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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John Denver Bailey

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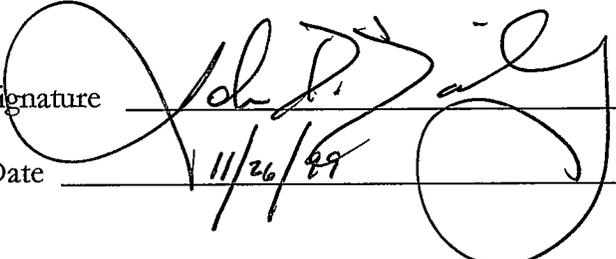
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I came to Montana an empty vessel...just a shell of man...my beautiful wife, Jana, filled up the inside and now I overflow. Thank you, my love, for giving me back something that was already mine but somehow lost...my heart.

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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.

Figure 2. Hierarchical fortification of trace minerals to ruminants.

Supplementary Sources of Copper and Zinc

Forage. Ruminant animals throughout the world consume forage-based diets that are commonly deficient in one or more essential trace elements. With the possible exception if phosphorus (P), Cu is likely the most frequently deficient trace mineral in ruminant diets (McDowell, 1992a). Thirty-four countries of Latin America, Africa and Asia have reported Cu deficiencies, more than any other element besides P (McDowell et al., 1984). In a recent study, Corah and Dargatz (1996) reported results from an 18-state forage sample collection and analysis project. These researchers found that 64% of the samples analyzed were either marginal or deficient in Cu. Moreover, these researchers found Cu antagonists (Fe and Mo) to be high (Fe > 400 ppm; Mo > 3 ppm) in 57.8% of the samples analyzed. These results indicate both Fe and Mo might be often present at levels that can cause a reduction in the bioavailability of Cu to animals consuming these types of diets.

In general, forage mineral content may be extremely variable, owing to soil mineral levels, soil pH, climatic factors and plant species and maturity (Spears, 1994). Knowing the concentration of minerals in the forage supply is of little value without considering environmental parameters (Spears, 1994). When comparing legumes and grass species grown in the same location, legumes have been shown to be higher in Ca, Cu, Zn and Co than grasses (Greene et al., 1998). Cereal grains typically contain 4-8 ppm Cu and leguminous seeds and oilseed meals are generally quite concentrated in Cu (15-30 ppm; Davis and Mertz, 1987). Research from Montana State University indicates that while

forage mineral content may not vary tremendously from year to year (Clark et al., 1994), there are quite often notable deficiencies for Cu and Zn (Paterson et al., 1999).

Soil. The availability of Cu and Zn to forage is affected by and best understood at the soil/plant interface. Soil pH, soil management, forage maturity and species, and climate affect the trace mineral content of forage (McDowell, 1992a). These factors have been extensively reviewed by McDowell (1985) and Spears (1994). Deliberate ingestion of soil by livestock is common, however, it may be quite detrimental if extreme. From a physical standpoint, animals ingesting soil have shown excessive tooth decay, increased digestive tract epithelial damage and gut impaction when underfed (McDowell, 1992c). Suttle et al. (1975) and Russell et al. (1988) concluded Mo and Zn from soil is available and may be an important factor in causing hypocuprosis in cattle or enzootic ataxia in neonatal lambs. Sillanpaa (1982) evaluated the effects of soil pH on the concentration of several trace elements in wheat plants. Although levels of Mo would not be antagonistic to Cu, the relationship is clear. The levels of Mo in the forage increased with increasing soil pH while the levels of Cu remained constant. This situation could be quite detrimental to cattle either ingesting the alkaline soils or forage in this type of area. It is well accepted that ingestion of soil is likely to be negative on certain trace minerals in the diet of grazing ruminants.

Water. McDowell (1992c) has reviewed the trace element content of water sources and the possibility of water serving as a viable source of minerals for farm animals. The most relevant information to this discussion is that water could provide only 1-2% of the

requirement for both Cu and Zn in most farm animal species (McDowell, 1992c). In some areas, water may contain substantial amounts of Cu and Zn antagonists and many nutritionists and veterinarians advocate water quality analyses as a tool in determining the trace mineral status and supplemental requirement for livestock. When the sulfate content of water was substantially reduced, beef cattle showed improved Cu status (Smart et al., 1986). Furthermore, naturally high concentrations of salt in drinking water may affect mineral supplement intake, (McDowell, 1992c) as most free-choice supplements are salt based. Reformulation of the supplement would be a necessity in this situation, as decreased mineral consumption would reduce the bioavailability of the micronutrients consumed by the animal.

Parenteral, Slow-release and Oral Drench Trace Mineral Supplements. There has been considerable research investigating the effectiveness of different trace mineral supplementation strategies. Researchers have evaluated these modes of supplementation for their effectiveness in meeting the Cu and Zn requirements for grazing ruminants. Baker and Ammerman (1995a) suggest three major categories of supplementation; plethoric, physiological and slow-release supplementation. McDowell (1992c) defines supplementation techniques by clarifying the distinction between direct and indirect methods. However, Graham (1991) limits his review to two supplementation methods, parenterally and orally.

Both Cu and Zn solutions have been directly administered parenterally with some success, however, there are serious considerations concerning their use. Copper glycinate, copper ethylenediaminetetraacetic acid (EDTA), or copper-methionate can be

injected sub-cutaneously to alleviate hypocuprosis in animals (Hemingway et al., 1970; Boila et al., 1984; Bohman et al., 1987). Zinc has been administered as an oral drench and as an intramuscular injection of Zn oxide (McDowell, 1992). Copper glycinate (Bohman, et al., 1987) and copper methionate (Suttle, 1981) may lead to increased injection site blemishes and abcessation, indications which are increasingly costly to the beef cattle industry. There is further evidence that injectable Cu supplements may increase incidence of Cu toxicosis and possibly death (Bulgin et al., 1986; Bohman et al., 1987). Graham (1991) suggested that mature cattle are more likely to become Cu toxic with use of parenteral supplementation compared to oral dosing. Other major limitations in the use of parenterally administered trace element supplements is their variability of effectiveness and the necessity for frequent readministration (Baker and Ammerman, 1995a).

There has been interest in supplementing cattle directly with slow-release, Cu containing boluses or particles. Theoretically, these constituents enter the reticulorumen and solublize steadily or, upon translocation into the abomasum, release ionic particles under the influence of this acidic pH. Is has been shown that periodic administration of soluble glass boluses (Knott et al., 1985), copper oxide wires (Judson et al., 1981; Richards et al, 1985; Deland et al., 1986) and compressed copper oxide powder/multi-element boluses (Lawson et al., 1990) have effectively maintained the Cu status of both sheep and cattle. There has been no direct scientific reports on the possible pathological effects of such plethoric supplementation of these Cu sources and, furthermore, there is substantial evidence that suggests copper oxide has very limited bioavailability to animals

(Cromwell et al., 1989; Baker et al., 1991; Kegley and Spears, 1994). When compared to copper sulfate, copper oxide showed little value as a growth promoter for weanling pigs (Cromwell et al., 1989). This source of Cu has also been shown to be unavailable to chicks (Baker et al., 1991) and an *in vitro* solubility study (Kegley and Spears, 1994) revealed that feed-grade copper oxide was essentially unavailable and should not be used as the sole source of Cu in diets for beef cattle.

Free-Choice Trace Mineral Supplements. The most preferred supplementation methods for beef cattle are either as free-choice trace mineral methods (grazing cattle) or as a component of a total mixed ration (feedlot animals). In terms of strategic supplementation, these modes provide the manager with the greatest amount of control with smaller opportunity for toxicosis. Traditionally, trace mineral supplements have been provided as inorganically bound oxides, sulfates, chlorides and carbonates. The concept of organically bound trace elements was deduced by O'Dell and Savage (1957) and there has been increased use and investigation of these forms of trace minerals.

Inorganic vs. Organic Forms of Supplementary Copper and Zinc

The American Association of Feed Control Officials (AAFCO) has classified the specific categories of organically bound trace minerals (Table 2; AAFCO, 1994; Greene, 1995). According to Greene (1995), organic trace mineral sources will differ in basic biochemical properties compared to the inorganic counterparts and, thus, may be absorbed, transported and retained to different degrees. Trace minerals *in vivo* are usually found in organic biomolecules or ligands and not as free inorganic ions (Greene, 1995).

Theoretically, organic forms of trace elements are more similar to those forms of trace elements absorbed naturally.

Table 2. Classification of organically bound trace minerals (Greene, 1995).

Name	Description
Metal amino acid complex	The product resulting from complexing a soluble metal salt with amino acids. The product must contain a specific metal.
Metal specific amino acid complex	The product resulting from complexing a soluble metal salt with a specific amino acid. The product must contain a specific metal as well as a specific amino acid.
Metal amino acid chelate	The product resulting from the reaction of a metal ion from a soluble metal salt with amino acids to contain a mole ratio of one mole of metal to one to three moles of amino acids to form coordinate covalent bonds. Average molecular weight must not exceed 800 AMU.
Metal proteinate	The product resulting from the chelation of a soluble metal salt with amino acids and (or) partially hydrolyzed protein. It must be declared as an ingredient as the specific metal proteinate.
Metal polysaccharide complex	The product resulting from complexing a soluble metal salt with a polysaccharide.

Use of organic trace minerals has been on the increase as researchers report improved feed efficiency, growth, reproduction and immunity. There is, however, very limited research on the specific mechanisms involved in molecular activity differences between inorganic and organic trace mineral supplements. Organic minerals may be absorbed more efficiently, however, in certain circumstances this may allow absorption of other antagonists into systemic circulation (Greene, 1995). It would be advantageous to

formulate trace mineral supplements that incorporate both inorganic and organic forms of trace elements. The more absorbable forms could maintain trace mineral status while the less available foms could preferentially bind to antagonists, lessening their systemic effects.

Apparent Bioavailability of Inorganic vs. Organic Copper and Zinc. Researchers have investigated the bioavailability of organically bound trace minerals and compared them to their inorganic counterparts. There is evidence that supports the idea that organically bound trace minerals may have increased bioavailability, however, there are contrasting data. Hemken et al. (1993) fed Cu sulfate or organic Cu sources to experimental rats, with or without high dietary Zn. They discovered that Cu proteinate supplemented animals exhibited greater Cu concentration in the liver, spleen and heart when compared to animals supplemented with copper sulfate. They also reported animals supplemented with Cu proteinate had significantly greater hepatic Zn as well, which led to the conclusion that organically bound Cu may be absorbed differently than inorganic Cu, which, like Zn, is absorbed via metallothionein. Based on hepatic Cu concentrations, copper proteinate and copper lysine increased hepatic Cu in rats when compared to copper sulfate (Du et al., 1996). Plasma Cu was maintained more effectively in cattle fed Cu-proteinate and high levels of the Cu antagonist Mo (Ward et al., 1996). When copper oxide was fed to Cu-deficient calves, it failed to improve Cu status when compared to calves fed no additional Cu (Kegley and Spears, 1994). More importantly, copper sulfate and copper lysine supplemented calves showed a rapid improvement in Cu status over calves supplemented with copper oxide. Nockles et al. (1993a) reported that calves fed

copper lysine exhibited greater apparent Cu absorption and increased Cu retention during a repletion phase compared to calves fed copper sulfate. Other data suggests copper lysine is of equal bioavailability compared to copper sulfate in chicks (Baker et al., 1991) and in steers (Ward et al., 1993).

Chicks fed corn-soybean meal diets had greater apparent bioavailability of Zn when fed as zinc methionine compared to zinc sulfate (Wedekind et al., 1992). In contrast, Nockles et al. (1993a) found no differences in apparent absorption or retention of Zn between zinc methionine and zinc sulfate in stressed calves. Spears (1989) reported that apparent Zn absorption was similar for zinc methionine compared to zinc oxide, but lambs receiving zinc methionine had greater Zn retention. Feeding either zinc lysine or zinc methionine resulted in equal or greater bioavailability than feeding zinc sulfate to sheep (Rojas, et al. 1995). Power et al. (1994) came to similar conclusions in rat metabolism studies.

Immunologic Response to Supplementary Trace Minerals. The immune system of mammals is comprised of two general mechanisms, commonly referred to as specific and non-specific. Within these two broad classifications, there exist three main sub-categories of mechanistic immune function; gut-associated lymphoid tissue (mucosal barrier) immunity, humoral immunity, and cell-mediated immunity. Cole (1995) suggested that mucosal barrier immunity is a non-specific process whereas both humoral and cell-mediated immune responses tend to be specific to a particular pathogen. While it is common to discuss these factors separately, they are thoroughly intertwined and

influenced by both physiological homeostatic systems and overall nutritional status (Cole, 1995).

Copper is integral to proper immune function through energy production, neutrophil function and activation, antioxidation, development of antibodies and lymphocyte proliferation (Nockles, 1994). Dietary Cu has been implicated in maintenance of major immune systems and its importance has been documented. Results comparing inorganic and organic trace element effects on immune function, however, have produced extremely variable results. This may be a consequence of attempting to measure such a resistant part of the internal environment of mammals. The immune system, regardless of whether humoral or cell-mediated, has preferential use of nutrient stores, including trace minerals. This fact is a difficult restraint in comparing effects of trace mineral form on immune function.

Attack and killing capability of neutrophils is compromised with Cu deficiency (Boyne and Arthur, 1986; Babu and Failla, 1990). In rats fed marginally low Cu diets, *in vitro* activities of T-lymphocytes and neutrophils were significantly depressed (Hopkins and Failla, 1995). Smart et al. (1981) noted drastic immune system dysfunction in Cu-deficient animals. Both cell-mediated and humoral immunity were compromised in steers (Jones and Suttle, 1981; Xin et al., 1991) and mice (Lukasewycz and Prohaska, 1983) with low stores of Cu. Copper supplementation enhanced bacterial resistance in sheep genetically predisposed to Cu deficiency (Wooliams et al., 1986). Organs associated with the immune system had significantly higher concentrations of Cu in animals supplemented with Cu (Stabel et al., 1993). In contrast, Ward et al. (1993)

indicated there was no effect on morbidity of Cu adequate calves supplemented with an additional 5 ppm Cu. Niederman et al. (1994) concluded there were no effects on either antibody proliferation or phagocytosis in mature beef cows offered supplementary Cu. There were no observed differences in immune function due to supplementation of Cu in calves infected with Infectious Bovine Rhinotracheitis (IBRV) and *Pasteurella hemolytica* (Stabel et al., 1993). Although these results seem contradictory, these researchers note that challenging cattle with either viral or bacterial pathogens causes a transient increase in serum ceruloplasmin and plasma Cu of Cu-repleted animals. This clearly demonstrates a major protective role for this trace element in immune function (Stabel et al., 1993).

There have been several attempts to study the different roles organic and inorganic trace minerals may play in immune function. There has also been interest in determining whether or not the presence of Cu antagonists plays additive roles in reduced immunocompetence. There is evidence accruing that suggests supplementing organic forms of trace minerals may have a therapeutic advantage on the immune system over inorganic supplements. Footrot, which is detrimental to animal production, was diminished with the use of zinc methionine to dairy cattle (Moore, 1988; Muirhead, 1992). Reiling et al. (1992) concluded that zinc methionine enhanced hoof strength and integrity over zinc sulfate. Nockels et al. (1993b) reported results with stressed feeder calves previously receiving high Mo diets. Calves receiving copper proteinate had greater IBRV antibody titers compared to calves receiving copper sulfate. Results from another study by Nockles et al. (1993a) led to the conclusion that steers fed copper lysine had

greater apparent absorption and retention of Cu compared to calves fed inorganic Cu. This enhanced balance of Cu from copper lysine could potentially reduce disease susceptibility compared with less bioavailable sources. Chirase et al. (1991) investigated the effects of dietary Zn on feed intake and rectal temperature in steers challenged with IBRV. Zinc methionine supplemented steers showed the smallest decrease in dry matter intake (DMI) and had lowest mean rectal temperatures. They concluded from these data that the recovery rate of steers fed zinc methionine was enhanced over steers receiving inorganic Zn. When compared to heifers receiving no trace minerals or inorganic trace minerals, cell-mediated immune response to intradermal injection of phytohemagglutinin (PHA) occurred sooner and to a greater extent in heifers receiving organic minerals (Ansotegui et al., 1995).

