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Effects of Supplemental Trace Minerals as Amino Acid Complexed or Inorganic Sources for Beef Cattle from Receiving through Finishing

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Effects of Supplemental Trace Minerals as Amino Acid Complexed or Inorganic Sources for Beef Cattle from Receiving through Finishing

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ABSTRACT

The objective of experiments was to investigate the effects of inorganic or amino acid-complexed sources of trace minerals on health, gain performance, liver mineral concentrations, and carcass characteristics of beef heifers from receiving through finishing. To investigate the effects of inorganic or amino acid-complexed sources of trace minerals (zinc, copper, manganese, and cobalt) on performance and morbidity of beef heifers during the receiving period, crossbred beef heifer calves arriving on 3 delivery dates were used in a 42-day receiving trial. Treatments consisted of supplemental zinc (360 mg/d), copper (125 mg/d), manganese (200 mg/d), and cobalt (12 mg/d) from complexed (Availa-4, Zinpro Corp. Eden Prairie, MN) or inorganic sources (sulfates). Replacing inorganic sources of zinc, copper, manganese, and cobalt with amino acid complexed supplementation resulted in greater body weights and average daily gain. Cattle supplemented with inorganic trace mineral sources had a greater percentage of bovine respiratory disease (BRD) morbidity than cattle supplemented with complexed trace mineral sources and medication costs were lower for heifers supplemented with complexed trace mineral sources. Prolonged stress indicated by serum haptoglobin concentrations decreased throughout the receiving trial, and cattle supplemented with complexed trace mineral sources tended to have lower haptoglobin concentrations by d 28 of supplementation. However, the source of trace mineral had no effect on liver mineral concentrations. This study provided evidence for supplementing cattle for the first 42 days after arrival with amino acid complexed trace mineral sources improved heifer performance as compared to heifers supplemented with inorganic trace minerals.

A second experiment was performed to determine the effects of inorganic or amino acid-complexed sources of trace minerals (zinc and copper) during a grazing period following a receiving trial on growth, feedlot, and carcass performance. Any cattle that failed to gain at least 0.45 kg/d, and(or) received 3 doses of antibiotic therapy during the receiving trial were removed from the study. Cattle grazed at 2 different locations in Arkansas; in Fayetteville, cattle grazed 6-acre stockpiled mixed grass pastures; in Batesville, cattle grazed 5-acre stockpiled novel-endophyte fescue pastures. Treatments consisted of supplemental zinc (540 mg/day) and copper (90 mg/day) from complexed (Availa, Zinpro Corp.) or inorganic sources (sulfates). A subset of calves was liver biopsied at the end of receiving and grazing phases to compare liver mineral concentrations. Following grazing, calves were removed from treatments and sent to a Kansas feedlot where they were commingled and fed in a single pen for 141 days. Morbidity and mortality data were recorded, and after slaughter, carcass data were collected. Overall, during grazing, growth performance nor liver mineral comparisons were not different between dietary treatments. Following the grazing period, there were no differences in feedlot morbidity, mortality, or carcass characteristics. In conclusion, although complexed sources of trace minerals (zinc, copper, manganese, and cobalt) improved body weight gain and decreased morbidity treatments during the receiving phase, there were no differences from grazing to slaughter when supplementing amino acid complexed versus sulfate mineral sources of zinc and copper during the grazing period.

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CHAPTER I

INTRODUCTION

It is evident in beef production that for cattle to perform at their greatest genetic potential their nutritional demands must be met. Trace minerals take part in important physiological functions in beef cattle and must be supplemented to beef cattle diets when forages and rations are deficient or have incorrect proportions (Paterson and Engle, 2005). Mineral inclusion to livestock diets must be sufficient to ensure the maintenance of body reserves and to provide appropriate concentrations in products that are edible. Various sources, concentrations, and combinations of trace mineral supplements exist commercially for cattle and their effects on immune function, growth, and performance measures have been evaluated in recent years. Livestock diets are often delivered with trace minerals supplemented in the form of inorganic salts, usually oxides, chlorides, sulfates, and carbonates (Hilal et al., 2016). These inorganic forms have been used in cattle diets for years because they are widely available and represent an inexpensive form of supplementation. However, organic trace mineral supplements are being used in replace of inorganic salts due to potentially greater bioavailability and functionality (Mohanta and Garg, 2014). There are different forms of organic trace mineral supplementation commercially available. One form, being a metal amino acid complex, used in this study, is a product resulting from complexing a soluble metal salt with a single amino acid (AAFCO, 2000).

Bovine respiratory disease (BRD) has been the most economically important disease costing producers in North America $800 to $900 million every year (Sanchez, 2022). Such loss remains a continuing problem in cattle production systems despite the widespread use of antibiotics and vaccines (Ellis, 2001). Morbidity accompanied with poor performance in receiving cattle is being addressed by nutritional intervention with hope to lower costs associated

with antibiotic treatments. Supplementing amino acid complex sources of trace minerals in replace of inorganic forms (sulfates) to receiving beef cattle is present in literature, but results are variable regarding growth performance and incidence of BRD. However, the effects on cattle performance following a receiving period have yet to be investigated.

Thus, the purpose of this series of experiments was to investigate the effects of inorganic or amino acid-complexed sources of trace minerals on health, gain performance, liver mineral concentrations, and carcass characteristics of beef heifers from receiving through finishing.

CHAPTER II

REVIEW OF LITERATURE

Bovine Respiratory Disease

The beef industry in the United States is comprised of numerous segments including seedstock, cow/calf, stocker/feedlot, and packers that work together to produce various products for the consumer. The initial segments, seedstock and cow/calf, consist of thousands of producers across diverse landscapes charged with breeding and producing calves (Thomas et al., 2018). The USDA National Agricultural Statistics Service (NASS) reported that the United States calf crop was estimated at 35.1 million head for the year of 2022 (USDA, 2023). Additionally, the average herd size in the U.S. is quite small (average = 43.5 head). The majority of cow calf producers do not produce enough calves to comprise a full truckload annually so calves are comingled from various locations. The calves that are produced by such operations are usually weaned from the dam between 6 to 8 months of age at which time they are often sold through local sale barns. When calves go through this process, they are exposed to various foreign pathogens due to the commingling of calves from other sources. This, combined with dehydration, changes in nutrition, and transportation could potentially leave their immune system negatively impaired.

As ownership changes frequently with each beef industry segment, an informational disconnect exists on calf performance regarding efficiency and health status as they move from one segment to another. The 5 consequences of stressors in newly received feedlot calves are: 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 according to Loerch and Fluharty (1999). Immunosuppression

resulting from concurrent management stress and viral infection in calves allow otherwise commensal bacteria inhabiting the respiratory tract to defeat a compromised immune response and proliferate extensively in the nasopharynx and bovine lung resulting in “shipping fever” pneumonia (Hodgson et al., 2005).

Stress- and virus-induced immunosuppression are considered major risk factors for the development of this respiratory issue in beef cattle (Kaufman, 2018). Most of the immunological changes are related to the immune cell numbers in the blood, in lung tissues, and the functional status of the cells involved (Earley et al., 2017). The immune system is divided into innate and adaptive responses. These responses can work together in an integrated manner to provide protection from such pathogens and diseases. The responses correlated with innate immunity are evolutionary conserved and non-specific, and therefore, effective immediately upon exposure against a wide variety of potential pathogens (Sordillo, 2016). The adaptive immune system provides specific responses to infectious pathogens, and therefore, takes several days to mount a response (Sordillo, 2016). The stress these calves are undergoing between segments of the industry and various ownership can alter their susceptibility to diseases by disrupting their cellular (Simensen et al., 1980) and humoral (Murata et al., 1987) immune responses. The overlap of these two mechanisms can occur which causes disruption of balancing the components of the immune system. One stressor could enhance the cell-mediated immune responses while simultaneously suppressing humoral responses. This has important implications for weaned beef calves developing respiratory complications and diseases.

It is important to note the defense mechanisms and immune responses within the respiratory tract. The respiratory tract is a mucosal surface that differs from other bodily surfaces where infectious agents have opportunity to interact with the host and diseases may develop

(Elis, 2001). There is close contact with the external and internal environments when considering gas exchange in the respiratory system. The mucosal surface has a filtering system that can remove small particles before they have opportunity to reach the alveoli, where gas exchange occurs. This mucosal lining is coated by mucous containing antimicrobial soluble factors like lysozyme and immunoglobulin A, and it performs trapping and antiseptic functions (Tizard, 1987). Viral agents could alter or destroy the structural integrity of the filtering system such as cilia and the epithelial cells lining the respiratory tract (Ellis, 2001).

Bovine respiratory disease (BRD) is a multifaceted syndrome involving physiological stress, commingling, and several viral and bacterial pathogens (Richeson, 2011). This disease is mostly characterized by an initial viral infection that is followed by a secondary bacterial infection. The pathogenesis of BRD typically occurs in a distinctive stepwise manner (Richeson, 2011) due to the experiences the calves are undergoing between each segment of production in the United States cattle industry. The 4 common viral pathogens associated with BRD include: 1) bovine herpesvirus (also known as infectious bovine rhinotracheitis virus; IBRV), 2) bovine viral diarrhea virus (BVDV), 3) parainfluenza$_3$ virus (PI3V), and 4) bovine respiratory syncytial virus (BRSV). There is evidence supporting that both cell-mediated and antibody responses are associated with disease sparing in bovine herpesvirus-1 (BHV-1) infections (Hasoksuz, 1999). For evidence regarding BVDV antigens, it is reported that they act as a respiratory pathogen primarily in the context of local and systemic immunosuppression by virtue of infection of mononuclear phagocytes, including bronchoalveolar macrophages (Ellis, 2001). It is known that PI3V infects bronchoalveolar macrophages and is likely to have immunosuppressive effects in the bovine respiratory system (Keles, 1998). The role of BRSV infection and the specific immune responses involved has been proposed in pathogenesis of atypical interstitial pneumonia

(AIP) in feedlot cattle, largely based on the similarity of gross and histologic lesions in BRSV-infected cattle and in cattle with AIP (Andrews, 1997; Baker, 1997). Importantly, the co-infection of bacterial and viral respiratory pathogens results in viral-bacterial synergy (Ohmann and Babiuk, 1985).

Additionally, there are 3 commonly known bacterial species that are described today in their involvement with BRD. The most frequent bacterial pathogen established to be associated with BRD is *Mannheimia haemolytica* (Ellis, 2001). Other pathogens, for example, *Pasteurella multocida* and *Histophilus somni* are not as persistent with BRD incidences. It can be concluded that the variety of bacteria and the immune responses of the host can result in inflammation responses that have opportunity to lead to severe lung damage.

Despite the recent advances in technology, biological, and pharmaceutical products designed to mitigate the disease, BRD remains the leading cause of feedlot morbidity and mortality (Woolums et al., 2005). Vaccinations with a multivalent respiratory vaccine containing antigen strains listed previously can be highly effective at preventing BRD if administered to an immunocompetent animal (Perino, 1996). BRD has been the most economically important disease costing producers in North America $800 to $900 million every year (Blakebrough-Hall et al., 2020). This is continuing to cause economic loss in cattle production systems, despite the widespread use of antibiotics and vaccines (Ellis, 2001).

Mineral Nutrition in Beef Cattle

It is evident throughout advances in beef production that for cattle to perform at their greatest genetic potential, it is important that their nutritional demands are met. Optimizing cattle performance is dependent on the greater nutritional demands for energy, protein, vitamins, and

lastly minerals. It is known that even though minerals make up a small portion of an animal's diet, they play an important role in their health and aid in growth performance.

Minerals are important because of their responsibility for the various functions within the body. Suttle (2010) summarized 4 broad types of functions as: structural, physiological, catalytic, and regulatory. Certain minerals can form structural components in organs and tissues. For physiological functions, minerals are present in bodily fluids and tissues as electrolytes to aid in the maintenance of osmotic pressure, acid base balance, membrane permeability, and transmission of nerve impulses. Additionally, minerals can act as catalysts in enzyme and endocrine systems. Certain activities involved with these catalysts can be anabolic or catabolic, life enhancing (oxidant) or life protecting (antioxidant). Lastly, minerals aiding in regulatory functions are assisting in the regulation of cell replication and differentiation. In the animal, multiple functions can be performed simultaneously by a single mineral. These incidences can also take place in plants for example or other sources on which livestock depend (Suttle, 2010).

Essential minerals can be delivered to the animal from various sources. Minerals can be found in practical feedstuffs, although certain minerals can be insufficient in diets fed to cattle; therefore, supplementation is necessary to optimize animal performance or health (NRC, 1996). However, there are natural sources of mineral consumption by livestock. Mineral concentrations in plants on which livestock graze are largely dependent on 4 factors: plant genotype, soil environment, climate, and stage of maturity (Suttle, 2010). Fertilization will alter mineral concentrations. Crop by-products are usually enriched with minerals, but there is increased variability in concentrations. Drinking water is not usually a major source of minerals, although there are exceptions (Shirley, 1978). Sulfur concentrations vary with water sources especially considering deep aquifers.

When considering natural sources of minerals for livestock, it is evident that natural sources alone do not always provide a complete feed for beef cattle to meet their nutritional requirements for production. For any animal to maintain long-term production, mineral supplementation is necessary (Greene, 2000). Essential mineral requirements for beef cattle are dependent on their purpose in production and the producer's goals. It is stated in the Nutrient Requirements of Beef Cattle that requirements for minerals are dependent on the stage of life and the level of production (NRC, 1996). When only considering net requirements, the functions performed by minerals can only be performed if enough are absorbed and retained to keep pace with growth, development, and reproduction (Suttle, 2010). Supplementing cattle to meet their maintenance requirements varies with each element and species, but the goal is to provide the amount the animal is "losing" or secreting out of the body. Minerals are lost largely via the feces as sloughed mucosal cells, microbial residues, and unabsorbed digestive secretions. Lastly, the net mineral requirement for beef production is determined by the mineral content of each unit of production for weight gain. These requirements are affected by the productive capacity of the breed, rate of production allowed by other constituents- notably energy- and the environment (Suttle, 2010).

Trace Mineral Nutrition

Minerals are generally divided into 2 different classes, 1) macro-minerals and 2) micro or trace minerals. Macro minerals, which include calcium, phosphorus, magnesium, potassium, and sulfur are required in large amounts and are usually expressed in percentages of the total diet or grams per day when supplemented. Microminerals, also referred to as trace minerals, are required in smaller amounts and typically expressed as parts per million (ppm) or milligrams per kilogram body weight or dry matter intake (mg/kg), which are concentration units. Macro

minerals are known to have important physiological functions in beef cattle and must be supplemented to beef cattle diets when forages and rations are deficient or have the incorrect proportions (Paterson and Engle, 2005). If cattle are not supplied with minerals in the correct amounts and proportions, metabolic diseases or toxicities can inhibit performance. However, Patterson and Engle (2005) stated that several of the mineral imbalances commonly observed in beef cattle are due to imbalances in trace minerals. Such deficiencies, toxicities, and imbalances of trace minerals require the animal to metabolically compensate for the nutrient deviation.

Assessment of Trace Mineral Status

The interrelationship of metabolic processes and physiological functions persists within ruminants where an assessment of trace mineral status could provide additional insight. The reasons for assessing trace mineral status include: 1) to determine whether a nutrient deficiency exists, 2) to assess the prevalence of a deficiency, or 3) estimate the endogenous reserves of trace minerals (Kincaid, 1999). There are several methods that can be used to assess trace mineral status within ruminants, so it is important to utilize the appropriate method to determine which nutrient is deficient for correction. Choosing the appropriate measurement criteria in the animal varies depending on the trace mineral in question.

Analyzing the diet or forages being grazed for the trace mineral in question, as well as other minerals that can affect the requirement of the mineral can provide useful supporting data (Spears et al., 2022). It should be noted that trace mineral content in forages and by-product feeds varies considerably, and a representative sample should be obtained for analysis. With diet and forage analysis, chemical analysis is required and should include elements with important interactions such as, Mo, Se, and Fe for example (Kincaid, 1999). However, to determine deficiencies using dietary concentrations of minerals alone is inadequate due to the variation of

factors that can affect the amount of minerals in the diet that are absorbed by the animal (Suttle, 2010). As mentioned, water can be a natural source that provides certain minerals for livestock. In some areas, mineral analysis of water can also be useful in assessing trace mineral status (Spears et al., 2022).

