Influence of form of supplementary copper and zinc on mineral status and performance of beef heifers during and after mineral antagonism
by John Denver Bailey

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University
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Abstract:
Experiment 1 evaluated the effects of supplementary mineral form on performance and hepatic trace mineral status in beef heifers consuming antagonists. Experiment 2 provided additional information on the effects of supplementary mineral form on performance and hepatic trace mineral status in beef heifers consuming antagonists and reared in drylot conditions. In each experiment, thirty yearling, Angus x Hereford heifers were randomly assigned to 1 of 5 treatments that were 1) basal supplement with no additional Cu or Zn (CON), 2) 25 ppm Cu and 50 ppm Zn as 50% organic complex, 50% sulfate form (2-Way), 3) same levels of Cu and Zn but as 50% organic complex, 25% sulfate and 25% oxide (3-Way), 4) same levels of Cu and Zn all in sulfate form (LoSulf), or 5) 50 ppm Cu and 100 ppm Zn in sulfate form (HiSulf). All heifers were individually fed daily the Cu antagonists Mo (10 ppm), S (3,000 ppm) and Fe (500 ppm). In Experiments 1 and 2, CON heifers had less (P < .05) hepatic Cu when compared to supplemented heifers from d 25 through d 100. In Experiment 1, HiSulf heifers had greater (P < .05) hepatic Cu from d 50 through d 100 when compared to 2-Way, 3-Way and LoSulf heifers. By d 100, hepatic accumulation of Mo was similar (P > .10) for CON and HiSulf heifers. In both Experiments, 3-Way heifers had accumulated less (P < .05) hepatic Mo than all other treatments by d 100. In Experiment 2, Mo accumulation was greater (P < .05) over time for CON heifers than for supplemented heifers. In Experiment 3, sixty Angus x Hereford heifers were used to evaluate the effects of supplementary mineral form on performance and hepatic trace mineral status in beef heifers previously consuming antagonists. For 77 d heifers were individually fed on an alternate day basis treatment supplements that were 1) CON 2) 2-Way, or 3) AllSulf (same as LoSulf in Experiments 1 and 2). From d 50 through d 75, heifers consuming the CON treatment accumulated less (P < .05) hepatic Cu than supplemented heifers.

At d 75, 2-Way heifers had less (P < .05) hepatic Mo than CON heifers and tended (P = .08) to have less hepatic Mo than AllSulf heifers. The data from Experiments 1 and 2 suggest forms of supplementary trace minerals interact differently in the presence of dietary antagonists and it appears a combination of inorganic and organic Cu and Zn including complex, sulfate and oxide forms may be used strategically to limit hepatic accumulation of Mo while conserving hepatic Cu. Results from Experiment 3 suggest supplementing trace minerals to incoming feedlot cattle previously consuming dietary mineral antagonists replenishes lost mineral stores more effectively than no supplementation. A combination of complex and sulfate Cu and Zn may decrease hepatic residence time of Mo in beef cattle.

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DURING AND AFTER MINERAL ANTAGONISM

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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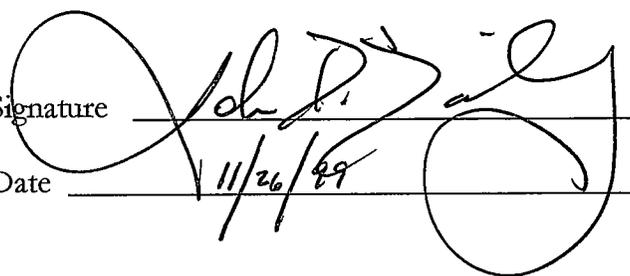
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I came to Montana an empty vessel...just a shell of man...my beautiful wife, Jana, filled up the inside and now I overflow. Thank you, my love, for giving me back something that was already mine but somehow lost...my heart.