Research from Montana State University revealed that dietary Cu antagonists may influence cell-mediated immune response to intradermal injection of PHA. Swenson et al. (1996) reported there were no apparent benefits on cell-mediated immunity among animals receiving either organic or inorganic trace minerals in the presence of Mo, S and Fe. Bailey et al. (1999) found similar results in heifers supplemented with Cu antagonists and either inorganic or a combination of inorganic and organic Cu and Zn. Niederman et al. (1994) reported adverse effects on the immune status of cows receiving excessive dietary Fe. Ward et al. (1993) found that prolonged exposure to dietary Mo and S decreased *in vivo* cell-mediated immunity in feeder cattle. These researchers further deduced cell fragility from the lower *in vitro* viability of lymphocytes collected from the steers receiving antagonists and concluded that cell-mediated immunity is more

susceptible to decreased Cu bioavailability than humoral immunity (Ward et al., 1993). Boyne and Arthur (1986) suggested that the negative impacts on neutrophil function are greater when Mo and Fe are excessive than when Cu content in the diet is merely low. These results suggest that decreased immunocompetence in Cu deficient animals is potentially worse if the deficiency is a result of the antagonistic relationship between Cu and Mo, S, and Fe.

Production Responses to Supplementary Trace Minerals. Researchers and nutritionists have studied the positive attributes of organic forms of supplementary trace minerals on performance. It has been suggested that the effects of stressors common to the production animal can be lessened with incorporation of organic trace minerals into the diet regimen (Herd, 1994). Ward et al. (1992) reported differences in ADG and DMI for incoming feedlot cattle consuming organic trace minerals and concluded a potential for improved performance with their use. Spears and Kegley (1991) found improved weaning weights of calves whose dams were supplemented with organic Zn and manganese.

As Herd (1994) suggested, trace mineral requirements of beef cattle may be simplistically met, given proper levels of elements in forage and low production goals. Indeed, supplement packages consisting of inorganic forms of trace elements could provide the best overall mineral nutrition for livestock given constraints on bioavailability are absent. Greene (1995) suggested the additive role of organic trace minerals has its greatest potential in strategically incorporating both inorganic and organic forms of trace elements into one dynamic supplementation tool. The

experiments outlined in the remainder of this thesis are of the first investigations into this methodology.

CHAPTER 3

EFFECTS OF SUPPLEMENTAL TRACE MINERAL FORM ON LIMITING COPPER ANTAGONISM IN YEARLING BEEF HEIFERS – EXPERIMENT 1

Summary

An experiment was conducted to determine if combinations of inorganic and organic sources of Cu and Zn in the presence of antagonistic minerals affect average daily gain (ADG), dry matter intake (DMI), feed efficiency (gain/feed; G/F), weight loss due to shipping stress (shrink weight; SW), cell-mediated immunity (CMI), or hepatic trace mineral status in beef heifers. Thirty yearling, Angus x Hereford heifers (avg 291 kg BW) were randomly assigned to treatments and housed in 1 of 8 open-shed pens. For 112 d heifers were individually fed diets that contained 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 both in sulfate form (LoSulf); 5) 50 ppm Cu and 100 ppm Zn both in sulfate form (HiSulf). In addition, all animals were individually fed daily the Cu antagonists Mo (10 ppm), S (3,000 ppm) and Fe (500 ppm). A basal diet of chopped, mixed-grass prairie hay and a barley-based concentrate was formulated to achieve .7 kg ADG. Heifers were weighed every 14 d to measure ADG.

Individual DMI was calculated daily whereas G/F was calculated every 14 d. Shrink weight was calculated on d 112 after induction of transportation stress.

Phytohemagglutinin (PHA) was injected intradermally at two sites on the neck to observe CMI. After taking an initial skinfold measurement, skinfold swelling response to PHA was measured at 3, 6, 9, 12 and 24 h post-injection on study d 30. Liver biopsies were taken on d 0, 25, 50, 75 and 100 and analyzed for trace minerals. All data were analyzed using individual animal as the experimental unit in this completely randomized design experiment. Overall ADG, mean daily DMI, overall G/F and SW were similar ($P > .10$) among treatments. Skinfold swelling response to PHA did not differ ($P > .10$) among treatments. Control heifers had less ($P < .05$) hepatic Cu when compared to supplemented heifers from d 25 through d 100. Heifers supplemented with HiSulf had greater ($P < .05$) hepatic Cu from d 50 through d 100 when compared to 2-Way, 3-Way and LoSulf heifers. Heifers supplemented with 2-Way, 3-Way, or LoSulf had similar ($P > .10$) hepatic Cu concentrations from d 25 through d 100. Hepatic Zn concentration differed ($P < .05$) over time but was similar ($P > .10$) among treatments. Molybdenum accumulation was greater ($P < .05$) from d 75 through d 100 for CON heifers than for 2-Way, 3-Way and LoSulf heifers. By d 100, hepatic accumulation of Mo was similar ($P > .10$) for CON and HiSulf heifers. In addition, 3-Way heifers had accumulated less ($P < .05$) hepatic Mo than CON, 2-Way, LoSulf, and HiSulf heifers from d 75 through d 100. Trace mineral supplements containing either all inorganic or a combination of inorganic and organic Cu and Zn reduced hepatic Cu loss and suppressed hepatic accumulation of Mo. When a combination of Cu and Zn 50% amino-acid complexes, 25% sulfates, and

25% oxides was supplemented, it provided the best scenario between both hepatic conservation of Cu and suppression of accumulation of Mo.

Introduction

Trace mineral supplementation continues to be a vital part of beef cattle production. Trace minerals such as Cu and Zn serve important roles in the growth and immunity of beef cattle (McDowell, 1992; Baker and Ammerman, 1995a). Dietary antagonists such as Mo, S, and(or) Fe limit the bioavailability of Cu to beef cattle, compromising Cu dependent systems of the animal. Antagonistic minerals have been implicated for their part in inducing hypocuprosis by forming insoluble Cu complexes in the digestive tract, bloodstream, and tissues of ruminants (Dick et al., 1975; Suttle and Field, 1983; Howell and Kumaratilake, 1990). It would be advantageous to develop trace mineral strategies that limit secondary trace mineral deficiencies without increasing dietary levels of essential trace elements. Simply increasing dietary levels of trace metals may lead to other deleterious mineral interactions or environmental contamination (Greene, 1995). There has been considerable interest in developing strategic supplementation protocols utilizing organically bound sources of trace minerals because of reported increased bioavailability with their use (Kincaid, et al., 1986; Manspeaker, 1987).

According to Greene (1995), organic trace mineral sources differ in their basic biochemical properties compared to their inorganic counterparts and may be absorbed, transported and retained to different degrees. Trace minerals *in vivo* are usually found in organic biomolecules or ligands and not as free inorganic ions (Greene, 1995). Organic

forms of supplementary trace elements are more similar to forms occurring naturally, which may allow alternative absorption pathways (Greene, 1995; Du et al., 1996).

Swenson et al. (1996) found evidence that complexed (organic) minerals could be used to develop strategic supplementation programs for beef cattle. These researchers found that liver levels of Cu increased in the presence of antagonists when cows were supplemented with complexed minerals. Additionally, liver Cu was maintained more effectively in the complex supplemented cows 150 d after supplementation ended (Swenson et al., 1996). Greene (1995) suggested strategies that combine inorganic and organic forms of Cu may help lessen the effects of dietary Cu antagonists. The objectives of this experiment were to determine the effects of combining inorganic and organic forms of Cu and Zn on ADG, DMI, G/F, SW, CMI and hepatic trace mineral status in beef heifers receiving dietary trace mineral antagonists, Mo, S, and Fe. A secondary objective was to determine if simply increasing the level of supplementary Cu and Zn was an alternative to feeding supplements containing half as much Cu and Zn in the presence of antagonists.

Materials and Methods

The experiment was conducted at the Montana State University Teaching and Research Livestock Experiment Station, Bozeman. Thirty yearling, Angus x Hereford heifers originating from the Montana State Prison Ranch (Deer Lodge) beef herd with an average initial body weight of 291 ± 9 kg were used in this 112 d experiment. All

procedures and protocols were approved by the Montana State University Institutional Animal Care and Use Committee.

A 14 d acclimation period was used to adapt animals to pens, individual feeding gates (American Calan, Northwood, NH), and waterers. On May 6, 1998, 14 d prior to the initiation of the experiment, heifers were randomly assigned to trace mineral treatments, pens and Calan Gate stalls within pen. After randomization, there were two pens that had only three animals. Two "phantom" animals were randomly placed in the two unbalanced pens to help minimize possible pen effects therein. These animals were not included in any analysis. There were 8 pens with 4 animals per pen and 6 animals per treatment in this completely randomized design experiment. Starting on d 0 (May 20, 1998) and continuing through d 112 (September 9, 1998) heifers were individually fed hay at approximately 1200. Starting on d 1 (May 21, 1998) and continuing through d 112 experimental heifers were individually fed diets daily (Table 3) that contained 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 both in sulfate form (LoSulf); 5) 50 ppm Cu and 100 ppm Zn both in sulfate form (HiSulf).
In addition, all animals were individually fed daily the antagonists Mo (10 ppm) from $Na_2MoO_4 \cdot 2H_2O$, S (3,000 ppm) from $CaSO_4$, and Fe (500 ppm) from $FeSO_4$. These levels of antagonists are thought to occur under numerous situations in beef cattle diets (Corah and Dargatz, 1996; Paterson et al, 1999). The concern has been the effect of these antagonists on the bioavailability of Cu to beef cattle and it would be beneficial to

develop trace mineral supplements that minimize Cu loss during secondary mineral deficiencies. It has been proposed that a Cu:Mo ratio of no less than 4:1 must be achieved in beef cattle diets in order to maintain Cu homeostasis (Alloway, 1973). In this experiment, our goal was to test this biological system of mineral interaction by combining different forms of Cu and Zn in an attempt to overcome antagonism. The levels of trace mineral supplements and antagonists were varied daily according to individual DMI to maintain treatment levels of Cu, Zn, Mo, S, and Fe in the total diet. Target treatment levels were 25 ppm of Cu and 50 ppm Zn for LoSulf, 2-Way and 3-Way while target treatment levels were 50 ppm of Cu and 100 ppm of Zn for HiSulf. Target treatment levels of antagonists were 10 ppm Mo, 3,000 ppm S, and 500 ppm Fe. Within 7 d of study initiation, all animals were consuming 100% of their respective treatment supplements.

A basal diet (Table 4) of chopped, mixed-grass prairie hay and a barley/soybean meal (SBM) based concentrate was formulated to achieve .7 kg ADG. The barley/SBM portion of the basal diet was individually fed at approximately 0900 h and the hay portion of the basal diet was individually fed at approximately 1200 h. All diets met or exceeded all nutrient requirements for medium frame, growing beef heifers with the exceptions of Cu and Zn (NRC, 1984). Due to availability, the mixed-grass prairie hay was changed 3 times over the course of the experiment. Analyses for the 3 different hay sources are presented in Table 4. The barley and SBM came from the same source throughout the experiment; analyses for these feedstuffs are presented in Table 4. When the hay portion of the basal diet was changed, new diet formulations were calculated to

maintain target levels of trace minerals in the diet. The concentrate ration served as the carrier for the trace mineral treatments and antagonists. Trace mineral treatments and antagonists were individually weighed (to the nearest mg), mixed in plastic containers and individually fed with the concentrates to heifers in rubber tubs. Automatic water troughs supplied water via continuous circulation from a common source and waterers were cleaned once per week to minimize pen effects on water consumption.

Table 3. Dietary trace mineral concentration for treatments in Experiment 1.

	Dietary concentration per treatment				
Mineral	CON	2-Way	3-Way	LoSulf	HiSulf
Copper[1]	7.33	28.67	28.88	27.64	55.96
Zinc[1]	31.23	62.76	63.17	63.57	117.32
Molybdenum[1]	12.18	12.18	12.18	12.18	12.18
Iron[1]	500.60	500.60	500.60	500.60	500.60
Sulfur[2]	.29	.29	.29	.29	.29
Cu:Mo	.6:1	2.4:1	2.4:1	2.3:1	4.6:1

[1] mg/kg DM
[2] % of total DM

To calculate ADG, heifers were weighed every 14 d at approximately 2 h before individual supplementation and 5 h before hay feeding. Initial weights were an average of two weights; one obtained one day prior to study initiation and one obtained on d 0. Final weights were an average of two weights obtained on d 111 and d 112, respectively. Dry matter intake was measured daily and G/F was measured every 14 d.

On d 112 heifers were loaded onto a cattle truck and shipped to Glendive, MT from Bozeman and then from Glendive back to Bozeman (approximately 1,400 km round trip). The heifers remained on the truck at Glendive for an amount of time sufficient to

bring the entire round trip time to approximately 24 h. This process was conducted to simulate typical stress incurred by feedlot cattle traveling to the Midwestern United States from Montana. Weight loss due to shipping (SW) was calculated for each animal. Pre-shipping weight was an average of two weights; one obtained 24 h before shipping and one obtained at shipping. Post-shipping weight was a single weight obtained when heifers arrived back at Bozeman. Shrink weight was determined mathematically by subtracting the post-shipping weight from the average of the pre-shipping weights.

Table 4. Basal diet composition for Experiment 1[a]

Item	Mixed-grass hay			Barley	SBM
Lot	1[b]	2[c]	3[d]		
DM (%)	89.56	86.78	87.17	88.0	89.1
CP (%)	9.75	11.9	11.1	13.0	44.0
ADF (%)	45.2	43.8	43.6	5.8	10.0
NE_m (Mcal/kg)	1.07	1.18	1.11	1.95	2.06
NE_g (Mcal/kg)	.60	.62	.62	1.30	1.40
Cu (ppm)	5.0	6.0	5.0	5.0	20.0
Zn (ppm)	13.0	15.0	13.0	23.0	62.0
Mo (ppm)	.92	.37	.55	1.25	4.0
Fe (ppm)	45.0	41.0	36.0	138.0	147.0
S (% DM)	.11	.16	.15	.14	.19

[a] Based on dry weight
[b] Fed from d 0 to d 56
[c] Fed from d 57 to d 87
[d] Fed from d 88 to d 112

In order to stimulate a CMI response, phytohemagglutinin (PHA, Sigma Chemical, St. Louis, MO) was injected intradermally at two locations (75 μg PHA in 0.1 mL physiological saline per injection site) on the neck on d 30. The injections were made immediately cranial to the *scapula* on the left side of the animal. The sites were shaved with clippers fitted with a surgical grade blade attachment and an initial skin-fold

measurement was taken. Skin-fold swelling response to PHA was measured with micrometer calipers (RCBS Sporting Equip. Div. of Blount, Inc., Oroville, CA) at 3, 6, 9, 12 and 24 h post-injection.