Samples of blood, urine, feces, saliva, and hair are used for assessment of trace minerals and have the advantage of accessibility by utilizing minimal invasive procedures (Suttle, 2010). Blood sampling is the most common method used for ruminants. Trace mineral analysis can be determined by sampling of the blood components including whole blood, plasma, and serum concentrations (Suttle, 2010). Blood measures are commonly used because they are significantly correlated to the nutritional status of some trace minerals (Kincaid, 1999). Spears and colleagues (2022), mention in an invited review that red blood cell concentrations of iron (Fe), zinc (Zn), manganese (Mn), and selenium (Se) can be detected in plasma. However, there are also limitations to consider when performing blood analysis. If measuring concentrations of trace minerals in plasma and serum, if hemolysis of red blood cells occurs, this will result in falsely elevated levels (Spears et al., 2022). Additionally, red blood cells in cattle have a life span of about 160 days and concentrations of minerals in whole blood (erythrocytes) often change slowly which could produce invalid results (Kincaid, 1999). Minerals can be incorporated as functional units in the immature erythrocyte after the release in the bloodstream, and the mineral content of younger erythrocytes singly reflect recent mineral nutriture (Suttle, 2010). Furthermore, mineral excretion via urine and feces may correlate with either mineral intake or mineral status of the animal. Such concentrations though are greatly influenced by digestibility of the diet and water intake, which vary between each animal. However, if the animal has prolonged exposure to low intakes, fecal mineral concentrations may contribute to a diagnosis

(Suttle, 2010). Hair samples are rarely taken due to the slow response of mineral intake and contamination risks.

Minerals are typically transported from the serosal side of the mucosa to the liver in either free or bound forms by the portal blood stream (Suttle, 2010). From the liver, the minerals are transported with the intent to be absorbed by different organs and tissues. Additionally, the absorption rates are variable as they depend on the transporter mechanisms in cell membranes and organelles in order to meet the intracellular needs (Suttle, 2010). Of the body tissues, liver and bone serve as these storage organs for several minerals which can be sampled for mineral composition. Liver is the organ that often represents the status of various trace elements in animals (McDowell, 1992). Mineral turnover rates can vary from tissue to tissue but are the greatest in the liver and slowest in the bone. Samples of the liver can be collected by biopsy techniques, but collection is more invasive and time consuming compared to the other measures used for mineral status previously mentioned. Liver trace mineral concentrations can be expressed on a DM basis or wet weight basis. It is recommended in literature that liver mineral concentrations should be analyzed on a DM basis because dehydration and other factors that make liver moisture content variable (Spears et al., 2022). Concludingly, liver concentrations can assist in indicating early dietary deprivation and depletion of certain elements.

Zinc

The essential trace mineral zinc is crucial for many physiological processes and is one of the most studied factors in nutrition and health (Weyh et al., 2022). Zinc plays various roles that are important regarding immune responses, enzyme systems, DNA, RNA, and protein production (Ward and Lardy, 2005). It is known that zinc plays elementary roles as being a regulator or being presented as a coenzyme for a great number of enzymes. Additionally, zinc is

required in diets for the sake of structural integrity of over 2,000 transcription factors (Beattie and Kwun, 2004). Zinc can act as an antioxidant within the body thus influencing the stability of biological membranes and the arrangement of multiprotein complexes, such as the T-cell receptor (Weyh et al., 2022).

Adequate zinc uptake is essential for the performance of both the innate and the adaptive immune system (Bonaventura et al., 2015). When referring to zinc's role with innate immune responses, it plays a central role in the activity of nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase of neutrophil granulocytes (DeCoursey et al., 2003). This enzyme is important for the body's defense mechanism against microbes by producing superoxide anions (Babior, 1999). Zinc also has a major role in natural killer (NK) cells where if undergoing deficiency, can induce NK cell counts and lead to impaired functionality (Weyh et al., 2022). Furthermore, regarding zinc's influence on adaptive immune responses, it is involved with the formation, maturation, and function of T cells as mentioned previously (Prasad, 2008). Specifically, T-cell development and function is compromised if a zinc deficiency is present and can inhibit maturation in the thymus affecting cytokine production.

In addition to zinc's effect on selective immune functions, zinc status is associated with the overall regulation of the immune system, normal growth rates, water and cation balance, and vitamin A metabolism (Weyh et al., 2022; McDowell, 2003). Increases in oxidative stress and systemic inflammatory responses are associated with zinc deficiencies and can influence the production and signaling of inflammatory cytokines. Furthermore, zinc plays a role in antioxidant defense intracellularly and extracellularly by ZnCu superoxide dismutase acting with vitamin E to provide a protection mechanism against iron-induced lipid peroxidation and by inducing metallothionein (Suttle, 2010).

The recommended requirement for zinc in beef cattle diets is 30 mg of Zn/kg dietary DM (NASEM, 2016). Specific requirements vary with age and growth rate as zinc absorption and growth rate decreases with age (McDowell, 2003). Requirements are also dependent on the production and use of the animal for example reproduction, lactation, growth, or remaining at maintenance. Zinc is the third most common trace mineral that is deficient for grazing cattle (Arthington and Ranches, 2021). If calves at an early age undergo zinc deficiencies, it can lead to reduced feed intake, growth rate, and feed efficiency (McDowell, 2003). Clinical consequences of zinc deprivation include anorexia, abnormalities of skin and appendages, skeletal disorders, and reproductive disorders (Suttle, 2010). The amount of zinc necessary to cause toxicity is much greater than requirements (NASEM, 2016). The maximum tolerable limit of zinc in ruminants is 500 mg/kg in the diet which will result in toxicity (NRC, 1996).

Zinc status in the ruminant animal can be determined using several assessment methods. However, it lacks a reliable tissue pool and well-defined enzyme for indicating status (Arthington and Ranches, 2021). Analyzing diets and forages for zinc is important in predicting the adequacy of the dietary zinc offered to the animal (Spears et al., 2022). Blood sampling can be done to determine plasma and serum zinc concentrations. Normal zinc concentrations in serum and plasma range between 0.6 and 1.2 mg/L (Suttle, 2010). Cattle undergoing acute stress or infection may result in a decrease in zinc concentrations representing a normal physiological response (Spears et al., 2022). Additionally, liver zinc concentrations are sensitive to zinc intake, even though the concentrations are affected by age of the animal (Kincaid, 1999). Specifically, calves readily absorb and bind large amounts of zinc as metallothionein in the liver as a result of increased zinc intakes (Kincaid et al., 1976). Zinc concentrations in the liver of cattle are considered adequate between 25 to 200 µg/g on a DM basis and is considered as toxic if

concentrations exceed 1,000 µg/g (Puls, 1988). Cattle are considered marginal in zinc when liver concentrations are between 25 and 40 µg/g of dry liver and deficient if concentrations fall under 20 µg/g dry liver (Puls, 1988).

Manganese

Manganese is considered an essential dietary element for ruminants (Hidiroglou, 1979). Conversely, there is limited literature focusing on this trace mineral, making it one of the least understood minerals in ruminant nutrition (Hansen et al., 2006). Suttle (2010) states that manganese is essential for various functions in the body, such as amino acid metabolism, cholesterol metabolism, and activation of enzyme systems involved in oxidative phosphorylation, bone formation, growth and reproduction. The main functions of manganese that are known can be linked to metalloenzymes which are activated by the element (Suttle, 2010). Manganese functions as both an enzyme activator as well as a constitute of enzymes (McDowell, 2003).

Manganese-containing enzymes include pyruvate carboxylase, arginase, and superoxide dismutase. Pyruvate carboxylase was discovered to be a manganese metalloprotein, confirming a specific biochemical role for manganese in energy metabolism persists (Suttle, 2010). Arginase's importance derives from being the final enzyme in the urea cycle. Mn-superoxide dismutase is strictly located in the mitochondria but acts similarly to CuZn-superoxide dismutase by protecting cells from damage from reactive oxygen species, specifically the superoxide radical O_2^- (Suttle, 2010). Furthermore, the Cu-Zn enzyme is responsible for scavenging O_2^- generated in the cytosol by oxidation-reduction reactions, however the manganese enzyme scavenges the O_2^- generated in the mitochondria. Manganese also can activate various hydrolases, kinases, transferases, and decarboxylases (McDowell, 2003). There are specific enzymes that need

manganese for activation. For example, manganese is needed for mucopolysaccharide synthesis in cartilage through the activation of the enzyme glycosyltransferase (Suttle, 2010).

The majority of manganese literature concludes that manganese plays an important role in growth and reproduction. As dietary concentrations of manganese increase, the concentration of the mineral also increases in reproductive tissues, providing a direct link between manganese and fertility (Ward and Lardy, 2005). Additionally, Hidiroglou (1975) found that manganese uptake was greater in the ovine graafian follicle and corpus luteum suggesting that manganese deficiency is associated with anestrus in cattle. The enzymatic properties of this trace mineral support it is essential for growth development in ruminants.

As with other minerals, manganese requirements vary depending on the stage and purpose of production. The manganese requirement for growing and finishing cattle is approximately 20 mg of Mn/kg diet (NASEM, 2016). However, manganese requirements for reproduction are greater than for growth and skeletal development, and the recommended concentration for breeding cattle is 40 mg/kg (NASEM, 2016). Suttle (2010) states that few pastures or forages will fail to meet NRC requirements. Consequently, Arthington and Ranches (2021) state that although variable, manganese concentrations of forages are commonly at or above the recommended 40 mg/kg of DM. The maximum tolerance for this mineral can be as high as 1,000 mg/kg before it causes noticeable negative effects, but it may interact with many different minerals that can alter the animal's tolerance (Ward and Lardy, 2005).

According to Hidiroglou (1979), all ruminant tissues contain manganese in low concentrations and variability within tissues is small. It is not common for manganese deficiencies to be reported in ruminants; however, there are no reliable biomarkers for declaring diagnosis (Arthington and Ranches, 2021). Manganese concentrations in the blood, bones, and

liver decline in animals when undergoing deprivation, but such markers are not as reliable as liver for elements like copper and selenium (Hansen et al., 2006). Diet analysis is generally considered the best indicator for dietary manganese adequacy (Underwood, 1981; Puls, 1994). For assessing manganese status in the animal, several criteria have been used including concentrations in plasma or serum, whole blood, liver, hair, and Mn-dependent superoxide dismutase activity in ruminants (Spears et al., 2022). Typical manganese concentrations are 5 to 10 ng/mL of plasma in cows (Gibbons et al., 1976). Kincaid (1999) mentions in a review article that manganese concentrations in red blood cells are greater when compared to plasma. It is important to note that the liver efficiently removes manganese from plasma, whether the manganese is as Mn^{2+} or is bound to α_2 macroglobulin, but not Mn^{3+} bound to the transferrin complex (Gibbons et al., 1976). When assessing liver manganese concentrations, it must be considered that the manganese that is being taken up by the liver is excreted endogenously through bile, and the accumulations of manganese left in liver tissues is not reflecting dietary intakes of manganese (Kincaid, 1999). For assessing the Mn-dependent superoxide dismutase activity in ruminants, only activity has been found significantly correlated in the heart to manganese intake (Masters et al., 1988), making it difficult to assess on a live animal.

Copper

In the body, copper is present and essential for the activity of various enzymes, cofactors, and reactive proteins (Suttle, 2010). Functions of copper in the body such as cellular respiration, protection from oxidants, and iron transport are important for red-blood cell health, collagen development, reproduction, and immune function of ruminants (Suttle, 2010; Ward and Lardy, 2005).

With many diverse functions of this trace mineral, one major role of copper is its involvement as an integral constituent of several metalloenzymes (Richardson and Cromwell, 1997). Cellular respiration for example, copper serves as a cofactor in the respiratory chain participating in the transfer of elections to oxygen and is important for the oxidative balance (Husain and Mahmood, 2019). Copper is associated with intracellular and extracellular oxidases (Richardson and Cromwell, 1997). One of the most important enzymes involved is Cytochrome c oxidase, which is responsible for the terminal electron transfer in the respiratory chain including energy generation in all tissues (Suttle, 2010). This enzyme catalyzes the reduction of oxygen to water and is required for normal myelination of brain cells and spinal cord aiding in the integrity of the nervous system (Richardson and Cromwell, 1997).

Additionally, superoxide dismutase is also an important copper-containing enzyme that is involved with the disposal of potentially damaging superoxide anions (Richardson and Cromwell, 1997). Copper, via action of ZnCu superoxide dismutase, has effect on neutrophils and macrophages' ability to kill foreign cells through the respiratory burst (Suttle, 2010). ZnCu superoxide dismutase and ceruloplasmin are the leading copper proteins in plasma that scavenge free radicals serving as antioxidants. Arthington and Ranches (2021) state that ceruloplasmin is associated with approximately 90% of copper found in the blood and is considered a major bovine acute phase protein. If copper-adequate cattle are undergoing inflammatory distress, ceruloplasmin concentrations increase instantly. However, in copper-deficient cattle this response will result in an alteration of this acute phase reaction by increasing haptoglobin and fibrinogen concentrations (Arthington and Ranches, 2021).

Availability of copper has impact on maintenance of immune competence, where a deficiency leads to reduced humoral and cellular immune function (Weyh et al., 2022). Copper

deficiency amongst cattle is one of the most widespread trace mineral deficiencies, specifically in grazing cattle (Spears et al., 2022). Additionally, copper is essential for microbial pathogens and the body can inhibit the growth of pathogens by limiting their copper availability (Weyh et al., 2022).

The recommended requirement for copper in beef cattle diets is 10 mg Cu/kg DM but can vary depending on the concentration of dietary molybdenum and sulfur (NASEM, 2016). This requirement was increased from 8 to 10 mg Cu/kg DM from the sixth to seventh NRC revision and has remained unchanged. With sulfur, molybdenum, and iron being copper antagonists, the copper requirement may vary greatly depending on such dietary factors (Arthington and Ranches, 2021; McDowell, 2003). Evidence suggests that molybdate and sulfide interact to form insoluble complexes to form thiomolybdates within the rumen (NASEM, 2016). Molybdenum and sulfur each can bind with copper independently within the rumen forming complexes that are poorly absorbed or completely not absorbed (Spears, 2002). Furthermore, in the presence of ruminal H^+ ions, sulfur is reduced to sulfide, which reacts with molybdenum to create thiomolybdates (Mason, 1986). Absorption is affected in the gastrointestinal tract by these thiomolybdates binding to copper. These thiomolybdates that are associated with solid rumen digesta (bacteria, protozoa, and indigested feed particles) form insoluble complexes that do not release copper in the abomasum where the environment is acidic (Allen and Gawthorne, 1987). To conclude, copper bioavailability can be reduced up to 70% when molybdenum levels are not corrected when sulfide concentrations increase (Suttle, 1975). Therefore, it is important to have a balance of molybdenum and copper within diets. The 10 mg Cu/kg requirement provides adequate copper if the diet does not exceed 0.25% S and 2 mg Mo/kg (NASEM, 2016).

Copper is distributed throughout the body in muscle tissue, internal organs, blood, bones, and hair (Richardson and Cromwell, 1997). When assessing status of animals, liver copper concentrations are the best indicator in ruminants (Spears et al., 2022). Following copper being absorbed, it is bound to albumin, amino acids, or the protein transcuprein for transport to the liver (Richardson and Cromwell, 1997). In the liver it is either incorporated into ceruloplasmin (or other liver proteins), bound to metallothionein, or excreted out of the body via bile (Richardson and Cromwell, 1997). The liver is known to be the major storage organ for copper, and the copper storage can readily be transported to the blood for biochemical functions (Spears et al., 2022). Normal liver copper concentrations for cattle range from 125 to 600 µg/g dry liver and are considered marginal if concentrations fall between 33 and 125 µg/g dry liver (Puls, 1988). If liver concentrations are below 33 µg/g dry liver, that animal is considered deficient (Puls, 1988). It is important to note that these concentrations can be affected by dietary copper, age, species, breed, and potentially sex and gestation (Spears et al., 2022).

Plasma and serum copper concentrations can also be utilized to assess copper status in ruminants. It is recommended that copper should be measured in plasma rather than serum because in the process of clotting some of the copper present is sequestrated into the clots (Laven and Smith, 2008). Erythrocytes have a representable fraction of copper that is loosely bound to protein and a copper fraction that includes superoxide dismutase (Kincaid, 1999). However, activity of superoxide dismutase is not an accurate measure of copper status because copper and ceruloplasmin activity is not consistently reduced until liver copper is low (Kincaid, 1999). Other copper-containing enzymes can be assayed to measure copper status, but is difficult to standardize (Spears et al., 2022).

Cobalt

Cobalt is considered an essential trace mineral for ruminants even though the only known nutritional function is it being a component of vitamin B12. Plants are not capable of synthesizing this vitamin, so most monogastric animals require cobalt in its active form, vitamin B12 (González-Montaña et al., 2020). However, ruminants produce vitamin B12 from cobalt by the microbes within the rumen. In mature ruminants, this is produced during the microbial fermentation of food in the stomach, but largely in the rumen (Stemme et al., 2006). It is worth noting that vitamin B12 production only occurs in certain bacteria and algae.

