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Effect of Supplemental Trace Mineral (Zinc, Copper, and Manganese) Source on Growth Performance, Morbidity, and Trace Mineral Status in Beef Cattle

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ABSTRACT

A series of studies were conducted to determine the effect of supplemental trace mineral source on growth performance, morbidity, and trace mineral status in growing beef cattle. The first experiment evaluated supplemental trace minerals from sulfate, organic, or hydroxy sources on growth performance and morbidity. Crossbreed beef calves were assigned to 1 of 3 treatments consisting of supplemental zinc (360 mg/d), copper (125 mg/d), and manganese (200 mg/d) from inorganic, organic, or hydroxy sources fed daily over a 42 to 45-d backgrounding phase. After removal of chronic and dead calves from the data set, trace mineral source had no effect on final or intermediate weights ($P > 0.55$) or average daily gain ($P = 0.51$). For all calves, dietary treatments had no effect on any morbidity measurements ($P \geq 0.53$). Overall, trace mineral source had no effect on total weight gain, average daily gain, or morbidity during the receiving phase in shipping stressed cattle.

A second experiment was conducted to study the effect of trace mineral source on trace mineral status, superoxide dismutase activity, and performance in beef heifers fed diets high in sulfur. Crossbreed heifers were stratified into 3 treatments consisting of 1) no supplemental trace minerals; 2) supplemental copper (55 mg/d), zinc (165 mg/d), and manganese (110 mg/d) from sulfate sources; or 3) supplemental zinc, copper, and manganese at isolevels to treatment 2 from hydroxy sources fed daily over a 55-d trial. Final and intermediate weights ($P = 0.73$), average daily gain ($P = 0.70$), and plasma copper and zinc concentrations ($P \geq 0.37$) were not affected by treatment. Liver copper concentrations on d 55 were greater for the sulfate treatment ($P > 0.004$) compared to control and hydroxy treatments, however, liver zinc concentrations were not affected ($P > 0.29$). Treatment had no effect on ($P \geq 0.36$) on total- or manganese-superoxide dismutase activity, however, a day effect ($P \geq 0.002$) was observed. Overall, trace mineral source

had no effect on growth performance, plasma mineral concentration, liver zinc concentrations, or superoxide dismutase activity. However, liver copper concentrations on d 55 were affected by trace mineral source.

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CHAPTER I

INTRODUCTION

In the United States, beef calves are often weaned between 6 and 8 months of age at which time calves are often sold through local auction barns. During this process, calves are exposed to a variety of stressors including food and water deprivation, dietary changes, and commingling with calves from other sources, potentially exposing calves to foreign pathogens. Stressors that are common during this period have been strongly implicated in the bovine respiratory disease (**BRD**) complex (Blecha et al., 1984), a multifaceted disease with major economic impacts on the beef cattle industry. In addition to expenses directly associated with mortality as well as labor and medicine to treat morbid animals, morbid cattle generally grow slower during the feedlot phase, have greater feed to gain conversions, and exhibit lighter weight and lower quality carcasses after slaughter (McNeill, 1995; Gardner et al., 1999), magnifying the potential economic consequences. Thus, researchers have explored many avenues to reduce morbidity from BRD during this period. One such avenue that has been explored is trace mineral supplementation as trace minerals play vital roles in the immune system (Wan et al., 1989; Erickson et al., 2000; Spears, 2000) as well as other roles for health and performance in beef cattle.

Mineral supplements exist in several chemical forms, including inorganic (ex. sulfates, oxides, chlorides, hydroxy, etc.) and organic (ex. amino acid complexes, metal polysaccharide complexes, etc.) forms. Considerable differences in bioavailability have been noted among different inorganic and organic mineral sources (Wedekind et al., 1992; Kegley and Spears, 1994; Spears et al., 2004). In addition to mineral source, bioavailability is also affected by other dietary components, most namely concentration of mineral antagonists in the diet. Antagonists

can bind with minerals within the rumen, forming insoluble complexes that cannot be absorbed in the small intestine.

The purpose of this series of experiments was to examine the effect of supplemental trace mineral source on growth performance, morbidity, and trace mineral status in shipping-stressed beef cattle and growing beef cattle fed diets high in a copper antagonist.

CHAPTER II

REVIEW OF LITERATURE

Overview of Minerals in Beef Cattle Nutrition

In the United States, beef calves are often weaned between 6 and 8 months of age at which time calves are often sold through local auction barns. During this process, calves are exposed to a variety of stressors including food and water deprivation, dietary changes, and commingling with calves from other sources, potentially exposing calves to foreign pathogens. Loerch and Fluharty (1999) acknowledged 5 consequences of stressors in newly arrived feedlot calves: 1) transient endocrine responses, 2) altered products of energy and protein metabolism, 3) changes in appetite and growth rate, 4) possible limited compromise of digestive and rumen function, and 5) a challenged immune system. Stressors that are common during this period have been strongly implicated in the bovine respiratory disease (**BRD**) complex (Blecha et al., 1984), a multifaceted disease with major economic impacts on the beef cattle industry. In addition to expenses directly associated with mortality as well as labor and medicine to treat morbid animals, morbid cattle generally grow slower during the feedlot phase, have greater feed to gain conversions, and exhibit lighter weight and lower quality carcasses after slaughter (McNeill, 1995; Gardner et al., 1999), magnifying the potential economic consequences. Thus, researchers have explored many avenues to reduce morbidity from BRD during this period. One such avenue that has been explored is trace mineral supplementation as trace minerals play vital roles in the immune system (Wan et al., 1989; Erickson et al., 2000; Spears, 2000) as well as other roles for health and performance in beef cattle.

Minerals required in beef cattle diets are divided into 2 categories, macrominerals and microminerals. Macrominerals, which include calcium, phosphorus, magnesium, potassium, and

sodium, are required in larger amounts by the animal with requirements being expressed as a percentage of the diet. Microminerals, also referred to as trace minerals, encompass minerals required in smaller amounts by the animal, including iron, manganese, zinc, copper, cobalt, iodine, and selenium. Micromineral requirements are expressed in milligrams per kilogram of diet. Beef cattle requirements for minerals are dependent on stage of life and level of production (NRC, 1996).

Many essential minerals can be found in sufficient concentrations in practical feedstuffs, however, certain minerals can be insufficient in diets fed to cattle, and supplementation is necessary to optimize animal performance or health (NRC, 1996). In addition, forages from certain regions of the United States have been found to be marginal or deficient in 1 or more essential trace minerals (Galyean et al., 1999; Coffey et al., 2005), thus necessitating supplementation to prevent deficiencies. Beef cattle can also obtain minerals from drinking water, soil ingestion, and atmospheric inputs (Suttle, 2010).

Minerals are responsible for many different functions within the body, all of which can be summarized into 4 broad types: structural, physiological, catalytic, and regulatory (Suttle, 2010). Under the first function, minerals can form structural components in body tissues, organs, and the skeletal system. In addition, some minerals can contribute to the structural stability of the molecules and membranes in which they are present. Secondly, minerals are present as electrolytes in body fluids and tissues to maintain osmotic pressure, acid-base balance, membrane permeability, and transmission of nerve impulses. Minerals also act as catalysts, either as components of the structure of metalloenzymes and hormones or as coenzymes within enzyme and endocrine systems. Finally, minerals function in the regulation of cell replication and differentiation.

Mineral supplements exist in many different forms. The most common mineral supplements include inorganic molecules such as sulfates, oxides, and chlorides. Minerals also exist in organic forms in which the mineral is bound to an organic molecule, typically an amino acid or carbohydrate (Kellogg and Kegley, 2002). Trace minerals are naturally present in both inorganic and organic forms in plant material. In addition, a variety of inorganic and organic forms are produced commercially for use in livestock diets. There is considerable variability in the bioavailability of different sources of inorganic and organic minerals (Wedekind et al., 1992; Kegley and Spears, 1994; Spears et al., 2004). Bioavailability is defined as the proportion of the mineral ingested that is absorbed, transported to site of action, and transformed to a physiologically active form (O'Dell, 1984). In addition to mineral form, bioavailability can also be affected by other dietary components including protein level and concentration of other minerals in the diet. To be absorbed, a mineral must be soluble in the gastrointestinal tract; however solubility in the rumen may be associated with formation of insoluble complexes that render the trace mineral unavailable in the small intestine (Kellogg and Kegley, 2002).

There are several classifications of organic trace minerals as defined by Kellogg and Kegley (2002) from the 2001 Association of American Feed Control Officials, Feed Ingredient Definitions. One form is the metal amino acid complex which results from complexing of a soluble metal salt with 1 or more amino acids. A second form results when a soluble metal salt is complexed with a specific amino acid (i.e. zinc methionine), referred to as a metal specific amino acid complex. A third organic form is a metal amino acid chelate, a product resulting from the reaction of a metal ion from a soluble metal salt with amino acids with a mole ratio of 1 mole metal to 1 to 3 moles of amino acids to form coordinate covalent bonds. Metal polysaccharide complexes result from complexing a soluble salt with a polysaccharide solution. Lastly, metal

proteinates are formed by the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein.

Assessment of Trace Mineral Status

Due to the interrelationship of metabolic processes and physiological functions, a trace mineral deficiency, especially in the early stages, has similar symptoms to deficiencies of other trace minerals as well as other dietary components such as vitamins. Therefore it is necessary to utilize appropriate assessment methods to determine which nutrient is deficient in order to correct the deficiency. One method of trace mineral status assessment is diet analysis (Kincaid, 1999). Chemical analysis of feedstuffs can provide useful supporting data to determine deficiency provided representative samples can be obtained. Data from diet analysis can determine minerals that may be marginal or deficient in concentration as well as concentrations of other minerals that may interact with the mineral of interest. However, dietary concentrations of minerals alone often are not enough to determine deficiencies as a variety of factors can affect the amount of minerals in the diet that are absorbed by the animal (Suttle, 2010).

Another method of mineral status analysis is the sampling of specific bodily tissues or fluids (Suttle, 2010). Blood measures including whole blood, plasma and serum concentrations are frequently used to assess mineral status as they are significantly correlated to nutritional status of some, but not all, minerals and are less invasive than other measures (Kincaid, 1999). However, limitations exist with blood measures as minerals are often incorporated as functional units of immature erythrocytes prior to release (Suttle, 2010). Erythrocytes have a lifespan of approximately 160 d in cattle, thus whole blood mineral concentrations change very slowly and the animal can be deficient for some time before concentrations show a deficiency (Kincaid, 1999). In addition, homeostatic mechanisms can act to limit the changes in plasma concentration

of certain minerals until endogenous reserves are depleted substantially (Miller, 1975). Finally, in the case of zinc, stress or infection can cause a redistribution of zinc in the body, leading to temporarily low plasma zinc concentrations characteristic of severe deficiency when no deficiency is present (Spears, 2002).

Liver tissue is commonly sampled for assessment of trace mineral status as it serves as a storage organ for several minerals (McDowell, 2003). Since it serves as a storage organ, liver concentrations can indicate early dietary deficiency and depletion of certain minerals before other measures (Suttle, 2010). However, obtaining liver samples is a relatively invasive and time-consuming procedure compared to other measures. More recently, measuring activity or concentrations of essential enzymes or compounds dependent on a particular mineral have been used to assess mineral status (McDowell, 2003). However, these tests can be plagued with complications from standardization as well as maintaining enzyme activity from time of sampling until analysis (Kincaid, 1999; Suttle, 2010). Finally, analysis of mineral concentration in feces, urine, saliva and appendages such as hair and hoof can be useful in determining mineral status (Suttle, 2010).

Zinc

Zinc plays an essential role in a multitude of catalytic, structural, and regulatory functions within the body (Holt et al., 2012). Zinc has been confirmed as an important component of over 50 enzymes representing all 6 International Union of Biochemistry classes of enzymes (Holt et al., 2012). Zinc is also required for the structural and functional integrity of more than 2,000 transcription factors and almost every signaling and metabolic pathway in the body is dependent on 1 or more zinc-requiring proteins (Beattie and Kwun, 2004). Zinc-binding domains comprise approximately 3 to 10% of the human genome (Cousins et al., 2006). A brief list of zinc-

dependent cofactors along with their function is presented at the end of the chapter (Table 1). Due to its rapidly proliferating cell system, the immune system is highly dependent on zinc (McDowell, 2003). Zinc influences T-cell development and function, modulation of cellular functions of cells, especially cytokine production from T and B cells, and has profound effects on lymphoid tissues including the thymus, tonsils, and lymph nodes. In addition, zinc plays a role in antioxidant defense both intracellularly and extracellularly via ZnCu superoxide dismutase, by acting with vitamin E to protect against iron-induced lipid peroxidation and by inducing metallothionein (Suttle, 2010). Zinc is also essential for normal growth rates, skin and wound healing, water and cation balance and vitamin A metabolism (McDowell, 2003).