Hepatic change in Cu, Zn, and Mo was measured by conducting liver biopsies on d 0, 25, 50, 75 and 100 using a modification of surgical procedures described by Corah and Arthington (1994). Upon entering a cattle chute, heifers were restrained with the head catch apparatus and a moderate amount of pressure was applied to the animal to disallow excessive movement. On the right aspect of the animal, an angular line was subjectively perceived distal and cranial from the *tuber coxae* to the proximal epiphysis of the *humerus*. At the point where this line transects the intercostal space between the 10th and 11th ribs and approximately 10-15 cm ventral to the dorsal ridge of the thoracic vertebrae, the biopsy procedure was performed. A 10 cm^2 area encompassing the biopsy site was completely clipped of hair using electric clippers fitted with a surgical grade blade attachment. Once the area was depilated and brushed free of debris, 5 mL lidocaine (20 mg/mL; Lido-epi, Radix Labs, Eau Claire, WI) was injected into the biopsy site, both subcutaneous and intramuscular in a fan-like motion. Surgical scrub (Povidine, .75% scrub, RXV Products, Porterville, CA) was directly applied to the biopsy area and, using a stiff-bristled scrub brush and dilute iodine, a circular scrubbing motion was applied, starting at the center (biopsy site) and moving outward to the edges of the shaved area. The step was repeated until the area was sufficiently field-sterile. The final prepatory step is administration of tincture of iodine (7%, RXV Products, Porterville, CA) and isopropyl alcohol (70%), both in separate plastic spray type containers, to the surgery

site. Application of one spray of alcohol followed by iodine was administered three times. Surgical instruments were kept in a sterilized (via autoclave), covered, stainless steel pan, submerged in Nolvasan™ solution. The surgical instruments used for this procedure were 1) sterile #22 scalpel blades and 2) a biopsy needle(s) (Tru-Cut™ biopsy needle, 14 gauge, 15 cm, 20 mm specimen notch, VWR Scientific Produnts, Batavia, IL). After field preparations were complete, the gloved, aseptically secure surgeon swiped the biopsy area with sterile gauze then, using the scalpel, made a puncture incision through the epidermis at the biopsy site noted above. The biopsy needle was inserted at an approximate 45° angle in such a way that an imaginary line was envisioned from the tip of the biopsy needle to the left side tip of the opposite *scapula*. The needle was pushed through the puncture incision, the tunicae, intercostal muscles, and then into the liver where a core sample of tissue was taken and placed on the anterior surface of the gloved hand not operating the needle. This procedure was repeated until the sample weight was approximately 40-60 mg (4-5 successful biopsy core samples). When a sufficient amount of hepatic tissue was collected, the core samples were combined on the anterior surface of the sterile collection glove and this conglomerate was then placed in a labeled, borosilicate glass, 12 x 75 mm, disposable culture tube (Fisher Scientific, Denver, CO), capped and frozen at -6° C until overnight shipping to Michigan State University, Animal Health and Diagnostic Laboratory for analysis via inductively coupled plasmology atomic emission spectroscopy. Before release from the restraining chute, heifers received three sprays of tincture of iodine over the biopsy site and an intramuscular injection in the neck of penicillin at a dose rate of 3 ml•68 kg^{-1} of body weight.

Elemental analysis of the liver tissue was conducted at Michigan State University, Animal Health Diagnostic Laboratory using inductively coupled plasmology atomic emission spectroscopic methodology (ICP-AES; Braselton, et al., 1997). Braselton et al. (1997) explain ICP-AES is an instrumental technique that nebulizes a liquified tissue sample into an argon plasma sustained by high frequency oscillating magnetic field. By utilization of this technique, virtually all chemical interference is eliminated, resulting in greater sensitivity and accuracy (Braselton et al., 1997). Upon arriving at Michigan State University, Animal Health Diagnostic laboratory, each biopsy sample was placed into a 15 mL Teflon container (Savillex, Minnetonka, MN), dried for 4 hours at 95°C, and cooled to room temperature. Dried samples, which weighed between 1 and 10 mg, were weighed with an analytical scale to the nearest .01 mg by transferring to a tared weigh paper after which samples were returned to the original Teflon container. Concentrated nitric acid (.25 mL) was added to the Teflon container and capped tightly. The samples were digested in a 95° C oven overnight. After digestion, the samples were cooled to room temperature, quantitatively transferred with water (18 meg ohm; Millipore four-bowl purification system, Millipore, Bedford, MA) to a 5 mL volumetric flask and brought to volume with water. Bovine liver tissue samples were analyzed via ICP-AES (Thermo Jarrell-Ash Polyscan 61E Simultaneous/Sequential ICP; Thermo Jarrell-Ash, Franklin, MA) interfaced to an ultrasonic nebulizer (Cetac U-5000 Ultrasonic Nebulizer with ATX-100 auto-tuning power supply; Cetac Technologies, Omaha, NE). All biopsy samples were compared with three blank digests and with a standard (Bovine Liver SRM 1577a; National Institute of Standards and Technology [NIST], Gaithersburg, MD)

digested in a manner to provide tissue-sample size and element amounts equivalent to those of the biopsy samples. The accuracy of the calibration curve was verified by analysis of certified reference material (NIST multielement Mix A-1 SRM 3171a and multielement Mix B SRM 3172) diluted to 1 mg•L^{-1} in 5% (vol•vol^{-1}) nitric acid.

Statistical Analyses

Experiment 1 was a completely randomized design. Animal served as the experimental unit because trace mineral treatments were imposed on the individual animal and daily supplement amount was determined by individual DMI.

The pre-set α level for all statistical analyses in this experiment was .05. Means were separated after a significant F-value was determined and evaluated by LSD tests. Least square means and *P*-values are reported. Where appropriate, least square means were compared using single degree of freedom orthogonal contrasts (Neter et al., 1996). Contrasts of interest were CON vs. supplemented, combinations vs. all inorganic (same level), 25 ppm Cu/50 ppm Zn vs. 50 ppm Cu/100 ppm Zn.

Overall ADG, daily DMI, overall G/F and SW were analyzed using the General Linear Models (GLM) procedure of SAS (1996). The models included the main effects of pen and treatment. Animal within treatment was used as the error term to test for treatment effects, whereas residual error was used to test for the effect of pen. Pen effect was not significant ($P > .10$) in any analysis and was taken out of the models and the data were reanalyzed. The reported data are from the analyses using the reduced models containing treatment.

Skin-fold swelling (mm) response to intradermal injection of PHA over time and hepatic concentrations (ppm) of Cu, Zn and Mo over time were analyzed as repeated measures using the GLM procedure of SAS (1996). The models included the main effects of pen and treatment. Pen effect was not significant ($P > 0.10$) in any analysis and was taken out of the models and the data were reanalyzed. Due to possible treatment differences in initial skin-fold thickness, the model for skin-fold swelling response to intradermal injection of PHA included initial skin-fold thickness as a covariable. Similarly, initial hepatic mineral concentration was included as a covariable in the models for liver concentration of Cu, Zn, and Mo, respectively. Initial skinfold thickness was similar ($P > .10$) among treatments and the covariable was taken out of the model and the data were reanalyzed; the data reported are from the reduced model. Initial hepatic concentrations of Cu, Zn, and Mo were significant as between subject effects ($P < .0001$) in the models for hepatic mineral status. Initial Cu and Zn were significant ($P < .05$) as covariates at all time periods. Initial Mo was significant ($P < .05$) as a covariate at all time periods except for d 100. Initial hepatic Mo was removed from the model for hepatic Mo at d 100 and reported data at this time period are from the reduced model.

Results and Discussion

At the initiation of this experiment, heifers weighed 291 ± 9 kg. Pooled mean initial weights were similar ($P > .10$) among treatments (Table 5). Mean ADG (Table 5) over the 112 d period did not differ among treatments ($P > .10$). Copper supplemented steers

have shown increased ADG compared to animals fed antagonists (Ward and Spears, 1997). These data support the idea that in the presence of trace mineral antagonists, ADG will not increase with additional Cu supplementation. Daily DMI, overall G/F and SW were similar ($P > .10$) among treatments in this experiment (Table 5). Copper supplementation improved DMI and gain:feed ratios in the study by Ward and Spears (1997). Wittenburg and Boila (1988) reported increased gain:feed ratios but not ADG in growing steers supplemented with Cu and consuming 10 mg Mo/kg DM. It was not apparent in this experiment that supplementation of Cu and Zn affected DMI, ADG, G/F or SW in beef heifers consuming antagonists.

Table 5. Pooled mean initial weights, daily dry matter intake, average daily gain, feed efficiency and weight loss due to shipping for heifers in Experiment 1[a].

Item	CON	2-Way	3-Way	LoSulf	HiSulf	SEM
Initial weight (kg)	291.3	292.2	291.3	294.3	285.2	9.02
Daily DMI (kg)	8.7	8.7	9.3	9.0	8.9	.49
ADG (kg/d)	.70	.69	.76	.68	.68	.03
Feed efficiency (G/F)	.080	.079	.081	.078	.079	.005
SW (kg)	-35.2	-36.0	-31.2	-37.3	-34.3	2.72

[a] n=6 animals per treatment

Skin-fold swelling response to PHA changed significantly ($P < .05$) over time but did not differ ($P > .10$) among treatments over the 24 h observation period on study d 30 (Figure 3). It appears that dietary antagonists may affect CMI, including intradermal injection of PHA. Swenson et al. (1996) reported that CMI response to PHA did not differ ($P > .10$) among cows supplemented with either organic or inorganic sources of Cu, Zn, Mn and Co and consuming the antagonists, Mo, S and Fe. With no

supplemental antagonists, CMI response to intradermal injection of PHA was less variable and occurred sooner and to a greater extent when heifers were fed organic forms of Cu, Zn, Co and Mn compared to heifers receiving either sulfated forms of these minerals or no additional minerals (Ansotegui et al., 1995). Ward et al. (1993) reported decreased *in vivo* CMI in feeder cattle exposed to high dietary Mo and S for a prolonged period of time.

There were treatment effects ($P < .05$) for hepatic Cu concentration (Figure 4). Orthogonal comparison of CON vs. Supplemented heifers indicated CON heifers had lower ($P < .05$) hepatic Cu concentrations from d 25 through d 100. This indicates that supplementing heifers with the sulfate forms of Cu and Zn or with combinations of inorganic and organic forms of Cu and Zn inhibits the loss of hepatic Cu in the presence of antagonists. LoSulf, 2-Way and 3-Way heifers had similar ($P > .10$) hepatic Cu levels from d 25 through d 100. Heifers supplemented with HiSulf had greater ($P < .05$) hepatic Cu from d 50 through d 100 when compared to LoSulf, 2-Way, and 3-Way heifers. This suggests that beef cattle consuming a trace mineral supplement with 50 ppm Cu from $CuSO_4$ may have greater hepatic Cu conservation in the presence of these levels of antagonists, when compared to combinations of inorganic/organic supplements containing 25 ppm Cu. Over the course of this experiment, hepatic Cu concentration was similar ($P > .10$) between heifers fed the same levels of either all inorganic or a combination of inorganic and organic Cu and Zn. This supports previous studies in which organic sources of Cu fed at similar levels were equally as effective in improving Cu status as $CuSO_4$ in cattle (Wittenberg et al., 1990; Ward et al., 1993).

Hepatic concentration of Zn differed ($P < .05$) over time but was similar ($P > .10$) among treatments (Figure 5) throughout the entire experiment. Hepatic Zn concentration is not a reliable indicator of overall Zn status of beef cattle (Puls, 1994) and tissue levels of Zn do not always reflect dietary Zn intakes (Underwood, 1981). Although feeding either Zn lysine or Zn methionine resulted in equal or greater availability than feeding Zn sulfate to sheep (Rojas, et al., 1995), it was not apparent in this experiment that either all inorganic or a combination of inorganic and organic Zn in the presence of antagonists affected hepatic Zn concentration in these beef heifers under these study conditions.

There were treatment effects ($P < .05$) for hepatic Mo concentration (Figure 6) for d 50 through d 100. At d 50, mineral supplements containing inorganic and organic Cu and Zn reduced ($P < .05$) hepatic accumulation of Mo compared to all sulfate supplements. At d 75, hepatic Mo accumulation was reduced ($P < .05$) in the 3-Way heifers and similar ($P > .10$) among 2-Way, HiSulf, and LoSulf heifers. From d 75 through d 100 CON and HiSulf heifers had greater ($P < .05$) hepatic Mo when compared to LoSulf, 2-Way, and 3-Way heifers. In addition, 3-Way heifers accumulated less ($P < .05$) hepatic Mo than all other treatment groups from d 75 through d 100. In this experiment, trace mineral supplements containing 25 ppm Cu reduced hepatic accumulation of Mo by d 100 and the 3-Way treatment appeared to have the greatest potential for limiting hepatic Mo accumulation while conserving hepatic Cu. Hepatic accumulation of Mo reduces ceruloplasmin synthesis and accumulation of Cu in tissues (McDowell, 1992a; Arthington et al., 1996). If di- and tri- thiomolybdates are absorbed

(i.e. thiomolybdates not complexed with Cu), systemic Cu binding has been noted in sheep (Kelleher et al., 1983) and cattle (Hynes et al., 1985). The inclusion of Cu oxide in an inorganic/organic combination may have provided a proportion of substrate in the digestive tract sufficient to disallow absorption of di- and tri- thiomolybdates with the result being less hepatic Mo accumulation and conserved hepatic Cu.

Implications

Supplementing heifers with 25 ppm Cu as either all inorganic or a combination of inorganic and organic Cu reduced hepatic Cu loss and reduced hepatic accumulation of Mo in the presence of dietary antagonists. A supplement containing 50 ppm Cu from $CuSO_4$ failed to reduce hepatic Mo accumulation by 100 d, although it increased hepatic Cu conservation by this time. Hepatic Mo accumulation has been shown to negatively affect Cu homeostasis in cattle. Furthermore, supplementing heifers with a Cu amino acid complex, $CuSO_4$, and Cu oxide combination had no apparent adverse effects on hepatic Cu while reducing hepatic Mo accumulation. This may be caused by preferential Cu thiomolybdate binding with unavailable Cu oxide in the digestive tract, which would conceivably lessen the opportunity of other Cu sources to become bound in insoluble complexes plus reduce the amount of absorbed Mo. Although heifers supplemented with 50 ppm Cu from $CuSO_4$ had greater hepatic Cu by study d 100, this was at the sacrifice of greater hepatic Mo accumulation when compared to supplements containing 25 ppm Cu. These data indicate that trace mineral supplements containing either all inorganic or combinations of inorganic and organic Cu and Zn at 25 ppm Cu and 50

ppm Zn would be advantageous to beef cattle consuming dietary antagonists. An inorganic/organic combination including CuO may offer the greatest potential in reducing hepatic accumulation of Mo while conserving hepatic Cu in beef cattle consuming dietary antagonists.

Figure 3. Skinfold swelling response to intradermal injection of PHA for heifers in Experiment 1. There were no ($P >$ .10) overall treatment effects.

Figure 4. Hepatic Cu change for heifers in Experiment 1. Study days 25-100 are adjusted least square means using initial Cu concentration as a covariable. Study day 0 values represent means not adjusted for initial Cu. Bars with different letters differ ($P < .05$) within time.

Figure 5. Hepatic Zn change for heifers in Experiment 1. Study days 25 through 100 are adjusted least square means using initial Zn as a covariable. Study day 0 values represent means not adjusted for initial Zn. Bars with different letters differ ($P < .05$) within study day.