Vitamin B12 is an essential part of several enzymatic systems that partake in metabolic functions, energy metabolism, and cell replication processes (González-Montaña et al., 2020). Cobalt inclusion is important specifically in ruminants due to the rumen microbial population that needs vitamin B12 to produce propionate, which is a metabolite that is a major determinant of the host's cobalt responsiveness (Suttle, 2010). In further detail, cobalt has been shown to influence intermediate energy metabolism keeping the tricarboxylic acid cycle moving by helping methylmalonyl coenzyme A (CoA) mutase to form succinate from propionate (Suttle, 2010). This coenzyme plays an important, regulatory role in gluconeogenesis and fatty acid oxidation (Graulet et al., 2007). Additionally, methylmalonyl-CoA mutase participates in the degradation of odd-chain fatty acids and in metabolic pathways that involve branched-chain amino acids and cholesterol (González-Montaña et al., 2020). Vitamin B12 in ruminants also assists in methionine synthase by transferring methyl groups from 5-methyl-tetrahydrofolate to homocysteine via S-adenosyl methionine for the regeneration of methionine (Suttle, 2010). Thus, this reaction links the metabolism between cobalamin and folate which plays an essential role in

transfer of methyl groups (González-Montaña et al., 2020). To conclude, methionine synthetase is crucial for nucleic acid synthesis (Rizzo and Laganá, 2020).

Additionally, it is important to consider all interactions between other minerals. An interference has been shown with cobalt and iron absorption (González-Montaña et al., 2020). Within the rumen, there is an inverse relationship between cobalt and iron, where iron deficiency symptoms seem to improve cobalt absorption (McDowell, 2012). Furthermore, high concentrations of either mineral can reduce selenium absorption (Schwalfenberg and Genuis, 2015). Recent research indicates that cobalt can interact with several amino acids (González-Montaña et al., 2020). Cobalt ions are actively being absorbed via the lumen of the small intestine by a divalent cation transporter that is responsible for other divalent cations (González-Montaña et al., 2020). This could be the explanation for such interactions mentioned. For absorption of vitamin B12 to take place, it must be bound to intrinsic factor, which is produced by parietal cells in the abomasum (Suttle, 2010). Following absorption into the portal circulation, vitamin B12 and folic acid are transported to the liver where a portion will be used by the liver cells meanwhile the remaining is methylated and released into the bloodstream for later use by tissues (González-Montaña et al., 2020). Vitamin B12 is stored in the liver in a specific way where, when the reserves are at maximum, they are usually enough to satisfy all the animal's needs for periods of greater than a year (Herdt and Hoff., 2011).

If deficient of cobalt, vitamin B12 storage in the liver and kidney will deplete. Other deficiency signs include decreases in feed intake and body weights and even anemia (Suttle, 2010). Furthermore, biochemical changes during deficiency include a decreased clearance of propionate and acetate from the blood and excretion of methylmalonic acid in urine (Suttle, 2010). Even though cobalt holds an indirect role in such functions, without synthesis in the

rumen, energy and one-carbon metabolism are not efficient and will not result in optimal health and performance for beef cattle (Van Emon, 2020). The requirement of cobalt for cattle remains at 0.10 mg/kg DM, but concentrations in the diet are considered adequate between 0.07 and 0.11 mg Co/kg (NASEM, 2016). There has been a question if the requirements should be increased further to maximize vitamin B12 concentrations and overall cattle performance.

Due to the metabolic role of cobalt, assessment of the animal's nutriture often centers on measures of vitamin B12 status, even though liver concentrations of cobalt and performance responses to cobalt inclusion can also be used in assessment (McDowell, 1992). In most tissues, cobalt can be found in concentrations lower than 0.2 mg/kg and it is not accumulated in any specific organs or tissues, but the liver, heart, and bone have been shown to hold the highest concentrations (González-Montaña et al., 2020). Kincaid (1999) states that concentrations of vitamin B12 in liver, but not in serum, more accurately reflect the body's reserve. Serum concentrations have shown great variation and are subject to cobalt intake (Paterson and MacPherson, 1990). Formiminoglutamic acid (FIGLU) concentrations in the urine have also been used as an indicator for copper deficiencies due to its involvement in the enzyme methionine synthetase (Kincaid, 1999).

Supplementation of Various Sources

As stated, trace minerals play vital roles in immune function, growth, and overall performance of beef cattle. Mineral inclusion to livestock diets must be sufficient to ensure the maintenance of body reserves and avoid deficiencies and toxicities. Evaluating feedstuffs and mineral supplements for cattle is dependent on 1) mineral content, 2) potential availability, and 3) potential absorption of the minerals from the gastrointestinal tract and then the mineral's utilization by tissues (Ammermam, 1995). Additionally, absorption can be variable depending on

age, intake of mineral relative to the amount required, chemical form of the mineral, and mineral interactions (Bourne and Hazell, 1985). Various sources, concentrations, and combinations of trace mineral supplements for cattle and their effects on immune function, growth, and performance measures have been evaluated. The following discussion will be limited to studies which investigated the effects of different sources and chemical forms of supplemental zinc, manganese, copper, and cobalt within beef cattle diets.

Livestock diets are often delivered with trace minerals supplemented in the form of inorganic salts, usually oxides, chlorides, sulfates, and carbonates (Hilal et al., 2016). These inorganic forms have been used in cattle diets for years because they are widely available and represent an inexpensive form of supplementation.

In recent years, animal nutritionists have evaluated organic mineral supplements and indicated their greater bioavailability and functionality over inorganic salts. The Association of American Feed Control Officials (AAFCO) have listed the organic trace minerals in the United States that are available in the following forms: metal proteinate, metal amino acid chelates, metal amino acid complex, and metal polysaccharide complex (AAFCO, 2000). Organic mineral supplements are usually more expensive (averaging 68 cents/kg) than inorganic minerals (16 cents/kg) (Ward and Lardy, 2005). The main difference between inorganic trace minerals is that they undergo a process of chelation with transition elements, often involving reacting inorganic mineral salts with suitable bonding groups. Bonding groups such as peptides or amino acids are chosen so the mineral becomes a part of a biologically stable structure (Byrne et al., 2021). Roy and Misger (2008) determined that for chelation to be effective, the chelating agent should have a stronger stability for the metal than the metal binding substances within the feed ration, but smaller stability constant than the tissue system where the metal is specifically required. There

are numerous factors that can affect the metal ion uptake mechanism in the animal. Known variables include metal ion equilibria, kinetic factors, pH gradients, and redox equilibrium (Lyons et al, 1999). The aim for the following discussion will be exploring factors affecting bioavailability and functionality when using metal amino acid complexed in replace of inorganic sulfates for zinc, manganese, copper, and cobalt. In which, metal amino acid complexes are the products resulting from complexing a soluble metal salt with a single amino acid (AAFCO, 2000).

Zinc

Supplemental zinc in cattle diets has positive effects on both health and growth performance. Furthermore, organic zinc is metabolized differently than inorganic sources. The common commercially available metal complex for zinc supplementation is Zn-methionine and Zn-lysine (AAFCO, 2000). In four consecutive studies, zinc was shown to be better retained when added as Zn-methionine in lambs as well as heifers (Spears, 1989). However, in this study the observations were justified not on greater absorption, but on the lower zinc excretion in animals receiving Zn-methionine. A study conducted by Kegley et al. (2001) showed 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 source comparison with a negative control diet consisting of 25 mg zinc/kg DM. This study also reported an increased response to phytohemagglutinin injection in zinc supplemented calves versus the control. It was concluded that calves that were receiving the zinc-amino acid complexes had greater antibody response to a second vaccination for bovine respiratory syncytial virus in comparison to the zinc sulfate supplemented calves. Additionally, Chirase and Greene (2001) reported that when calves were stressed by transportation or challenged with infectious bovine rhinotracheitis virus, they tended

to have lower rectal temperatures, greater dry matter intake (**DMI**), and decreased loss in body weights (**BW**) when being fed organic zinc and manganese sources than the corresponding oxide sources. A recent study suggested a role for organic trace mineral supplementation in reducing digital dermatitis (**DD**) and increasing overall performance of beef cattle (Anklam, 2022).

Hilal et al. (2016) stated in a review article that there is difficulty evaluating zinc availability from different sources as it relies on the fact that zinc is being absorbed according to the animals needs and homeostasis in ruminants is achieved by control of intestinal absorption. Although there is evidence supporting that organic zinc supplementation has been shown to increase growth performance and immunity, not all studies were able to report similar results. Nunnery et al. (2007) reported that heifers receiving diets consisting of 52.5 mg/kg DM zinc had greater growth performance by day 35 on treatments compared to heifers receiving 75 mg/kg of DM supplemental zinc from sulfate, propionate, and methionine sources. Dorton et al. (2010) found in a comparative study that there were no differences in liver zinc and plasma zinc concentrations of steers receiving $ZnSO_4$ or Zn amino acid complexes. Given that these were feedlot steers, the mineral status of the cattle at the start of the study could account for such confounding effects.

Manganese

There are different sources of manganese that are currently available and used as supplements in livestock diets, however the most common are manganese carbonate ($MnCO_3$), hausmannite (Mn_3O_4), manganese oxide (MnO), manganese dioxide (MnO_2), manganite (Mn_2O_3), manganous chloride ($MnCl_2 \cdot 4H_2O$), and manganese sulfate ($MnSO_4$) which are inorganic sources (Hilal et al., 2016). The effects of supplemental manganese on performance and health of beef cattle have been investigated, however not as extensively as other minerals

such as zinc. Spears and Hansen (2008) stated that only a few studies have been conducted comparing bioavailability of different sources in ruminants fed physiological concentrations of manganese. However, there are organic options for manganese supplementation which include Mn-methionine, Mn-proteinate, and Mn-polysaccharide (Hilal et al., 2016). Kratzer (1986) stated that some chelates, and complexes might improve mineral bioavailability greater than the soluble inorganic forms. It has been mentioned that only organic manganese sources with moderate to strong chelation strength can provide greater bioavailability due to their ability to resist calcium antagonists during digestion (Hilal et al., 2016).

Although there are limited studies investigating manganese alone, there are studies that have compared a combination of trace minerals which include manganese. For example, Marques et al. (2016) investigated inorganic and organic sources of cobalt, copper, manganese, and zinc supplementation to late gestating beef cows. From this, it was reported that the amino acid complexed sources resulted in greater weaning weights of calves produced and calves had reduced incidences of disease in the feedlot. It was collectively stated that supplementing late gestating beef cows with an organic complexed source of trace mineral seemed to optimize offspring productivity.

Copper

Various technologies, including proteinates, amino acid chelates, amino acid complexes, and polysaccharide complexes are currently available for copper supplementation in livestock (Hilal et al., 2016). The effectiveness of copper being supplemented in livestock diets for optimal performance seems to be controversial. A study using an amino acid complexed source, Cu-lysine, compared to the inorganic sulfate reported greater retention of copper within steers supplemented with the organic source (Nockels et al., 1993). Additionally, Arthington et al.

(2003) reported lower DMI in calves supplemented with organic copper compared to inorganic copper and molasses-based supplements. It is important though in all studies that copper antagonists are considered. In a meta-analysis, assessing the benefits of amino acid complex source, replacement of sulfates showed marginal improvements in milk production, milk fat and milk protein were found in lactating dairy cows (Rabiee et al., 2010). Additionally, they found that the organic sources did not affect somatic cell count and pregnancy rates.

Cobalt

Literature on cobalt sources is limited regarding bioavailability, even though it is considered an essential trace mineral providing vitamin B12. Because cobalt is usually supplemented simultaneously with other trace minerals it is difficult to explain the individuality of the mechanisms involved with cobalt in ruminants. The common inorganic supplementation inclusion is cobalt sulfate ($CoSO_4$). Suttle (2010) states that inorganic cobalt sources must be readily soluble within the rumen to be of nutritional value to ruminants when used in diet supplementation.

Combinations of trace minerals: Zn, Mn, Cu, Co

Trace minerals mentioned throughout this discussion are rarely supplemented in livestock diets individually due to the interactions and known antagonists. Physiological pathways are often affected by more than one mineral at a time and that metabolism of minerals is interrelated. Numerous studies have investigated the effects of multiple trace minerals from different sources. Specifically, improvements in animal performance have been shown when supplementing amino acid complexed sources in replacement of common sulfates.

In 1997, George and coworkers investigated the effects of supplementing zinc (106 mg/kg), copper (37 mg/kg), manganese (58 mg/kg), and cobalt (7 mg/kg) from sulfate (1x

amount) or amino acid complexed sources (1x amount or 3x amount for first 14-days followed by 1x amount) on feedlot performance and immune function using stressed heifers. When feeding the 3x/1x treatment, there was a 17.2% reduction in the incidence of respiratory disease. Additionally, there was an improvement in primary and secondary humoral, and cell-mediated immune responses compared to the sulfate supplemented heifers. Considering this, there were no differences between treatments reported for DMI, ADG, or feed efficiency.

The effects of supplementing basal diets with manganese, zinc, and copper, as sulphate, glycine or methionine salts, on colostrum and milk performance, blood immunity indices and blood minerals of pre- and post-partum Holstein cows was investigated by Roshanzamir and coworkers (2020). Organic supplementation for 60 days prior to calving increased calf serum total antioxidant capacity and immunoglobulin A and M concentrations compared to calves from other treatments. This study also found that neutrophils, lymphocytes, and circulating concentrations of copper, manganese, and zinc were not altered by supplementation. Although all parameters did not support the hypothesis, the organic source of supplementation had an advantage over the sulphate forms in terms of the blood immunoglobulins. Here, it was concluded that supplementation of individual trace minerals could provide more specific benefits to immune function of offspring.

A study investigating complexed amino acid sources verses amino acid chelates of trace minerals in high-risk calves during receiving suggested that there were differences in the magnitude of health-related responses based on the percentage of calves requiring BRD treatments (Goodall and Schuetze, 2019). This study showed no difference in overall morbidity nor mortality percentages, but the percentage of calves receiving BRD treatments was less for calves receiving chelated sources. The overall receiving performance data were similar for both

treatments. The calves receiving the complexed sources seemed to compensate and this resulted in greater DMI in the second half of the trial.

Kegley et al. (2012) investigated the effects of supplemental zinc (360 mg/day), copper (125 mg/day), manganese (200 mg/day), and cobalt (12 mg/day) from sulfate or amino acid complexed sources in calves over a receiving phase. There was an increase in final body weights and ADG for the calves supplemented with complexed minerals. This was supported by observing a tendency for a decrease in percentages of the calves receiving a second antibiotic treatments for BRD compared to the sulphate supplemented calves. Furthermore, there was an increase in the antibody response to infectious bovine rhinotracheitis virus vaccination in calves supplemented with the sulfates.

Additionally, Lippolis et al. (2017) investigated the production and health effects of supplementing complexed and inorganic sources of copper, cobalt, manganese, and zinc in a preconditioning period. During the preconditioning period, the mean liver concentrations of cobalt, zinc, and copper were greater for the complexed treatment. There were no treatment differences to report for ADG, feed efficiency, antibodies against *Mannheumia haemolytica*, or haptoglobin concentrations. However, calves supplemented with inorganic sources resulted in greater mean plasma cortisol concentrations during the period comprising transport and feedlot entry. Even though liver concentrations were affected by mineral source, trace mineral source did not impact cattle performance or immune responses.

In conclusion although there is evidence that organically complexed mineral sources might occasionally have different effects on feedlot performance and immune function, effects are variable making it difficult to recommend feeding different sources. Therefore, the objective of this thesis research was to investigate the effects of inorganic or amino acid-complexed

sources of trace minerals (zinc, copper, manganese, and cobalt) on performance and morbidity of beef heifers during a receiving period.