The recommended requirement of zinc in beef cattle diets is 30 mg Zn/kg diet (NRC, 1996). Actual requirements vary according to age and growth rate as zinc absorption and growth rate decrease with age (McDowell, 2003). Dietary zinc requirements also vary depending on the criteria used to evaluate requirements. For example, it is generally recognized that zinc requirements are greater for optimal fertility compared to requirements for normal growth (McDowell, 2003). In ruminants, early stages (subclinical) of zinc deficiency lead to reduced feed intake, growth rate, and feed efficiency (McDowell, 2003). As deficiency progresses to the clinical stages, symptoms include listlessness, excessive salivation, reduced testicular growth, swollen feet with lesions, parakeratotic lesions on the legs, neck, head and around the nostrils, failure of wounds to heal and alopecia (NRC, 1996). The amount of zinc required to cause toxicity in ruminants is much greater than requirements with a maximum tolerable limit of 500 mg/kg diet (NRC, 1996).

Zinc status can be determined using several assessment methods. Plasma zinc concentrations are reduced during zinc deficiency with severely deficient diets eliciting

depressed concentrations within 36 h (Kincaid, 1999). Normal plasma zinc concentrations range from 0.8 to 1.4 mg/L with marginal levels ranging from 0.5 to 0.8 mg/L. Beef cattle are considered deficient in zinc with plasma concentrations from 0.2 to 0.4 mg/L (Kincaid, 1999). Concentrations of zinc in plasma can fluctuate with age, stress, infections, and feed restriction. In addition, plasma zinc is part of the acute phase response and is initially reduced by infection followed by elevated concentrations a few days later (Kincaid, 1999). Liver zinc concentrations are also sensitive to zinc intake, although the relationship between zinc intake and liver zinc concentrations is affected by age of the animal (Kincaid, 1999). Adequate liver concentrations of zinc range from 25 to 200 mg/kg of dry liver. Cattle are considered marginal in zinc when liver concentrations fall between 25 and 40 mg/kg of dry liver and animals are considered deficient when liver concentrations are from 40 to < 20 mg/kg dry liver (Kincaid, 1999).

Copper

Within the body, copper is present in and essential for the activity of multiple enzymes, cofactors, and reactive proteins (Suttle, 2010). The metal centers of these proteins bind oxygen and produce water, superoxide, or hydrogen peroxide along with other organic compounds (Prohaska, 2012). These copper-containing proteins, also referred to as cuproenzymes, are involved in functional processes such as energy production, iron utilization, maturation of the extracellular matrix, activation of neuropeptides, and neurotransmitter synthesis (McDowell, 2003). Like zinc, copper plays vital roles in the immune system. Copper metabolism affects T and B cells with deficiency affecting both number of cells as well as impairing cell function (McDowell, 2003). Copper, through action of ZnCu superoxide dismutase, affects both neutrophils' and macrophages' ability to kill foreign cells via the respiratory burst (Suttle, 2010). In addition, ZnCu superoxide dismutase along with ceruloplasmin, the predominant copper

protein in plasma, serve as antioxidants by scavenging free radicals. Finally, Prohaska et al. (1983) reported an impaired humoral immune response in copper deficient mice. The number of copper-dependent enzymes and proteins is second only to zinc-dependent proteins; therefore all enzymes will not be discussed in the text. A brief list of cuproenzymes as well as functions is presented at the end of the chapter (Table 2).

The recommended requirement for copper in beef cattle diets is 10 mg Cu/kg diet (NRC, 1996). However, actual copper requirements can vary greatly depending on dietary factors such as iron, molybdenum, sulfur, zinc, and protein source (McDowell, 2003). Molybdenum and sulfur can each bind with copper on their own in the rumen to form complexes that are either poorly absorbed or not absorbed at all (Spears, 2002). This antagonistic action is greater when both molybdenum and sulfur are present at high levels in the rumen resulting in the formation of thiomolybdates. Thiomolybdates can cause copper to be tightly bound to plasma albumin and not available for biochemical functions (Spears, 2002). High levels of zinc and iron also depress copper absorption (McDowell, 2003). Copper requirements can also vary by breed as differences have been reported in biliary excretion of copper as well as susceptibility to copper deficiency between breeds of beef cattle (Goonerate et al., 1994; Ward et al., 1995).

Copper deficiency causes poor growth and appetite (though not as conspicuous as seen in zinc deficiency), anemia, diarrhea, cardiac failure, fragile bones, depigmentation of hair, and temporary infertility (McDowell, 2003). The maximum tolerable limit for copper in the diet is estimated to be 100 mg/kg (NRC, 1996). This upper limit is dependent on concentrations of sulfur, molybdenum, iron, and zinc as well as age of animal as younger cattle are more susceptible to copper toxicity than adult cattle (NRC, 1996).

Assessment of copper status can be examined using several methods. The most sensitive indicator of copper status in beef cattle is liver copper concentration. Liver copper concentration in ruminants is correlated to the bioavailable copper in the diet (Kincaid, 1999). In addition, when copper intake is below physiological need copper stored in the liver is released, thus a reduction in liver copper is a sign of low copper intake. Normal liver copper concentration ranges from 125 to 600 mg/kg dry liver with animals considered marginal in copper when liver concentration falls between 33 and 125 mg copper/kg dry liver. When liver copper falls below 33 mg/kg dry liver, an animal is considered deficient (Kincaid, 1999). Copper concentration and ceruloplasmin activity in plasma can also be used to assess copper status, although concentrations of copper and ceruloplasmin activity is not consistently reduced until liver copper is < 40 mg/kg (Kincaid, 1999). In addition, plasma copper can also be affected by estrus, infection, breed, and concentrations of copper antagonists, thus these must be taken into consideration (Kincaid, 1999). Normal plasma levels range from 0.7 to 0.9 mg/L with deficient plasma levels being < 0.5 mg/L (Kincaid, 1999).

Manganese

Like other trace minerals, manganese functions as both an enzyme activator and as a constituent of enzymes (McDowell, 2003). Manganese-containing enzymes include pyruvate carboxylase, arginase, and a third form of superoxide dismutase, Mn-superoxide dismutase (Spears, 2002). Mn-superoxide dismutase acts similarly to ZnCu-superoxide dismutase as it functions to scavenge free radicals; however, Mn-superoxide dismutase is only present in the mitochondria versus in the intracellular and extracellular matrixes (Suttle, 2010). Manganese can activate a number of hydrolases, kinases, transferases, and decarboxylases; however, magnesium can partially substitute for manganese with little to no loss of enzymatic activity for most

enzymes (McDowell, 2003). Enzymes that are manganese-specific for activation include glycosyltransferases, glutamine synthetase, farnesyl pyrophosphate synthetase, and phosphoenolpyruvate (Nielsen, 2012). Manganese-containing and activated enzymes play roles in bone growth, reproduction, lipid and carbohydrate metabolism, cell function and structure, and immune and brain function (McDowell, 2003).

The manganese requirement for growing and finishing cattle is approximately 20 mg Mn/kg diet (NRC, 1996). The requirement for reproduction is greater than for growth and skeletal development and thus the requirement for breeding cattle is 40 mg/kg (NRC, 1996). Manganese requirements can be affected calcium and phosphorus levels in the diet with greater levels resulting in increased manganese requirements (McDowell, 2003). Manganese deficiency in ruminants causes ataxia, decreased soft tissue and skeletal growth, weak and abnormally shaped bones, reduced milk production, and reproductive disorders (McDowell, 2003; Suttle, 2010). The maximum tolerable limit for manganese in diets has been set at 1,000 mg/kg of diet; however, manganese has been fed at levels equal or greater than the maximum tolerable limits for extended periods of time without adverse effects, making manganese toxicity not likely to occur (NRC, 1996; Spears, 2002).

Compared to copper and zinc, assessment of manganese status in beef cattle is relatively difficult and often requires assessment of more than one criterion to diagnose a manganese deficiency (McDowell, 2003). The liver efficiently removes manganese from the plasma, but manganese is excreted from the liver endogenously via the bile and accumulations of manganese in the liver do not reflect dietary accumulations (Kincaid, 1999). Liver concentration of manganese is considered adequate at >13 mg/kg dry liver and considered deficient when < 7 mg/kg dry liver (Kincaid, 1999). Whole blood concentration represents a second assessment

method for manganese status. Manganese is considered deficient when whole blood concentration is below 20 μg/L and considered adequate from 70 to 200 μg/L (Kincaid, 1999). It has been concluded that manganese deficiency in ruminants can be best diagnosed using a combination of liver (< 6 mg/kg) and diet (< 20-40 mg manganese/kg diet) analysis (McDowell, 2003).

Trace Mineral Supplementation from Different Sources

As stated previously, trace minerals play a vital role in immune function as well as growth and performance of beef cattle. Numerous sources, concentrations and combinations of various trace minerals and their effects on immunity, growth, and performance measures have been evaluated in the past. Due to the vast amount of research in this area, the following discussion will be limited to studies which investigated the effect of zinc, copper, and manganese, or combinations of these minerals in growing beef cattle. With the exception of potassium, actual mineral requirements of stressed beef calves do not seem to be greater than those of unstressed calves (Galyean et al., 1999). However, decreased feed intake that accompanies stress necessitates the need to increase concentrations of minerals in the receiving diet in order to meet requirements (NRC, 1996).

Zinc

Potentially low zinc concentrations for grazing cattle have been observed by researchers in various regions, thus, zinc deficiency may be common in practice (Galyean et al., 1999). Several studies have shown supplemental zinc to have positive effects on both health and growth performance of cattle. Kegley and coworkers (2001) reported an increase in average daily gain (**ADG**) from d 15 to 28 in calves supplemented with 360 mg zinc/d from sulfate and amino acid complex sources versus a negative control diet containing 25 mg zinc/kg DM. The study also

observed a greater response to phytohemagglutinin injection in zinc supplemented calves versus control calves. Finally, it was reported that calves supplemented with zinc-amino acid complexes exhibited a greater antibody response to a second vaccination for bovine respiratory syncytial virus than control or zinc sulfate supplemented calves. However, Kegley and coworkers (2001) found similar ADG among treatments for the entire 28 d trial. Engle et al. (1997) also reported a greater response to phytohemagglutinin injection in calves fed 40 mg/kg of zinc versus calves fed 17 mg zinc/kg DM with no changes in plasma or liver zinc concentrations. The investigators concluded that immune response may be decreased before functional zinc deficiency symptoms are present. Mandal et al. (2007) reported greater cell mediated and humoral immune responses in bulls supplemented with zinc propionate at 35 mg zinc/kg diet versus bulls supplemented with equal levels of zinc sulfate. Spears and Kegley (2002) observed greater ADG in steers supplemented with 25 mg/kg DM of zinc regardless of source during an 84-d growing phase versus control calves receiving basal diets with 33 mg/kg DM of zinc. During the finishing phase of the same study, steers supplemented with zinc from either of 2 zinc proteinate sources exhibited a tendency for greater ADG and gain to feed ratios (**G:F**) than calves supplemented with zinc oxide at equal levels (Spears and Kegley, 2002).

Despite the fact that zinc supplementation has been shown to increase immunity and growth performance in cattle, not all studies observed similar results. Nunnery et al. (2007) observed greater body weights and G:F on d 35 of the receiving period in control heifers fed diets containing 52.5 mg/kg DM zinc versus heifers that received similar diets with 75 mg/kg of DM supplemental zinc from sulfate, methionine, or propionate sources. During the finishing phase of that same study, the authors reported no difference in dry matter intake (**DMI**) or ADG between treatments, although control heifers tended to have lower G:F than calves that received

supplemental zinc which the authors attributed to lack of supplemental zinc for an extended period of time (Nunnery et al., 2007). Nunnery and coworkers (2007) also reported similar immune responses from ovalbumin injection on d 0 and 14 across treatments. Kincaid and others (1997) reported no difference in cell-mediated immunity in Holstein heifer calves supplemented with various levels of zinc oxide, zinc methionine, or zinc lysine. In a study during which gain differences were observed due to zinc supplementation, Spears and Kegley (2002) observed no differences in cell-mediated or humoral immune responses in growing steers supplemented with zinc oxide, 2 types of zinc proteinate, or control calves. Malcolm-Callis and colleagues (2000) observed no difference in feedlot performance among steers supplemented with inorganic or organic zinc.