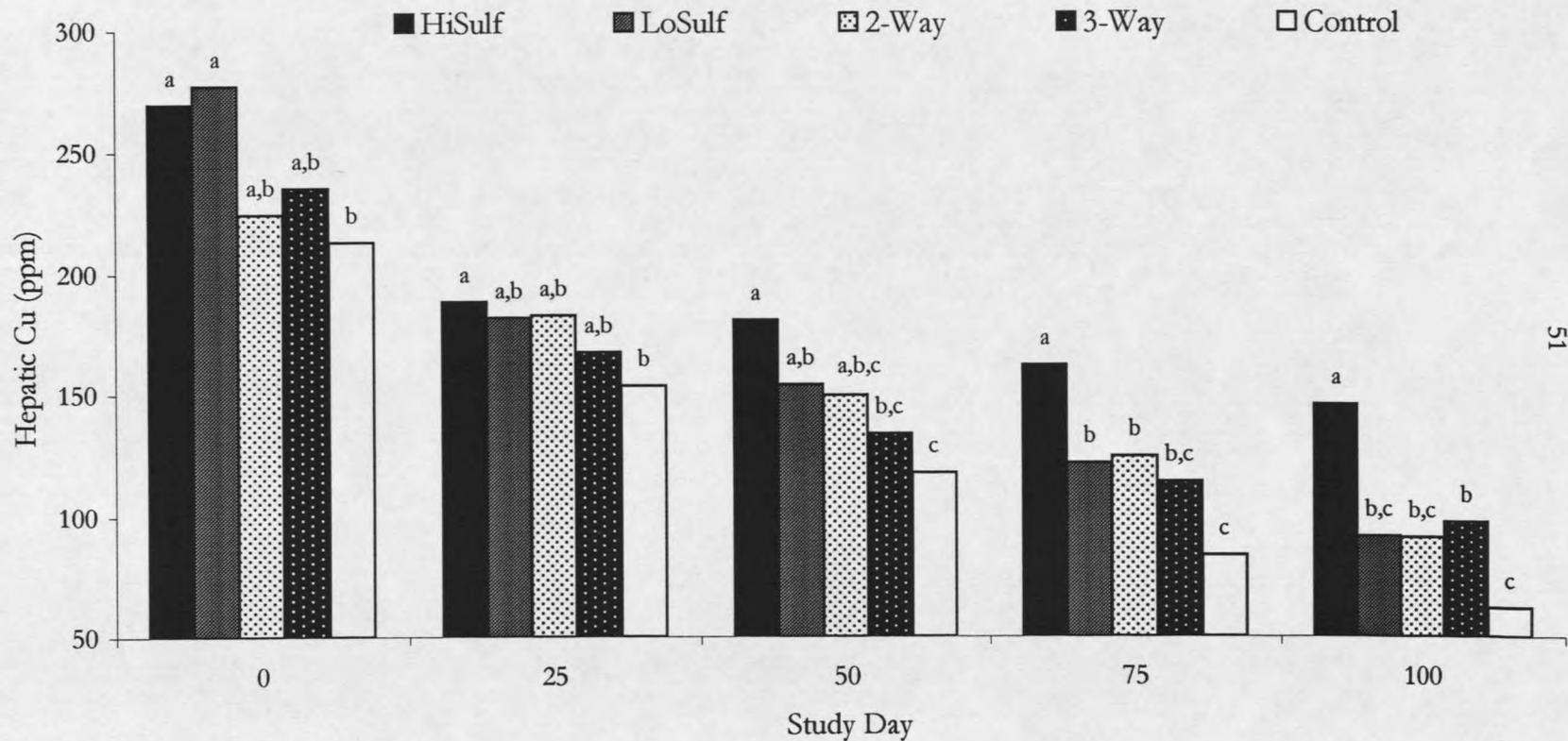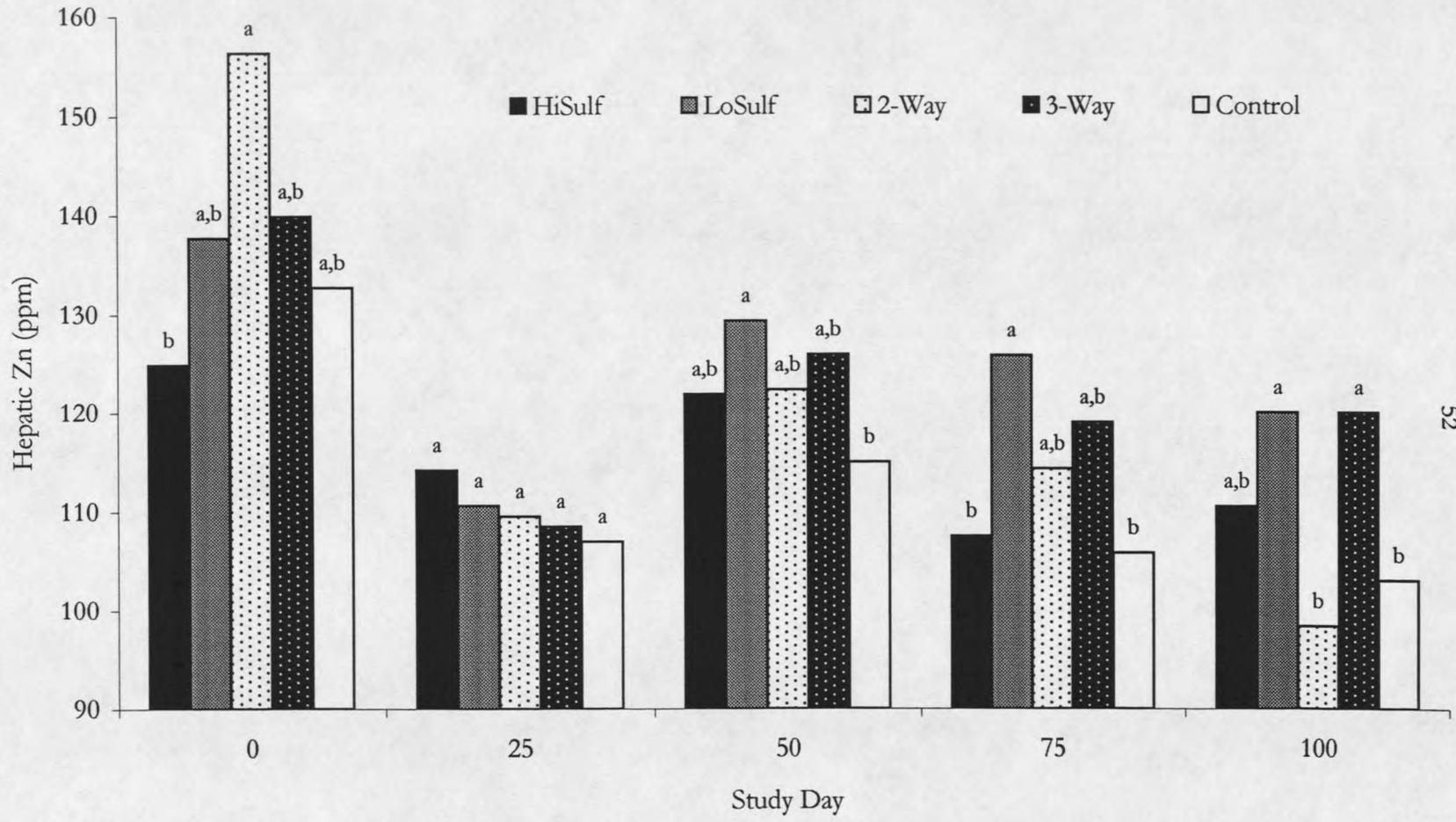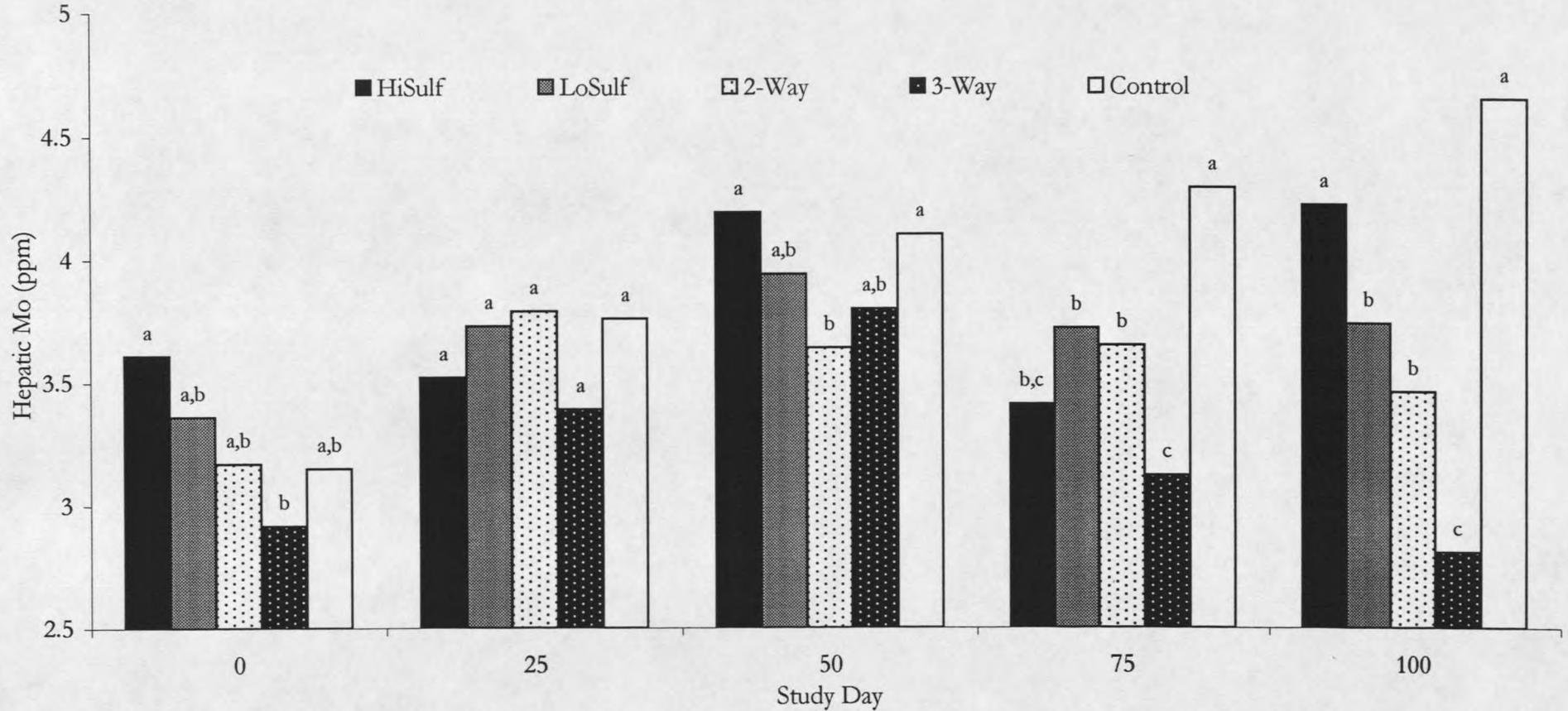Figure 6. Hepatic Mo change for heifers in Experiment 1. Study days 25-75 are adjusted least square means using initial Mo concentration as a covariable. Initial Mo was not significant ($P > .10$) as a covariable at study d 100. Study days 0 and 100 values represent means not adjusted for initial Mo. Bars with different letters differ ($P < .05$) within time

CHAPTER 4

EFFECTS OF SUPPLEMENTAL TRACE MINERAL FORM ON LIMITING COPPER ANTAGONISM IN YEARLING BEEF HEIFERS – EXPERIMENT 2

Summary

A companion experiment was conducted to provide additional information on supplementing combinations of inorganic and organic forms of Cu and Zn in the presence of antagonistic minerals. Objectives were to determine effects on average daily gain (ADG) cell-mediated immunity (CMI), weight loss due to shipping stress (shrink weight; SW) or trace mineral status in beef heifer calves reared in drylot conditions. Thirty yearling, Angus x Hereford heifers (avg 293 kg BW) were randomly assigned to treatments and blocked in 1 of 6 drylot pens. For 112 d heifers were fed diets that contained 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); 5) 50 ppm Cu and 100 ppm Zn in sulfate form (HiSulf). In addition, all animals were individually fed daily the antagonists Mo (10 ppm), S (3,000 ppm) and Fe (500 ppm). A basal diet of chopped, mixed-grass prairie hay and a barley-based concentrate was formulated to achieve .7 kg ADG. Heifers were

weighed every 14 d to measure ADG. Shrink weight was calculated on d 112 after induction of transportation stress.

Phytohemagglutinin (PHA) was injected intradermally at two sites on the neck. After taking an initial skinfold measurement, response to PHA was measured at 3, 6, 9, 12 and 24 h post-injection on study d 30. Liver biopsies were taken on d 0, 25, 50, 75 and 100 and analyzed for trace minerals. Average daily gain did not differ ($P > .10$) among treatments. Skin-fold swelling response to PHA did not differ ($P > .10$) among treatments. Hepatic Cu concentration was greater ($P < .05$) over time for 2-Way, 3-Way, LoSulf, and HiSulf heifers when compared to CON heifers. Hepatic Zn concentration differed ($P < .05$) over time but was similar ($P > .10$) among treatments. Molybdenum accumulation was greater ($P < .05$) over time for CON heifers than for 2-Way, 3-Way, LoSulf, and HiSulf heifers. As in Experiment 1, 3-Way heifers accumulated less ($P < .05$) hepatic Mo when compared to CON, 2-Way, LoSulf, and HiSulf heifers by d 100. Hepatic Mo accumulation was similar ($P > .10$) between heifers consuming 2-Way, HiSulf and LoSulf mineral treatments throughout the experiment. Trace mineral supplements containing either all inorganic or a combination of inorganic and organic Cu and Zn reduced hepatic Cu loss and suppressed hepatic accumulation of Mo. When a combination of Cu and Zn 50% amino-acid complexes, 25% sulfates, and 25% oxides was supplemented, it provided the best scenario between both hepatic conservation of Cu and suppression of accumulation of Mo.

Introduction

Appropriate trace mineral nutrition of beef cattle is essential to maintaining proper growth and immune function (Cousins, 1985). When Cu and(or) Zn become deficient to beef cattle, either through primary or secondary means, it is likely that systems which rely on them will become less efficient, resulting in losses in production (O'Dell, 1989). It would be advantageous to develop supplementation programs that could limit antagonism while conserving essential trace elements. There has been interest in using organically bound trace minerals to improve mineral status over their inorganic counterparts and there have been noted increases in apparent bioavailability when supplementing organic forms of Cu to cattle (Nockels et al., 1993a; Ward et al, 1996). Herd (1994) and Greene (1995) suggest, however, that while organic forms of trace elements may reduce intestinal mineral interactions, this may allow further absorption of antagonists that may interfere with systemic mineral metabolism. Combining inorganic and organic trace elements in a manner that would maintain trace element stores while reducing antagonism both in the digestive tract and systemically would be a strategic alternative to simply increasing the amount of the essential trace metals in the diet (Greene, 1995). Increasing trace metal concentration in beef cattle diets could lead to other mineral imbalances and environmental contamination (Graham, 1995). Objectives of this experiment were to provide additional information on the effects of combining inorganic and organic forms of Cu and Zn in the presence of Cu antagonists on ADG, CMI, and hepatic trace mineral status in beef heifers managed in drylot conditions.

Materials and Methods

This experiment was conducted at the Montana State University Teaching and Research Livestock Experiment Station, Bozeman. Thirty yearling, Angus x Hereford heifers originating from the Montana State Prison Ranch (Deer Lodge) beef herd with an average initial body weight of 293 ± 11 kg were utilized to determine if combinations of inorganic and organic forms of Cu and Zn in the presence of Cu antagonists (Mo, S, and Fe) influence ADG, CMI, or hepatic trace mineral status. A secondary objective was to determine if simply increasing the concentration of Cu in a supplement could affect hepatic trace mineral status in the presence of Cu antagonists. The experiment was conducted for 112 d and all procedures and protocols were approved by the Montana State University Institutional Animal Care and Use Committee.