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ABSTRACT

Experiment 1 evaluated the effects of supplementary mineral form on performance and hepatic trace mineral status in beef heifers consuming antagonists. Experiment 2 provided additional information on the effects of supplementary mineral form on performance and hepatic trace mineral status in beef heifers consuming antagonists and reared in drylot conditions. In each experiment, thirty yearling, Angus x Hereford heifers were randomly assigned to 1 of 5 treatments that were 1) basal supplement with no additional Cu or Zn (CON), 2) 25 ppm Cu and 50 ppm Zn as 50% organic complex, 50% sulfate form (2-Way), 3) same levels of Cu and Zn but as 50% organic complex, 25% sulfate and 25% oxide (3-Way), 4) same levels of Cu and Zn all in sulfate form (LoSulf), or 5) 50 ppm Cu and 100 ppm Zn in sulfate form (HiSulf). All heifers were individually fed daily the Cu antagonists Mo (10 ppm), S (3,000 ppm) and Fe (500 ppm). In Experiments 1 and 2, CON heifers had less ($P < .05$) hepatic Cu when compared to supplemented heifers from d 25 through d 100. In Experiment 1, HiSulf heifers had greater ($P < .05$) hepatic Cu from d 50 through d 100 when compared to 2-Way, 3-Way and LoSulf heifers. By d 100, hepatic accumulation of Mo was similar ($P > .10$) for CON and HiSulf heifers. In both Experiments, 3-Way heifers had accumulated less ($P < .05$) hepatic Mo than all other treatments by d 100. In Experiment 2, Mo accumulation was greater ($P < .05$) over time for CON heifers than for supplemented heifers. In Experiment 3, sixty Angus x Hereford heifers were used to evaluate the effects of supplementary mineral form on performance and hepatic trace mineral status in beef heifers previously consuming antagonists. For 77 d heifers were individually fed on an alternate day basis treatment supplements that were 1) CON 2) 2-Way, or 3) AllSulf (same as LoSulf in Experiments 1 and 2). From d 50 through d 75, heifers consuming the CON treatment accumulated less ($P < .05$) hepatic Cu than supplemented heifers. At d 75, 2-Way heifers had less ($P < .05$) hepatic Mo than CON heifers and tended ($P = .08$) to have less hepatic Mo than AllSulf heifers. The data from Experiments 1 and 2 suggest forms of supplementary trace minerals interact differently in the presence of dietary antagonists and it appears a combination of inorganic and organic Cu and Zn including complex, sulfate and oxide forms may be used strategically to limit hepatic accumulation of Mo while conserving hepatic Cu. Results from Experiment 3 suggest supplementing trace minerals to incoming feedlot cattle previously consuming dietary mineral antagonists replenishes lost mineral stores more effectively than no supplementation. A combination of complex and sulfate Cu and Zn may decrease hepatic residence time of Mo in beef cattle.

CHAPTER 1

INTRODUCTION

The beef cattle industry has a multitude of areas where improper trace mineral nutrition may diminish optimal production and decrease profitability. This production cycle is continually challenged by the necessity of profitability for all involved. In these experiments, our interest was to examine the influence of trace mineral supplementation on important areas of beef cattle production, specifically, growth, immunity, and trace mineral status.

Copper (Cu) and zinc (Zn) are trace minerals that are intimately involved with growth, both cell-mediated and humoral immunity, and reproductive success of livestock. A deficiency of Cu and(or) Zn at the cellular level will lead to suboptimal functioning of these systems that utilize Cu and Zn as enzymatic cofactors. Trace mineral deficiencies can occur as a consequence of inadequate mineral intake (primary deficiency) or as a result of poor absorption through post-ingestive influences (secondary deficiency; Graham, 1991). Primary deficiencies occur due to dietary mineral content and form, plant species and maturity, soil dynamics, and climate (Ammerman and Goodrich, 1983). Secondary deficiencies are most likely incurred due to pre-existing disease or trace mineral antagonism that negatively affects absorption, retention or metabolism of the trace elements (Graham, 1991).

Biological availability (bioavailability) of trace elements is an interactive process involving extrinsic and intrinsic factors of both the animal and the nutritional environment. The bioavailability of a nutrient is defined as the amount of the ingested nutrient that is absorbed, transported to an action site, and converted to an active form (O'Dell, 1984) sufficient enough to invoke its biological role(s). Copper bioavailability is affected by dietary levels of molybdenum (Mo), sulphur (S) and(or) iron (Fe). Sulfur and Mo have been shown to interact with Cu in the rumen and form insoluble Cu-thiomolybdates (Suttle, 1991). Unbound oxythiomolybdates, if absorbed, may result in further systemic, insoluble copper complexes (Howell and Kumaratilake, 1990; Suttle, 1991).