Copper

As is the case with zinc, both supplemental copper concentration and source have been studied extensively with mixed results, as has been observed in zinc research. Arthington et al. (2003) observed a tendency for greater ADG as well as an increase in ceruloplasmin in heifers supplemented with 100 mg/d (equaling approximately 10 mg copper/kg DM assuming a DMI of 2.5% BW) copper in molasses-based supplements from sulfate or amino acid complex sources compared to controls. In a second experiment that utilized steers receiving supplemental copper from either an organic at 10 mg/kg, tribasic chloride at 10 mg/kg or 30 mg/kg, or 30 mg/kg copper from 50:50 ratio of organic and tribasic chloride sources in molasses supplements, Arthington et al. (2003) reported lower DMI in calves supplemented with 10 mg/kg organic copper with similar ADG across all treatments. Engle and Spears (2000) observed similar ADG among growing steers individually fed a corn silage-based diet containing 10.2 mg copper/kg DM and supplemented with various levels and sources of copper. Treatments included control

(no supplemental copper), sulfate (20 or 40 mg copper/kg DM), citrate (20 mg copper/kg DM), proteinate (20 mg copper/kg DM), or tribasic chloride (20 mg copper/kg DM) sources. The authors also observed an increase in liver copper in steers that received supplemental copper versus control steers. However, in the finishing phase of the same study, Engle and Spears (2000) reported a decrease in ADG, feed intake and G:F in steers supplemented with copper versus control steers. Cheng et al. (2011) reported increased plasma superoxide dismutase in finishing sheep fed a basal diet containing 6.74 mg copper/kg DM and supplemented with 10 or 20 mg copper/kg DM from either copper lysine or tribasic copper chloride compared to control sheep that received no supplemental copper.

With the antagonistic interaction of molybdenum and sulfur in the rumen, many studies have investigated the effects of supplemental copper in diets along with supplemental molybdenum and sulfur. Arthington and Spears (2007) examined the effect of supplemental copper from either sulfate or hydroxy sources in growing heifers and reported similar ADG among treatments. The authors did report a decline in liver copper when supplements were administered via molasses-based diets, in spite of copper being supplemented at approximately 3 times greater than NRC (1996) requirements. These results are similar to those reported by Arthington and Pate (2002). In these experiments, the authors also fed supplemental copper in corn-based diets with similar levels of copper antagonists to the molasses-based diets. However, in contrast to the molasses-based diet, cattle receiving corn based diets experienced increased concentrations of liver copper. Thus, Arthington and Spears (2007) concluded that corn- and molasses-based supplements appear to affect copper metabolism differently which may affect voluntary forage DMI. Arthington and colleagues (1996) studied the effect of molybdenum-induced copper deficiency on acute-phase protein concentrations, superoxide dismutase activity

and leukocyte numbers in beef heifers inoculated with bovine herpesvirus-1 (**BHV-1**). In this study, control heifers were fed a diet containing 8 mg copper/kg DM with molybdenum-supplemented heifers receiving the same diet with added molybdenum to obtain a 2.5:1 molybdenum to copper ratio and sulfur at 0.3% of the diet. After 129 d, molybdenum-supplemented heifers were considered deficient and all heifers were inoculated with BHV-1. Overall, Arthington and colleagues (1996) concluded that copper deficiency alters acute-phase protein response and may affect lymphocyte responsiveness to mitogen stimulation.

Manganese

The effect of supplemental manganese on performance of growing cattle has also been investigated, however, not to the extent of copper and zinc. Therefore, relatively little research has been published regarding the subject. Legleiter et al. (2005) reported similar ADG, DMI, G:F, and final weights during growing and finishing phases when manganese was supplemented at 0, 10, 20, 30, 120, or 240 mg/kg diet from manganese sulfate to diets containing 29 mg manganese/kg DM (growing diet) or 8 mg manganese/kg DM (finishing diet). Similar results were reported by Hansen and colleagues (2006) in growing heifers when manganese was supplemented to a diet containing 15.8 mg manganese/kg DM at 0, 10, 30 or 50 mg/kg of DM from manganese sulfate.

Combinations of trace minerals

With physiological pathways often being influenced by more than 1 trace mineral and metabolism of minerals often interrelated, it makes logical sense to supplement trace minerals in combination with each other, especially when dealing with cattle of unknown trace mineral status. Various combinations of separate trace minerals, concentrations and sources have been studied expansively with varying results. Kegley and coworkers (2012) investigated the effects

of supplemental zinc (360 mg/d), copper (125 mg/d), manganese (200 mg/d), and cobalt (12 mg/d) from either sulfate or amino acid complex sources in newly received male calves over a 42-d backgrounding phase. The authors observed increased final weights and ADG as well as a tendency for a decrease in percentage of calves that received a second antibiotic treatment in calves that were supplemented with amino acid complexed trace minerals. Kegley et al. (2012) also observed an increase in the antibody response to infectious bovine rhinotracheitus virus vaccination in calves supplemented with sulfate sources. However, Sharmen et al. (2008) reported no differences in ADG in newly received steers supplemented with sulfate or amino acid complexed trace minerals at levels equal to Kegley et al. (2012) over a 28-d receiving period. Sharmen et al. (2008) also investigated the effects of trace mineral source on morbidity and reported similar results across all treatments. The authors did however report a tendency for an increase in percentage repulls (defined as when an animal was treated more than once for morbidity) in calves supplemented with amino acid complexes. Rhoads and coworkers (2003) concluded that source and concentration above NRC recommendations of copper, zinc, manganese and cobalt had minimal effects on performance and immunity in crossbred steers during both growing and finishing periods. George and coworkers (1997) investigated the effect of zinc (106 mg/kg), copper (37 mg/kg), manganese (58 mg/kg), and cobalt (7 mg/kg) from sulfate (1x amount) or amino acid complex sources (1x amount or 3x amount for first 14-d followed by 1x amount) on feedlot performance and immunity in stressed heifers. For the 3x/1x treatment, the authors observed a 17.2% reduction in incidence of respiratory disease along with significant improvements in primary and secondary humoral, and cell-mediated immune responses compared to the sulfate treatment. However, no differences in DMI, ADG or feed efficiency were observed among treatments in the study. Dorton and coworkers (2006) examined

the effects of trace mineral supplementation (360 mg/d zinc, 200 mg/d manganese, 125 mg/d copper, and 12.5 mg/d cobalt) and source in steers beginning 30-d post weaning on farm backgrounding period followed by a 28-d feedlot receiving period. Control diets contained 45 mg zinc/kg DM, 16 mg copper/kg DM, and 33 mg manganese/kg DM (backgrounding period) and 51, 16, and 28 mg/kg DM of zinc, copper and manganese (receiving period). The authors observed no difference in ADG during the 30-d post weaning period but reported a greater ADG for calves receiving organic trace minerals during the 28-d feedlot receiving period versus calves supplemented with inorganic trace minerals. No differences in morbidity or mortality rate were observed during either period (Dorton et al., 2006).

With the advent of trace minerals in injectable forms, several researchers have investigated the effect of injectable trace minerals on subsequent health and performance of cattle. Richeson and Kegley (2011) investigated the effect of 2 different injectable trace minerals containing zinc, manganese, copper, and selenium on health and performance of highly stressed, newly received beef heifers. The researchers observed greater ADG and DMI as well as improved G:F in heifers receiving trace mineral injections compared to control calves. Bovine respiratory disease morbidity rates were reduced in calves receiving one of the formulations compared to control calves with the second formulation being intermediate. Finally, Richeson and Kegley (2011) noted a lower antibiotic cost for cattle receiving trace mineral injections compared to the control treatment. Arthington and Havenga (2012) investigated the effect of injectable zinc, copper, manganese, and selenium administered at the time of vaccination on humoral immune response to a multivalent vaccine in beef calves. The authors reported an increase in neutralizing antibody titers to bovine herpes virus-1 on d 14, 30, and 60 after vaccination in calves receiving trace mineral injections compared to saline injected control

calves. However, there was no effect of treatment on antibody titer response to either bovine viral diarrhea virus type-1 or type-2 observed in the study (Arthington and Havenga, 2012). Arthington et al. (2014) investigated the effects of trace mineral injections on performance and trace mineral status of pre- and postweaned beef calves. In 1 of 3 experiments included in the publication, Arthington et al. (2014) observed greater ADG and porcine red blood cell (**PRBC**) antibody titers after PRBC injection in heifers injected with trace minerals versus saline injected heifers. In a second experiment, Arthington et al. (2014) reported a greater acute phase protein concentration in heifers that were injected with trace minerals at birth, 100 and 200 d of age and upon arrival to the feedlot versus saline injected calves after being transported 1,600 km to a feedlot post weaning.

As is evident in the previously reviewed literature, trace mineral research is plagued by variability in response to both levels and source. A portion of this variability can be attributed to factors that affect requirements and bioavailability of trace minerals, which have been reviewed by Kegley et al. (2012). Therefore, research in this area continues in an attempt to gain clarity about the effects of trace mineral concentration and source in shipping stressed and growing cattle.

Objectives

Two experiments were conducted to determine the effects of trace mineral source on growth performance and immunity in growing cattle. The first experiment examined the effects of supplemental zinc, copper, and manganese from sulfate, amino acid complexed, and hydroxy sources on growth performance, morbidity, and antibody titer response to vaccination in newly received, shipping stressed cattle. The second experiment was conducted to determine the effect

of supplemental zinc, copper and manganese from sulfate or hydroxy sources on growth, trace mineral status, and superoxide dismutase status in growing beef heifers fed a high sulfur diet.

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Table 1. Partial list of zinc metalloenzymes and function found in mammalian tissues

Enzyme	Function
Alcohol dehydrogenase	NAD^+-linked interconversion of alcohol to aldehyde
Alkaline phosphatase	Freeing phosphate from bound forms
Carbonic anhydrase	Facilitation of CO_2 transport
Carboxypeptidases A and B	Hydrolysis of C-terminal amino acids from polypeptides
Collagenase	Degradation of collagen fibrils
Leucine aminopeptidase	Liberation of amino acids from N- terminal end of proteins and polypeptides
Mannosidase	Hydrolysis of mannose
Superoxide dismutase	Destruction of free radical O^{2-}

Source: Underwood and Suttle (1999).

Table 2. Mammalian copper-dependent enzymes

Enzyme	Function
Amine oxidases	Oxidative deamination
Ceruloplasmin	Fe^{2+} oxidation
Cytochrome c oxidase	Oxygen electron transfer
Dopamine-β-monooxygenase	Norepinephrine synthesis
Extracellular superoxide dismutase	Superoxide disproportionation
Hephaestin	Fe^{2+} oxidation
Lysyl oxidase	Elastin cross-linking
Peptidylglycine α-amidating monooxygenase	Peptide C-terminal α-amidation
Superoxide dismutase 1	Superoxide disproportionation
Tyrosinase	Melanin synthesis
Zyklopen	Fe^{2+} oxidation

Source: Prohaska (2012).