A 14 d acclimation period was utilized to adapt animals to pens, feed bunks, and waterers. On May 6, 1998, 14 d prior to the initiation of the experiment, heifers were randomly assigned to trace mineral treatments and randomly blocked by pen. There were 5 animals per pen and 6 animals per treatment in this completely randomized block experiment. Starting on d 0 (May 20, 1998) and continuing through d 112 (September 9, 1998) heifers were pen-fed hay at approximately 1300. Starting on d 1 (May 21, 1998) and continuing through study d 112 heifers were removed from their pens and individually fed daily trace mineral supplements at approximately 0900 h (Table 6). Diets contained 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 both in sulfate form (LoSulf); 5) 50 ppm Cu and 100 ppm Zn both in sulfate form (HiSulf). In addition, all animals were individually fed daily the antagonists Mo (10 ppm) from $Na_2MoO_4 \cdot 2H_2O$, S (3,000 ppm) from $CaSO_4$, and Fe (500 ppm) from $FeSO_4$. These levels of antagonists, especially Mo, are thought to occur under numerous situations in beef cattle diets (Corah and Dargatz, 1996; Paterson et al, 1999). There is evidence these antagonists reduce the bioavailability of Cu to beef cattle (Gooneratne, 1994) and it would be beneficial to develop trace mineral supplements that would minimize Cu loss in these animals. In this experiment, it was our goal to test this biological system of mineral interaction by combining inorganic and organic Cu and Zn in an attempt to overcome antagonism. Within 7 d, all animals were consuming 100% of their respective treatment supplements.

Table 6. Dietary trace mineral concentration for treatments in Experiment 2.

Mineral	Dietary concentration per treatment				
	CON	2-Way	3-Way	LoSulf	HiSulf
Copper[1]	7.33	28.67	28.88	27.64	55.96
Zinc[1]	31.23	62.76	63.17	63.57	117.32
Molybdenum[1]	12.18	12.18	12.18	12.18	12.18
Iron[1]	500.60	500.60	500.60	500.60	500.60
Sulfur[2]	.29	.29	.29	.29	.29
Cu:Mo	.6:1	2.4:1	2.4:1	2.3:1	4.6:1

[1] mg/kg DM
[2] % of total DM

The level of trace mineral supplement was varied daily according to pen averaged DMI in an attempt to maintain treatment levels of Cu, Zn, Mo, S, and Fe in the total diet. Target treatment levels were 25 ppm of Cu and 50 ppm Zn for LoSulf, 2-Way and

3-Way while target treatment levels were 50 ppm of Cu and 100 ppm of Zn for HiSulf.

Target treatment levels of antagonists were 10 ppm Mo, 3,000 ppm S, and 500 ppm Fe.

A basal diet (Table 7) of chopped, mixed-grass prairie hay and a barley/soybean meal (SBM) based concentrate was formulated to achieve .7 kg ADG. Due to availability, the mixed-grass prairie hay was changed 3 times over the course of the experiment. Analyses for the 3 different hay sources are presented in Table 7. The barley and SBM came from the same source throughout the experiment (Table 7).

Table 7. Basal diet composition for Experiment 2[a]

Item	Mixed-grass hay			Barley	SBM
Lot	1[b]	2[c]	3[d]		
DM (%)	89.56	86.78	87.17	88.0	89.1
CP (%)	9.75	11.9	11.1	13.0	44.0
ADF (%)	45.2	43.8	43.6	5.8	10.0
NE_m (Mcal/kg)	1.07	1.18	1.11	1.95	2.06
NE_g (Mcal/kg)	.60	.62	.62	1.30	1.40
Cu (ppm)	5.0	6.0	5.0	5.0	20.0
Zn (ppm)	13.0	15.0	13.0	23.0	62.0
Mo (ppm)	.92	.37	.55	1.25	4.0
Fe (ppm)	45.0	41.0	36.0	138.0	147.0
S (% DM)	.11	.16	.15	.14	.19

[a]Based on dry weight.
[b] Fed from study d 0 to study d 56.
[c] Fed from study d 57 to study d 87.
[d] Fed from study d 88 to study d 112.

When the hay portion of the basal diet was changed, new diet formulations were calculated to maintain target levels of trace minerals in the diet. The concentrate ration served as the carrier for the antagonists and trace mineral treatments. Automatic water troughs supplied water via continuous circulation from a common source and waterers were cleaned once per week to minimize pen effects on water consumption.

To calculate ADG, heifers were weighed every 14 d at approximately 2 h before individual supplementation. Initial weights were an average of two weights, one obtained one day prior to initiation of the experiment and one obtained on study d 0. Final weights were an average of two weights obtained on study d 111 and 112, respectively.

In order to stimulate a CMI response, phytohemagglutinin (PHA, Sigma Chemical, St. Louis, MO) was injected intradermally at two locations in the same manner as in Experiment 1.

On study d 112 heifers were subjected to shipping stress induction in the same manner as in Experiment 1. Weight loss due to shipping (shrink weight; SW) was calculated for each animal. Pre-shipping weight was an average of two weights; one obtained 24 h before shipping and one obtained at shipping. Post-shipping weight was a single weight obtained when animals arrived back at the experiment station in Bozeman. Shrink weight was determined mathematically by subtracting the post-shipping weight from the average of the pre-shipping weights.

Hepatic change in Cu, Zn, and Mo was measured by conducting liver biopsies on study d 0, 25, 50, 75 and 100 using a modification of surgical procedures described by Corah and Arthington (1994). The procedure was conducted in the same manner as in Experiment 1.

Elemental analysis of the liver tissue was conducted at Michigan State University, Animal Health Diagnostic Laboratory using inductively coupled plasmology atomic emission spectroscopic methodology (ICP-AES) (Braselton, et al., 1997). The procedure utilized was described in Experiment 1.

Statistical Analyses

This experiment was a completely randomized block design with animal serving as the experimental unit and pen serving as the block. Experimental treatments were imposed daily on the individual animal. The pre-set α level for all statistical analyses in this experiment was .05. Means were separated after a significant F-value was determined and evaluated by LSD tests. Least square means and *P*-values are reported. Where appropriate, least square means were compared using single degree of freedom orthogonal contrasts (Neter et al., 1996). Contrasts of interest were CON vs. supplemented, combinations vs. all inorganic (same level), and 25 ppm Cu/50 ppm Zn vs. 50 ppm Cu/100 ppm Zn.

Overall ADG was analyzed using the General Linear Model (GLM) procedure of SAS (1996). Pen effect and the interaction of treatment x pen were not significant (*P* > .10) in this model and were taken out and the data were reanalyzed; the reported data are from the reduced model.

Skin-fold swelling (mm) response to intradermal injection of PHA over time and hepatic concentrations (ppm) of Cu, Zn and Mo over time were analyzed as repeated measures using the GLM procedure of SAS (1996). The models included the main effects of pen and treatment and the interaction of pen x treatment. Pen effect and the interaction of pen x treatment were not significant (*P* > .10) in the analyses and were taken out of the models and the data were reanalyzed; reported date are from the reduced models. Due to possible influences of initial skin-fold thickness differences, the model for skin-fold swelling response to intradermal injection of PHA included initial

skin-fold thickness as a covariable. Similarly, initial hepatic mineral concentration was included as a covariable in the models for liver concentration of Cu, Zn, and Mo, respectively. Initial skinfold thickness was similar ($P > .10$) among treatments and the covariable was taken out of the model and the data were reanalyzed. Initial hepatic concentrations of Cu, Zn, and Mo were significant ($P < .0001$) as between subject effects in the models for hepatic mineral status. Initial Cu and Zn were significant ($P < .05$) as covariates at all time periods. Initial Mo was significant ($P < .05$) as a covariate at all time periods except for d 100. Initial hepatic Mo was removed as a covariable from the model for hepatic Mo at d 100 and reported data at this time period are from the reduced model.

Results and Discussion

At the initiation of this experiment, heifers weighed 293 ± 11 kg. Pooled mean initial weights were similar ($P > .10$) among treatments (Table 8). Average daily gain and SW did not differ among treatments ($P > .10$) (Table 8). These results are similar to those in Experiment 1 and support the idea that in the presence of antagonists, ADG of beef cattle will not increase with additional trace mineral supplementation (Ward and Spears, 1997). It was not apparent in this experiment that supplementation of Cu and Zn affected SW in heifers consuming dietary mineral antagonists.

Table 8. Pooled initial weights, average daily gain and shrink weight for Experiment 2[a].

	HiSulf	LoSulf	2-Way	3-Way	CON	SEM
Initial weight (kg)	291.9	303.1	297.2	290.6	279.4	10.8
ADG (kg/d)	.75	.77	.70	.65	.68	.05
SW (kg)	-36.	-38.5	-37	-40.3	-35.3	2.49

[a] n=6 animals per treatment

Skin-fold swelling response to PHA increased significantly ($P < .05$) over time but did not differ ($P > .10$) among treatments (Figure 7). These results are similar to those in Experiment 1 and support the hypothesis that dietary trace mineral antagonists may influence skin-fold swelling response to intradermal injection of PHA in beef cattle (Ward et al., 1993; Swenson et al., 1996).

There were ($P < .05$) treatment effects for hepatic Cu concentration over time (Figure 8). Hepatic Cu loss was greater over time ($P < .05$) for CON heifers than for 2-Way, 3-Way, HiSulf, and LoSulf heifers. Copper loss was similar for 2-Way, 3-Way, and LoSulf heifers over the 100 d. At study d 25, heifers supplemented with either 2-Way or 3-Way had greater ($P < .05$) hepatic Cu concentrations when compared to HiSulf. At study d 50, hepatic Cu tended ($P = .08$) to be greater for 2-Way heifers than for HiSulf heifers but was similar to 3-Way and LoSulf heifers, respectively. By study d 75 and continuing through study d 100, hepatic Cu concentration was similar among 2-Way, 3-Way, LoSulf and HiSulf heifers. The results of this experiment agree with those in Experiment 1 in that they indicate supplementing heifers with the sulfate forms of Cu and Zn or with combinations of inorganic and organic forms of Cu and Zn inhibited the

loss of hepatic Cu in the presence of Cu antagonists. In contrast to Experiment 1, however, there was no significant inhibition of Cu antagonism in heifers fed 50 ppm Cu as $CuSO_4$ when compared to heifers fed 25 ppm Cu as either all inorganic or a combination of inorganic/organic Cu. This suggests simply offering a trace mineral supplement with a higher concentration of Cu under these conditions did not adequately relieve severe Cu antagonism. These data also support previous studies in which organic sources of Cu fed at the same level were equally as effective in improving Cu status as Cu sulfate in cattle (Wittenberg et al., 1990; Ward et al., 1993).

Changes in liver Zn differed ($P < .05$) over time but were similar ($P > .10$) among treatments (Figure 9). These results are similar to those in Experiment 1 and further indicate the need for more reliable metabolic indicators of Zn status in beef cattle.

There were ($P < .05$) treatment effects for hepatic Mo accumulation (Figure 10). Molybdenum accumulation tended to be greater ($P = .08$) for CON heifers at d 75 when compared to supplemented heifers. By d 100, hepatic Mo accumulation was greater ($P < .0001$) for CON heifers than for supplemented heifers; these results agree with Experiment 1. Also in agreement with Experiment 1, 3-Way heifers accumulated less ($P < .05$) hepatic Mo when compared to CON, 2-Way, LoSulf, and HiSulf heifers at d 100. Hepatic Mo accumulation was similar ($P > .10$) between heifers consuming 2-Way, HiSulf and LoSulf mineral treatments throughout the experiment. These results support the idea that a combination of complex, sulfate and oxide forms of Cu and Zn interact with antagonists in such a way as to limit accumulation of hepatic Mo while conserving hepatic Cu.

Implications

In contrast to Experiment 1, Cu loss was similar in heifers fed 50 ppm Cu from $CuSO_4$ compared to heifers fed 25 ppm Cu from either combinations of inorganic and organic Cu and Zn or $CuSO_4$. In the presence of high Cu antagonists, simply increasing the concentration of Cu did not adequately improve Cu status above supplements containing half as much Cu under these study conditions. It appears there is a need for better understanding of the underlying biological mechanisms associated with plethoric supplementation of trace minerals.

As in Experiment 1, trace mineral supplements containing either all inorganic or a combination of inorganic and organic Cu and Zn reduced hepatic Mo accumulation over time. This is an important finding because systemic thiomolybdate formation is related to an available source of Mo and reduces viable Cu to tissues and cells (McDowell, 1992a). Trace mineral supplements can apparently be used to lessen the effect of Cu antagonism in beef cattle consuming Mo, S, and Fe.

In Experiment 1, d 100, beef heifers consuming the 3-Way supplement showed the least hepatic accumulation of Mo when compared to all other treatment groups. Similarly, at d 100 in Experiment 2, heifers consuming the 3-Way supplement also reduced hepatic accumulation of Mo. It should be noted, however, the time course and pattern of reduction in hepatic Mo accumulation differed between these experiments; this could be related to the different management protocols between the experiments. Nevertheless, the overall trend was the same between Experiments 1 and 2. Trace mineral supplements containing either all inorganic Cu and Zn or an inorganic/organic