Some studies suggest organic sources of Cu and Zn are more bioavailable compared to their inorganic counterparts (Kincaid, et al., 1986; Manspeaker, 1987). Ward et al., (1996) reported blood plasma Cu was maintained more effectively in cattle fed Cu-proteinate and high dietary Mo. Feeding either Zn lysine or Zn methionine resulted in equal or greater availability than feeding Zn sulfate to sheep (Rojas et al., 1995). Our studies were designed to evaluate the influence of Cu depletion and subsequent repletion on growth performance, cell-mediated immune response and hepatic trace mineral status of yearling beef heifers fed either all inorganic or a combination of inorganic and organic Cu and Zn.

CHAPTER 2

LITERATURE REVIEW

Metabolic Functions of Copper

Copper is a metal element required for proper functioning of a variety of metabolic and physiological processes. All classes of farm animals require Cu and diminished intake or bioavailability of this element can result in detrimental clinical and pathological maladies. Early scientific investigations revealed disorders related to hypocuprosis such as enzootic ataxia (swayback) of lambs, bovine falling disease, decreased pathogen resistance and aortic rupture in rabbits, cattle, swine, guinea pigs, and chickens (McDowell, 1992a). Other ailments such as achromotricia of hair and wool, reduced growth rates, anemia, severe diarrhea, and bone disorders in all classes of livestock have been observed and successfully treated with Cu supplementation (Baker and Ammerman, 1995a).

The primary role of Cu at the cellular level is as an enzymatic cofactor, activator and constituent. Cellular respiration, bone formation, proper cardiac function, connective tissue keratinization, spinal cord myelination, and tissue pigmentation are all dependent upon proper enzymatic integrity and on adequate Cu status of these enzyme systems (McDowell, 1992a). Examples of Cu metalloenzymes include ceruloplasmin, needed for

proper Fe incorporation into hemoglobin; cytochrome c oxidase, the terminal electron acceptor in the electron transport chain; lysyl oxidase for formation of collagen and elastin; superoxide dismutase as an oxygen free radical scavenger; dopamine-β-hydroxylase used to insure neurotransmitter integrity in the central nervous system and tyrosinase used in the conversion of tyrosine to melanin in the pigmentation process (Corah and Ives, 1991). Thus, the major problems associated with a deficiency of Cu to ruminant animals are these decreases in enzymatic activity, resulting in less than optimal productivity and metabolic health.

Metabolic Functions of Zinc

Zinc, like Cu, is an element that plays integral roles in proper functioning of many enzymatically controlled metabolic and physiological processes. Zinc is involved with carbohydrate and energy metabolism, protein synthesis, nucleic acid metabolism, hormone biosynthesis, and function, immunocompetence and Vitamin A transport (Corah and Ives, 1991). Early studies revealed dietary Zn helped treat and alleviate porcine parakaratosis, a condition that commonly occurred in commercial swine diets high in Ca (Tucker and Salmon, 1955). Other ailments such as footrot, reduced growth rates, delayed sexual development, dermatitis, bone and cartilage disorders and failed reproductivity in both males and females have been noted during Zn deficiency in all animals (McDowell, 1992b).

Zinc has its primary function at the cellular level where it is an enzymatic cofactor, activator and constituent, similar to Cu. However, Zn is involved with a wider array and

greater number of enzymatic processes; this may explain its' universal requirement by all organisms. As a structural component of biomolecules, Zn provides stability for quaternary structures of enzymes and is central to RNA, DNA, and ribosome synthesis (McDowell, 1992b). Cellular replication, skeletal formation, proper immune function, connective tissue keratinization and sexual development are all dependent upon proper enzymatic integrity within these processes and on adequate Zn status of the animal (Baker and Ammerman, 1995b). Examples of Zn metalloenzymes include alkaline phosphatase; liver, retinal and testicular alcohol dehydrogenase; fetal and connective tissue thymidine kinase; pancreatic carboxypeptidase A; liver nuclear DNA-dependant RNA-polymerase (McDowell, 1992b); glutamic, lactic, and malic dehydrogenase (Cousins, 1985). It has even been suggested that Zn plays an exceptionally important role in gluconeogenesis, as it is a potent allosteric inhibitor of fructose-1,6 bisphosphatase (Pedrosa et al., 1975).