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CHAPTER III

SUPPLEMENTAL TRACE MINERALS AS AMINO ACID COMPLEXED OR INORGANIC SOURCES FOR BEEF CATTLE DURING THE RECEIVING PERIOD

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ABSTRACT: To investigate the effects of inorganic or amino acid-complexed sources of trace minerals (zinc, copper, manganese, and cobalt) on performance and morbidity of beef heifers during the receiving period, crossbred beef heifer calves (n = 287, initial body weight = 231 ± 0.49 kg) arriving on 3 delivery dates were used in a 42-day receiving trial. Heifers were processed after arrival and stratified by d -1 body weights and allocated randomly to 8 pens (11 to 13 heifers/pen; 24 pens total). Within truckload, pens were assigned randomly to dietary treatment (12 pens/treatment). Calves were housed on 0.42-ha grass paddocks, provided ad libitum access to bermudagrass hay and water, and fed grain supplements that served as the carriers of the dietary treatments. Treatments consisted of supplemental zinc (360 mg/d), copper (125 mg/d), manganese (200 mg/d), and cobalt (12 mg/d) from complexed (Availa-4, Zinpro Corp. Eden Prairie, MN) or inorganic sources (sulfates). Cattle were observed daily for clinical bovine respiratory disease (BRD). If presenting symptoms of BRD and if rectal temperature was ≥ 40°C, cattle were deemed morbid and treated with an antibiotic according to a standard preplanned protocol. Six heifers/pen were bled to determine serum haptoglobin concentrations on days 0, 14, and 28. Statistical analyses were performed using the MIXED and GLIMMIX procedures of SAS 9.4 with truckload as a random effect and pen within truckload specified as subject. There tended to be a treatment by day interaction for body weights ($P = 0.07$). Body weights were similar on day 0 ($P = 0.82$) and day 14 ($P = 0.36$), but heifers supplemented with complexed trace mineral sources had greater body weights on day 28 ($P = 0.04$) and day 42 ($P = 0.05$; 264 vs. 260 kg, SE = 1.8). Overall average daily gains were greater for heifers supplemented with the complexed trace mineral sources ($P = 0.05$; 0.78 vs. 0.70 kg, SE = 0.03). Cattle supplemented with inorganic trace mineral sources had a greater percentage of BRD morbidity than cattle supplemented with complexed trace mineral sources ($P = 0.03$; 58 vs. 46%,

SE = 3.6). Medication costs were lower for heifers supplemented with complexed trace mineral sources (P = 0.05; $11.01 vs. $14.90, SE = 1.33). Haptoglobin concentrations decreased throughout the trial (day, P = 0.0002), and cattle supplemented with complexed trace mineral sources tended to have lower haptoglobin concentrations (treatment, P = 0.07). In conclusion, supplementing cattle for the first 42 days after arrival with amino acid complexed trace mineral sources improved heifer performance as compared to heifers supplemented with inorganic trace minerals.

INTRODUCTION

Trace minerals take part in important physiological functions in beef cattle and must be supplemented to beef cattle diets when forages and rations are deficient or have incorrect proportions (Paterson and Engle, 2005). Mineral inclusion to livestock diets must be sufficient to ensure the maintenance of body reserves and to provide appropriate concentrations in products that are edible. Various sources, concentrations, and combinations of trace mineral supplements exist commercially for cattle and their effects on immune function, growth, and performance measures have been evaluated in recent years. Livestock diets are often delivered with trace minerals supplemented in the form of inorganic salts, usually oxides, chlorides, sulfates, and carbonates (Hilal et al., 2016). These inorganic forms have been used in cattle diets for years because they are widely available and represent an inexpensive form of supplementation. However, organic trace mineral supplements are being used in replacement of inorganic salts due to potentially greater bioavailability and functionality (Mohanta and Garg, 2014). There are different forms of organic trace mineral supplementation commercially available. One form, being a metal amino acid complex, used in this study, is a product resulting from complexing a soluble metal salt with a single amino acid (AAFCO, 2000).

Issues associated with health and management of newly received cattle continue to pose significant animal welfare and economical challenges for the beef industry. Bovine respiratory disease (BRD) has been the most economically important disease costing producers in North America $800 to $900 million every year (Sanchez, 2022). Such loss remains a continuing problem in cattle production systems despite the widespread use of antibiotics and vaccines (Ellis, 2001). Morbidity accompanied with poor growth performance in receiving cattle is being addressed by nutritional intervention. Thus, the objective of this experiment is to investigate the effects of inorganic or amino acid-complexed sources of trace minerals (zinc, copper, manganese, and cobalt) on growth performance and morbidity percentages of beef heifers during a receiving period.

MATERIALS AND METHODS

Animal methods were approved by the University of Arkansas Animal Care and Use Committee (Approval #21142).

Two hundred-eighty-seven crossbred beef heifers (231 ± 0.49 kg) were obtained from a cooperating producer, who purchased calves in regional sale barns and then shipped cattle to the University of Arkansas Beef Cattle Research Facility near Fayetteville, AR. Heifers arrived in 3 shipment sets (block) with arrival dates of October 6, 2021 (block 1, n = 94), October 26, 2021 (block 2, n = 95), and November 23, 2021 (block 3, n = 98). Upon arrival (d -1), 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 (d 0), calves were administered respiratory (Pyramid 5, Boehringer Ingelheim

Vetmedica, Duluth, GA) and clostridial (Covexin 8, Intervet, In., Madison, NJ) vaccinations, and dewormed (Ivomec Plus, Boehringer Ingelheim Vetmedica, Duluth, GA). Calves received booster vaccinations on d 14. In addition to administering vaccinations, all calves were branded with a hot iron on the right hip and weighed. The weights recorded on both days (d -1, d 0) were averaged to represent initial weight values.

Within each block, animals were stratified by d -1 BW and allocated randomly to 1 of 8 pens (11 to 13 calves/pen). After the 3 truckloads (block) were delivered, there were 12 replicate pens per dietary treatment total. Within each block, pens were assigned randomly to a dietary treatment. Calves were housed on 0.45-hectare grass paddocks and were fed grain-grain by-product supplements (Table 1) that served as the carrier of the treatments. Treatments consisted of supplemental zinc (360 mg/d), copper (125 mg/d), manganese (200 mg/d), and cobalt (12 mg/d) from amino acid-complexed (Zinpro Availa-4, Zinpro Corp. Eden Prairie, MN; n = 4 pens/block) or inorganic sources (sulfates; n = 4 pens/block). Calves were offered supplement formulated for feeding at 0.91 kg/d (as-fed-basis) on d 0. When most of the calves in the pen were consuming the supplement at this rate, the pen was switched to a supplement with the appropriate mineral treatment that was formulated for feeding at a 1.36 kg/d (as-fed basis) rate (d 6, block 1; d 4, block 2; d 3, block 3). As per prior changes, pens were moved to supplements (with the appropriate mineral treatment) that were formulated to be fed a 1.82 kg/d (as-fed basis) rate (d 10, block 1; d 8, block 2; d 7, block 3). Changes in the supplement were formulated so the new supplement was approximately equal in nutrients to the original diet, but the percentage of soybean meal was reduced (Table 1). Calves received the1.82 kg/d supplements for the remainder of the 42-d trial. Bunk readings were evaluated each day to determine when to increase feed delivered. When feed bunks were examined each morning, any refusals from the

previous day were collected, weighed, and subsamples frozen for later analyses of DM. Supplement disappearance from the bunk was calculated if there were any refusals. Calves had ad libitum access to bermudagrass hay (7% CP, 93% NDF, 75% ADF, 61 mg Zn/kg, 124 mg Mn/kg, 10 mg Cu/kg: DM basis). Grab samples of supplement and hay were taken for each representing block throughout the trial and were frozen at -20°C until analysis dry matter (Table 2 and 3).

Cattle were observed daily (0800 h) by trained personnel for signs of bovine respiratory disease (BRD) beginning the morning of the day after processing. Signs of BRD included depression, ocular or nasal discharge, cough, poor appetite, and respiratory distress. Cattle were given a Clinical Attitude Score (CAS) of 0 to 4 by the pen checker who was blinded to dietary treatment (0 = normal, 1 = mild BRD, 2 = moderate BRD, 3 = severe BRD, 4 = moribund). The individual checking pens and scoring cattle remained the same for the entirety of this study. Cattle with a score > 0 were brought to the chute and a rectal temperature was taken. Cattle with a CAS ≥ 1 and a rectal temperature of ≥ 40° C, were treated according to a preplanned antibiotic protocol. The BRD therapy 1 (Nuflor, Merck Animal Health, Rahway, NJ) was administered at 5 mL/45.45 kg BW subcutaneously in the neck and returned to their home pen. Calves receiving BRD therapy 1 were sent back to their home pen. If the calf scored a CAS ≥ 1 following BRD therapy 1 and if rectal temperature was ≥ 40°C, calves would receive BRD therapy 2 (Baytril, Elanco Animal Health, Shawnee, KS) at a rate of 5.7 mL/45.45 kg BW subcutaneously in the neck and be sent back to their home pen. At time of reevaluation, if rectal temperature was ≥ 40°C, the calf would receive BRD therapy 3 (Excenel, Zoetis, Florham Park, NJ) administered 2 mL/45.45 kg BW dosage subcutaneously in the neck for 3 consecutive days. During the 3 days, the calf would be placed in a hospital pen to be monitored. After the 3 days, if the calf remained

in the same state of health and rectal temperature was ≥ 40°C, calves received a final BRD therapy 4 (Draxxin, Zoetis) dosed at 1.1 mL/45.45 kg BW subcutaneously in the neck. After administering BRD therapy 4, if the CAS was ≥ 2 and rectal temperature was ≥ 40°C, then the calf was considered nonresponsive, and no further treatments were given. If BRD symptoms were present > 21 days after administered the previous therapy, symptoms were considered a new BRD episode and treatment began with BRD therapy 1. Records were kept of all calves pulled from each pen, their CAS, rectal temperatures, all antibiotics administered, and medication costs. The medication costs reported were the drug cost with no additional fees assessed. If a calf was treated ≥ 3 times with antibiotics and failed to gain > 0.45 kg/d for the 42-d period, then it was considered and recorded as a chronic calf. If a calf did not live for the duration of the 42-d trial, this was recorded, and the calf was necropsied to determine cause of death.

Weights were recorded initially (d -l and 0) and before supplement feeding in the mornings of d 14, 28, 41, and 42 for each load of calves delivered. Average daily gain was calculated for interim and final periods based on the averages of initial and final weights that were recorded on the 2 consecutive days. Blood samples were collected from 6 randomly selected animals in each pen to evaluate serum haptoglobin concentrations. Blood was collected via jugular venipuncture from the same animals on d 0, 14, and 28 using tubes containing a clot activator (BD Inc., Franklin Lakes, NJ) The samples were allowed to sit at room temperature for at least 30 min to allow clot formation. The serum was separated by centrifugation at 2,060 × g for 20 min. Serum was then decanted and stored at -20°C until time of analysis. Samples were analyzed for haptoglobin concentrations using a commercial ELISA (Immunology Consultants Laboratory Inc., Portland, OR).

Liver biopsies were taken on d 5 ± 2 and 43 ± 1 from 3 calves selected randomly from each pen. Animals were restrained in a hydraulic squeeze chute, and the 10th intercostal space on the right side of the animal was identified on the abdomen. An area approximately 10 cm × 10 cm was clipped using an electric clipper to remove the hair for incision. An aseptic technique was used by scrubbing the area with chlorhexidine gauze sponges followed by scrubbing with 70% isopropyl alcohol gauze sponges and a final scrub of an iodine surgical solution. At the site of incision, animals were injected with 5 mL of 2% lidocaine solution under the skin and into the intercostal muscle for numbness. A 5 min wait period was given after the injection to allow the lidocaine to take effect within the surgical area. Following this wait period, a sterile #15 scalpel was used to make a 1 cm incision through the skin. A biopsy needle (16 ga × 10.2 cm or 14 ga × 16.2 cm Tru-Cut Biopsy Needles, Jorgensen Labs Inc., Loveland, CO) was inserted through the incision previously made to obtain liver samples. The same biopsy needle was used to obtain multiple samples from the same calf, until the minimum sample weight for analysis reached 0.05 g. A sterile transfer pipette was used to remove the liver sample from the biopsy needle, and it was carefully placed into individual microtubes and promptly placed on ice. Biopsy needles underwent cold sterilization in between animals. Samples from each collection day were submitted to the Michigan State University Veterinary Diagnostic Laboratory for mineral analysis using mass spectroscopy.

Grain supplements per batch made and hay offered were sampled to analyze for DM, ash, NDF, ADF, CP, and mineral composition. Samples 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. Fiber analyses were determined using Ankom 200 Fiber analyzer (ANKOM Technology Corp., Macedon, NY). Nitrogen percentages

were used to determine crude protein percentages (ECS 8020 CHNSO dual furnace, NC Technologies). Mineral analyses were performed in duplicate for hay samples and in triplicate for grain samples. To start, 1 ± 0.01 g of hay and 0.5 ± 0.01 g of grain were weighed into 50 mL centrifuge tubes. Then 15 mL of trace mineral grade nitric acid was added to each tube containing sample. Samples underwent wet ash digestion that was performed by covering the tubes with plastic watch glasses and placing them into a heating block. With the heating block, the temperature was set at 80°C for 15 min, or until all brown gasses had escaped and foaming was not present. After all brown gas had escaped, the temperature was set at 115°C for 1 h. Following, the tubes were allowed to cool and were filled to a 45 mL volume with deionized water, inverted, and capped. Samples were analyzed by inductively coupled plasma (ICP) atomic emission spectroscopy (CIROS. Fitchburg, MA) at the University of Arkansas System Division of Agriculture Agricultural Diagnostic Laboratory, Fayetteville, AR.

Statistical Analysis

Data were analyzed as a randomly complete block design with pen identified as the experimental unit. Each block (date of shipment) was treated as the random block effect and treatment was a fixed effect in the model. The subject was identified as the pen within each block. All data were analyzed using various programs of SAS 9.4 (SAS Inst. Inc., Cary, NC). Heifer body weights, average daily gains, and haptoglobin concentrations were analyzed using the MEANS procedure for overall pen averages and the MIXED procedure. Body weights, haptoglobin concentrations, and liver mineral concentrations were analyzed as repeated measures. Kenward-Rogers were specified as the degrees of freedom selection, with compound symmetry as the covariance structure. The model included treatment, day, and the day by

treatment interaction. Significance was declared when $P \leq 0.05$, with tendencies declared when $P > 0.05$ and ≤ 0.10.

If there were any interactions that were significant ($P \leq 0.10$), treatment means were separated with a t-test using the PDIFF option in SAS. Haptoglobin concentrations were transformed using logarithmic transformation to achieve normal distribution of values. Number of antibiotics administered, and medication costs were also analyzed using the MIXED model. Morbidity data included percentage treated once, twice, trice, or more; calves deemed chronic, relapses, and mortality. Morbidity data were analyzed using the GLIMMIX procedure with block and treatment in the class statement. Block was considered a random variable and pen within block was the subject. The model included treatment within shipment as a random variable and the subject as pen within shipment. Grain supplement and hay sample data were generated using the MEANS procedure. Supplement disappearance averages were analyzed in the MIXED procedure with the subject as pen within block and block as the random effect.

RESULTS AND DISCUSSION

Growth Performance and Health

Initial body weights (Table 4) were similar for both treatment groups (231 ± 1.26 kg). There tended to be a treatment by day interaction for body weights ($P = 0.07$). Body weights were not different on d 0 ($P = 0.82$) and d 14 ($P = 0.36$), but heifers supplemented with complexed trace mineral sources had greater body weights on d 28 ($P = 0.04$) and d 42 ($P = 0.05$; 264 vs. 260 kg, SE = 1.8). Calves receiving the complexed trace mineral sources were 4 kg heavier than those receiving inorganic sources by d 42 (Figure 1). There were no treatment differences in supplement disappearance from the bunks ($P = 0.98$). Supplement refusals within the first week were similar for both treatments (Table 4; $P = 1.00$). Supplementing cattle for the

first 42 d after arrival with amino acid complexed trace mineral sources resulted in greater (P = 0.05) overall ADG when compared to supplementing inorganic sources (0.78 vs. 0.70 ± 0.03 kg/d). Supportive results were reported by Kegley et al. (2012) investigating the effects of supplemental zinc (360 mg/d), copper (125 mg/d), manganese (200 mg/d), and cobalt (12 mg/d) from sulfate or amino acid complexed sources in calves over a receiving phase. This study reported an increase in final body weights and ADG for the calves supplemented with complex trace mineral sources. In agreement, Dorton et al. (2007) reported by the end of a 28-d feedlot receiving phase, steers that were supplemented with organic (amino acid complexes) trace minerals had greater ADG than steers supplemented with inorganic (sulfates) trace minerals. However, previous research regarding trace mineral supplementation from sulfate or organic sources, growth performance results have not been consistent. A study similar investigated the effects of zinc (360 mg/d), manganese (200 mg/d), and copper (125 mg/d) from inorganic (sulfates), organic (amino acid complex), and hydroxy sources during a receiving period (Ryan et al., 2015). This study reported that trace mineral sources had no effect on body weights or ADG during the receiving period.

Cattle supplemented with inorganic trace mineral sources had greater (P = 0.03) BRD morbidity incidence (Table 5). The percentage of calves treated at least once for BRD was lower for those supplemented with complexed trace mineral sources (46.2% vs. 57.7% ± 3.6). The timing of antimicrobial usage did not differ between treatments ($P \leq 0.60$). Deaths occurred in both treatments during the receiving trial (complexed, n = 2; inorganic, n = 4). Bacteria cultures from necropsies revealed diagnosis of BRD (complexed, n = 2; inorganic, n = 3) or acute acidosis (inorganic, n = 1). Rectal temperatures at time of antimicrobial usage remained the same for both treatments ($P \geq 0.12$). Medication costs were lower (P = 0.05) for calves supplemented

with complexed trace mineral sources ($11.01 vs. $14.90). Kegley et al. (2012) also reported a tendency for a decrease in percentages of the calves receiving a second antibiotic treatments for BRD compared to the sulfate supplemented calves.