combination of Cu and Zn reduced hepatic Cu loss and suppressed hepatic Mo accumulation. Furthermore, an inorganic/organic supplement containing complexed, sulfate and oxide forms of Cu and Zn limited hepatic Mo accumulation while conserving hepatic Cu most effectively. These results further indicate the need for understanding the underlying biological framework behind the interaction of combinations of inorganic and organic trace mineral supplements and nutritional mineral antagonists. Further research into strategic supplementation of combinations of inorganic and organic trace minerals may help investigators elucidate ways to overcome mineral antagonism while conserving trace mineral stores in cattle. These results also demonstrate the need for more repeatable and predictable trace mineral indices in beef cattle research.
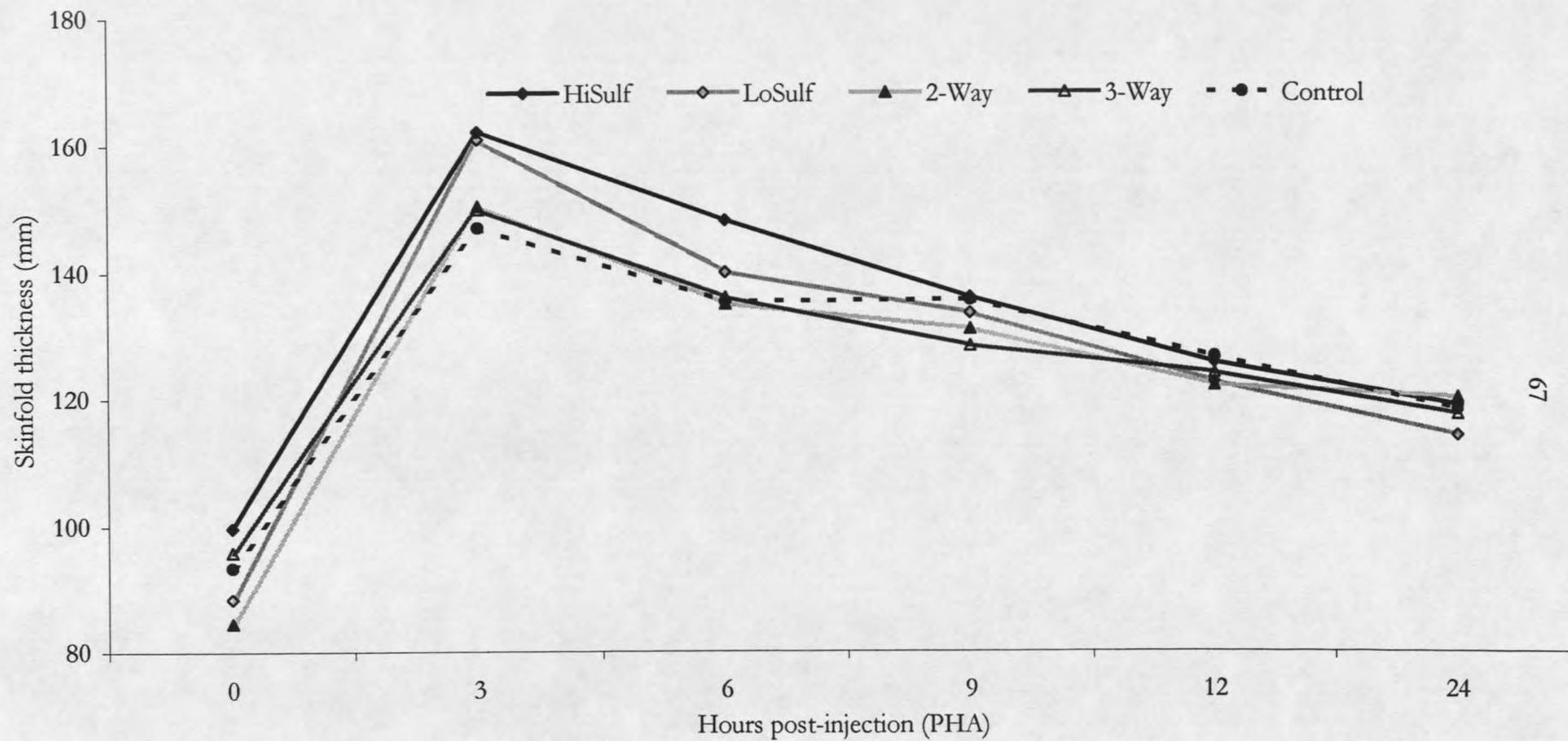
Figure 7. Skinfold swelling response to intradermal injection of PHA for heifers in Experiment 2. There were no ($P > .10$) overall treatment effects.
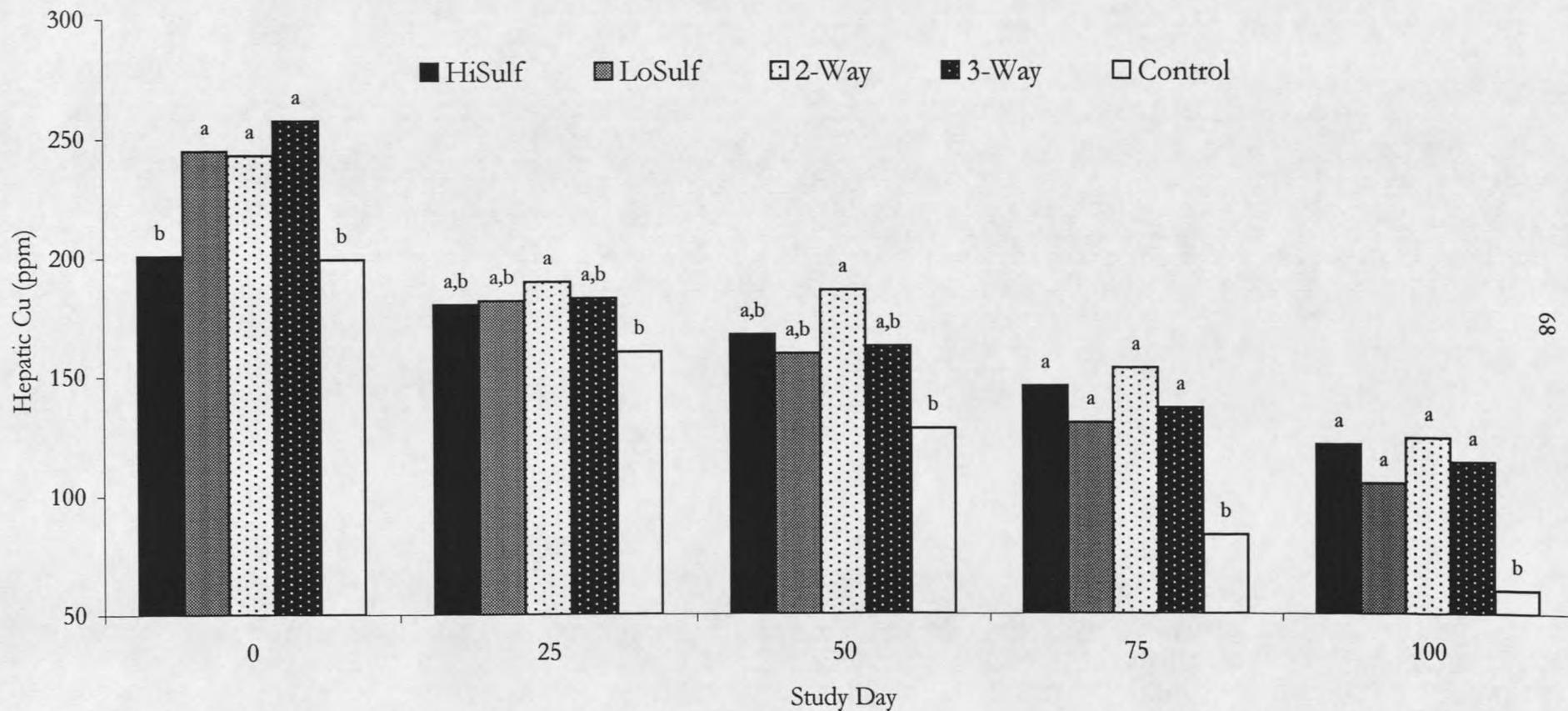
Figure 8. Hepatic Cu change for heifers in Experiment 2. Study days 25-100 are adjusted least square means using initial Cu concentration as a covariable. Study day 0 values represent means not adjusted for initial Cu. Bars with different letters differ ($P < .05$) within time.

Figure 9. Hepatic Zn change for heifers in Experiment 2. Study days 25 through 100 are adjusted least square means using initial Zn as a covariable. The overall effect of treatment was not significant ($P > .05$). Bars with unlike letters differ ($P < .05$) within time.

Figure 10. Hepatic Mo change for Experiment 2. Study days 25, 50, and 75 are adjusted least square means using initial Mo concentration as a covariable. Initial Mo was not significant ($P > .05$) as a covariable at study day 100. Study day 0 and 100 values represent means not adjusted for initial Mo. Bars with different letters differ ($P < .05$) within study day.
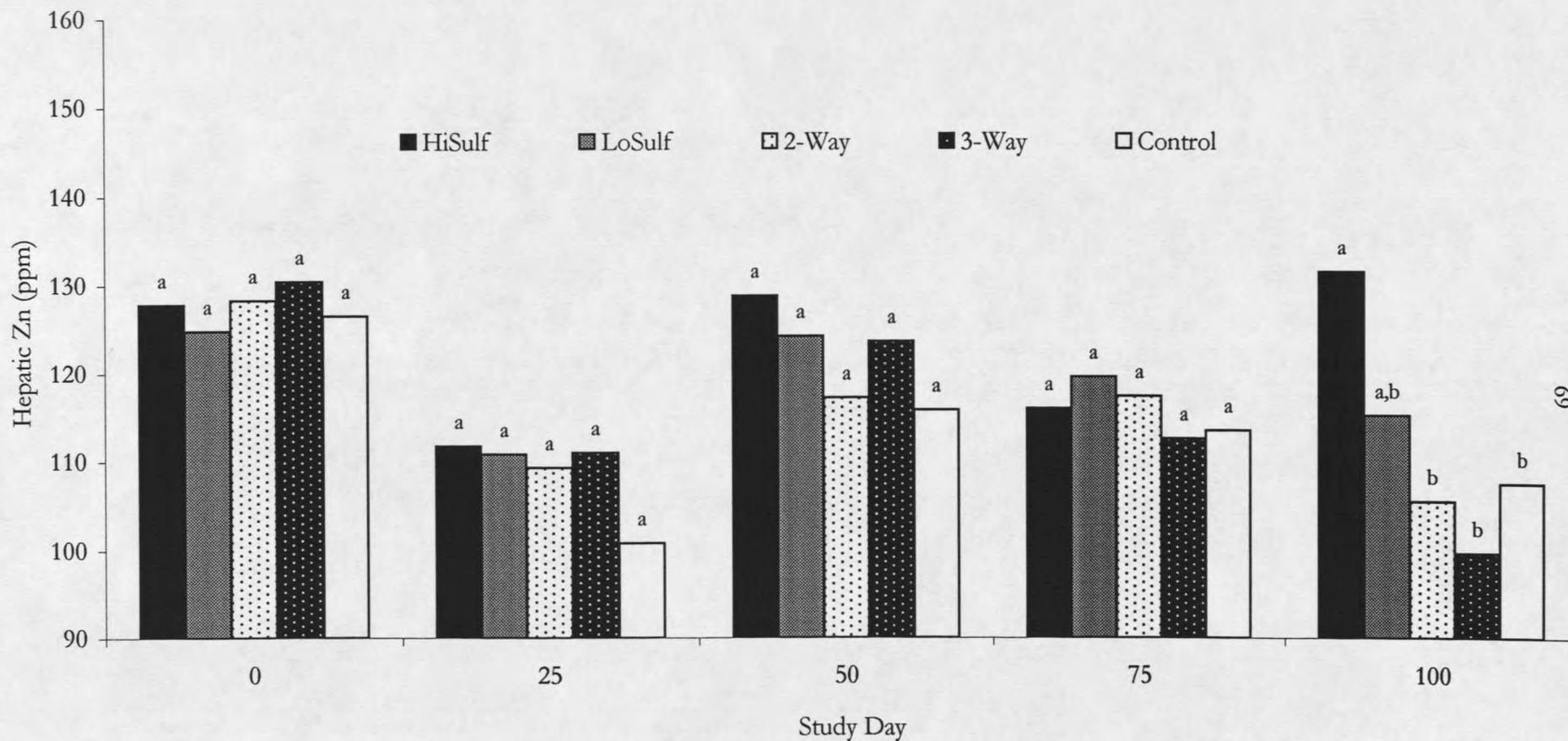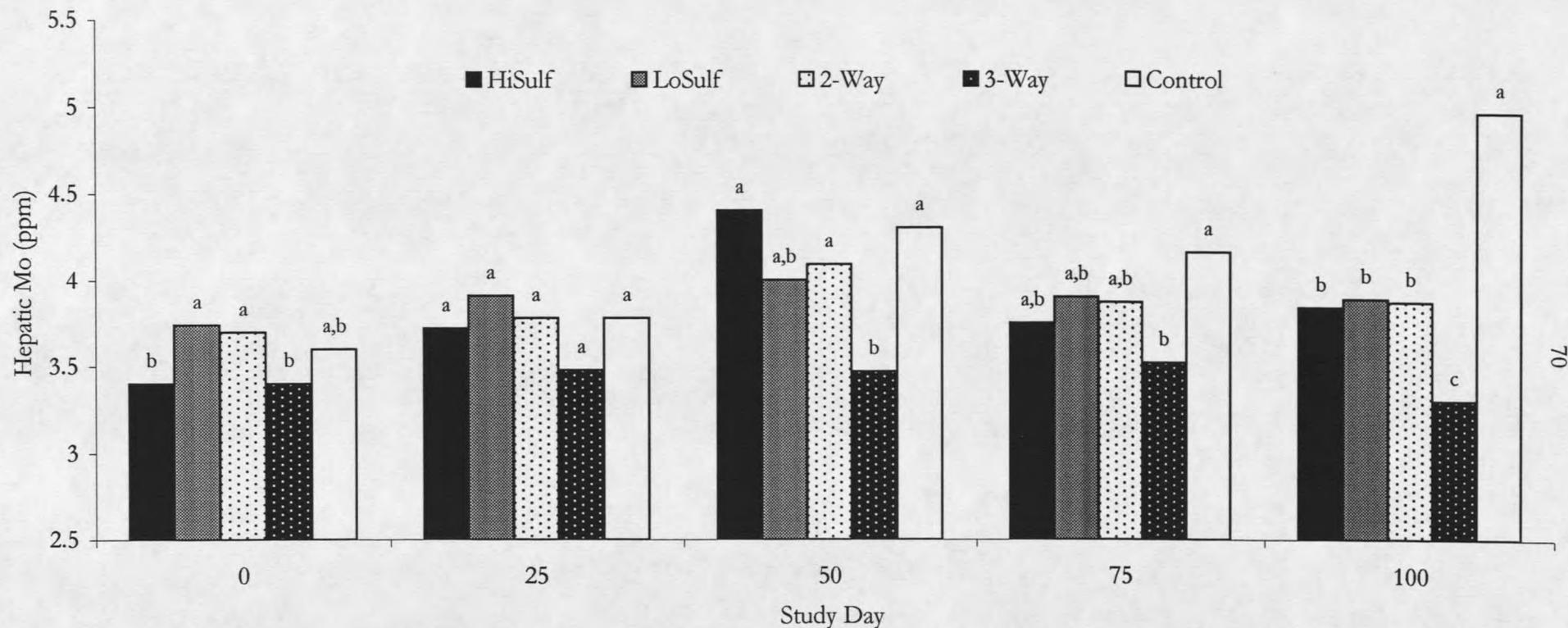
CHAPTER 5

FEEDLOT PERFORMANCE AND HEPATIC TRACE MINERAL STATUS OF
BEEF HEIFERS PREVIOUSLY CONSUMING DIETARY COPPER
ANTAGONISTS - EXPERIMENT 3

Summary

This experiment was conducted to determine if a combination of inorganic and organic

Cu and Zn affect average daily gain (ADG), cell-mediated immunity (CMI), dry matter

intake (DMI), feed efficiency (Gain/Feed; G/F), carcass characteristics (hot carcass

weight (CW), rib-eye area (REA), fat thickness at the 13th rib (FT), USDA quality grade

(QG), and USDA yield grade (YG)) or hepatic trace mineral status in feedlot heifers

previously consuming Cu antagonists. Sixty yearling, Angus x Hereford heifers (avg 372

kg BW) were stratified according to BW, initial hepatic Cu, and previous treatment (i.e.

treatments from Experiments 1 and 2) across 12 feedlot pens. For 77 d heifers were fed

diets that contained 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 both in sulfate form (AllSulf). Heifers were weighed every 14 d to

measure ADG. Phytohemagglutinin (PHA) was injected intradermally at two sites on

the neck. Response to PHA was measured at 0, 3, 6, 9, 12 and 24 h post-injection on

study d 50. Dry matter intake was measured daily on a per pen basis. Liver biopsies

were taken on d 0, 25, 50, and 75 and analyzed for trace minerals. Average daily gain, DMI, and G/F were analyzed using pen as the experimental unit. Average daily gain, DMI, G/F did not differ (P >.10) among treatments. Carcass weight (CW), rib-eye area (REA), fat thickness at the 13th rib (FT), quality grade (QG), and yield grade (YG) were similar (*P* > .10) among treatments. Skin-fold swelling response to intradermal injection of PHA did not differ (P > .10) among treatments. Hepatic repletion of Cu was not different (*P* > .10) among treatments until study d 50. From study d 50 through study d 75, heifers consuming the CON treatment accumulated less (*P* < .05) hepatic Cu than heifers consuming either the 2-Way or AllSulf supplements. Throughout the entire experiment, heifers consuming the 2-Way or AllSulf supplements accumulated similar (*P* < .05) concentrations of hepatic Cu. Changes in liver Zn differed (*P* < .05) over time but were similar (*P* > .10) among treatments. Changes in hepatic Mo differed (*P* < .05) over time but were similar (*P* > .10) among treatments until d 75. 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.

Introduction

Feedlot cattle are exposed to a variety of stress during shipping and upon arrival at a feedyard. Transportation, feed and water deprivation and immunologic challenges are some factors that can influence feedlot animal production (Cole, 1995). Proper trace mineral status may help an animal adapt to these factors, as trace minerals are involved with immunologic response and other metabolic health issues (Nockles, 1994). Dietary

Cu antagonists negatively affect hepatic stores of Cu and cattle consuming them may have compromised ability to respond to pathogens or other stress (Jones and Suttle, 1981; Xin et al., 1991). The bioavailability of Cu can be low in diets for cattle, especially when Mo, S, and(or) Fe are present in moderate to high concentrations in the diet (Davis and Mertz, 1987). Animals previously consuming high dietary antagonists have shown decreased Cu status (Ward and Spears, 1997; Ward et al, 1993; Suttle and Field, 1983), which has been shown to negatively impact systems utilizing Cu, such as growth, immunity, and reproduction. During periods of stress, immune function can be depressed (Cole, 1995) and in response to this depression, plasma Cu and ceruloplasmin ordinarily increase in an attempt to modulate inflammatory response and scavenge oxygen free-radicals (Cousins, 1985). Sufficient plasma Cu and ceruloplasmin activity are dependant on proper hepatic stores of Cu (Cousins, 1985). In Cu deficient animals, increases in plasma Cu and ceruloplasmin activity are compromised (Gengelbach et al., 1997) and it is possible that a deficiency of Cu may alter the interaction of stress and immunity.