Zinc plays a biological role in several hormonal systems, from biosynthetic processes to end-organ responsiveness (McDowell, 1992b). The most notable decreases in hormone activity associated with Zn deficiency are with testosterone, insulin and the glucocorticoids. Inadequate Zn status has resulted in impaired maturation of spermatozoa and decreased testosterone levels *in vivo* (Apgar, 1985; Puls, 1994). Zinc is intimately related to pancreatic concentration of insulin. When rats were fed Zn deficient diets, plasma insulin and pancreatic release of insulin were significantly depressed (McDowell, 1992b). Adrenocorticotropic hormone (ACTH) function is apparently dependent on Zn. Flynn et al. (1972) showed that even with ACTH

administration, corticosteroid synthesis was defunct in animals maintained on a Zn free

diet. Zinc has been supplemented in excess of requirement especially if animals are

weak, stressed or immunodeficient because of its therapeutic properties. Though Zn has

a large window of safety, Zn can interact with other trace elements and reduce their

bioavailability to animals. Many researchers are diligently attempting to ascertain ways to

feed trace elements strategically, maintaining their beneficial properties while reducing

animal excretion of these elements and trace mineral antagonism.

Beef Cattle and Their Requirement for Copper and Zinc

Copper and Zn metabolism and supplementation of growing and finishing beef cattle

continue to be active areas of research and interest, as trace elements are so widely

involved with factors such as growth, immunity, and reproduction. The mineral

requirements for beef cattle set by either the National Research Council (NRC) or

Agricultural Research Council (ARC) represent recommendations for minimum dietary

levels for disease free animals, serving low production roles (Graham, 1991). National

Research Council recommendations (Table 1) do not reflect requirement shifts due to

increased nutrient demand (i.e. growth, pregnancy, sickness etc.). Furthermore, trace

minerals are known to participate in a number of interactions that may change the

relative bioavailability of the trace elements involved. Ruminant nutritionists are

challenged by balancing mineral requirements according to kind and level of production

in accordance with forage levels and sources of both trace minerals and antagonists. At

times, forages may supply all essential minerals to beef cattle (Herd, 1994; Spears, 1994).

However, if there are inadequate mineral levels in the forage or if there is a propensity for trace mineral antagonism, a mineral supplement must be administered in order to maintain trace element homeostasis. In contrast, arbitrarily feeding excessive amounts of trace minerals is not justified because of the possibility of both antagonism, toxicosis or environmental contamination (Greene, 1995). Though published recommendations may be appropriate in some situations, proper dietary and animal assessment must be achieved before deducing trace element deficiency or adequacy (Graham, 1991).

Table 1. Current U.S. Government recommendations for dietary Copper and Zinc for beef cattle (adapted from Graham, 1991).

	NATIONAL RESEARCH COUNCIL	AGRICULTURAL RESEARCH COUNCIL
Copper	4-10 mg/kg DM	---
Growth	---	8-15 mg/kg DM
Pregnancy	---	13-20 mg/kg DM
Lactation	---	8-14 mg/kg DM
Zinc	20-40 mg/kg DM	---
Growth	---	26-35 kg/mg DM
Pregnancy	---	13-21 mg/kg DM
Lactation	---	18-31 mg/kg DM