Additionally, Chirase and Greene (2001) concluded from a mineral study, with high density confinement rearing of livestock, an additional important role of nutrition has been that animals are not only fed for optimal productive or reproductive performance but must also be fed to decrease infectious diseases and their concomitant stresses. This ultimately concluded that appropriate mineral nutrition could lower the use of antibiotics and other anti-infection drugs in livestock production. From this, both treatments included in this receiving study provided iso levels of adequate trace mineral supplementation to meet requirements which gave opportunity to reveal bioavailability differences between treatments. However, results in previous literature have not been consistent when investigating trace mineral supplementation source. Lippolis et al. (2017) observed that supplementing beef cattle with an inorganic and complexed source of cobalt, copper, manganese, and zinc during a 58-d receiving period did not impact cattle performance and health responses. Even though Dorton et al. (2007) reported a difference in ADG, there were no effects of trace mineral source on morbidity or the number of treatments per morbid animal throughout the receiving period. Accompanied with growth performance, Ryan et al. (2015) reported that trace mineral source had no effect on BRD incidence, average antibiotic cost per calf, or the percentage of calves that relapsed.

Haptoglobin Concentrations

Haptoglobin concentrations decreased (day, $P < 0.0002$) throughout the 42-d trial (Figure 2). By d 28, cattle supplemented with complexed trace mineral sources had ($P = 0.03$) lower concentrations [8.5 vs. 9.91 ng/ml(log)]. Previous research states that copper-deficient cattle

have a suppressed response resulting in an alteration of the normal acute phase reaction showing noticeable increases in haptoglobin concentrations (Arthington and Ranches, 2021). The results reported in this receiving trial align with previous studies using haptoglobin as a stress biomarker. Harvey et al. (2021) found while supplementing amino acid complexed sources of trace minerals in replace of inorganic sources that there was an increase in plasma cortisol and haptoglobin concentrations in those receiving the inorganic supplement. Supporting data suggests that supplementing amino acid complexed sources of trace minerals results in lower haptoglobin concentrations over time.

Liver Mineral Concentration

In this receiving trial, the source of trace mineral supplementation had no effect on liver mineral concentrations (Figure 3) and there was no treatment × day interactions. Although organic trace mineral forms are expected to have enhanced absorption, retention, and biological activity compared with sulfate minerals (Spears, 1996), similar liver mineral concentrations in cattle suggests that treatments were biologically irrelevant. Additionally, the effects of supplementing organic or inorganic sources of trace mineral on liver mineral status of beef cattle have been variable. On arrival, liver concentrations of selenium, copper, zinc, and manganese were all below levels that are considered adequate by Puls (1988). Galyean et al. (1999) stated that most minerals need to be increased in receiving diets to compensate for low feed intake by stressed calves. By d 43 of calves receiving supplement, day effects were present showing liver concentrations of copper, cobalt, and selenium had increased (day, $P < 0.01$).

Liver copper concentrations increased (day, $P < 0.01$) by d 43 exceeding adequate concentration, suggesting that both organic and inorganic sources provided calves with efficiently absorbed copper. The concentration range considered adequate in dry liver by Puls

(1988) is from 125 to 600 µg/g, marginal if concentrations are between 33 to 125 µg/g and considered deficient if concentrations fall below 33 µg/g. Spears et al. (2022) also stated that liver copper concentrations are the best indicator of status in ruminants. To support, McDowell (1992) stated that the concentration of copper in the liver of ruminants is directly correlated to the bioavailability of copper in the diet. It is important to note that intakes of zinc, molybdenum, iron, and sulfur affect copper utilization (McDowell, 1992). Nockels et al. (1993) conducted a study determining whether copper or zinc balance would be affected by feeding either organic (ZnMet and CuLys) or inorganic ($ZnSO_4$ and $CuSO_4$) sources of zinc and copper before and after stressing calves. Calves fed CuLys had 53% greater apparent copper absorption and increased retention during repletion when compared to the calves fed $CuSO_4$. Copper, involved in immune response, a deficiency or imbalance can alter the activity of certain enzymes and function of specific organs thus impairing specific metabolic pathways including overall immune function (Paterson and Engle, 2005).

Liver zinc concentrations decreased (day, $P < 0.01$) by d 43, but remained within the adequate concentration range for the duration of the trial. Both treatments decreased similarly; calves supplemented with amino acid complexed sources dropped from 162 µg/g to 111 µg/g and inorganic calves dropped from 197 µg/g to 121 µg/g. The concentration range considered adequate by Puls (1988) is 25 up to 200 µg/g. However, Arthington and Ranches (2021) state that zinc lacks a reliable tissue pool for indicating status. It is also stated that liver zinc concentrations are sensitive to zinc intake (Kincaid, 1999). In the liver, calves readily absorb and bind large amounts of zinc as metallothionein in response to elevated zinc intakes (Kincaid et al., 1976). Spears et al. (2022) explained in a review that cattle undergoing acute stress or infection may result in a decrease in zinc concentrations suggesting the values reported with this study

represent a normal physiological response. It has been reported that zinc retention becomes negative during stress caused by feed and water deprivation (Nockels et al., 1993). Furthermore, Nockels et al. (1993) reported that copper and zinc retention was decreased in steers injected with a stressor accompanied with feed and water restriction. This physiological response may be taken into consideration of the decrease in zinc concentrations in this experiment.

Manganese concentrations tended to decrease (day, $P = 0.09$) and calves were considered marginally deficient during the 43 days. In cattle, if manganese concentrations in the liver are below 7 µg/g it is considered deficient according to Puls (1988). Calves on both treatments were slightly above deficient concentrations (complexed = 7.1 µg/g, inorganic = 7.57 µg/g) when sampled initially. By the end of the receiving trial both treatments had decreased manganese concentrations. However, Kincaid (1999) stated more recently that when assessing liver manganese concentrations, it should be considered that the manganese is being absorbed in the liver, but then excreted endogenously through the bile. Thus, literature and this study suggests that the accumulation of manganese remaining in liver tissue does not directly reflect dietary intake.

Cobalt concentrations increased ($P < 0.01$) revealing a day effect for both treatments during the 42 d. Cobalt liver concentrations were 0.96 µg/g for amino acid complex supplemented calves and 0.68 µg/g for inorganic supplemented calves at the conclusion of the trial. There were no differences between treatments. Due to the metabolic role of cobalt, assessment of the animal's nutriture often centers on measures of vitamin B12 status. Difficulty assessing tissue cobalt status persists and current literature was not able to provide values for adequate tissue concentrations.

Although selenium concentrations increased (day, $P = 0.01$), the values remained below concentrations considered adequate for both treatments. Selenium was delivered at the same rate to both treatment groups as sodium selenite resulting in similar liver concentrations. Although selenium concentrations increased with a day effect, both treatments remained below adequate concentrations according to Puls (1988). Adequate concentrations of selenium in the liver are above 1.25 µg/g, which in this study the highest concentration reported was 1.11 µg/g within the 42-d trial. Cattle are considered marginally deficient in selenium if concentrations fall between 0.60 and 1.25 µg/g. The concentrations were not severely deficient values, but marginally deficient calves may display weakness and low vigor according to Palomares (2022).

Manganese liver concentrations were similar for both treatments during the receiving trial (Figure 3). Both treatments resulted in a tendency for manganese concentrations to decrease similarly by day (day, $P = 0.09$), but there were no treatment or treatment × day interactions. Manganese concentrations were considered deficient for both treatments according to Puls (1988).

CONCLUSION

To conclude, replacing inorganic sources with amino acid complexed sources of trace minerals (zinc, copper, manganese, and cobalt) improved growth performance and decreased morbidity treatments and medication costs during this receiving phase. Source of trace mineral supplementation had no effect on liver mineral concentrations by the end of this receiving period. Hence, additional research is warranted to further assess and potentially validate such impacts of supplementing inorganic or organic complexed sources of zinc, copper, manganese, and cobalt to beef calves.

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Table 1. Ingredient composition of grain supplement (as-fed basis)

	Fed at 0.91 kg/d		Fed at 1.36 kg/d		Fed at 1.82 kg/d	
Ingredient	Inorganic	Complexed	Inorganic	Complexed	Inorganic	Complexed
Corn-cracked, %	39.7	39.7	52.4	52.4	58.5	58.5
Dried distillers' grains, %	30	30	30	30	30	30
Soybean meal, %	21	21	10	10	4.7	4.7
Salt, white, %	2.0	2.0	1.5	1.5	1.0	1.0
Molasses, %	2.0	2.0	2.0	2.0	2.0	2.0
Limestone, %	2.4	2.4	2.0	2.0	1.8	1.8
Fat, %	1.0	1.0	1.0	1.0	1.0	1.0
Corn/Rumensin premix[a], %	0.8	0.8	0.53	0.53	0.4	0.4
Vitamin A, D, E premix[b], %	0.2	0.2	0.14	0.14	0.1	0.1
Vitamin E[c], %	0.1	0.1	0.07	0.07	0.05	0.05
Availa-4, %	-	0.8	-	0.5	-	0.4
Zinc sulfate (35.5% Zn), g/ton	1011	-	676	-	507	-
Manganese sulfate (32% Mn), g/ton	623.5	-	416.7	-	312.5	-
Copper sulfate (25.2% Cu), g/ton	494.7	-	330.7	-	248	-
Cobalt carbonate (46% Co), g/ton	25.9	-	17.4	-	13	-
Sodium selenite (0.99% Se), g/ton	100.8	100.8	67.3	67.3	50.5	50.5

[a] Premix provides 22 kg monensin/kg

[b] ADE premix contains 880,000 IU/kg Vitamin A, 1760,000 IU/kg Vitamin D, and 1,100 IU/kg Vitamin E

[c] Vitamin E contains 44,000 IU/kg

Table 2. Analyzed nutrient composition of hay and grain supplements for receiving phase, DM basis[a].

Item	Hay	Fed at 0.91 kg/d		Fed at 1.36 kg/d		Fed at 1.82 kg/d	
		Inorganic	Complexed	Inorganic	Complexed	Inorganic	Complexed
DM, %	95	96	96	97	97	97	97
Zn, mg/kg	61 ± 17	445 ± 1	526 ± 12	400 ± 88	644 ± 39	466 ± 154	387 ± 6
Mn, mg/kg	124 ± 8	133 ± 13	271 ± 12	191 ± 18	199 ± 11	99 ± 38	193 ± 4
Cu, mg/kg	10 ± 2	214 ± 107	173 ± 4	123 ± 6	133 ± 22	107 ± 16	120 ± 3

[a]LSMeans ± SEM; Hay, n = 2; 0.91 kg/d, n = 2; 1.36 kg/d, n = 2; 1.82 kg/d, n =3

Table 3. Forage and grain supplement analysis for receiving phase, DM basis.

Item	Hay	Fed at 0.91 kg/d		Fed at 1.36 kg/d		Fed at 1.82 kg/d	
		Inorganic	Complexed	Inorganic	Complexed	Inorganic	Complexed
DM, %	95	96	96	97	97	97	97
CP, %	7	19	21	20	19	17	19
Ash, %	6.82	9.27	9.56	7.96	6.72	6.69	7.54
NDF, %	93	70	68	69	70	69	69
ADF, %	75	60	59	59	60	59	59

Table 4. Effect of complexed amino acid or inorganic trace mineral supplementation on growth performance of newly received calves.

Item	Complexed	Inorganic	SEM[2]	*P*-value
BW, kg				
d 0	231	231	1.78	0.82
d 14	241	239	1.78	0.36
d 28	253	249	1.78	0.04
d 42	264	260	1.78	0.05
ADG, kg/d				
d 0 to 14	0.70	0.61	0.06	0.32
d 14 to 28	0.85	0.70	0.08	0.20
d 28 to 42	0.76	0.72	0.05	0.58
d 0 to 42	0.78	0.70	0.03	0.05
Supplement Disappearance, kg/d				
d 0 to 7	0.95	0.95	0.03	1.00
d 0 to 42	1.37	1.37	0.02	0.98

Table 5. Effect of complexed amino acid or inorganic trace mineral supplementation on health of newly received cattle.

Item	Organic	Inorganic	SEM[2]	*P*-value
Cattle treated at least once, %	46.2	57.7	3.6	0.03
Day 1st treated	5	6	0.7	0.60
Rectal temperature at 1st treatment, °C	40.78	40.71	0.14	0.50
Cattle treated at least twice, %	14	21.5	3.3	0.12
Day 2nd treated	14	16	1.1	0.34
Rectal temperature at 2nd treatment, °C	40.44	40.64	0.22	0.24
Relapse, %	30.7	38.2	5.9	0.38
Cattle treated at least 3 times, %	9.7	9.6	2.7	0.98
Day 3rd treated	18	21	2.3	0.42
Rectal temperature at 3rd treated, °C	40.79	40.40	0.31	0.12
Cattle treated 4 times, %[a]				
Chronic cattle, %	7.6	9.6	2.4	0.58
Dead cattle, %[b]				
Number of antibiotic treatments	0.75	0.93	0.09	0.18
Medical cost, $	11.01	14.90	1.33	0.05

[a]GLIMMIX model did not converge: n=3 for organic vs. n=8 for inorganic

[b]GLIMMIX model did not converge: n=2 for organic vs. n=4 for inorganic

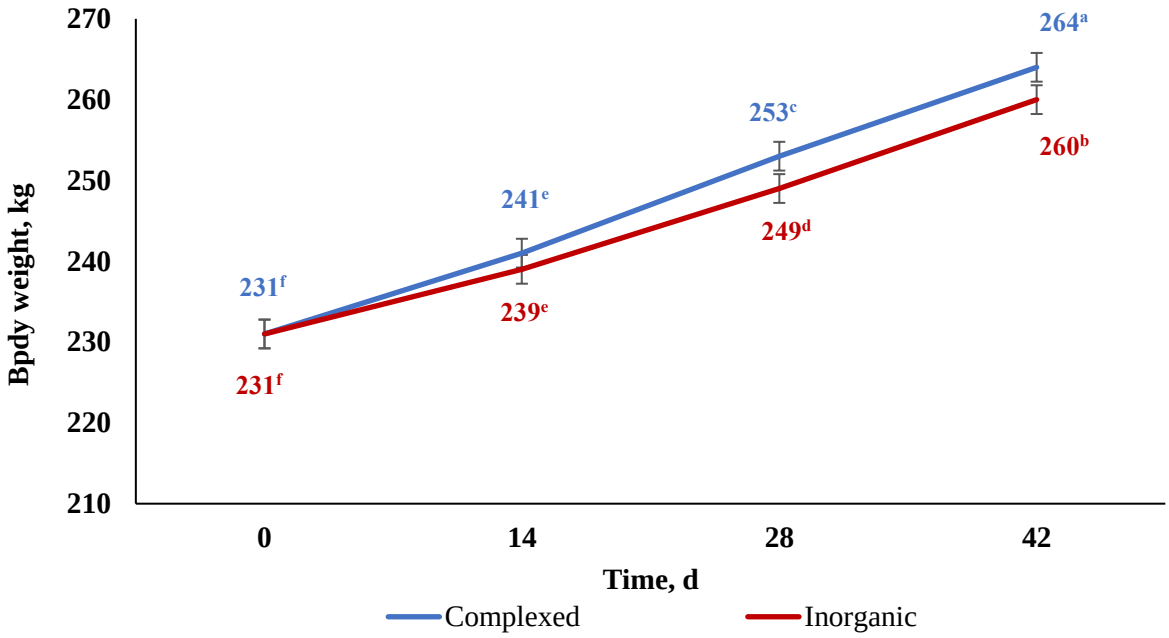

Figure 1. Effects of complexed amino acid or inorganic trace mineral supplementation on body weights during 42-d receiving trial. Treatment × day ($P = 0.07$); Day ($P < 0.0001$); Treatment ($P = 0.14$). Means within a day without a common superscript differ ($P \leq 0.05$).