CHAPTER III

Supplemental trace minerals (zinc, copper, and manganese) as sulfates, organic amino acid complexes, or hydroxy trace mineral sources for shipping-stressed calves

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Abstract

Crossbreed calves (n = 350; average BW 240 ± 1 kg) were obtained from regional livestock auctions. Within each set (n = 4), calves were stratified by BW and arrival gender into 1 of 8 0.42-ha pens (10 to 12 calves/pen). Pens were randomly assigned to 1 of 3 treatments consisting of supplemental Zn (360 mg/d), Cu (125 mg/d), and Mn (200 mg/d) from inorganic (zinc sulfate, manganese sulfate, and copper sulfate; n = 2 pens/block), organic (zinc amino acid complex, copper amino acid complex, and manganese amino acid complex; Availa-4, Zinpro Corp., Eden Prairie, MN; n = 3 pens/block) and hydroxy (Intellibond-Z, Intellibond-C, and Intellibond-M; Micronutrients, Indianapolis, IN; n = 3 pens/block) sources. During the 42 to 45-d backgrounding period calves had ad libitum access to bermudagrass hay, and were fed grain-grain by-product supplements that served as carrier for the treatments. After removal of chronic (n = 6) and deceased (n = 1) calves from the data set, trace mineral source had no effect on final or intermediate weights ($P > 0.55$) or ADG ($P = 0.51$). With all calves included in the analysis, dietary treatments had no effect on the number treated once ($P = 0.93$), twice ($P = 0.71$), or 3 times ($P = 0.53$) for bovine respiratory disease, or on the number of calves classified as chronic ($P = 0.55$). Based on these results, trace mineral source had no effect on total weight gain, ADG, or morbidity during the receiving phase in shipping stressed cattle.

Key words: beef cattle, copper, manganese, trace mineral, zinc

Introduction

In the beef cattle industry, calves are often weaned between 6 and 8 mo of age. At or soon after weaning, calves are often sold through local auctions barns during which time they are exposed to a variety of stressors, including food and water deprivation and potentially dramatic dietary changes from forage- to concentrate-based diets. Additionally, calves from multiple

sources are typically commingled after purchase, thus potentially exposing calves to foreign pathogens. Stress experienced by calves during transportation and weaning increases their susceptibility to infection (Breazile, 1988). In addition to medical costs due to morbidity, morbid cattle in general grow slower during the feedlot phase, are less efficient at converting feed to gain, and have both lighter weight and lower quality carcasses after slaughter (McNeill, 1995; Gardner et al., 1999). Several factors can affect immune function, one of those being trace mineral status (Wan et al., 1989; Erickson et al., 2000; Spears, 2000). However, different sources of trace minerals may vary in price and have been shown to differ in bioavailability (Wedekind et al., 1992; Kegley and Spears, 1994; Spears et al.. 2004). In addition, Kegley and coworkers (2012) reported an increase in growth performance in calves supplemented with amino acid complexed trace minerals compared to inorganic sulfate trace minerals. However, trace minerals from hydroxy sources have not been evaluated as a trace mineral supplement in shipping stressed cattle. Therefore, our objective was to evaluate the effect of trace mineral supplementation from sulfate, organic amino acid complex or hydroxy sources on growth performance, morbidity, and immune response to bovine viral diarrhea virus vaccination in newly-received stocker cattle.

Materials and Methods

Three hundred-fifty crossbreed beef calves (89 heifers, 129 steers, and 132 bulls; 240 ± 1 kg BW) were obtained from regional livestock auctions and were shipped to the University of Arkansas Beef Cattle Facility at Savoy. Calves arrived in 4 shipment sets (block) with arrival dates of February 8 (n = 87), March 1 (n = 88), May 10 (n = 89), and September 26, 2013 (n = 86). Upon arrival, calves were tagged in the left ear with a unique identification number, weighed, ear notched, and housed overnight in a holding pen with access to hay and water. Ear notches were sent for persistent infection with bovine viral diarrhea virus (**PI-BVDV**) testing

(Cattle Stats, LLC, Oklahoma City, OK) within 48 h of cattle arrival with no calves testing positive for PI-BVDV. The following morning, calves were administered respiratory (Pyramid 5, Boehringer Ingelheim Vetmedica, Ridgefield, CT) and clostridial (Covexin 8, Intervet, Inc., Omaha, NE) vaccinations, dewormed (Ivomec Plus, Merial Limited, Duluth, GA), and bulls were castrated by banding (California Bander, Inosol Co. LLC, El Centro, CA). All animals were branded with a hot iron on the right hip and weighed.

Within each block of calves, animals were stratified by BW and arrival gender into 1 of 8 pens (10 to 12 calves/pen). Pens were assigned randomly to treatment. Calves were housed on 0.42-ha grass paddocks. Calves were fed grain-grain by-product supplements (Tables 1 and 2) that served as carriers of mineral treatments. Treatments consisted of supplemental zinc (360 mg/d), copper (125 mg/d), and manganese (200 mg/d) from sulfate (n = 2 pens/block), organic amino acid complex (Availa-4, Zinpro Corp., Eden Prairie, MN; n = 3 pens/block), and hydroxy (Intellibond, Micronutrients, Inc., Indianapolis, IN; n = 3 pens/block) trace mineral sources. Calves were offered supplement formulated for feeding at 0.9 kg/d (as-fed basis) on d 0. When the majority of the calves in each pen were consuming the supplements, the pen was switched to supplements with the appropriate mineral treatment formulated for feeding at the 1.4 kg/d (as-fed basis) rate (d 8, block 1; d 8, block 2; d 9, block 3; d 8, block 4), and then to supplements formulated for feeding at the 1.8 kg/d (as-fed basis) rate (d 16, block 1; d 12, block 2; d 11, block 3; d 11, block 4), with calves receiving this supplement for the remainder of the 42 (block 4) to 45-d (block 1, 2, and 3) trial. During block 1, intakes of the 0.9 and 1.4 kg/d supplements were deemed inadequate and thus the supplement composition was changed before block 2. Changes in the supplement were formulated so that the new supplement was approximately equal in nutrients to the original diet but the percentage of dried distiller's grain was reduced. Calves had

ad libitum access to bermudagrass hay (12.85% CP, 70% NDF, 38% ADF, 134 mg Mn/kg, 52 mg Zn/kg, 9 mg Cu/kg and 0.25% S; DM basis). Grab samples of supplement and hay were taken throughout the trial and were frozen at -20°C until analysis. Any supplement refusals were collected, weighed, and a subsample was frozen at -20°C until DM analysis. Calves received booster vaccinations on d 14 (block 4) or d 16 (block 1, 2, and 3).

Cattle were observed daily by trained personnel for signs of bovine respiratory disease (**BRD**) beginning the day after processing. Signs of BRD included depression, nasal or ocular discharge, cough, poor appetite, and respiratory distress. Cattle were given a clinical illness score of 1 to 5 (1 = normal to 5 = moribund). Calves with a score > 1 were brought to the working facility and a rectal temperature was taken. If the rectal temperature was ≥ 40°C, the calf was treated according to a preplanned antibiotic protocol (therapy 1 = Micotil, Elanco Animal Health, Indianapolis, IN) administered at 3 mL/45.45 kg BW. Sick calves were returned to home pen for convalescence and were re-evaluated in 72 h. If rectal temperature was ≥ 40ºC during re-evaluation, the calf received therapy 2 (Nuflor, Intervet, Inc., Omaha, NE) at a rate of 6 mL/45.45 kg BW. Calves receiving therapy 2 were re-evaluated in 72 h and if rectal temperature was ≥ 40ºC, calves received a final therapy 3 (Excenel, Zoetis, Florham Park, NJ) administered at 2 mL/45.45 kg BW dosage for 3 consecutive days. After administering therapy 3, if the clinical illness score was greater than time 0 or ≥ 2 and rectal temperature was ≥ 40°C, then the calf was considered nonresponsive and no further treatments were given. If BRD symptoms occur > 21 days after administering the previous therapy symptoms were considered a new BRD episode and treatment began with therapy 1.Records were kept of all antibiotics administered, and medication cost reported is the drug cost with no additional fees assessed. Calves that received all 3 drug therapies and gained less than 0.23 kg/d were deemed chronic (n = 6).

Weights were recorded initially (d -1 and 0) and before supplement feeding on d 14, 28, 41 and 42 (block 4) or d 16, 30, 44 and 45 (block 1, 2, and 3). Average daily gain was calculated for interim and final periods based on averages of initial and final weights that were taken on 2 consecutive days. All calves were bled on d -1 and the final day (d 42 or d 45) via jugular venipuncture for plasma trace mineral analysis. Blood was collected into vacuum tubes specifically made for trace mineral analysis (Kendall Monoject 307014, Tyco Healthcare Group, Mansfield, MA), inverted to mix, and placed on ice. Calves in the final 2 blocks were bled on d -1, 16, 30, and 45 (block 3) or d -1, 14, 28 and 42 (block 4) for antibody titer response to vaccination. Blood was collected via jugular venipuncture in tubes containing a clot activator (BD Vacutainer 367985, BD, Franklin Lakes, NJ) and allowed to sit at room temperature for at least 30 min to allow clot formation. All blood was spun at 2,060 × g for 20 min, and plasma and serum was stored at -20°C. Plasma was deproteinated by mixing 1 mL plasma with 7 mL of 1 N trace metal grade nitric acid for 24 h, and then centrifuged at 2,060 × g for 20 min. The supernatant was taken to the University of Arkansas-Division of Agriculture Altheimer Laboratory for trace mineral analysis by inductively coupled plasma spectroscopy (ICP). Serum was analyzed for BVD type 1 antibody titers at the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA).

Samples of supplements (Table 3) and hay were dried at 50°C in a forced air oven until a constant weight to determine dry matter. Dried samples were ground in a Wiley Mill (Arthur H. Thompson, Philadelphia, PA) through a 1-mm screen. Samples were analyzed for CP via total combustion (Rapid Combustion Method, Elementar Americas Inc., Mt. Laurel, NJ) and sequentially for NDF and ADF (Van Soest method, ANKOM Technology Corp., Fairport, NY). Mineral concentrations were determined via ICP at the University of Arkansas-Division of

Agriculture Altheimer Laboratory after wet ashing duplicate 0.5 g samples with 15 mL of trace metal grade nitric acid (J.T. Baker 9598-34, Avantor Performance Materials, Center Valley, PA) in 50 mL centrifuge tubes. Samples were predigested in a heating block at 80°C for 30 min followed by digestion at 115°C for 1 h. Samples were brought to a constant 45 mL volume with deionized water following digestion.

Pen was used as the experimental unit and incorporated in a randomized complete block design. The model included treatment as a fixed effect and block as a random effect. Growth performance, morbidity data (if calves were treated 1, 2, or 3 times with antibiotics) and medical costs were analyzed using MIXED procedures of SAS (SAS Institute Inc., Cary, NC). Plasma minerals, antibody titer response, and BW were analyzed using MIXED procedure with repeated measure statement. The covariance structure of the repeated measure was variance components (VC) and the subject was pen within block. Data reported as least square means with standard errors. The LIFETEST procedure was used to compare the day when calves received their first, second, third or last antibiotic treatment with calf as the experimental unit.

Results and Discussion

For growth performance data, after removal of chronics (n = 6) and animals that died (n = 1), trace mineral source had no effect on d 14, 28, or final weights ($P = 0.86$) or ADG ($P \geq 0.24$) in this trial (Table 4). This concurs with Sharman et al. (2008), who reported no differences in ADG during a 28-d receiving period in newly received steers supplemented with either sulfate or organic amino acid complex trace minerals at levels equal to those used in the present study. Since a hydroxy source was not included, a comparison for the hydroxy source is not possible. Engle and Spears (2000) also found no difference in ADG among growing steers individually fed various levels of copper from sulfate, citrate, hydroxy, or proteinate sources over a 56-d growing

phase. Arthington and Spears (2007) investigated the effects of copper supplemented at 100 mg/d from either sulfate or hydroxy sources in growing heifers and likewise reported similar ADG for both treatments. However, in regards to trace mineral supplementation from sulfate or organic sources, results have not been consistent. Kegley et al. (2012) observed an increase in ADG and final weight over a 42 d backgrounding period in newly received calves supplemented with organic trace minerals versus calves supplemented with sulfate sources at levels identical to those fed in the current study. Average DM consumption of grain-grain by-product supplement did not differ ($P \geq 0.35$) for any period during the experiment (Table 5).

Sixty-two percent of the calves on this trial were treated with the initial antibiotic for BRD (Table 6). Dietary treatment had no effect on the number of calves treated once ($P = 0.95$), twice ($P = 0.71$), or 3 times ($P = 0.55$) for BRD. Numerically, 1 calf fed sulfate sources was deemed chronic versus 2 and 3 calves fed organic and hydroxyl trace mineral sources respectively, however, the difference was not significant ($P = 0.81$). One calf fed hydroxy trace minerals died. Dietary treatments also had no effect ($P = 0.81$) on average antibiotic cost per calf or percentage of calves that relapsed ($P = 0.64$; Table 6). The day calves received their first, second, third or last antibiotic treatment was not affected ($P \geq 0.39$) by trace mineral source (data not shown).