Incoming feedlot cattle are subjected to many different stressors. Among these, transportation, feed and water deprivation and immunologic challenge are some factors that can influence feedlot animal production (Cole, 1995). Proper trace mineral status may help an animal adapt to these factors, as they are involved with immunologic response and other metabolic health issues (Nockles, 1994). Strategic supplementation of organic trace minerals upon arrival at the feedyard may offer some pharmacological benefits (Greene, 1995) or help replenish reduced mineral stores due to antagonism

(Spears, 1995). Objectives of this experiment were to determine if a combination of inorganic and organic Cu and Zn affect ADG, DMI, G/F, CW, REA, FT, QG, YG, CMI, or hepatic trace mineral status in feedlot heifers previously consuming Cu antagonists and different trace mineral supplements.

Materials and Methods

This experiment was conducted at the Montana State University Teaching and Research Livestock Experiment Station, Bozeman. Sixty, 15 month old Angus x Hereford heifers from Experiments 1 and 2 (average body weight 372 ± 6 kg) were utilized in a one-factor nested design finishing phase experiment to determine if a combination of inorganic and organic Cu and Zn influences ADG, DMI, G/F, CW, REA, FT, QG, YG, CMI, or hepatic trace mineral status in heifers previously consuming dietary Cu antagonists and different trace mineral supplements. The experiment was conducted for 77 days and all procedures and protocols were approved by the Montana State University Institutional Animal Care and Use Committee.

After shipping stress induction in Experiments 1 and 2, heifers arrived back at Bozeman and were processed as if arriving at a typical feedyard; in addition, an initial liver biopsy was taken. Liver biopsies were obtained on study days 0, 25, 50, and 75 using the same biopsy procedure outlined in Experiment 1. Liver biopsy samples were analyzed by the same laboratory using the same ICP-AES methodology explained in Experiment 1. On September 19, 1998 (Study Day 0) heifers were stratified across 12 feedlot pens by liver Cu status (hepatic Cu at study d 100, Experiments 1 and 2), pre-

shipping BW and previous treatment so that all heifers within a pen 1) had relatively similar pre-shipping body weights and pre-shipping hepatic Cu and 2) had no pen mate in the same previous treatment. Six feedlot pens had northern exposure and 6 feedlot pens had southern exposure. Pens within each side were then randomly assigned to a trace mineral treatment. Starting on study day 1 and continuing through study d 77 (December 14, 1998), all heifers were individually fed on an alternate day basis trace mineral supplements (Table 9). Total diets contained 1) basal supplement with no additional Cu or Zn (CON)' 2) 25 ppm Cu and 50 ppm Zn as a 50% organic complex, 50% sulfate form (2-Way); 3) 25 ppm Cu and 50 ppm Zn all in sulfate form (AllSulf).

Table 9. Dietary trace mineral concentrations for treatments in Experiment 3.

	Trace mineral concentration per treatment		
Mineral	2-Way	AllSulf	CON
Copper[1]	25.3	23.5	6.0
Zinc[1]	54.8	53.5	16.7

[1] mg/kg DM

Heifers received *ad libitum* access to a typical finishing feedlot ration (Table 10) for 77 days at approximately 1000 h. The total mixed finishing diet was comprised of mixed-grass prairie hay, barley, corn, and a 30% CP supplement; it was formulated to provide an approximate gain of 1.14 kg/d. Step-up rations (Table 11) were formulated on a per pen basis and fed when pen intakes of the the previous ration reached 100% *ad libitum* consumption. Treatment supplements were formulated from 2 d pen-averaged DMI sufficient to maintain target treatment levels of 25 ppm Cu and 50 ppm Zn (2-Way and AllSulf) in the total diet. Heifers were individually fed treatment supplements on

alternate days at approximately 0700 h. The specific level of trace mineral supplements reflected the 2 d total DMI on supplementation days so as to compensate for the alternate day feeding protocol. Barley from the basal diet was used as the carrier for the treatment supplements.

Table 10. Basal finishing ration composition for experiment 3[a]

Item	Mixed-grass hay	Barley	Soybean meal	Protein Supplement
DM (%)	87.0	88.0	89.1	92.0
CP (%)	9.65	13.0	44.0	33.0
ADF (%)	45.7	5.8	10.0	10.0
NE_m (Mcal/kg)	1.03	1.95	2.06	-
NE_g (Mcal/kg)	.57	1.30	1.40	-
Cu (ppm)	5.0	5.0	20.0	-
Zn (ppm)	17.0	23.0	62.0	-
Mo (ppm)	1.30	1.25	4.0	-
Fe (ppm)	89.0	138.0	147.0	-
S (% DM)	.13	.14	.19	-

[a]Based on dry weight.

To calculate ADG, heifers were weighed every 14 d at approximately 2 h before daily basal diet feeding. Initial weights were an average of two weights obtained on study days 0 and 1 respectively. Similarly, ending weights were an average of two weights obtained on study days 76 and 77, respectively. Dry matter intake was measured daily on a per pen basis and G/F was measured every 14 d.

Hepatic changes in Cu, Zn, and Mo were measured by conducting liver biopsies on study day 0, 25, 50 and 75 using surgical procedures described in Experiment 1.

Elemental analysis of the liver tissue was conducted at Michigan State University, Animal Health Diagnostic Laboratory, East Lansing, MI using inductively coupled

plasmology atomic emission spectroscopic methodology (ICP-AES) (Braselton, et al., 1997). The procedure utilized was described in Experiment 1.

Table 11. Step-up rations for experiment 3[a]

Item	Start-up[b]	Step-up 1[c]	Step-up 2[d]	Step-up 3[e]
DM (%)	86.5	87.0	87.7	88.2
CP (%)	12.0	12.7	12.6	13.1
NE_m (Mcal/kg)	1.51	1.62	1.75	1.87
NE_g (Mcal/kg)	.95	1.04	1.15	1.24

[a]Based on dry weight.
[b]Fed for 4 d
[c]Fed for 3 d
[d]Fed for 3 d
[e]Fed for 67 d

In order to stimulate a CMI response, phytohemagglutinin (PHA, Sigma Chemical, St. Louis, MO) was injected intradermally at two locations (75 μg PHA in 0.1 mL physiological saline per injection site) on the neck on d 50. The prepatory and injection procedures were done in the same fashion as in Experiments 1 and 2. After obtaining an initial skin-fold thickness, skin-fold swelling response to PHA was measured with the same micrometer calipers as in Experiments 1 and 2 (RCBS Sporting Equip. Div. of Blount, Inc., Oroville, CA) at 3, 6, 9, 12 and 24 h post-injection.

On study d 77, all heifers were shipped to Monfort Packing Plant, Greeley, CO for harvesting. Hot carcass weight (CW), rib-eye area (REA), fat thickness at the 13th rib (FT), quality grade (QG) and yield grade (YG) were obtained for each animal and recorded by Monfort personnel.

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Statistical Analyses

Experiment 3 was a one-factor nested design with treatment nested within pen. It was suspected that initial BW, initial liver Cu and previous treatments could influence the outcome of our variables of interest, therefore, heifers were stratified across the 12 feedlot pens according to initial BW, initial liver Cu and previous treatment. Treatments were then randomly assigned to pens within each side. Pen served as the experimental unit for ADG, DMI, and G/F because the basal diet was fed on a per pen basis and DMI was calculated daily for each pen. Individual animal served as the experimental unit for hepatic trace mineral status because animals were removed from their respective pens and individually fed trace mineral treatments based on pen-averaged basal diet intake. The pre-set α level for all statistical analyses in this experiment was .05. Means were separated after a significant F-value was determined and evaluated by LSD tests. Least square means and *P*-values are reported.

Overall ADG, DMI, G/F, CW, REA, FT, QG, and YG were analyzed using the General Linear Models (GLM) procedure of SAS (1996). Treatment nested within pen was used as the error term to test for treatment effects, whereas residual error was used to test for the effects of side and the interaction of treatment x side. Side and the interaction of treatment x side was not significant ($P > .10$) for any of the variables of interest and were taken out of these models and the data were reanalyzed. The reported data are from analyses using the reduced models.

Skin-fold swelling response (mm) to intradermal injection of PHA and hepatic concentrations (ppm) of Cu, Zn, and Mo were analyzed as repeated measures using the

GLM procedure of SAS (1996). Initial skinfold thickness was included as a covariable in the model for skinfold swelling response to PHA. Similarly, the models for hepatic trace mineral status included study day 0 biopsy as a covariable. Initial skinfold thickness was similar ($P > .10$) among treatments and the covariable was taken out of the model and the data were reanalyzed. Initial liver status was significant ($P < .05$) as a covariate at all time periods for all three trace elements. Least square means for these variables are reported using the covariate models. Where appropriate, least square means were compared using single degree of freedom orthogonal linear contrasts (Neter et al., 1996).

Results and Discussion

At the initiation of this experiment, heifers weighed 372 ± 6 kg. Pooled, mean initial BW were similar ($P > .10$) among treatments (Table 12). Average daily gain over the 77 d period did not differ among treatments ($P > .10$) (Table 12). Additionally, DMI and G/F were similar ($P > .10$) among treatments throughout the study (Table 12). As in Experiments 1 and 2, there was no apparent effect of form of supplementary Cu and Zn on heifer feedlot performance under these conditions. These results agree with a previous study in which Cu supplementation had no effect on ADG, DMI and G/F in beef cattle (Ward et al., 1993). Hot CW, REA, FT, QG, and YG were similar ($P > .10$) among treatments (Table 12). Little research has been done to determine the effects of trace mineral supplementation on carcass characteristics of cattle. Ward and Spears (1997) reported Cu supplemented steers produced carcasses with less ($P = .06$) backfat

and slightly larger ($P = .09$) rib-eye areas. In this experiment, it was not evident that dietary Cu form or concentration altered carcass characteristics in these beef heifers.

Table 12. Pooled initial weights, average daily gain, dry matter intake, feed efficiency, carcass weight, rib-eye area, fat thickness, quality grade and yield grade for heifers in Experiment 3[a].

	2-Way	AllSulf	CON	SEM
Initial weight (kg)	372	372	368	6.1
ADG[b]	1.06	1.0	1.02	.2
DMI[b]	9.84	10.12	9.7	.8
Feed efficiency (G/F)[b]	.107	.099	.106	.004
CW (kg)	248.5	260.2	258.6	7.8
REA (cm²)	28.6	27.9	28.2	.69
Fat thickness (cm)	.86	.92	.84	.046
Quality Grade				
Prime	5%	5%	<0>	
Choice	60%	60%	70%	
Select	35%	35%	30%	
Yield Grade				
1	5%	5%	<0>	
2	90%	95%	85%	
3	5%	<0>	15%	

[a] n=20 animals per treatment
[b] Pen averaged

Skin-fold thickness response to PHA increased significantly ($P < .05$) over time but did not differ ($P > .10$) among treatments over the 24 h observation period on study d 30 (Figure 11). Other research indicates organic trace minerals may have a positive influence on CMI in cattle (Ansotegui et al., 1995).

Hepatic Cu was affected ($P > .10$) by treatments (Figure 12). By d 50 and through d 75, heifers consuming the CON treatment accumulated less ($P < .05$) hepatic Cu than heifers consuming either the 2-Way or the AllSulf supplements. Sufficient Cu stores are

necessary for responding to pathological challenge and stress (Nockles, 1994; Cole, 1995) which are commonly incurred by incoming feedlot cattle. These data indicate that trace mineral supplements containing either all inorganic or a combination of inorganic and organic Cu and Zn at 25 ppm and 50 ppm respectively, enhanced hepatic repletion of Cu after mineral antagonism over animals receiving no supplemental Cu and Zn. Throughout the entire experiment, heifers consuming either the 2-Way or AllSulf supplements accumulated similar concentrations ($P > .10$) of hepatic Cu. Although 2-Way heifers tended to be numerically higher than the AllSulf heifers throughout the experiment, this combination of inorganic and organic Cu and Zn provided no ($P > .10$) increase in hepatic Cu repletion when compared to heifers consuming the AllSulf treatment. These results agree with previous studies in which organic forms of Cu were similar in apparent bioavailability when compared to inorganic $CuSO_4$ (Ward et al., 1993; Baker et al., 1991).

Changes in hepatic Zn differed ($P < .05$) over time but were similar ($P > .10$) among treatments (Figure 13). Hepatic Zn concentration is not a reliable indicator of overall Zn status of beef cattle (Puls, 1994) and tissue levels of Zn do not always reflect dietary Zn intakes (Underwood, 1981). It was not apparent in this experiment that organic or inorganic sources of Zn affected hepatic Zn concentration in these beef heifers under these study conditions. These results agree with previous studies in which hepatic Zn was not affected by either organic or inorganic Zn supplementation (Engle et al., 1997) and further demonstrate the need for alternative Zn status indicators in beef cattle.

There were treatment effects ($P < .05$) for hepatic Mo change (Figure 14). By d 75, heifers consuming the 2-Way treatment had lower ($P < .05$) levels of hepatic Mo when compared to CON heifers. By the end of the experiment, heifers consuming the 2-Way treatment tended ($P = .08$) to have lower hepatic Mo when compared to AllSulf heifers. These results indicate that incoming feedlot heifers supplemented with a combination of inorganic/organic Cu and Zn had an advantage over CON heifers with respect to hepatic residence time of Mo. Hepatic accumulation of Mo reduces ceruloplasmin synthesis and accumulation of Cu in tissues (McDowell, 1992a; Arthington et al., 1996). It is unknown whether this aspect was a direct result of reduced accumulation of dietary Mo, increased systemic Mo complexing by supplemented animals, or as a result of possible carryover effects of previous supplemental treatments.

