120 ppm
- liver copper concentrations of greater than 6 ppm mg Cu/kg) wet weight basis are unlikely to be associated with a responsive situation
- liver copper concentrations of less than 6 ppm usually associated with a responsive situation (<3 ppm - severely deficient)

**Table 4. Reference Values for Copper in Pastures**

**Copper**

- >15 ppm Potential copper toxicity - sheep
- 5-15 ppm Normal providing molybdenum and sulfate in acceptable range
- 2-5 ppm Possibility of copper deficiency if molybdenum or sulfate concentration are marginally high
- <2 ppm Probable primary copper deficiency

**Sulfur**

Greater than 0.2% will cause interference with copper absorption with marginal copper intakes.

**Copper:Molybdenum Ratios**

These are probably of more value in determining the copper deficiency potential of a pasture than are copper concentrations except in situations of severe copper deficiency with pasture values of copper (<2 ppm).

Canadian studies show that Cu:Mo of less than 2 often associated with clinical or responsive copper deficiency.

British studies suggest that Cu:Mo ratios should be greater than 5 to avoid problems.

Our studies support the above and suggest that ratios less than 5 are suspect problem areas with a variable response on treatment response trials. Pastures with ratios of less than 2 have in general been problem pastures.

**Iron**

Experimental and field data suggest that 500 ppm or greater of iron in the diet can be associated with impaired copper absorption.

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