Dorton and coworkers (2006) reported similar morbidity results in calves supplemented with either sulfate or organic copper, zinc, manganese and cobalt sources beginning at ranch post-weaning and continuing through a 28-d feedlot receiving phase. Likewise, Sharmon and others (2008) observed no effect on total morbidity in newly received steers supplemented with copper, zinc, or manganese from either sulfate or amino acid complexes. However, these authors reported a tendency for an increase in percentage repulls (defined as when an animal is treated

more than once for morbidity) in steers supplemented with amino acid complex trace minerals compared to no difference in percentage relapse (defined as when an animal is treated more than once for morbidity) observed in the current study. It is important to note that Sharmon and coworkers (2008) used a point scoring system to assess morbidity in which 1 point was assigned for exhibiting each of the following respiratory symptoms: ocular discharge, nasal discharge, coughing, rapid breathing and depressed appetite. In addition, 2 additional points were assigned if rectal temperature exceeded 39.5°C and any steers with a total of 4 or more points were considered morbid and treated with an antibiotic. In the current study, animals were not considered morbid and treated with an antibiotic unless their rectal temperature was ≥ 40ºC regardless of the type or number of symptoms exhibited. These differences in morbidity scoring systems could play a role in the difference between the studies. As was the case with growth performance, morbidity results have not been consistent across trials. In a previous study, Kegley and others (2012) reported a tendency for a decrease in the percentage of calves receiving a second antibiotic treatment and a tendency for the second treatment to be administered 1 day later in calves that were supplemented with amino acid complex trace minerals versus those supplemented with sulfate sources.

One hundred seventy-five calves were measured for BVD type 1 antibody titer response to respiratory vaccination. Antibody titer response was compared in all calves as well as the subpopulation that had no detectable antibody titers on d 0 (naïve calves; n = 117). There was a day of sampling effect ($P < 0.0001$) in all groups as most calves developed antibodies in response to vaccination. However, dietary treatment had no effect on antibody titer response in all cattle ($P > 0.70$) or in naïve cattle ($P > 0.83$) nor was there a treatment × day interaction in either group ($P \geq 0.95$; Figure 1). Bovine viral diarrhea type 1 virus is only 1 of 5 viral agents

that were present in the respiratory vaccine, which also included BVD type 2, infectious bovine rhinotracheitis virus (IBR), bovine parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV); all viral agents present in the vaccine have been associated with respiratory tract disease in feedlot calves (Plummer et al., 2004). Thus, BVD type 1 antibody titer response alone cannot be used to describe trace mineral source effect on vaccine response. Kegley and others (2012) reported no difference in BVDV (which encompasses both BVD type 1 and 2), BRSV, or PI3 after vaccination but did observe increases in IBR antibody titers in calves supplemented with sulfate sources of copper, zinc, manganese and cobalt compared to those supplemented with amino acid complex sources of copper, zinc and manganese and cobalt glucoheptonate. Since the current study did not examine any virus other than BVD type 1 titers, a direct comparison cannot be made. However, George et al. (1997) reported improved antibody titer response 14 and 28 d post vaccination to IBRV vaccination in calves supplemented with organic trace minerals versus inorganic minerals. Thus, as seen in previously discussed results, the variability that exists in growth performance and morbidity results for supplemental trace mineral sources extends to antibody titer response to vaccination.

Trace mineral source had no effect on plasma copper ($P > 0.92$) or zinc ($P > 0.83$) concentrations (Table 7). All dietary treatments exceeded current NRC (1996) recommendations for zinc and copper, therefore, differences in plasma concentrations of these trace minerals were not anticipated. Both copper and zinc concentrations were in the adequate range (0.7 to 0.9 mg/L for Cu, and 0.8 to 1.4 mg/L for Zn; Kincaid, 1999) on both sampling days. However, plasma concentrations of copper are not particularly sensitive to deficient copper intake as plasma concentrations are not consistently reduced until liver copper is < 40 mg/kg (Kincaid, 1999). Thus it is possible that animals can be marginal in copper, especially in the short term, without

changes in plasma copper. Plasma zinc concentrations are sensitive to zinc intake, especially if fed at extremely low or extremely high levels, but zinc can also be affected by age, stress, infections and feed restriction (Kincaid, 1999). In addition, Engle et al. (1997) reported a reduced cell-mediated response to phytohemagglutinin injection in calves fed 17 mg/kg zinc compared to calves fed 40 mg/kg zinc with no changes in either plasma or liver zinc concentrations, concluding that cell-mediated immune response may be decreased before functional zinc deficiency symptoms are present.

Implications

In the current study, trace mineral source had no effect on growth performance, morbidity, average antibiotic cost, or antibody titer response to bovine viral diarrhea virus vaccination in shipping-stressed cattle over a 42 to 45 d backgrounding phase. However, additional research is needed regarding hydroxy trace minerals effect on morbidity and antibody titer response as little research currently exists.

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Table 1. Ingredient composition of grain supplements, as fed basis

	Fed at 0.9 kg/d[a]			Fed at 1.4 kg/d[a]			Fed at 1.8 kg/d[b]		
Ingredient	**Sulfate**	**Organic**	**Hydroxy**	**Sulfate**	**Organic**	**Hydroxy**	**Sulfate**	**Organic**	**Hydroxy**
Dried distillers grains with solubles, %	46.2	46.2	46.2	49.6	49.6	49.6	47.2	47.2	47.2
Corn-cracked, %	37.8	37.2	37.8	43.2	42.9	43.3	46.5	46.2	46.5
Soybean meal, %	7.7	7.7	7.7	-	-	-	-	-	-
Limetone, %	3.3	3.3	3.3	3.2	3.2	3.2	2.9	2.9	2.9
Molasses, %	2	2	2	2	2	2	2	2	2
Salt, white %	1.7	1.7	1.7	1.1	1.1	1.1	0.9	0.9	0.9
Availa-4, g/907 kg	-	6,984	-	-	4,668	-	-	3,500	-
Zinc sulfate (35.5% Zn), g/907 kg	1012	-	-	676	-	-	508	-	-
Manganese sulfate (32% Mn), g/907 kg	628	-	-	418	-	-	313	-	-
Copper sulfate (25.2% Cu), g/907 kg	492	-	-	328	-	-	245	-	-
Intellibond Z, g/907 kg	-	-	652	-	-	436	-	-	349

Table 1. Ingredient composition of grain supplements, as fed basis (Cont.)

Intellibond M, g/907 kg	-	-	456	-	-	304	-	-	227
Intellibond C, g/907 kg	-	-	212	-	-	146	-	-	109
Cobalt glucoheptonate (2.5% Co), g/907 kg	504	-	504	336	-	336	251	-	251
Sodium Selenite (0.99% Se), g/907 kg	100	100	100	68	68	68	50.5	50.5	50.5
Vitamin ADE premix[c], %	0.2	0.2	0.2	0.14	0.14	0.14	0.1	0.1	0.1
Vitamin E premix[d], %	0.1	0.1	0.1	0.07	0.07	0.07	0.05	0.05	0.05
Rumensin mix[e], %	0.8	0.8	0.8	0.54	0.54	0.54	0.4	0.4	0.4

[a]Fed to blocks 2, 3 and 4.

[b]Fed to all blocks.

[c]Vitamin ADE premix contains 8,800,000 IU vitamin A, 1,760,000 IU vitamin D, and 1,100 IU vitamin E/kg.

[d]Vitamin E premix contains 44,000 IU/kg.

[e]To provide 160 mg monensin/d when supplement fed at listed rate.

Table 2. Ingredient composition of supplements used for block 1, as fed basis

	Fed at 0.9 kg/d			Fed at 1.4 kg/d		
Ingredient	**Sulfate**	**Organic**	**Hydroxy**	**Sulfate**	**Organic**	**Hydroxy**
Dried distillers grains with solubles, %	84.6	84.6	84.6	59.1	59.1	59.1
Corn-cracked, %	6.5	6	6.6	33.6	33.3	33.7
Limetone, %	3.9	3.9	3.9	3.3	3.3	3.3
Molasses, %	2	2	2	2	2	2
Salt, white %	1.7	1.7	1.7	1.1	1.1	1.1
Availa-4, g/907 kg	-	6,984	-	-	4,668	-
Zinc sulfate (35.5% Zn), g/907 kg	1012	-	-	676	-	-
Manganese sulfate (32% Mn), g/907 kg	628	-	-	418	-	-
Copper sulfate (25.2% Cu), g/907 kg	492	-	-	328	-	-
Intellibond Z, g/907 kg	-	-	652	-	-	436
Intellibond M, g/907 kg	-	-	456	-	-	304
Intellibond C, g/907 kg	-	-	212	-	-	146
Cobalt glucoheptonate (2.5% Co), g/907 kg	504	-	504	336	-	336

Table 2. Ingredient composition of supplements used for block 1, as fed basis (Cont.)

Sodium Selenite (0.99% Se), g/907 kg	100	100	100	68	68	68
Vitamin ADE premix[a], %	0.2	0.2	0.2	0.14	0.14	0.14
Vitamin E premix[b], %	0.1	0.1	0.1	0.07	0.07	0.07
Rumensin mix[c], %	0.8	0.8	0.8	0.54	0.54	0.54

[a]Vitamin ADE premix contains 8,800,000 IU vitamin A, 1,760,000 IU vitamin D, and 1,100 IU vitamin E/kg.

[b]Vitamin E premix contains 44,000 IU/kg.

[c]To provide 160 mg monensin/d when supplement fed at listed rate.

Table 3. Analyzed nutrient composition of supplements, DM basis

		Fed at 0.9 kg/d			Fed at 1.4 kg/d			Fed at 1.8 kg/d		
Nutrient	**Unit**	**Sulfate**	**Organic**	**Hydroxy**	**Sulfate**	**Organic**	**Hydroxy**	**Sulfate**	**Organic**	**Hydroxy**
DM	%	90	90.2	90.1	90.2	90.2	89.7	90.1	89.8	90
CP	%	22	21.1	22.3	19.8	20.5	19.9	20.3	20.6	19.9
NE_m[a]	Mcal/45.4 kg	94	94	94	95	95	95	96	96	96
NE_g[a]	Mcal/45.4 kg	65	65	65	65	65	65	66	66	66
Zn	mg/kg	415	408	429	248	324	320	301	316	316
Mn	mg/kg	225	234	249	114	221	183	203	183	174
Cu	mg/kg	139	152	140	71	126	99	103	122	90
Co	mg/kg	17	16	17	8	13	12	10	13	11
S	%	0.47	0.43	0.43	0.39	0.44	0.41	0.44	0.44	0.42

[a]Values calculated with University of Arkansas Cattle Grower Ration Balancer software.

Table 4. Performance of cattle supplemented with sulfate, organic complexes or hydroxy trace minerals[a], LS means ± SE

				P-value		
	Sulfate	**Organic**	**Hydroxy**	**Treatment**	**Day**	**Treatment × Day**
Initial wt, kg	240 ± 4.3	240 ± 3.5	240 ± 3.5			
D 14 wt, kg	256 ± 4.3	260 ± 3.5	258 ± 3.5			
D 28 wt, kg	268 ± 4.3	272 ± 3.5	269 ± 3.5			
Final wt, kg[b]	280 ± 4.3	283 ± 3.5	280 ± 3.5	0.87	<0.0001	0.63
Average daily gain, kg						
D 0 to 14	1.07 ± 0.08	1.25 ± 0.07	1.17 ± 0.07	0.24		
D 14 to 28	0.86 ± 0.13	0.90 ± 0.11	0.81 ± 0.11	0.84		
D 28 to 42	0.86 ± 0.11	0.75 ± 0.09	0.78 ± 0.09	0.80		
D 0 to 28	0.97 ± 0.05	1.09 ± 0.05	1.00 ± 0.05	0.25		
D 0 to 42	0.94 ± 0.05	0.99 ± 0.04	0.93 ± 0.04	0.49		

[a]Data excludes measurements from cattle labeled as chronic[b] (n = 6) or dead (n = 1).

[b]D 42 (blocks 1, 2, and 3) or 45 (block 4).