Some of the most difficult nutritional demands to meet are the trace mineral requirements of beef cattle. Although severe trace mineral deficiencies are quite uncommon, marginal (sub-clinical) deficiencies occur quite frequently and are of significant importance. Recently, Herd (1997) demonstrated the growing concern that sub-clinical trace element deficiencies are limiting production to a greater extent than previously recognized. Identification of animals with a sub-clinical trace mineral deficiency is very difficult because they may not manifest specific clinical symptoms. In addition, these animals may have reduced metalloenzyme activity, fertility, feed efficiency

and sub-normal immunity (Wikse, 1992). Although beef cattle encountering sub-clinical deficiencies continue to grow and reproduce, they do so sub-optimally, lending themselves to the lower end of productivity and profitability for the producer. A review of factors to consider when diagnosing the trace mineral status of beef cattle is offered by Paterson et al. (1999). The mineral status of cattle is not always easily assessed. As Paterson et al. (1999) pointed out, assessing Cu status through serum can be erroneous and liver tissue seems to be the best indicator of Cu status over time. In our experiments, we used the liver as the indicator of Cu, Zn and Mo status of beef cattle.

Copper Antagonism in Ruminants

If a nutrient is somehow limited in the diet and another nutrient is antagonistic to the former, the bioavailability of the limiting nutrient is greatly decreased (O'Dell, 1989). Such is the case in many different metal ion interactions. This is especially important to ruminants as they typically consume diets high in charged micronutrients (i.e. ionic particles from soil, fibrous feed, etc.). Metal ion interactions may be synergistic or antagonistic, depending on the subsequent effect(s) on the bioavailability of the nutrient(s) (O'Dell, 1989). For example, excessive dietary Zn has been shown to be detrimental to the Cu status of rats used as a model of Zn/Cu metabolism (Oestreicher and Cousins, 1985) and in growing heifers (Wellington, et al., 1998). Synergistically, adequate Cu is crucial for Fe absorption and metabolism (O'Dell, 1989; McDowell, 1992a) as evidenced by hypocupraemic anemia. To further complicate the association between synergism and antagonism, studies done by Humphries et al (1983) support the

hypothesis that excessive dietary Fe may exert an independent antagonism toward Cu, the result being compromised soluble Cu. It is very clear that trace elements interact in a variety of ways which further supports the idea that capricious, one-element supplementation significantly above requirement is not justified in diets for beef cattle.

Thiomolybdate Speciation

One of the most hypothesized and studied ionic interactions involving trace mineral nutrition is that of thiomolybdate formation and the apparent negative impact on the Cu status of ruminants. Copper thiomolybdates have been particularly important to our understanding of not only trace mineral antagonism but also ruminant metabolism of dietary Cu. Thiomolybdates are a series of complexes that form during progressive substitution for S and oxygen (O) in the molybdate (MoO_4^{-2}) anion when hydrogen sulfide (H_2S) and MoO_4^{-2} interact *in vitro* at neutral pH (Aymonino et al., 1969). It has been suggested thiomolybdates form in the rumen (where H_2S is quite plentiful or readily formable) when dietary Mo and S are exceedingly high (Suttle, 1974; Dick, et al., 1975; Mason, 1978). Corroboration of these hypotheses has been challenging to quantify due to interference from other compounds and low concentrations of Mo in feed which is frequently the case (Allen and Gawthorne, 1987). However, when molybdate concentrations increase to even moderately high levels, absorption spectra typical of thiomolybdates have been found in washed suspensions of rumen micro-organisms (Dick et al., 1975; El Gallad et al., 1983) and in whole rumen contents (Dick et al., 1975). These compounds have been implemented for their part in inducing hypocuprosis by forming insoluble Cu complexes in the digestive tract, bloodstream, and tissues (Dick, et

al., 1975; Suttle and Field, 1983; Howell and Kumaratilake, 1990) of several species of animals.