Implications

In this experiment, by d 50, feedlot heifers previously consuming dietary antagonists and presently consuming trace mineral supplements containing either all inorganic or a combination of inorganic and organic Cu and Zn accumulated greater hepatic Cu compared to heifers receiving no supplemental Cu and Zn. There was no difference in hepatic Cu accumulation in heifers fed the 2-Way supplement when compared to heifers fed the AllSulf supplement. These results suggest for incoming feedlot cattle previously consuming dietary antagonists, supplements containing either all inorganic or a combination of inorganic and organic Cu and Zn replenish hepatic Cu stores more effectively than no supplemental Cu and Zn. Furthermore, there may be an advantage to

using an inorganic/organic combination to reduce hepatic Mo residence time, which may limit further systemic effects of Cu antagonists.
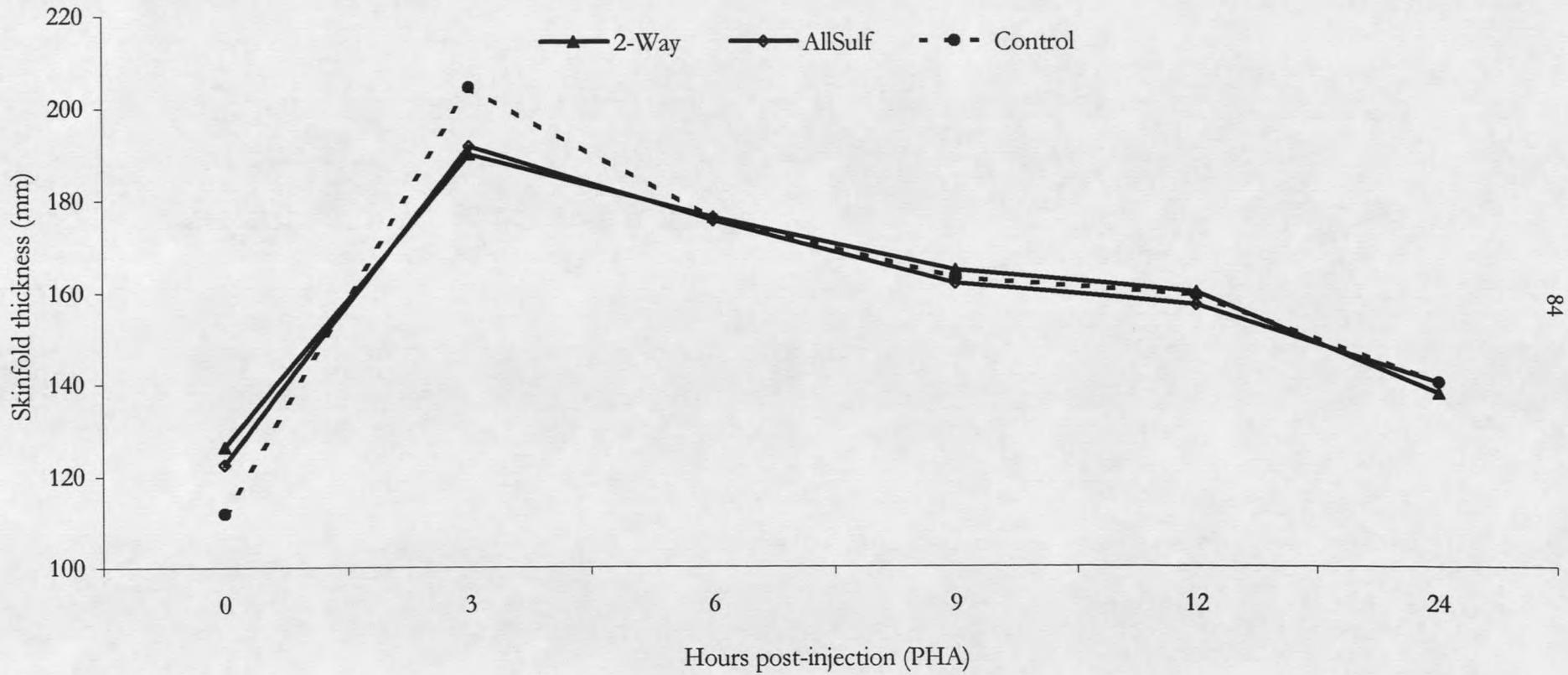
Figure 11. Skinfold welling response to intradermal injection of PHA for heifers in Experiment 3. There were no (*P* > .10) overall treatment effects.
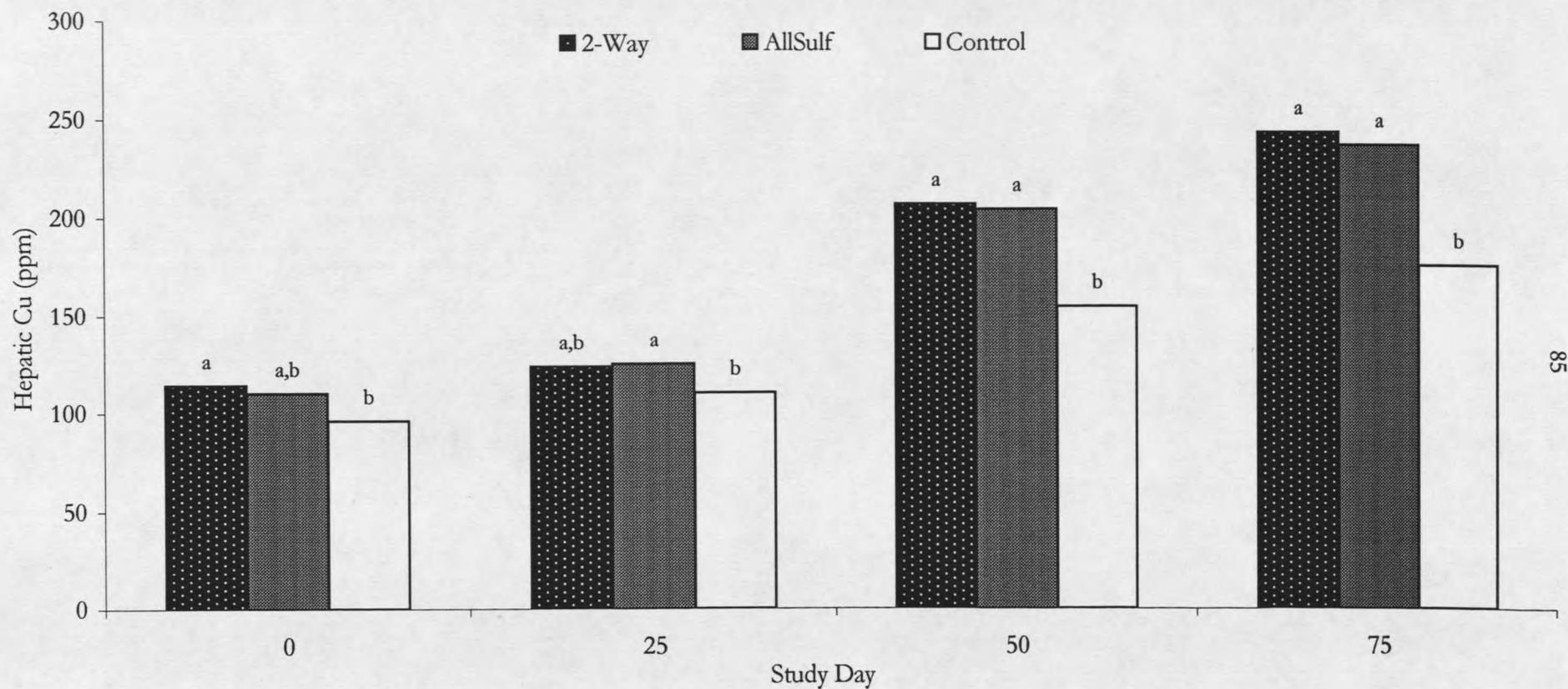
Figure 12. Hepatic Cu change for heifers in Experiment 3. Study days 25-75 are adjusted least square means using initial Cu concentration as a covariable. Study day 0 values represent means not adjusted for initial Cu. Bars with different letters differ ($P < .05$) within time.
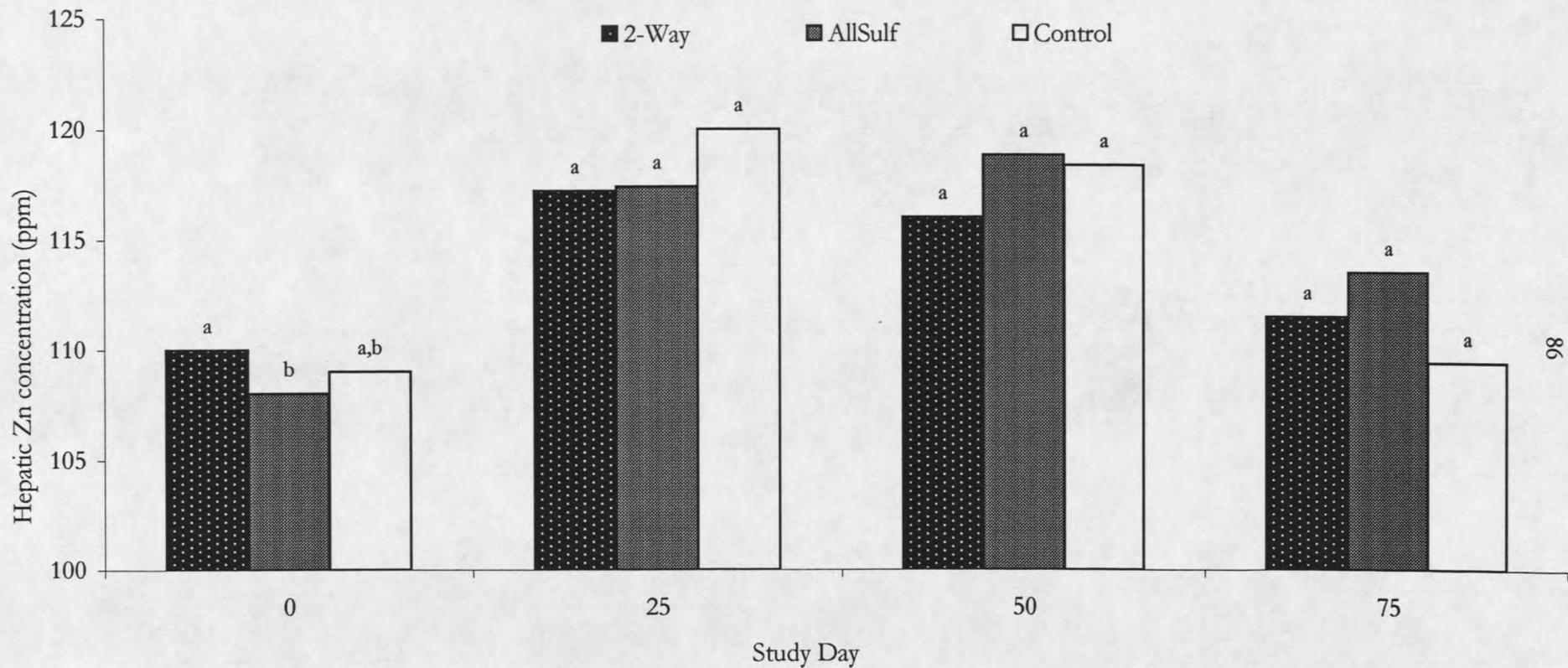
Figure 13. Hepatic Zn change for heifers in Experiment 3. The overall effect of treatment was not significant ($P >$ .10). Study days 25-75 are adjusted least square means using initial Zn concentration as a covariable. Study day 0 values represent means not adjusted for initial Zn.
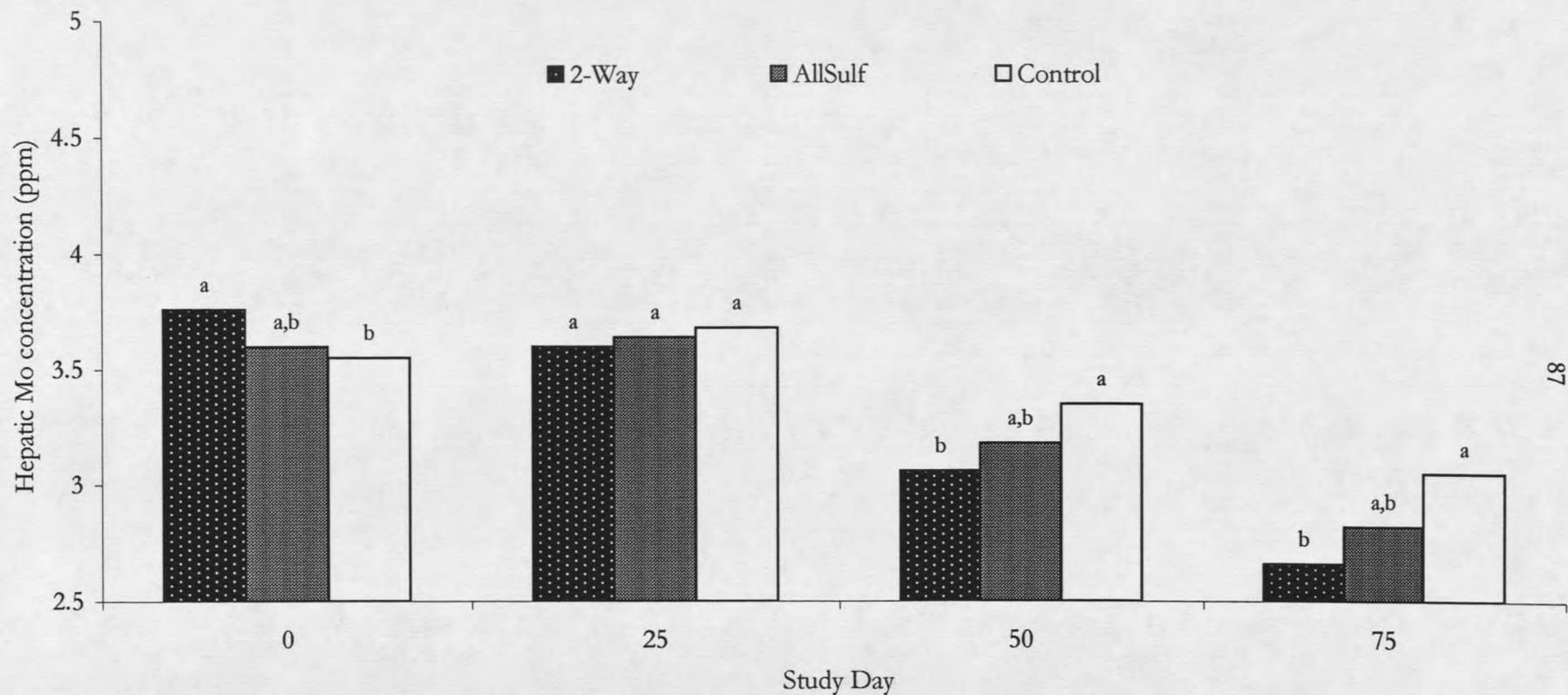
Figure 14. Hepatic Mo change for heifers in Experiment 3. Study day 25-75 are adjusted least square means using initial Mo concentration as a covariable. Study day 0 values represent means not adjusted for initial Mo. Bars with unlike letters differ ($P < .05$) within time.

CHAPTER 6

SUMMARY AND CONLUSIONS

The overall goal of these experiments was to provide information about combining inorganic and organic sources of Cu and Zn during and after trace mineral antagonism in beef cattle diets. The specific objectives were to examine possible influences of combination mineral supplements on the performance and hepatic trace mineral status of beef heifers.

The results of Experiment 1 demonstrated that a supplement containing 50 ppm Cu and 100 ppm Zn increased hepatic conservation of Cu over supplements containing half of these concentrations. This was, however, at the sacrifice of increased hepatic accumulation of Mo. This increase in accumulation of Mo would likely cause substantial systemic insoluble Cu complexes. A combination supplement containing Cu and Zn as 50% amino-acid complexes, 25% sulfates, and 25% oxides effectively relinquished the pattern of hepatic Mo accumulation while conserving hepatic Cu. The results of Experiment 2 also demonstrated that the pattern of hepatic accumulation of Mo is changed when using the 3-Way combination. However, with respect to hepatic conservation of Cu, there was no advantage to feeding a supplement containing 50 ppm Cu and 100 ppm Zn. Although the time course and patterns of hepatic trace mineral

change was dissimilar between the experiments, the overall effects on hepatic trace mineral status were the same. Trace mineral supplements containing either all inorganic or a combination of inorganic and organic Cu and Zn reduce hepatic Cu loss and suppress hepatic accumulation of Mo. When a combination of Cu and Zn 50% amino-acid complexes, 25% sulfates, and 25% oxides was supplemented, it provided the best scenario between both hepatic conservation of Cu and suppression of accumulation of Mo.

The overall objective of Experiment 3 was to examine the influence of trace mineral supplements in the absence of dietary antagonists. Results indicated that trace mineral supplements could be a useful tool in replenishing lost stores of minerals for feedlot cattle previously consuming dietary antagonists. There was an advantage to feeding a combination of 50% amino acid complex/50% sulfate Cu and Zn. The 2-Way combination supplement effectively reduced the time of hepatic residence of Mo. This supplement may provide a strategic tool in diminishing the effects of prolonged exposure to dietary mineral antagonists.

The relationship among inorganic and organic trace minerals with dietary antagonists and how they biochemically interact in beef cattle diets remains indistinct. Further understanding of the underlying biological mechanisms behind the interaction between mineral supplements and antagonists must be achieved in order to provide better nutritional management of beef cattle. It appears the idea of combining inorganic and organic minerals to lessen secondary trace mineral deficiencies warrants further scientific investigation.

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