Table 4. Performance of cattle supplemented with sulfate, organic complexes or hydroxy trace minerals[a], LS means ± SE (Cont.)

[c]Chronic animals defined as having received all drug therapies and gaining < 0.23 kg/d.

Table 5. Average grain-grain by-product supplement DMI in cattle supplemented with sulfate, organic complexes, or hydroxy trace minerals, LS Means ± SE

	Sulfate	Organic	Hydroxy	*P*-value
D 0 to 7, kg	0.60 ± 0.06	0.65 ± 0.05	0.65 ± 0.05	0.76
D 0 to 14, kg	0.89 ± 0.06	0.99 ± 0.05	1.00 ± 0.05	0.35
D 0 to 28, kg	1.23 ± 0.04	1.30 ± 0.03	1.30 ± 0.03	0.36
D 0 to Final[a], kg	1.37 ± 0.03	1.41 ± 0.02	1.41 ± 0.02	0.36

[a]D 42 (blocks 1, 2, and 3) or 45 (block 4).

Table 6. Morbidity from bovine respiratory disease for cattle supplemented with sulfate, organic complexes or hydroxy trace minerals, LS means ± SE

	Sulfate	Organic	Hydroxy	*P*-value
Morbidity, %	60.8 ± 6.6	60.7 ± 5.4	63.0 ± 5.4	0.95
Treated twice, %	29.6 ± 7.2	21.9 ± 5.8	25.2 ± 5.8	0.71
Treated thrice, %	8.0 ± 2.9	3.9 ± 2.3	5.3 ± 2.3	0.55
Relapse[a], %	44.3 ± 0.1	32.9 ± 0.1	39.6 ± 0.1	0.64
Chronic, %	1.1 ± 1.6	1.5 ± 1.3	2.4 ± 1.3	0.81
Medical cost, $/calf	18.66 ± 2.75	16.49 ± 2.24	17.95 ± 2.24	0.81

[a]Relapse defined as when animal is treated more than once for bovine respiratory disease.

Table 7. Plasma mineral concentrations for cattle supplemented with sulfate, organic complexes, or hydroxy trace minerals, LS means ± SE

				P-value		
	Sulfate	**Organic**	**Hydroxy**	**Treatment**	**Day**	**Treatment x day**
Copper, mg/L				0.92	0.45	0.96
D -1	0.88 ± 0.03	0.89 ± 0.02	0.89 ± 0.02			
Final d[a]	0.89 ± 0.03	0.90 ± 0.02	0.90 ± 0.02			
Zinc, mg/L				0.83	0.15	0.65
D -1	0.93 ± 0.07	0.85 ± 0.06	0.89 ± 0.06			
Final d[a]	0.82 ± 0.07	0.82 ± 0.06	0.86 ± 0.06			

[a]D 42 (blocks 1, 2, and 3) or 45 (block 4).

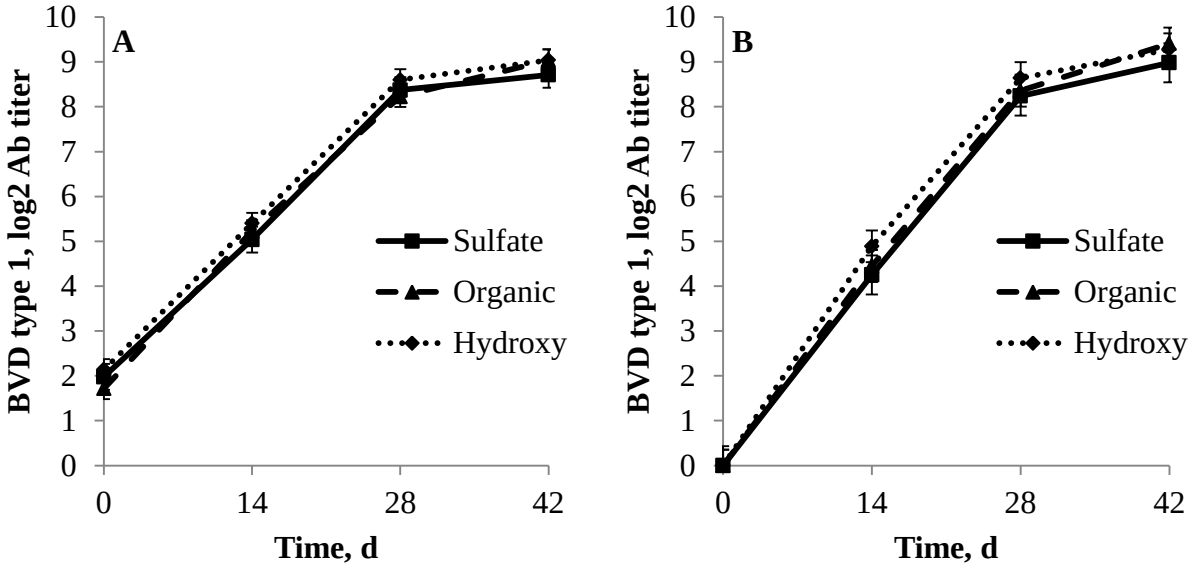

Figure 1. Bovine viral diarrhea type 1 antibody titer response to vaccination with modified live vaccine for respiratory virus, (A) in all cattle (n = 175; treatment × day interaction, *P* = 1); (B) in naïve cattle (n = 117; treatment × day interaction, *P* = 0.95).

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To Whom It May Concern:

Anthony W. Ryan is the first author of the article "Supplemental trace minerals (zinc, copper, and manganese) as sulfates, organic amino acid complexes, or hydroxy trace mineral sources for shipping-stressed calves". As part of his research project for his M.S. degree, Anthony Ryan completed over 51% of the work reported in this article.

Sincerely,

Beth Kegley, Ph.D.

Professor

CHAPTER IV

Effects of trace minerals (zinc, copper, and manganese) as sulfate or hydroxy sources on trace mineral status, superoxide dismutase activity, and performance in beef heifers fed diets high in a copper antagonist

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Abstract

Twenty-four crossbreed heifers (average BW 274 ± 5.5 kg) at the University of Arkansas Livestock and Forestry Research Station - Batesville were stratified by BW and plasma copper concentration into 1 of 9 pens (2 or 3 heifers/pen; 8 heifers/treatment). Pens were assigned randomly to 1 of 3 treatments consisting of 1) no supplemental trace minerals; 2) supplemental copper (55 mg/d), zinc (165 mg/d), and manganese (110 mg/d) from sulfate sources; or 3) supplemental zinc, copper, and manganese at isolevels to treatment 2 from hydroxy sources (Intellibond-Z, Intellibond-C, and Intellibond-M; Micronutrients, Indianapolis, IN). During the 55-d trial, heifers had ad libitum access to bermudagrass hay and pasture and were fed grain supplements that contained dried distillers grains and served as carriers for the treatments. Final and intermediate weights ($P = 0.73$), ADG ($P = 0.70$) and plasma zinc and copper concentrations ($P \geq 0.37$) were not affected by treatment. Liver copper concentrations on d 55 were greater for the sulfate treatment ($P > 0.004$) compared to the control and hydroxy treatments, however, liver zinc concentrations were not affected ($P > 0.29$). Treatment had no effect ($P \geq 0.36$) on total- or manganese-superoxide dismutase activity, however, a day effect ($P \geq 0.002$) was observed with total-superoxide dismutase decreased and manganese-superoxide dismutase increased on d 55 compared to d 0. Overall, trace mineral source had no effect on growth performance, plasma mineral concentration, liver zinc concentrations, or superoxide dismutase activity. However, heifers supplemented for 55 d with trace minerals from sulfate sources had greater liver copper concentrations compared to other treatments.

Key words: beef cattle, copper, manganese, zinc, trace mineral

Introduction

Trace minerals are required for normal growth, health, performance, and reproduction in cattle (Suttle, 2010). Many of these essential trace minerals can be found in sufficient concentrations in normal feedstuffs consumed by beef cattle (NRC, 1996). However, forages from certain regions of the United States have been found to be marginal or deficient in 1 or more essential trace minerals (Galyean et al., 1999; Coffey et al., 2005), thus requiring supplementation in order to prevent deficiencies. In addition to low dietary copper concentrations, copper deficiency can also be induced by high dietary concentrations of sulfur and molybdenum, both of which can form insoluble complexes with copper in the rumen that are not absorbed (NRC, 1996).

Different forms of trace minerals have been shown to differ in bioavailability (Wedekind et al., 1992; Kegley and Spears, 1994). In addition, different chemical forms of copper have shown differing bioavailability in the presence of copper antagonists (Ward et al., 1996; Spears et al., 2004). Current blood measures to assess trace mineral status are plagued by insensitivity as well as interference by homeostatic controls within the animal (Kincaid, 1999). Thus, new measures assessing functional forms of minerals are being studied. Superoxide dismutase, a functional form of copper and manganese in the blood, has been reported to be decreased by trace mineral deficiency (Arthington et al., 1996), however, the effects of different trace mineral sources on superoxide dismutase has not been evaluated. Therefore, the objective of this study was to evaluate the effect of trace mineral supplementation from sulfate or hydroxy sources on growth performance, trace mineral status, and superoxide dismutase activity in beef heifers fed diets high in sulfur.

Materials and Methods

Prior to initiation of this study, care, handling, and sampling of the animals were approved by the University of Arkansas Animal Care and Use Committee. Twenty-four crossbreed Angus-Charolais heifer calves (274 ± 5.5 kg BW) at the University of Arkansas Livestock and Forestry Research Station-Batesville were used in this experiment. Eight days prior (d -8) to the start of this experiment, heifers were weighed and bled via jugular venipuncture for plasma copper analysis. On d 0, heifers were stratified by BW and plasma copper concentration into 1 of 9 pens (2 or 3 calves/pen; 8 calves/treatment). Pens were assigned randomly to treatment. Calves were housed on 2.02-ha bermudagrass pastures where calves were fed grain supplements (Table 1) that served as carriers of mineral treatments. Treatments consisted of 1) control (no supplemental copper, zinc or manganese); 2) supplemental copper (55 mg/d), zinc (165 mg/d), and manganese (110 mg/d) from sulfate trace mineral sources; and 3) supplemental copper, zinc, and manganese at isolevels to treatment 2 from hydroxy (Intellibond, Micronutrients, Inc., Indianapolis, IN) trace mineral sources. Levels of trace minerals were formulated to meet NRC (1996) requirements at an estimated DMI of 2% BW/d. In addition, supplements were formulated to meet or exceed NRC (1996) requirements for CP, macrominerals and vitamins for all treatments. Supplements were fed at 1.4 kg/d beginning on d 0. Calves had ad libitum access to bermudagrass hay (8.8% CP, 71% NDF, 40.7 % ADF, 11.8 mg Zn/kg, 158 mg Mn/kg, 4.1 mg Cu/kg, 0.11% S) as well as standing forage (11.0% CP, 59.5% NDF, 32.3% ADF, 58.5 mg Zn/kg, 211.8 mg Mn/kg, 17 mg Cu/kg, 0.56% S) in the pastures. Grab samples of supplements were taken during the mixing process and hay samples were taken on d 0 of the experiment (March 26, 2014). Samples of supplements and hay were frozen at -20°C until analysis. Beginning on d 28 of the trial, standing forage was sampled at 14 d intervals by randomly obtaining 5 grab samples hand clipped 2.5 cm above soil surface in each pen.

Standing forage was composited by sampling day, dried and a subsample taken for analysis. Any feed refusals were collected, weighed, and a subsample frozen at -20ºC until DM analysis. On d 14, a reproductive tract score from 1 to 5 (1 = immature or infertile to 5 = currently cycling; Virginia Cooperative Extension, Blacksburg, VA) were assigned to individual animals and all animals were dewormed (Cydectin, Boehringer Ingelheim Vetmedica, Ridgefield, CT) and received a modified live vaccine (Bovi-Shield Gold FP VL5, Zoetis, Florham Park, NJ). On d 42, insecticide ear tags (XP 820, Y-Tex Corp., Cody, WY) were placed in each animal.