Suttle and Field (1983) indicated there are criticisms of the "thiomolybdate hypothesis." Some *in vitro* studies suggest that di- and tri- thiomolybdates (MoO_2S_2 and $MoOS_3$) rather than tetra-thiomolybdates (MoS_4) will predominate in the rumen (Clarke and Laurie, 1980); the tetra-thiomolybdate specie is the only one that impairs Cu absorption in experimental rats (Mills et al., 1981; Bremner et al., 1982). More recent evidence, however, have implicated di- and tri- thiomolybdate speciation in post-absorptive, endogenous Cu binding in sheep (Kelleher et al., 1983) and cattle (Hynes et al., 1985). One aspect of Cu metabolism in ruminants that has received additional attention is the apparent involvement of the solid phase of rumen digesta in limiting the bioavailability of Cu. Allen and Gawthorne (1987) found evidence that the solid phase of rumen digesta (that portion of digesta including micro-organisms and plant material) has implications in the facultative formation of tetra-thiomolybdates and molybdeno-proteins, both of which are strong chelators of cuprous ions. Tri-thiomolybdates and tetra-thiomolybdates were predominant in ruminal, duodenal, and illeal digesta and smaller amounts of di-thiomolybdates and tri-thiomolybdates were found in the liquid phase of duodenal digesta (Price et al., 1987). These researchers concluded that tetra-thiomolybdate species reduce Cu absorption while di- and tri-thiomolybdates may be the primary culprits in post-absorptive, insoluble Cu complexes. There is further evidence that the structure of tetra-thiomolybdate (Figure 1) is such that obligatory binding with ionic Cu in the gastrointestinal tract is most probable (Nicholson et al., 1983). Although

the fate of extraintestinal and extrahepatic binding of Cu by oxythiomolybdates is vague, most evidence clearly suggests that the occurrence of thiomolybdate speciation reduces Cu absorption and exhibits independent effects on endogenous Cu bioavailability. Research suggests ruminal tetra-thiomolybdates account for the majority of insoluble Cu complexes in the rumen and gastrointestinal tract, while absorbed species of di- and tri-thiomolybdates account for a good portion of the systemic insoluble Cu complexes. These systemic interactions could be the causal part of symptoms associated with secondary hypocuprosis or hypocupraemia in ruminants (Suttle, 1988) which would include decreased growth rate, fertility anomalies and immunodepression, all of which have direct influences on profitability in livestock production.

Figure 1. The chrystallographic structure of the assembly of complexed copper (I) ions about a tetrathiomolybdate (VI) moiety (adapted from Nicholson et al., 1983).
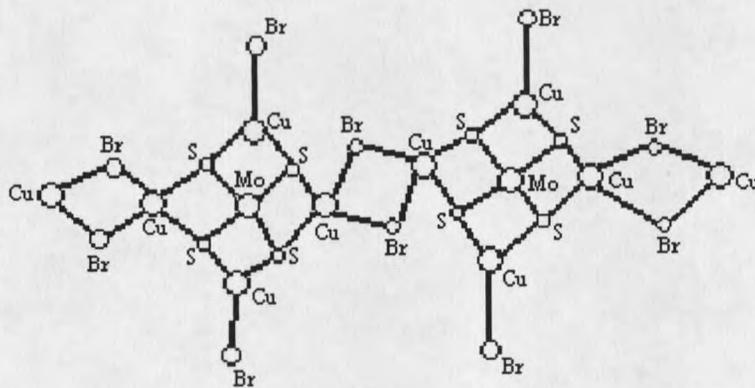
Additional Copper Antagonists

Other components may contribute to alteration of the metabolism, retention or requirement of Cu in beef cattle. Other trace metal ion interactions include dietary protein source and solubility, and even breed may affect the bioavailability or

requirement for Cu. Although these factors may play smaller independent roles than thiomolybdate speciation, in facultative situations, their influence on intensifying conditional trace mineral deficiencies are clear.

Metal Ion Interactions. As previously mentioned, excessive dietary Zn is antagonistic to Cu and leads to hypocupraemic anemia that can be alleviated by Cu supplementation. Smith and Larson (1946) first demonstrated Zn antagonism of Cu and more recent research support this theory in rats (Oestreicher and Cousins, 1985; Du et al., 1996) sheep (Saylor and Leach, 1980) and in beef cattle (Puls, 1994; Wellington et al., 1998). Baker and Ammerman (1995a) indicated there was apparent compensatory Cu accumulation in the absence of dietary Zn in some tissues. However, Zn antagonism on Cu is more frequent because Cu status is more sensitive to overabundance of dietary Zn; much more so than the reverse interaction (Baker and Ammerman, 1995a). Furthermore, in diets commonly consumed by beef cattle, forage levels of Zn tend to be naturally higher than forage levels of Cu, though forage levels tend to be marginal in unison.