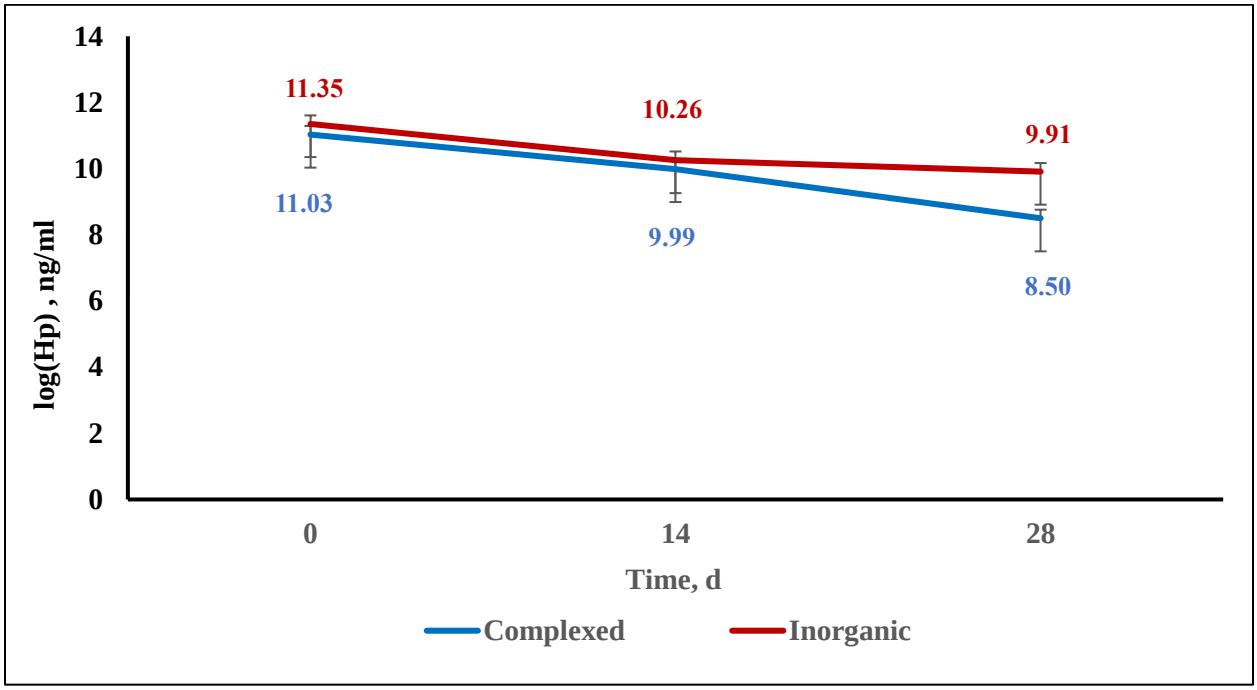

Figure 2. Effects of complexed amino acid or inorganic trace mineral supplementation on haptoglobin concentrations in calves. Day ($P = 0.0002$); Treatment ($P = 0.07$); Treatment × day ($P = 0.36$).

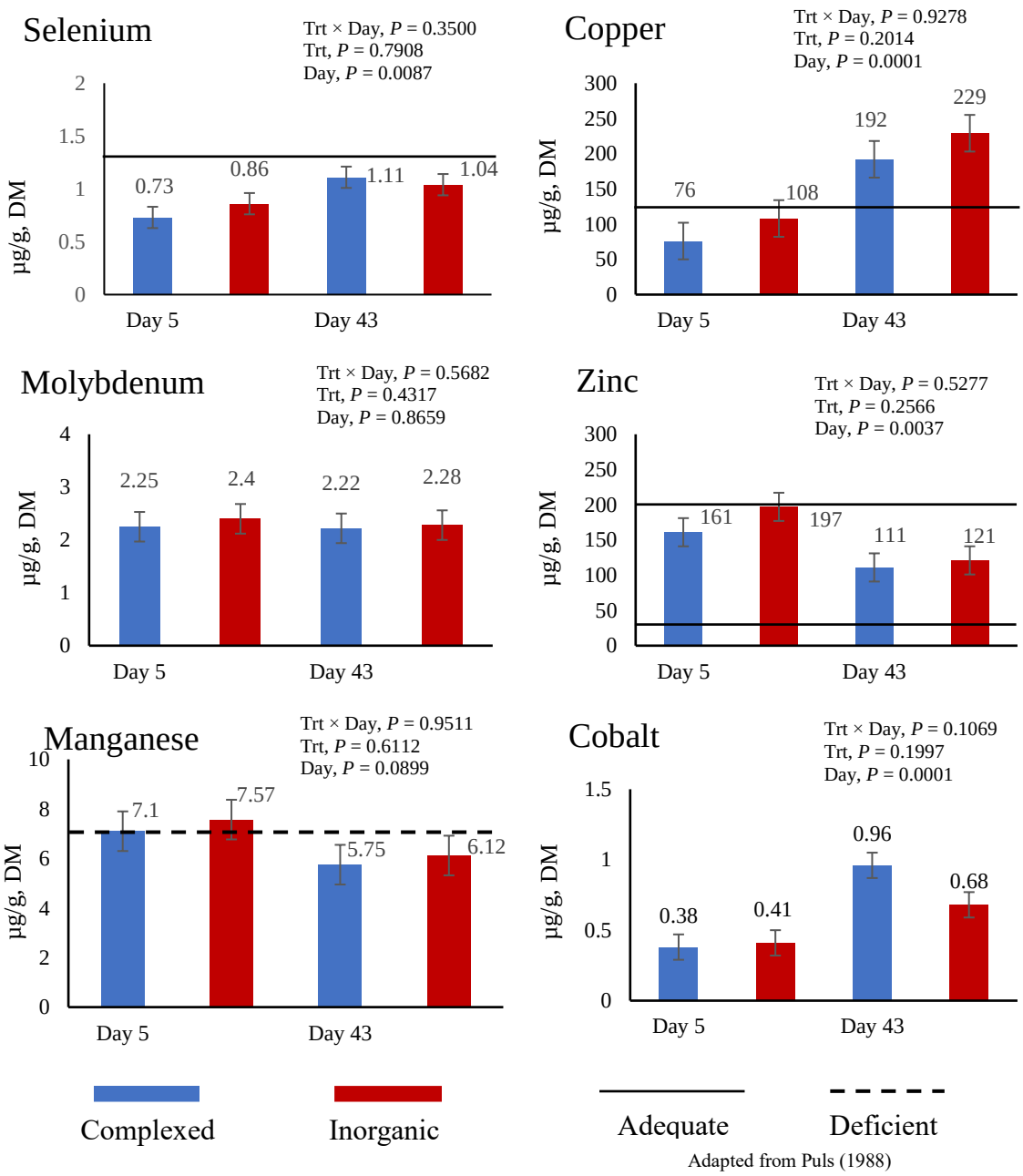

Figure 3. Liver mineral concentration comparison during the receiving period (n = 72).

CHAPTER IV

CONTINUED SUPPLEMENTATION OF AMINO ACID COMPLEXED OR INORGANIC TRACE MINERALS TO GRAZING CATTLE FOLLOWING A RECEIVING PHASE DIETARY TRACE MINERAL COMPARISON

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ABSTRACT: The objective of this study was to investigate the effects of inorganic or amino acid-complexed sources of trace minerals (zinc and copper) on performance of beef heifers during a grazing period following a receiving trial. Crossbred beef heifer calves (n = 287, initial body weight = 231 ± 0.49 kg) were used in a 42-day receiving trial. Any cattle that failed to gain at least 0.45 kg/d, and(or) received 3 doses of antibiotic therapy during the receiving trial were removed from the study. The remaining cattle were kept within their respective pen and treatment and were randomly retained for the grazing phase. If there were not enough eligible calves/pen, calves on the same treatment that had been removed randomly from other pens were added to achieve equal pen counts. Cattle (n = 204; initial body weight = 262 ± 1.44 kg) grazed at 2 different locations in Arkansas; in Fayetteville, cattle (12 pens; 6 pens/treatment) grazed 6-acre stockpiled mixed grass pastures (n = 8 calves/pasture); in Batesville, cattle (12 pens; 6 pens/treatment) grazed 5-acre stockpiled novel-endophyte fescue pastures (n = 9 calves/pasture). Treatments consisted of supplemental zinc (540 mg/day) and copper (90 mg/day) from complexed (Availa, Zinpro Corp. Eden Prairie, MN) or inorganic sources (sulfates). Cattle grazed for at least 114 days until reaching a weight goal of 341 kg. Cattle had ad libitum access to water and were offered bermudagrass hay when forage was limited. Body weights were measured on 28-day intervals. A subset of calves (3/pen) were liver biopsied at the end of receiving and grazing phases to compare liver mineral concentrations. Following grazing, calves were removed from treatments and sent to a Kansas feedlot (n = 198) where they were commingled and fed in a single pen for 141 days. Morbidity and mortality data were recorded, and after slaughter, carcass data were collected. Statistical analyses were performed using MIXED and GLIMMIX procedures of SAS 9.4 with location as a random effect and pen within location specified as subject. There was no treatment ($P = 0.14$) or treatment × day interaction (P

= 0.92) for body weights during the grazing phase. Overall average daily gain was not different between treatments ($P = 0.94$; 0.69 kg/d, SE = 0.04). Treatment did not affect liver mineral concentrations ($P \geq 0.17$). There were no differences in feedlot morbidity, mortality, or carcass characteristics ($P \geq 0.28$). In conclusion, although complexed sources of trace minerals (zinc, copper, manganese, and cobalt) improved body weight gain and decreased morbidity treatments during the receiving phase, there were no differences from grazing to slaughter when supplementing amino acid complexed versus sulfate mineral sources of zinc and copper during the grazing period.

INTRODUCTION

It is evident in beef production that for cattle to perform at their greatest genetic potential, it is important that their nutritional demands are met. Trace minerals take part in important physiological functions in beef cattle and must be supplemented to beef cattle diets when forages and rations are deficient or have incorrect proportions (Paterson and Engle, 2005). Various sources, concentrations, and combinations of trace mineral supplements exist for cattle and their effects on immune function, growth, and performance measures have been evaluated in recent years. Livestock diets are often delivered with trace minerals supplemented in the form of inorganic salts, usually oxides, chlorides, sulfates, and carbonates (Hilal et al., 2016). These inorganic forms have been used in cattle diets for years because they are widely available and represent an inexpensive form of supplementation. However, organic trace mineral supplements are now being used in replace of inorganic salts due to potentially greater bioavailability and functionality (Mohanta and Garg, 2014). There are different forms of organic trace mineral supplementation available. One form, a metal amino acid complex, used in this study, is a product resulting from complexing a soluble metal salt with a single amino acid (AAFCO, 2000).

Bovine respiratory disease (BRD) has been the most economically important disease costing producers in North America $800 to $900 million every year (Blakebrough-Hall et al., 2020). Morbidity accompanied with poor performance in receiving cattle is being addressed by nutritional intervention. The previous chapter reported that receiving cattle supplemented with amino acid-complexed trace mineral sources had greater BW, ADG, and fewer incidences of BRD. However, the effects on performance thereafter have yet to be investigated. Thus, the objective of this project was to investigate the effects of inorganic or amino acid-complexed sources of trace minerals (zinc and copper) on performance of beef heifers during a grazing period following a receiving trial.

MATERIALS AND METHODS

Animal methods were approved by the University of Arkansas Animal Care and Use Committee (Approval #21142).

Receiving

Two hundred-eighty-seven crossbred beef heifers (231 ± 0.49 kg) were obtained from a cooperating producer, who purchased calves in regional sale barns and then shipped cattle to the University of Arkansas System Division of Agriculture Beef Cattle Research Facility near Fayetteville. Heifers arrived in 3 shipment sets (block) with arrival dates of October 6, 2021 (block 1, n = 94), October 26, 2021 (block 2, n = 95), and November 23, 2021 (block 3, n = 98). All calves were used in a 42-d receiving trial investigating the effects of inorganic or amino acid-complexed sources of trace minerals on growth performance and morbidity. Calves were processed after arrival and stratified by d -1 body weights and allocated randomly to 8 pens (11 to 13 heifers/pen; total of 24 pens). Cattle were housed on 0.42-ha grass paddocks, provided ad libitum access to bermudagrass hay and water, and fed grain supplements that served as the

carriers of the dietary treatments. Treatments consisted of supplemental zinc (360 mg/d), copper (125 mg/d), manganese (200 mg/d), and cobalt (12 mg/d) from complexed (Availa-4, Zinpro Corp. Eden Prairie, MN) or inorganic sources (copper, manganese, and zinc sulfates; and cobalt carbonate). Calf growth performance, morbidity, and mortality were recorded during the 42 d. Additionally in the receiving trial, 3 calves per pen (n = 36/treatment) initial (d 5 ± 2) and ending (d 43 ± 1) livers were biopsied for mineral comparison.

Grazing

At the end of the 42-d receiving trial, final weights (the average of the 2 consecutive BW; d 41, d 42) were calculated. Any cattle that failed to gain 0.45 kg/d, and/or received 3 doses of antibiotic for BRD were removed from this study. Out of the 287 head used in the receiving trial, 6 heifers had died (complexed, n = 2; inorganic, n = 4) and 57 heifers (complexed, n = 30; inorganic, n = 27) were removed for chronic BRD and/or poor gain. The remaining cattle were kept within their respective pen with the appropriate dietary treatment until the start of grazing. Calves within pen then were selected randomly for retention for the grazing phase. If there were not enough eligible calves per pen (complexed, n = 2; inorganic, n = 3), calves on the same dietary treatment that had been removed randomly from other pens were added to achieve equal pen counts. Additionally, 20 (complexed, n = 9; inorganic, n = 11) were removed randomly from the project due to of lack of space.

Calves that continued to the grazing study (n = 204; initial BW = 262 ± 1.44 kg) grazed at 2 different beef research locations within the University of Arkansas System Division of Agriculture at the: 1) Beef Cattle Research Facility near Fayetteville and 2) Livestock and Forestry Research Station in Batesville. Because of this location change, calves were distributed to 4 blocks. Because calves completed the 42-d receiving trial on different dates, each block

started grazing at different dates. Cattle grazing in Batesville consisted of block 1 that was shipped and began grazing November 21, 2021 (n = 72; 4 pens/treatment) and block 2 that were shipped and began grazing December 12, 2021 (n = 36; 2 pens/treatment). Cattle grazing in Fayetteville consisted of block 3 that started grazing on December 8, 2021 (n = 32; 2 pens/treatment), and block 4 that started grazing on January 4, 2022 (n = 64; 4 pens/treatment).

In Batesville, cattle (12 pens; 6 pens/treatment) grazed 2.02-ha stockpiled novel-endophyte fescue pastures (n = 9 calves/pasture). In Fayetteville, cattle (12 pens; 6 pens/treatment) grazed 2.43-ha stockpiled mixed grass pastures (n = 8 calves/pasture). Cattle were fed grain-grain by-product supplements (Table 1) that served as the carrier of the treatments. Dietary treatments consisted of supplemental zinc (540 mg/d) and copper (90 mg/d) from complexed (Zinpro Availa Zn and Zinpro Availa Cu), or inorganic sources (sulfates). Cattle were receiving grain at a 1.81 kg/d rate by the end of the 42-d receiving trial. Cattle continued to receive 1.81 kg/d of grain supplement at the start of grazing and were increased to 2.73 kg/d according to bunk readings. When most of the calves in the pen were consuming the supplement at a 1.81 kg/d rate, the pen was switched to a supplement with the appropriate mineral treatment that was formulated for feeding at a 2.73 kg/d (as-fed basis) rate. The grain delivered was increased to achieve the targeted ADG (0.79 kg/d). Changes in the supplement were formulated so the new supplement was approximately equal in nutrients to the original diet, but the percentage of dried distiller's grain was reduced. There were no supplement refusals to collect. No free choice mineral was offered during this study. Cattle had ad libitum access to water and were offered stored forage when standing forage was limited. Samples of all supplements, stored forage, and pastures were composited monthly at each location and were frozen at -20°C until

analysis (Table 2, 3). Water samples were collected at each location during the grazing trial and were analyzed by the Arkansas Water Resources Center (Table 4).

All cattle grazing in Batesville received a pour on treatment for lice (block 1 = d 90; block 2 = d 68). All medications administered were recorded. Morbidity data, specifically instances of pinkeye and lameness were recorded. Three heifers were injured in Batesville, euthanized, and excluded from data analysis. Because the blocks of cattle started the grazing trial on different days, the length of grazing was different for each (block 1 = 153 d, block 2 = 131 d, block 3 = 138 d, block 4 = 105 d). The final weights recorded on d 41 and 42 of the receiving trial were considered d 0 for grazing. Body weights were measured at approximately 28-day intervals thereafter. The target weight for the completion of the grazing trial was 340 kg. At the conclusion of grazing, final body weights were calculated by an average of weights obtained on consecutive days.

A subset of calves (3/pen) were liver biopsied at the start of receiving, end of receiving, and end of grazing to compare liver mineral concentrations. In the receiving trial, initial (d 5 ± 2) and ending (d 43 ± 1) liver samples were biopsied for liver mineral comparison. If cattle were identified to be removed or had died by the d 43 sample in receiving, calves were replaced with another calf within the corresponding pen and treatment. A separate data set (n = 53) was used to analyze concentrations from all cattle that were able to be liver biopsied on all 3 collection days.