On d 55, liver biopsies were attempted on all animals with samples obtained on 23 out of 24 animals. To obtain liver samples, animals were restrained in a manual squeeze chute and the 10^{th} intercostal space between the hook and elbow on the right side of the animal was identified. An area approximately 10 cm × 10 cm was clipped using an electric clipper and size 40 surgical blade and brushed to remove loose hair and debris. The area was then scrubbed with chlorhexidine using a gauze sponge followed by scrubbing with 70% isopropyl alcohol, beginning at the expected site of incision and working outward to the periphery of the area. This series of scrubs was repeated 2 more times and followed by a single scrub of iodine surgical solution. The site of injection was then injected with 5 mL of 2% lidocaine solution (Phoenix Lidocaine Hydrochloride Injectable - 2%, Clipper Distributing, Inc., St. Joseph, MO) under the skin and into the intercostal muscle. After allowing 5 min for lidocaine to take effect, a sterile scalpel was used to make a 1 cm incision through the skin. A Tru-Cut biopsy needle (Cardinal Health, Dublin, OH) was then inserted through the incision to obtain liver samples. The same biopsy needle was used to obtain subsequent samples from the same animal until an adequate amount of sample was obtained. A sterile hypodermic needle was used to remove liver from the biopsy needle and samples were stored in acid-washed, pre-weighed borosilicate tubes, covered

with parafilm, and placed on ice. Liver samples were weighed to obtain a wet weight and then dried at 100ºC for 48 h to determine dry matter. Samples were then wet ashed with 2 mL trace metal grade nitric acid, predigested at 80°C for 30 min followed by 1 h digestion at 115ºC and brought to 5 g weight with deionized water after digestion. Samples were transported to the University of Arkansas-Division of Agriculture Altheimer Laboratory for zinc and copper analysis by inductively coupled plasma spectroscopy (ICP).

Body weights were recorded on d -8, 0, 14, 28, 42, and 55. Calves were bled on d -8, 0, 14, 28, 42, and 55 via jugular venipuncture for plasma trace mineral and erythrocyte lysate superoxide dismutase (SOD) analysis. Blood was collected into vacuum tubes specifically made for trace mineral analysis (Kendall Monoject 307014, Tyco Healthcare Group, Mansfield, MA), inverted to mix, and placed on ice. Blood was spun at 1,430 × *g* at 4ºC for 10 min., and plasma stored at -20°C. After removal of plasma, the buffy layer was removed and 2 mL of erythrocytes were lysed in 8 mL ice-cold HPLC- grade water. Lysed erythrocytes were then centrifuged at 10,000 × *g* at 4°C for 15 min with supernatant stored at -80ºC until analysis.

Plasma was deproteinated by mixing 1 mL plasma with 7 mL of 1 N trace metal grade nitric acid for 24 h, and then centrifuged at 2,060 × *g* for 20 min. The supernatant was then transported to the University of Arkansas-Division of Agriculture Altheimer Laboratory for trace mineral analysis by ICP. Erythrocyte lysate from d 0 and 55 was analyzed for total- and manganese-SOD activity using a commercial assay kit (Cayman Chemical Company, Ann Arbor, MI). Superoxide dismutase activity is expressed as unit of SOD activity per g of hemoglobin with hemoglobin being measured using a commercial assay kit (Teco Diagnostics, Anaheim, CA). A unit of SOD activity is defined as the amount of SOD enzyme needed to exhibit 50% dismutation of the superoxide radical.

Samples of supplements (Table 2), hay and standing forage were dried at 50°C in a forced air oven until a constant weight to determine dry matter. Dry samples were ground in a Wiley Mill (Arthur H. Thompson, Philadelphia, PA) through a 1-mm screen. Samples were analyzed for CP via total combustion (Rapid Combustion Method, Elementar Americas Inc., Mt. Laurel, NJ) and sequentially for NDF and ADF (Van Soest method, ANKOM Technology Corp., Fairport, NY). Mineral concentrations were determined via ICP at the University of Arkansas-Division of Agriculture Altheimer Laboratory after wet ashing duplicate 0.5 g samples with 15 mL of trace metal grade nitric acid (J.T. Baker 9598-34, Avantor Performance Material, Center Valley, PA) in 50 mL centrifuge tubes. Samples were predigested in heating block at 80ºC for 30 min followed by digestion at 115°C for 1 h. Samples were brought to a constant 45 mL volume with deionized water following digestion.

Pen was used as the experimental unit and incorporated in a randomized complete block design. The model included treatment as a fixed effect and pen as a random effect. Growth performance and liver mineral concentration were analyzed using MIXED procedures of SAS (SAS Institute Inc., Cary, NC). Plasma minerals, total- and manganese-SOD, and BW were analyzed using MIXED procedure with repeated measure statement. The covariance structure of the repeated measure was variance components (VC) and the subject was pen. Results reported as least square means with standard errors. If the interaction was significant ($P \leq 0.10$), treatment means were separated with a t-test using the PDIFF option in SAS.

Results and Discussion

For growth performance data, treatment had no effect on d 14, 28, 42, or final weights ($P = 0.73$) or ADG ($P \geq 0.67$; Table 3). These results are similar to those reported by Arthington

and Spears (2007) in which growing heifers were supplemented with 100 mg/d copper from either sulfate or tribasic copper chloride or received no supplemental copper (control) in either corn- or molasses-based diets. Engle and Spears (2000) also reported similar ADG among growing steers supplemented with various levels of copper over a 56-d growing period from sulfate, citrate, tribasic copper chloride, or proteinate sources. However, in the finishing period of the experiment, Engle and Spears (2000) reported reduced ADG, feed intake, and feed efficiency in steers supplemented with copper versus the control group. However, the high-concentrate finishing diets were fed for either 101 or 121 d and were corn-soybean meal based and thus had considerably lower amounts of copper antagonists than diets fed in the current study. Arthington et al. (2003) observed a tendency for increased ADG in heifers fed molasses-based diets supplemented with copper from either sulfate or organic source versus heifers that received no supplemental copper. The tendency for a difference in ADG compared to no difference observed in the current study could be partially due to the 84 d trial and thus longer exposure to copper antagonists used by Arthington et al. (2003) versus 55 d in the current study. Supplement DMI for trial tended to be lower (P =0.06) in the control group versus either supplemented groups, which were similar (Table 3). However, there was no difference in supplement DMI after d 3 with precipitation being recorded d 0 to d 3, potentially affecting feed intake. Thus, it is not likely the difference in supplement DMI was in response to trace mineral supplementation. Average reproductive tract scores were not different (3.5 for control, 3.89 for sulfate, and 3.78 for hydroxy; P = 0.70) among treatments.

Twenty-two heifers (8 control, 8 sulfate, and 6 hydroxy) were measured for liver copper and zinc concentrations. Two animals in the hydroxy treatment were not measured as a liver sample was not obtained on 1 animal and an inadequate amount of sample was obtained from the

second animal. Treatment had no effect on liver zinc ($P > 0.29$) concentrations (Table 4), and liver zinc was within the adequate range (25 to 200 mg/kg DM; Kincaid, 1999) for all treatments. For liver copper concentration, animals receiving supplemental trace minerals from sulfate sources had increased ($P > 0.004$) liver copper compared to the control and hydroxy treatments. Liver copper concentrations were in the deficient range (<33 mg/kg DM; Kincaid, 1999) for control and hydroxy treatments and marginal range (33 to 125 mg/kg DM; Kincaid, 1999) for the sulfate treatment. Due to equipment and facility malfunctions, initial liver samples and therefore initial trace mineral concentrations in liver were not obtained. However, all animals in the trial were managed as a single group since birth, thus potential for highly variable initial liver trace mineral concentrations should have been minimized. These results differ from several other published studies that reported an increase in liver copper concentration regardless of source (Arthington and Spears, 2007; Engle and Spears, 2000; Arthington et al., 2003) versus the current study in which liver copper concentration was only increased in the sulfate treatment. In addition, since initial copper concentrations are not known, it is impossible to discern if trace mineral supplementation increased the copper concentration in the sulfate treatment or merely reduced copper depletion. In the second of 2 experiments, Arthington and Spears (2007) reported increased liver copper concentration in heifers that received supplemental copper versus the control heifers but still noted decreased copper concentrations over the course of the 90-d trial. In that trial, Arthington and Spears (2007) fed a molasses-based diet that was relatively high in both sulfur and molybdenum, minerals that have been acknowledged as known copper antagonists. The current study utilized supplements and standing forage that had sulfur levels above the NRC recommended maximum tolerable level (0.4% S), thus it is possible to have results similar to

Arthington and Spears (2007) with the sulfate treatment being most effective at preventing copper depletion.

Plasma mineral concentrations were not affected ($P \geq 0.37$) by treatment during the 55-d trial (Table 5). With the exception of copper, zinc, and manganese, all other minerals were at levels equal to or greater than NRC recommendations in all dietary treatments and thus difference in plasma concentrations were not anticipated. Plasma concentrations of zinc and copper were in adequate ranges (0.8 to 1.4 mg/L for zinc, and 0.7 to 0.8 mg/L for copper; Kincaid, 1999) for all treatments on all sampling days. Plasma copper concentrations are not particularly sensitive to copper intake and plasma concentrations are not consistently reduced until liver concentrations are < 40 mg/kg (Kincaid, 1999). The control and hydroxy treatments had liver copper concentrations below 40 mg/kg, however, plasma concentrations were not reduced. This may be due in part to the relatively short 55 d trial, especially if liver concentrations were adequate on d 0 and were depleted over the course of the trial. If this is the case, it is possible liver copper concentrations did not fall below 40 mg/kg of copper until late in the trial and thus a reduction in plasma copper was not detected. However, since initial liver copper is not known, this cannot be confirmed. In addition, certain thiomolybdates and molybdates can be absorbed into the blood where they bind with endogenous copper and render it unusable for metabolic purposes (Kincaid, 1999). Kincaid (1980) observed a rise in plasma copper concentration even though liver copper concentration was depleted and ceruloplasmin activity was reduced in Holstein calves supplemented with various levels of molybdenum. A similar occurrence may have existed in the current trial with the high levels of sulfur consumed by the animals.

Treatment had no effect on either total- ($P = 0.36$; Figure 1) or manganese SOD ($P = 0.97$; Figure 2) activity over the course of the 55 d trial. However, there was a day effect ($P \leq 0.002$) for both total- and manganese-SOD with total-SOD being lower and manganese-SOD being greater on d 55 compared to d 0. Dietary concentrations of manganese were well above the NRC (1996) requirement of 20 mg/kg in supplements and forage, thus manganese deprivation was not an issue. The high level of manganese intake in this trial may also explain the rise in manganese-SOD as increasing dietary manganese supplementation has been show to increase manganese-SOD activity in both broilers and rats (Lu et al., 2006; Payter, 1980). Total-SOD, which encompasses copper-zinc SOD and manganese-SOD, was decreased over the course of this study. Since manganese-SOD was increased over the course of the study, the reduction in total-SOD came from the copper-zinc SOD fraction. Results from the current study are similar to those reported by Arthington et al. (1996) who observed a decrease in copper-zinc SOD activity in molybdenum-induced copper deficient beef heifers. In their study, Arthington et al. (1996) did not observe a difference in copper-zinc SOD activity until d 129, although liver copper concentrations were not deficient until d 90 of their experiment. While initial liver trace mineral concentrations are not known for the current trial, potentially lower liver copper concentrations could explain why SOD activity was reduced much sooner in the current trial. However, it has been reported that SOD activity does not fall with deficient copper intakes until plasma copper and ceruloplasmin are reduced (Kincaid, 1999). However, an explanation as to why total-SOD was reduced but plasma copper concentrations were not in the current trial remains unclear.

Implications

In the current study, trace mineral source had no effect on plasma mineral concentrations, total- or manganese-SOD activity, or growth performance over a 55 d trial in growing beef

heifers fed a high sulfur diet. However, liver copper concentrations were greater in heifers supplemented with trace minerals from sulfate sources versus the control and hydroxy treatments and total-SOD activity was reduced in all treatments on d 55 compared to d 0. Therefore, additional research is needed regarding the effect of trace mineral source on liver copper concentrations and high sulfur diets on total-SOD activity in beef cattle in order to better clarify the interaction between trace mineral sources and trace mineral antagonists.