The mechanism behind the Cu/Zn mutual interaction presumably has its basis at the intestinal level, through the absorption process. It has not been completely elucidated as to whether or not primary absorption of Cu and Zn is as ionic passage or as ligand-bound absorption. Using the vascularly perfused intestine of the rat, Oestreicher and Cousins (1985) found that high luminal Zn concentration decreased mucosal cell cytosolic Cu as well as Cu transferred to portal effluent. It has been proposed that the mediators for Cu and Zn absorption across the brush border of the small intestine are

one or more absorbable ligands (Cousins, 1985) and most likely a proteinous, metallophyllic biomolecule. Most attention has been given to the metal binding protein metallothionein. Metallothionein synthesis in the sub-mucosa of the small intestine is apparently mediated and enhanced by increased dietary Zn (Oestreicher and Cousins, 1985; Baker and Ammerman, 1995b). Metallothionein has a greater affinity for Cu than it has for Zn; when rats were fed high levels of Zn, dietary Cu replaced metallothionein-bound Zn (Hall et al., 1979; Fischer et al., 1983). The Cu bound to metallothionein was rendered essentially unavailable to these animals, as it was sloughed off with the mucosal cell layer and excreted.

There have been numerous reports of benefits associated with supplementing Zn in excess of requirements. Excessive dietary Zn (1,142 ppm) from zinc sulfate was added to the diet of feedlot steers and it reduced ruminal degradation of dietary protein, thus increasing abomasal protein passage (Froetschel et al., 1990). Cecava et al. (1993) treated soybean meal with Zn and found similar decreases in ruminal protein degradation. Certain proteolytic bacteria may be hampered in association with high ruminal concentrations of Zn (Karr et al., 1991). Spears (1995) concluded from these data that most performance or carcass quality responses to high dietary Zn are not a result of enhanced physiological responses. They are more likely related to pharmacological effects associated with Zn *in vivo* (Spears, 1995). Though these responses can be beneficial, care must be exercised in practical situations concerning excessive, single nutrient supplementation. Excessive supplementation of trace minerals may lead to

increased excretion of heavy metals into the environment and reduced animal performance due to deleterious mineral interactions or toxicoses.

In addition to interactions between Cu and Zn, other ionic metals may impair the bioavailability of Cu to ruminant animals. Several non-essential micronutrients interact with Cu, and under certain circumstances, amalgamate with soluble dietary Cu, limiting its bioavailability. Among these are lead (Pb), silver (Ag), and cadmium (Cd) and nickel (Ni) (Baker and Ammerman, 1995a), all of which have been implicated as possible Cu antagonists. However, these interactions are poorly understood due to extreme quantification complexities (Gawthorne, 1987).

There is increasing evidence that excessive dietary Fe exerts in independent, negative effect on Cu status in ruminants. Although the mechanism(s) behind iron's role in the antagonism of Cu remains elusive, Suttle et al. (1984) postulated the mechanism is involved with increased ferrous sulfide speciation in the rumen which disassociates in the abomasum, liberating sulfide. This sulfide may then complex with Cu, rendering a poorly absorbed complex. Campbell et al. (1974), Humphries et al. (1983), Bremner et al. (1987), and Gengelbach et al. (1994) have documented direct negative interference of dietary Fe on Cu metabolism in beef cattle. Others have observed similar occurrences in sheep (Suttle and Peter, 1984).

Other Dietary Components and Breed. Dietary protein content, solubility, and level have been investigated as to their potential for limiting Cu to ruminants. High levels of dietary protein have reduced Cu availability and retention (Ivan and Veira, 1981). Admittedly, this observation could have been related to sulphur containing peptides or

amino acids (Graham, 1991). Robbins and Baker (1980), and Aoyagi and Baker (1994) reported reduced Cu absorption in chicks supplemented with cysteine at 4,000 ppm in the total diet. Cysteine, as well as other sulphur containing compounds, may serve as a reducing agent in the rumen and gastrointestinal tract, providing binding sites for Cu through sulfhydryl and amine moieties (Baker and Ammerman, 1995a). When methionine and homocysteine were fed to rats, marked inhibition of Cu absorption occurred (Linder, 1991). In contrast, other studies have reported generally positive effects of high protein diets on Cu absorption in adult males (Greger and Snedeker, 1980) and pre-adolescent girls (Engel et al., 1967). Dietary phytate, ascorbic acid, various carbohydrates and some arsenicals have been shown to reduce the bioavailability of Cu to various animals (Baker and Ammerman, 1995a).