In Batesville, liver biopsies were performed on March 15, 2022 (block 1 = d 118, block 2 = d 96). In Fayetteville, liver biopsies were performed on April 12, 2022 (block 3 = d 124, block 4 = 91). Animals were restrained in a hydraulic squeeze chute, and the 10^{th} intercostal space on the right side of the animal was identified on the abdomen. An area approximately 10 cm × 10 cm was clipped using an electric clipper to remove the hair for incision. An aseptic technique

was used by scrubbing the area with chlorhexidine gauze sponges followed by scrubbing with 70% isopropyl alcohol gauze sponges and a final scrub of an iodine surgical solution. At the site of incision, animals were injected with 5 mL of 2% lidocaine solution under the skin and into the intercostal muscle for numbness. A 5 min wait period was given after the injection to allow the lidocaine to take affect within the surgical area. Following this wait period, a sterile #15 scalpel was used to make a 1 cm incision through the skin. A biopsy needle (16 ga × 10.2 cm or 14 ga × 16.2 cm Tru-Cut Biopsy Needle, Jorgensen Labs Inc., Loveland, CO) was inserted through the incision previously made to obtain liver samples. The same biopsy needle was used to obtain multiple samples from the same calf, until the minimum sample weight for analysis reached 0.05 g. A sterile transfer pipette was used to remove the liver sample from the biopsy needle, and it was carefully placed into individual microtubes and promptly placed on ice. Biopsy needles underwent cold sterilization in between animals. Samples from each collection day were submitted to the Michigan State University Veterinary Diagnostic Laboratory for mineral analysis using mass spectroscopy.

Grain supplements per batch made, baleage or hay offered, and standing forage of pastures were sampled to analyze for DM, ash, NDF, ADF, CP, and mineral composition. At time of analysis, samples were thawed and 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. Fiber analyses were determined using Ankom Fibers (ANKOM Technology Corp., Macedon, NY). Nitrogen percentages were used to calculate crude protein percentages (ECS 8020 CHNSO dual furnace, NC Technologies). Mineral analyses were performed in duplicate for hay, baleage, and forage samples and in triplicate for grain samples. To start, 1 ± 0.01 g of forage and 0.5 ± 0.01 g of grain were weighed into 50 mL centrifuge tubes.

Then 15 mL of trace mineral grade nitric acid was added to each tube containing sample. Samples underwent wet ash digestion performed by covering tubes with plastic watch glasses and placing them into a heating block. With the heating block, the temperature was set at 80°C for 15 min, or until all brown gases had escaped and foaming was not present. After all brown gas had escaped, the temperature was set at 115°C for 1h. Following, tubes were allowed to cool and were filled to a 45 mL volume with deionized water, inverted, and capped. Samples were analyzed by inductively coupled plasma (ICP) atomic emission spectroscopy (CIROS. Fitchburg, MA) at the University of Arkansas, Fayetteville, AR (Altheimer Laboratory).

Finishing

At the conclusion of the grazing trial, cattle were removed from dietary treatments and shipped to the Innovative Livestock Services-Ward Feed Yard (n = 198) in Larned, Kansas (Batesville = 887 km; Fayetteville = 625 km). If cattle were considered unfit for transportation, they were excluded from shipment and experiment analysis (n = 3). The 3 calves unfit for transportation were all from the complex treatment; 1 was injured on the final weighing day being lame, 1 had excessive warts, and 1 was pregnant. Upon entry to the feedlot, cattle received 2 identification tags. Cattle were commingled and fed in a single pen for 141 days. Diets delivered to the pen, morbidity, and mortality data were recorded. Any medications administered and diagnosis given were recorded.

Slaughter

After the feedlot, remaining cattle (n = 194) were sent to slaughter at Tyson Fresh Meats Beef Facility in Garden City, KS. West Texas A&M University trained individuals were present at time of slaughter to record kill order. At this time, carcasses were tagged to ensure the validity of data collected. Camera data were used for carcass characteristic analyses, which included

values for hot carcass weight, quality grade, yield grade, calculated yield grade, ribeye area, marbling score, backfat thickness, and if liver was considered edible. It was noted which calves had been liver biopsied to determine the effect on liver scores. Live weights were estimated by dividing the individual hot carcass weight by the standard average dressing percentage (63.5%).

Statistical Analysis

Treatment data were analyzed as a randomly complete block design with pen identified as the experimental unit. Cattle were distributed to 4 blocks being a random block effect. Treatment, day, and grazing location were placed in the class statement. Location was set as a random effect, treatment was fixed, and pen within location was specified as the subject. All data were analyzed by using various programs of SAS (SAS Inst. Inc., Cary, NC). Significance was determined when $P \leq 0.05$, with tendencies determined when $P > 0.05$ and ≤ 0.10.

For the grazing trial analysis, heifer body weights, and average daily gains were analyzed using the MEANS procedure for overall pen averages. Body weights and liver mineral concentrations were analyzed as repeated measures using MIXED procedures. Kenward-Rogers was specified as the degrees of freedom selection, with compound symmetry as the covariance structure. The model included treatment, day, and the day by treatment interaction. Grain supplement and forage sample data were generated using the MEANS procedure.

Data for the finishing phase were analyzed as in the grazing phase with the respective treatment and pens. Treatment, pen, and grazing location were placed in the class statement. Location was set as a random effect, treatment was fixed, and pen within grazing location remained specified as the subject. Feedlot morbidity (incidence of BRD or footrot) and mortality were analyzed using GLIMMIX and FREQ procedures. Carcass data were analyzed using MIXED, GLIMMIX, and FREQ procedures.

RESULTS AND DISCUSSION

Grazing

Performance. Body weights were similar for both treatments throughout the grazing phase (Figure 1). The removal randomly of calves after the receiving period resulted in calves that continued the inorganic treatment having a 5 kg greater body weight at the start of grazing, which was also apparent in later grazing weights (Table 5). There was no treatment ($P = 0.14$) nor treatment × day interaction ($P = 0.92$) for body weights during grazing (Figure 1). When calculated based on days grazing, overall ADG nor any approximate 28 d period ADG were different between treatments ($P \geq 0.40$). The average daily gain for the entire period the cattle grazed, ranging from 105 to 153 days (Table 5), did not differ due to dietary trace mineral source ($P = 0.94$; 0.69 kg/d, SE = 0.04). Neither treatment achieved the targeted gain of 0.79 kg/d.

There were no pinkeye or lameness incidences to report during grazing. One heifer, on the complexed supplemental trace mineral treatment, in Batesville was treated for BRD with Baytril on d 96 of grazing. Because of the removal process after the receiving trial, all cattle remaining in the continuing study were not expected to have high morbidity percentages.

Liver Mineral Concentrations. At the conclusion of grazing, dietary treatments did not affect liver mineral concentrations ($P \geq 0.17$) and there were no treatment × day interactions ($P \geq 23$). It is important to note that treatments only consisted of supplemental copper and zinc during the grazing trial. Day effects were present for increased selenium, molybdenum, and manganese, and a decrease in cobalt liver mineral concentrations by the end of the grazing period ($P \leq 0.03$).

Source of copper supplementation did not affect liver copper concentrations ($P = 0.41$). Both treatments had similar copper concentrations that remained above adequate concentrations during grazing. The concentration range considered adequate by Puls (1988) is from 125 to 600

μg/g dry liver and only considered deficient if concentrations fall below 33 μg/g. Spears et al. (2022) also stated that liver copper concentrations are the best indicator of status in ruminants. To support, McDowell (1992) stated that the concentration of copper in the liver of ruminants is directly correlated to the bioavailability of copper in the diet. Nockels et al. (1993) conducted a study determining whether copper or zinc balance would be affected by feeding either organic (ZnMet and CuLys) or inorganic ($ZnSO_4$ and $CuSO_4$) sources of zinc and copper before and after stressing calves. It was reported that calves fed CuLys had 53% greater apparent copper absorption and increased retention during repletion when compared to the calves fed $CuSO_4$.

Zinc liver concentrations were not affected by the type of trace mineral sources during the grazing period ($P = 0.17$). However, both treatments remained within the adequate concentration range. The concentration range considered adequate by Puls (1988) is 25 up to 200 μg/g. However, Arthington and Ranches (2021) stated that zinc lacks a reliable tissue pool for indicating status.

Manganese liver concentrations were similar for both treatments during grazing (Figure 2). Both treatment groups were receiving the same amounts of $MnSO_4$ for this duration. Both treatments increased similarly in manganese concentrations to above deficient concentrations (day, $P = 0.02$), but there were no treatment or treatment × day interactions.

Liver cobalt concentrations decreased similarly during the grazing period for both treatments (day, $P = 0.0001$). In receiving, diets consisted of 12 mg/d of cobalt from either complex amino acid or inorganic sources. For the duration of grazing, all cattle were supplemented with only 1.40 mg/d of $CoCO_3$, which may explain the decrease of concentrations in the liver. Due to the metabolic role of cobalt, assessment of the animal's nutriture often centers

on measures of vitamin B12 status. Difficulty assessing tissue cobalt status persists and current literature was not able to provide a value for adequate tissue concentrations.

Liver selenium concentrations increased to above adequate concentrations by the end of the grazing period for both treatments with a day effect ($P = 0.01$). Selenium was delivered at the same rate to both treatment groups as sodium selenite (0.99% Se) resulting in similar liver concentrations. Adequate concentrations of selenium in the liver are above 1.25 μg/g, which was exceeded by the end of the grazing period. Cattle of both treatments increased molybdenum concentrations in the liver during grazing with a day effect ($P = 0.03$). However, molybdenum concentrations were similar throughout the trial and cattle were not being supplemented with any forms of molybdenum.

A separate data set included all cattle that were able to be biopsied on all 3 collection dates (n = 53; Figure 3). Copper, being supplemented throughout receiving and grazing, liver concentrations increased (day, $P = 0.0001$) for both treatments similarly from the start of receiving to the conclusion of grazing. Copper concentrations were below adequate concentrations initially at the start of receiving but exceeded adequate concentrations by the end of receiving and grazing periods (Figure 3). Copper absorption in ruminants is poorly regulated because of the developed mechanisms for storing excess copper in the liver by decreasing the amount of copper being excreted through the bile. Acute copper toxicities can occur after administration of a large dose of copper but is usually a chronic process that occurs when excessive copper is supplied in the diet and hepatic copper reserves are overwhelmed (Suttle, 2010). The storage of excess copper in the liver potentially becomes high risk when ruminants are fed copper diets slightly above requirements and literature suggests that cattle are potentially being over supplemented in recent years (López-Alonso and Miranda, 2020).

Supplementing zinc in receiving through grazing revealed a tendency for zinc concentrations in the liver to be affected by a treatment × day interaction ($P = 0.09$). Zinc concentration means were the greatest at the start of receiving ($P = 0.69$) but were not different between treatments. By the end of receiving, zinc concentrations had decreased for both treatments ($P = 0.01$) and were similar at the conclusion of grazing ($P = 0.68$). Although concentrations had decreased, all means remained within the adequate concentration range according to Puls (1988).

Manganese liver concentrations tended to be affected by a treatment × day interaction throughout the receiving and grazing periods ($P = 0.06$). Manganese concentrations for both treatments decreased during receiving and then increased exceeding deficient concentrations by the end of the grazing period (day, $P = 0.03$). Cobalt liver concentrations of cattle on both treatments increased during receiving and decreased similarly by the end of grazing (Day, $P = 0.0001$). Manganese and cobalt were only supplemented as two different sources during the receiving trial and were fed as the same source during grazing, but there were no treatment differences to report for the duration of the project.

Feedlot Performance. There were no differences between treatments in feedlot morbidity or mortality ($P \geq 0.32$; Table 6). Incidences of BRD in the feedlot occurred for both treatments (complexed, n = 8; inorganic, n = 6). Additionally, 4 heifers, 2 from each treatment, died due to acute BRD. All deaths occurred after 91 days on feed in the feedlot.

Carcass Characteristics. There were no treatment differences in any carcass characteristics ($P \geq 0.28$; Table 7). Hot carcass weights (HCW) were similar ($P = 0.28$) at time of slaughter (complexed = 373 kg, inorganic = 378; SE = 3.3 kg). Live weights were estimated by dividing the individual HCW by the average dressing percentage (63.5%) and were not different

between treatments ($P = 0.62$). Quality grades were measured on a universal scale (1 = Prime, 2 = Choice, 3 = Select, 4 = No Roll). Quality grades for both treatments averaged as choice ($P = 0.88$). There were no treatment differences in either yield grade ($P \geq 0.41$). Cattle from both treatments averaged 95 cm^2 for ribeye area measurements ($P = 0.90$). Marbling scores from both treatments averaged 501 ($P = 0.99$). Thus, both cattle of both treatments resulted in marbling that was considered modest. Backfat thickness were similar for both treatments ($P = 0.44$). Lastly, there were no treatment differences in the percentage of livers that were considered nonedible ($P = 0.28$). However, the livers that had been biopsied negatively impacted the percentage of total edible livers. Of the calves that had livers that were considered nonedible, the percentage of calves that had previously been biopsied was greater (biopsied = 30%, not biopsied = 17%; $P = 0.05$).

CONCLUSION

In conclusion, although complexed sources of trace minerals (zinc, copper, manganese, and cobalt) improved body weight gain and decreased morbidity treatments during the receiving phase, there were no differences in growth performance when supplementing amino acid complexed in replace of inorganic sources of zinc and copper for the grazing period. Treatments did not reflect in liver mineral concentrations from receiving through grazing periods. There were no differences due to supplementation of complexed amino acid source nor inorganic sources of trace minerals during backgrounding on morbidity during the finishing phase or on carcass characteristics

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Table 1. Ingredient composition of grain supplement (as-fed basis)

	Fed at 1.82 kg/d		Fed at 2.73 kg/d	
Ingredient	Inorganic	Complexed	Inorganic	Complexed
Corn-cracked, %	58.6	58.6	59	59
Dried distillers' grains, %	30	30	30	30
Soybean meal, %	4.7	4.7	4.7	4.7
Salt, white, %	1.0	1.0	0.67	0.67
Molasses, %	2.0	2.0	2.0	2.0
Limestone, %	1.8	1.8	1.9	1.9
Fat, %	1.0	1.0	1.0	1.0
Corn/Rumensin premix[a], %	0.4	0.4	0.267	0.267
Vitamin A, D, E premix[b], %	0.1	0.1	0.07	0.07
Vitamin E[c], %	0.05	0.05	0.033	0.033
Availa-Zn 120, g/ton	-	2,250	-	1,500
Availa-Cu 100, g/ton	-	450	-	300
$ZnSO_4$ (35.5% Zn), g/ton	760	-	507	-
$CuSO_4$ (25.2% Cu), g/ton	178	-	119	-
$MnSO_4$, g/ton	277	277	185	185
$CoCO_3$, g/ton	1.5	1.5	1.0	1.0
Se (0.99%), g/ton	50.5	50.5	33.6	33.6
Ca $(IO_3)_2$, g/ton	3.6	3.6	2.4	2.4

[a] Premix provides 22 kg monensin/kg

[b] ADE premix contains 880,000 IU/kg Vitamin A, 1760,000 IU/kg Vitamin D, and 1,100 IU/kg Vitamin E

[c] Vitamin E contains 44,000 IU/kg

Table 2. Analyzed nutrient composition of supplements and forage during grazing phase, DM basis[a].

Item	Hay	Forage	Baleage		Fed at 1.82 kg/d		Fed at 2.73 kg/d	
					Inorganic	Complexed	Inorganic	Complexed
	F[b]	B[c]	F[b]	B[c]				
n	1	3	1	1	**2**	**2**	**6**	**6**
DM, %	95	95	94	93	97	97	96	97
Zn, mg/kg	25	25 ± 11	80	50	395 ± 9	293 ± 12	349 ± 72	262 ± 55
Mn, mg/kg	120	120 ± 25	160	106	136 ± 44	106 ± 36	108 ± 57	82 ± 25
Cu, mg/kg	4.40	4.40 ± 2	10	8.57	58 ± 12	59 ± 17	58 ± 17	48 ± 9
Fe, mg/kg	126	106 ± 14	150	175	163 ± 10	153 ± 64	142 ± 35	117 ± 27
Na, mg/kg	358	192 ± 137	301	93	4,836 ± 152	4,734 ± 1,549	4,204 ± 896	3,803 ± 506
S, %	0.14	0.19 ± 0.07	0.17	0.21	0.26 ± 0.01	0.26 ± 0.03	0.29 ± 0.03	0.27 ± 0.02
Mg, %	0.30	0.20 ± 0.02	0.20	0.15	0.20 ± 0.00	0.20 ± 0.013	0.21 ± 0.01	0.21 ± 0.01
Ca, %	0.51	0.46 ± 0.01	0.57	0.60	0.96 ± 0.10	0.91 ± 0.35	1.14 ± 0.31	0.96 ± 0.15
K, %	1.84	2.03 ± 1.19	2.33	2.57	0.89 ± 0.02	0.86 ± 0.03	0.95 ± 0.07	0.92 ± 0.06
P, %	0.32	0.23 ± 0.10	0.40	0.40	0.54 ± 0.01	0.53 ± 0.02	0.58 ± 0.04	0.58 ± 0.02

[a]LSMeans ± SEM
[b]F = Fayetteville
[c]B = Batesville

Table 3. Feed and forage analysis during the grazing phase, DM basis.