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Table 1. Ingredient composition of grain supplements, as fed basis

Ingredient	Control	Sulfate	Hydroxy
Dried distillers grains with solubles, %	88.3	88.2	88.2
Calcium sulfate, %	2.7	2.7	2.7
Salt, white %	1	1	1
Calcium carbonate,%	3	3	3
Zinc sulfate (35.5% Zn), g/907 kg	-	310	-
Manganese sulfate (32% Mn), g/907 kg	-	230	-
Copper sulfate (25.2% Cu), g/907 kg	-	146	-
Intellibond Z, g/907 kg	-	-	200
Intellibond M, g/907 kg	-	-	167
Intellibond C, g/907 kg	-	-	64
Sodium selenite (0.99% Se), g/907 kg	202	202	202
Cobalt carbonate, g/907 kg	4.4	4.4	4.4
Calcium iodate, g/907 kg	5.3	5.3	5.3
Vitamin ADE premix[a], %	2.8	2.8	2.8
Vitamin E premix[b], %	1.4	1.4	1.4
Rumensin mix[c], %	0.54	0.54	0.54

[a]Vitamin ADE premix contains 8,800,000 IU vitamin A, 1,760,000 vitamin D, and 1,100 IU vitamin E/kg.

[b]Vitamin E premix contains 44,000 IU/kg.

[c]To provide 160 mg monensin/d when supplement fed at 1.4 kg/d.

Table 2. Analyzed nutrient composition of supplements, DM basis

Nutrient	Unit	Control	Sulfate	Hydroxy
DM	%	90.9	91	90.9
CP	%	26.8	27.1	25.5
NE_m[a]	Mcal/45.4 kg	91	91	91
NE_g[a]	Mcal/45.4 kg	63	63	63
Zn	mg/kg	62	235	187
Mn	mg/kg	27	143	125
Cu	mg/kg	13	59	56
Co	mg/kg	2	2.5	2.7
S	%	1.1	1.1	1.2

[a]Values calculated with the University of Arkansas Cattle Grower Ration Balancer software.

Table 3. Performance data for beef heifers supplemented with sulfate or hydroxy trace minerals, LS means

					P-value		
	Control	**Sulfate**	**Hydroxy**	**SE**	**Treatment**	**Day**	**Treatment × Day**
Initial wt, kg	301	294	285	12.1			
D 14 wt, kg	299	296	287	12.1			
D 28 wt. kg	325	321	310	12.1			
D 42 wt, kg	360	355	346	12.1			
D 55 wt, kg	357	358	351	12.1	0.73	<0.0001	0.98
Average daily gain, kg							
D 0 to 28	0.87	0.97	0.88	0.12	0.83		
D 28 to 55	1.37	1.34	1.54	0.17	0.67		
D 0 to 55	1.12	1.15	1.21	0.07	0.70		
Supplement DMI, kg							
D 0 to 55	1.18	1.21	1.21	0.01	0.06		

Table 4. Liver copper and zinc concentrations in beef heifers supplemented with sulfate or hydroxy trace minerals for 55 d, DM basis, LS means

	Control	Sulfate	Hydroxy	SE	*P*-value
Copper, mg/kg	18.1[a]	50.5[b]	24.1[a]	4.29	0.004
Zinc, mg/kg	92	51.7	47.3	19.8	0.29

[a,b]Values within a row without a common superscript differ ($P \leq 0.05$).

Table 5. Plasma mineral concentrations for beef heifers supplemented with sulfate or hydroxy trace minerals, LS means

					P-value		
	Control	**Sulfate**	**Hydroxy**	**SE**	**Treatment**	**Day**	**Treatment × day**
Copper, mg/L					0.40	0.01	0.24
D 0	0.76	0.77	0.75	0.03			
D 14	0.73	0.72	0.72	0.03			
D 28	0.81	0.74	0.77	0.03			
D 42	0.78	0.77	0.72	0.03			
D 55	0.80	0.82	0.74	0.03			
Zinc, mg/L					0.81	0.007	0.31
D 0	0.77	0.85	0.92	0.09			
D 14	0.96	1.13	0.91	0.09			
D 28	1.04	1.02	1.00	0.09			
D 42	1.02	1.05	0.97	0.09			

Table 5. Plasma mineral concentrations for beef heifers supplemented with sulfate or hydroxy trace minerals, LS means (Cont.)

D 55	0.97	0.89	0.95	0.09

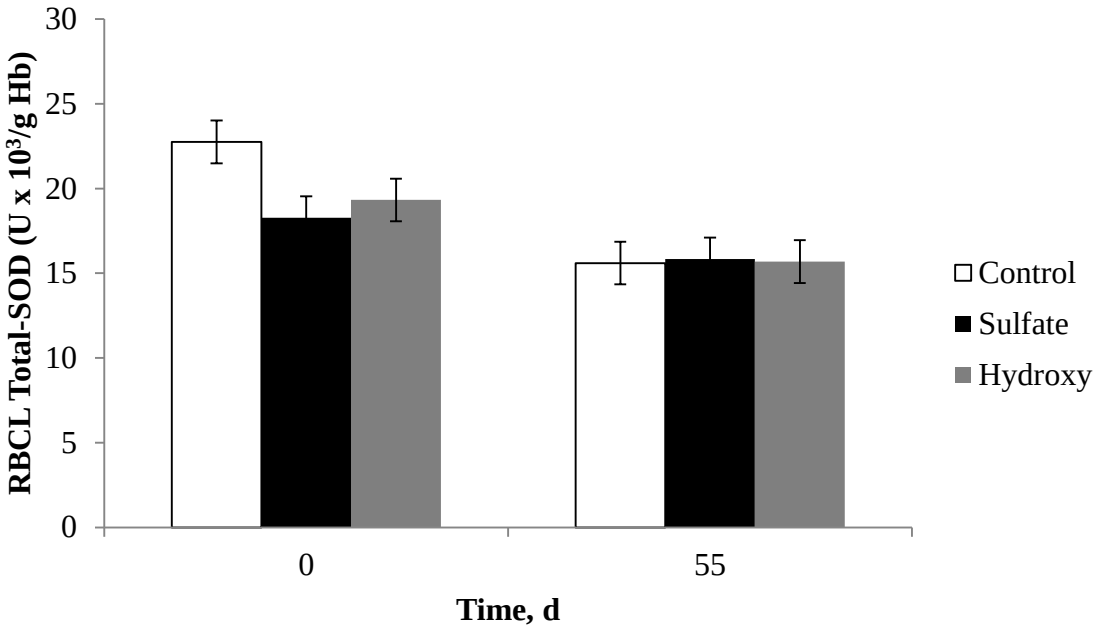

Figure 1. Effect of trace mineral source on red blood cell lysate (RBCL) total-superoxide dismutase (total-SOD) in beef cattle fed a high sulfur diet; values are LS means ± SE, n = 8 calves/treatment; treatment effect (P = 0.36), day effect (P = 0.0024).

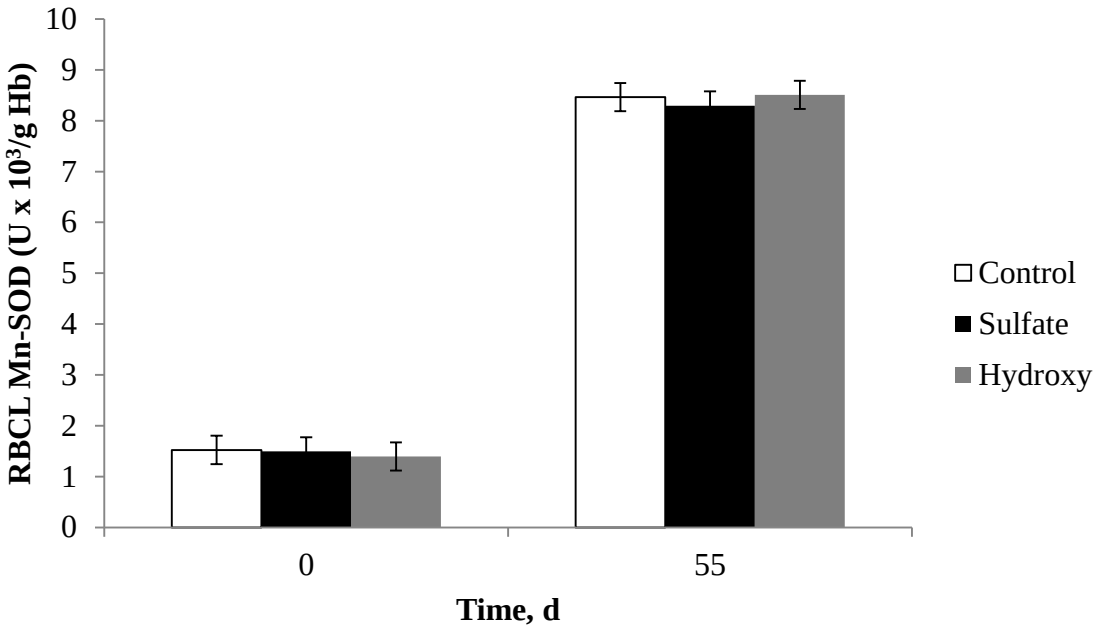

Figure 2. Effect of trace mineral source on red blood cell lysate (RBCL) manganese-superoxide dismutase (Mn-SOD) in beef cattle fed a high sulfur diet; values are LS means ± SE, n = 8 calves/treatment; treatment effect ($P = 0.97$), day effect ($P < 0.0001$).

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To Whom It May Concern:

Anthony W. Ryan is the first author of the article "Effects of trace minerals (zinc, copper, and manganese) as sulfate or hydroxy sources on trace mineral status, superoxide dismutase activity, and performance in beef heifers fed diets high in a copper antagonist". As part of his research project for his M.S. degree, Anthony Ryan completed over 51% of the work reported in this article.

Sincerely,

Beth Kegley, Ph.D.

Professor

CHAPTER V

CONCLUSION

A series of studies were conducted to determine the effect of supplemental trace mineral source on growth performance, morbidity, and trace mineral status in growing beef cattle. The first experiment evaluated supplemental trace minerals from sulfate, organic, or hydroxy sources on growth performance and morbidity. Crossbreed beef calves (n = 350) were obtained from regional livestock auctions. Within each set (n = 4), calves were stratified by body weight and gender into 1 of 8 pens (10 to 12 calves/pen). Pens were randomly assigned to 1 of 3 treatments consisting of supplemental zinc (360 mg/d), copper (125 mg/d), and manganese (200 mg/d) from inorganic, organic, or hydroxy sources fed daily over a 42 to 45-d backgrounding phase. After removal of chronic (n = 6) and dead (n = 1) animals from the data set, trace mineral source had no effect on final or intermediate weights ($P > 0.55$) or average daily gain ($P = 0.51$). For all calves, dietary treatments had no effect on the number of calves treated once ($P = 0.93$), twice ($P = 0.71$), or 3 times ($P = 0.53$) for bovine respiratory disease, or on the number of calves classified as chronic ($P = 0.55$). Overall, trace mineral source had no effect on total weight gain, average daily gain, or morbidity during the receiving phase in shipping stressed cattle.

A second experiment was conducted to study the effect of trace mineral source on trace mineral status, superoxide dismutase activity, and performance in beef heifers fed diets high in sulfur. Twenty-four crossbreed heifers were stratified by body weight and plasma copper concentration into 1 of 9 pens (2 or 3 heifers/pen). Pens were assigned randomly to 1 of 3 treatments consisting of 1) no supplemental trace minerals; 2) supplemental copper (55 mg/d), zinc (165 mg/d), and manganese (110 mg/d) from sulfate sources; or 3) supplemental zinc, copper, and manganese at isolevels to treatment 2 from hydroxy sources fed daily over a 55-d

trial. Final and intermediate weights ($P = 0.73$), average daily gain ($P = 0.70$), and plasma mineral concentrations ($P \geq 0.37$) were not affected by treatment. Liver copper concentrations on d 55 were greater for the sulfate treatment ($P > 0.004$) compared to control and hydroxy treatments, however, liver zinc concentrations were not affected ($P > 0.29$). Treatment had no effect on ($P \geq 0.36$) on total- or manganese-superoxide dismutase activity, however, a day effect ($P \geq 0.002$) was observed with total-superoxide dismutase decreased and manganese-superoxide dismutase increased on d 55 compared to d 0. Overall, trace mineral source had no effect on growth performance, plasma mineral concentration, liver zinc concentrations, or superoxide dismutase activity. However, heifers supplemented with trace minerals from sulfate sources had greater liver copper concentrations on d 55 compared to other treatments.