Genetic variation associated with Cu homeostasis is well documented in sheep (Wooliams et al., 1982; Harrison et al., 1987; Wiener et al., 1984). Although there is limited data availability concerning either actual heritability estimates or breed differences for Cu homeostatic traits (Rowlands et al., 1980; Wiener et al., 1983) there is evidence that Simmental cattle excrete greater biliary Cu than Angus cattle (Gooneratne et al., 1994) and may have greater requirement for this trace element. In a study comparing nine breeds of cattle, Littledike et al. (1995) reported significant differences in liver, serum and plasma concentrations of several microminerals, including Cu. Ward et al. (1995) reported that Angus cattle had over twice as much liver Cu than Simmental cattle reared at the same experiment station and fed the same diet. More importantly, based on liver Cu concentrations, half the Simmental cattle could be classified as deficient

(Underwood, 1981; Puls, 1994) whereas none of the Angus cattle were classified as deficient.

Factors Affecting the Bioavailability of Zinc

Trace elements are intertwined within their own biochemical properties that affect the overall amount of the element that is finally destined for a functional, biological role. Zinc bioavailability may be affected through various mechanisms. There are similarities between the proposed antagonisms of Cu and Zn, with many dietary and non-dietary factors having potential roles in limiting Zn to animals. Admittedly, Zn deficiency is not very common in most grazing animals, however, numerous sub-clinical and clinical cases have been observed in grazing ruminants (McDowell, 1992b). Numerous reviewers have published information which indicates Zn is often present at marginal or deficient levels (Corah and Dargatz, 1996; Spears, 1994; Paterson et al., 1999).

Chelating agents and ionic interaction have been cited as being the most important dietary factors affecting the bioavailability of Zn (Ammerman and Baker, 1995b). The requirement for dietary Zn can be affected by age, physiological state, environmental stress and health (McDowell, 1992b). Zinc metabolism, absorption and retention may be dependent on dietary levels of Cd, Ca, Cu, Fe, and selenium (Se). More importantly, Zn status in the animal can alter the efficiency of absorption of Zn from the diet, presumably through modification of metallothionein synthesis (Cousins, 1985) or other pre-absorptive ligands. Dietary polyphosphoinositols (phytates) show the clearest participation in disrupting normal Zn homeostasis. Recent information indicates the

hexa- and pentaphosphate derivatives of inositol are most relevant to Zn absorption (Lonnerdal et al., 1989). Dietary calcium, zinc, and inositol derivatives interact and form insoluble complexes, thus reducing the bioavailability of Zn. Although this interaction is affected by dietary levels of soluble Zn, it is almost wholly regulated by dietary levels of Ca (Davies et al., 1979; Morris et al., 1980).

Hierarchical Fortification of Trace Minerals to Ruminants

When assessing the trace mineral nutritional environment that cattle are subjected to, the level, source and form of the trace elements are all integral to this equation. The level of the trace element indicates at what concentration the element is occurring at in the diet while source refers to where or how the element is presented. Form of the trace element refers to the biochemical state(s) in which the micronutrient commonly exists. Nutritionists and veterinarians must consider all of this interactive information when evaluating the extrinsic nature of the trace mineral status of livestock. There exists a hierarchy (Figure 2) among the variables that dictates the overall trace mineral status of an animal or directly affects the bioavailability of essential micronutrients. It is within the sub-levels of this hierarchy in which strategic supplementation has its greatest use to managers of livestock. By developing efficacious techniques for evaluating the trace mineral status of animals in both an extrinsic and intrinsic manner, we may be better able to ascertain efficient supplementation strategies.