Item	Hay	Forage	Baleage		Fed at 1.82 kg/d		Fed at 2.73 kg/d	
					Inorganic	Complexed	Inorganic	Complexed
	F[a]	B[b]	F[a]	B[b]				
n	1	3	1	1	**2**	**2**	**6**	**6**
DM, %	95	95	94	93	97	97	96	97
CP, %	10	14	11	16	15	14	15	15
Ash, %	6.91	7.43	8.32	9.73	6.47	6.31	6.91	6.45
NDF, %	93	86	87	87	73	72	71	71
ADF, %	49	49	49	49	36	35	35	35

[a]F = Fayetteville
[b]B = Batesville

Table 4. Water analysis of all sources used.

Item	Fayetteville		Batesville
	Source #1	Source #2	
Arsenic, mg/L	0.000	0.003	0.003
pH	7.8	7.9	7.2
Total dissolved solids, mg/L	240.4	236	162.8
Conductivity, µS/cm	437	429	296
Cl^-, mg/L	19.4	22.5	6.2
F^-, mg/L	0.01	0.05	0.07
SO_4^{2-}, mg/L	2.0	2.6	4.0
$NO3^-$, mg/L	5.1	7.5	2.6
Ca, mg/L	64.7	58.5	45.9
Mg, mg/L	1.1	1.3	1.2
Na, mg/L	12.4	16.2	5.8
Cu, mg/L	0.03	0.04	0.004
Fe, mg/L	0.0	0.0	0.0
Mn, mg/L	0.0	0.0	0.0
SRP[a], mg/L	0.012	0.015	0.021

[a]SRP = Soluble reactive phosphorus

Table 5. Effects of complexed or inorganic trace mineral supplements on heifer performance during the grazing period and overall growth. LS means reported using pen means in MIXED procedure.

Item	Complexed	Inorganic	SEM	*P*-value
ADG[a], kg/d				
d 0 to 33	0.47	0.43	0.07	0.67
d 33 to 60	0.81	0.90	0.07	0.40
d 60 to 88	0.74	0.67	0.06	0.45
d 88 to 114	0.95	0.99	0.10	0.74
d 0 to 114	0.72	0.72	0.03	0.97
BW[b], kg				
Initial	267	272	1.63	0.05
Final	355	360	3.12	0.33
ADG[b], kg/d				
Initial to final	0.69	0.69	0.04	0.94

[a]This dataset includes the first 114 days of grazing for all cattle.

[b] Receiving final weights were used for initial body weights and the start of gain period. The final weights in this dataset were consecutive day weights taken before cattle were shipped to the feedlot. Cattle started/ended receiving trial at different times, thus their grazing periods varied in length: (Block 1) 71 calves grazed for 153 days, (Block 2) 34 calves grazed for 131 days, (Block 3) 31 calves grazed for 138 days, (Block 4) 63 calves grazed for 105 days.

Table 6. Effects of supplementing amino acid complex or inorganic (sulfates) during receiving and grazing phase on feedlot morbidity and mortality: number of incidences.

Item	Complexed	Inorganic	SEM	*P*-value
Bovine respiratory disease, n	8	6	0.61	0.57
Footrot[a], n	0	1		0.32
Any treatment[a], n	8	7		0.97
Dead[a], n	2	2		0.98

[a]GLIMMIX did not converge (only BRD converged), *P*- values from FREQ (chi-square).

Table 7. Effects of supplementing amino acid complex or inorganic (sulfates) during receiving and grazing phase on carcass characteristics.

Item	Complexed	Inorganic	SEM	P-value
Hot carcass weight, kg	373	378	3.3	0.28
Live weight[a], kg	590	594	11.4	0.62
Quality grade[b]	2.10	2.09	0.05	0.88
Yield grade	2.5	2.6	0.09	0.41
Calculated yield grade	3.0	3.1	0.10	0.44
Ribeye area, cm^2	95.5	94.8	0.16	0.90
Marbling score	501	501	12.4	0.99
Backfat, cm	1.73	1.78	0.05	0.44
Liver, % nonedible	25	19	4.2	0.28

[a]Live Weight (LW) = HCW/0.635 (average dressing percentage = 63.5%)

[b]Quality Grade (QG): 1 = Prime, 2 = Choice, 3 = Select, 4 = No Roll

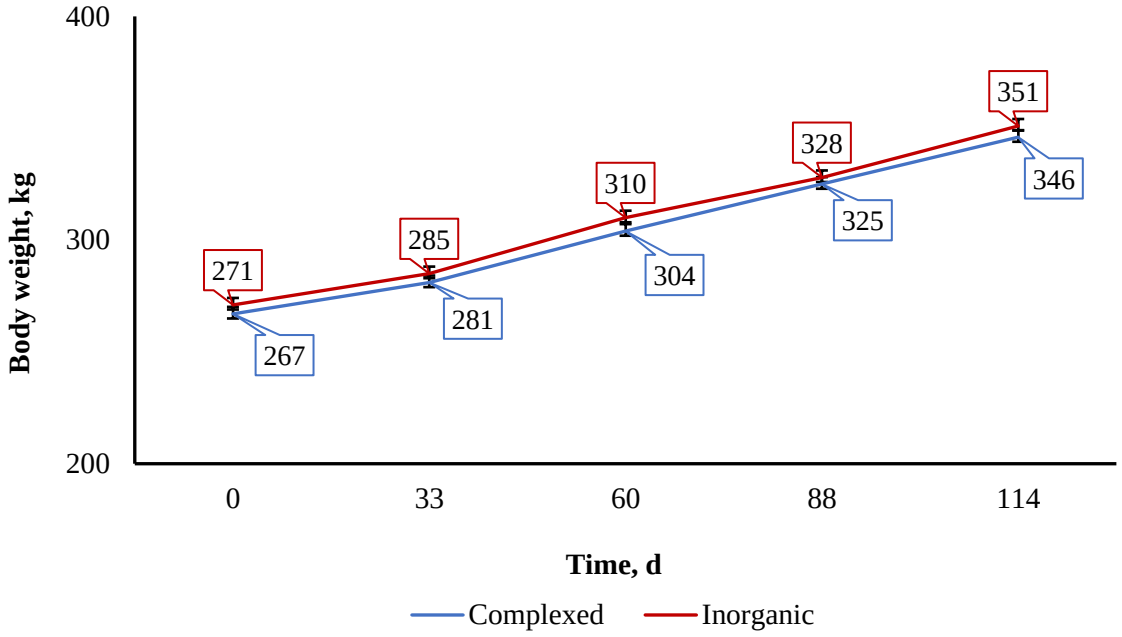

Figure 1. Effects of complexed or inorganic trace mineral supplements on heifer body weights during grazing period. LS means reported using pen means in MIXED procedure. This dataset includes the first 114 days of grazing for all cattle.

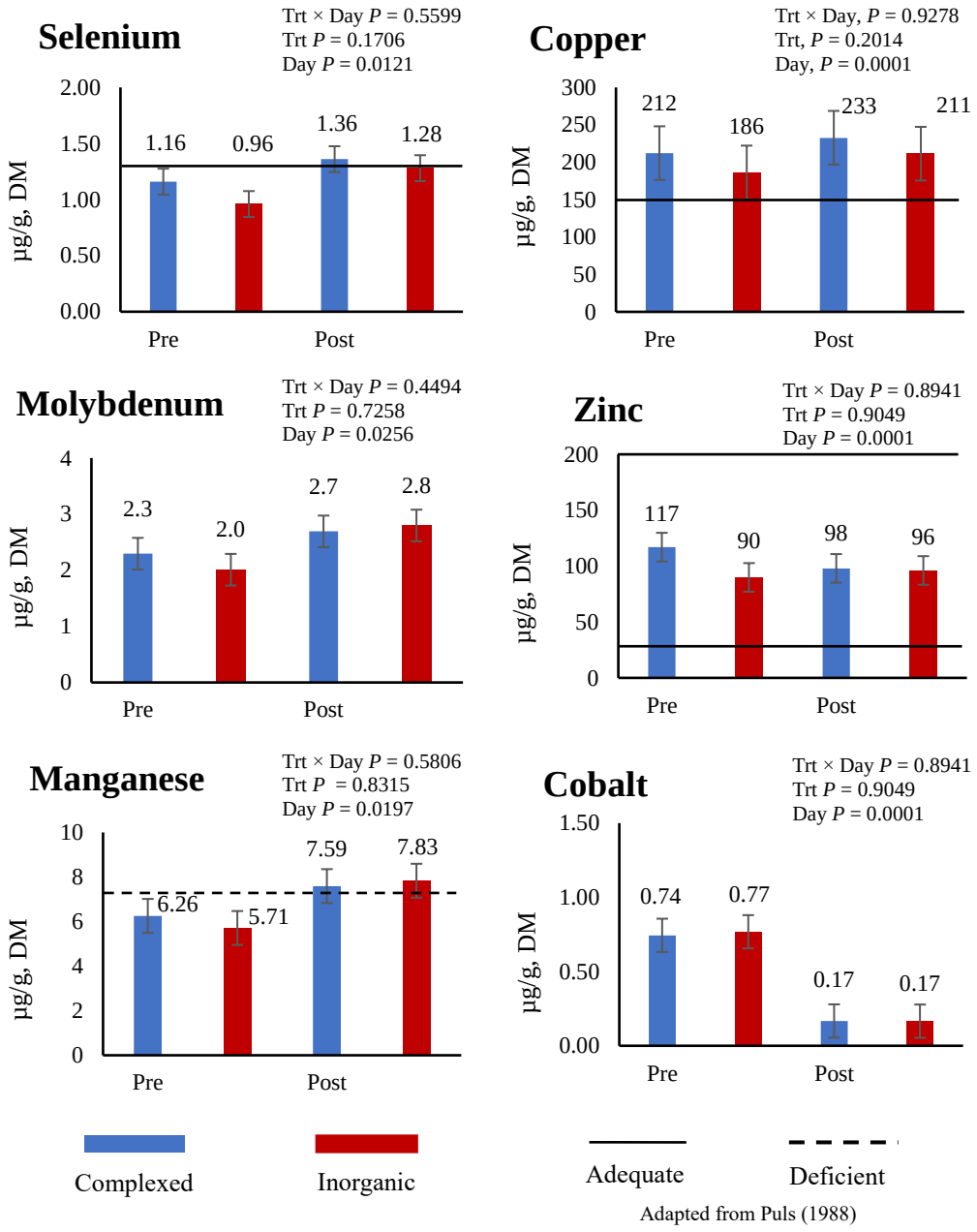

Figure 2. Effects of complexed-amino-acid or inorganic (sulfate) sources of zinc (540 mg/d) and copper (90 mg/d) on liver mineral concentrations at the conclusion of the grazing period (n = 72).

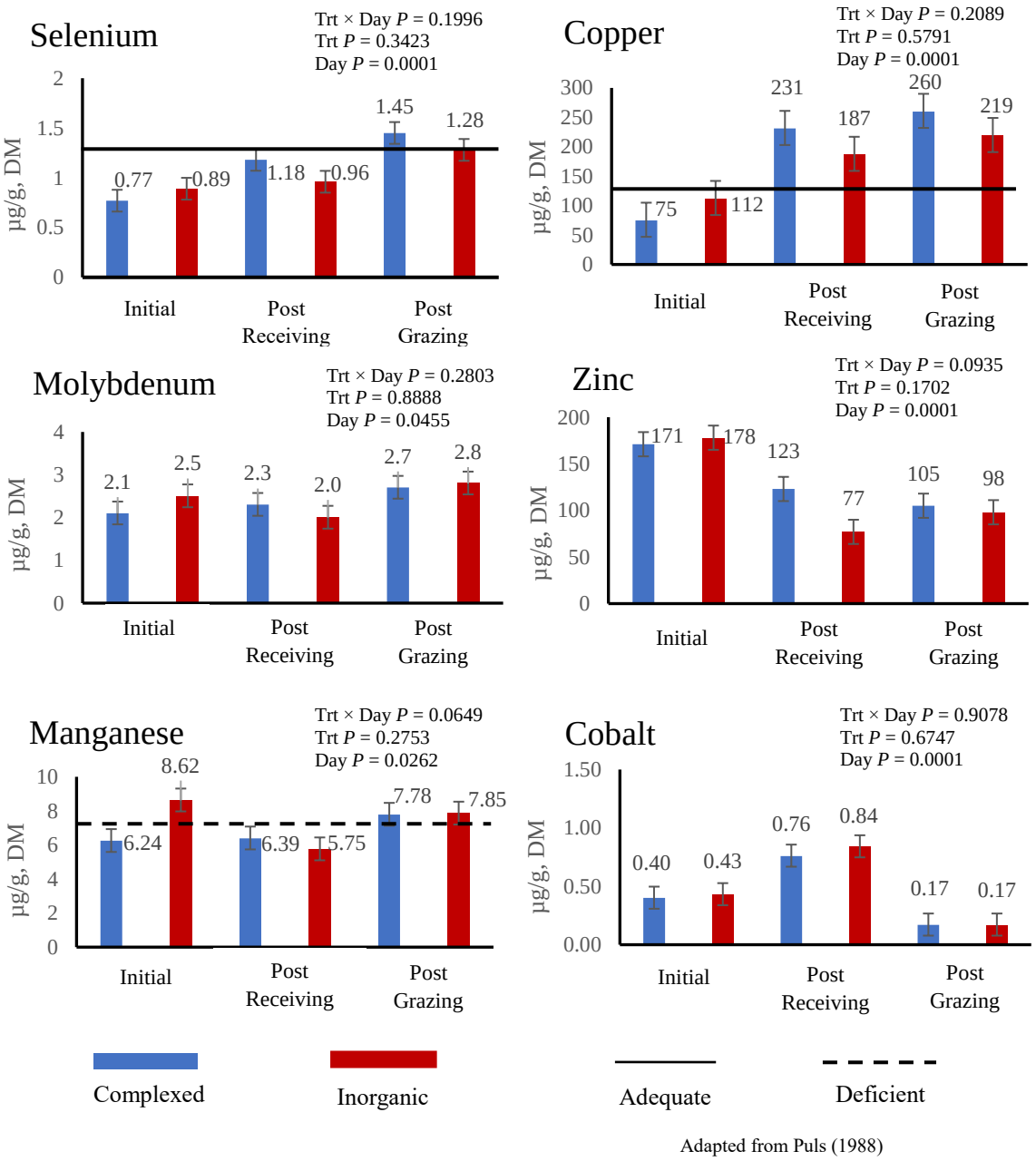

Figure 3. Effects of complexed-amino-acid or inorganic (sulfate) sources of zinc (540 mg/d) and copper (90 mg/d) on liver mineral concentrations of calves (n = 53) from the start of receiving (Day 5), end of receiving (Day 43), and end of grazing (Block 1, Day 196; Block 2, Day 174; Block 3, Day 181; Block 4, Day 148). Data represents the entire duration cattle received dietary treatments.

CHAPTER V

CONCLUSION

Two experiments were conducted to determine the effects of inorganic or amino acid-complexed sources of trace minerals on health, gain performance, liver mineral concentrations, and carcass characteristics of beef heifers from receiving through finishing. One experiment evaluated the effects of inorganic or amino acid-complexed sources of trace minerals (zinc, copper, manganese, and cobalt) on performance and morbidity of newly received stocker cattle. In the receiving period, treatments consisted of supplemental zinc (360 mg/d), copper (125 mg/d), manganese (200 mg/d), and cobalt (12 mg/d) from complexed (Availa-4, Zinpro Corp. Eden Prairie, MN) or inorganic sources (sulfates). Replacing inorganic sources of zinc, copper, manganese, and cobalt with amino acid complexed supplementation resulted in greater body weights and average daily gain. Cattle supplemented with inorganic trace mineral sources had a greater percentage of bovine respiratory disease (BRD) morbidity than cattle supplemented with complexed trace mineral sources and medication costs were lower for heifers supplemented with complexed trace mineral sources. Prolonged stress indicated by serum haptoglobin concentrations decreased throughout the receiving trial, and cattle supplemented with complexed trace mineral sources tended to have lower haptoglobin concentrations by d 28 of supplementation. However, the source of trace mineral had no effect on liver mineral concentrations. This study provided evidence for supplementing cattle for the first 42 days after arrival with amino acid complexed trace mineral sources improved heifer performance as compared to heifers supplemented with inorganic trace minerals. Hence, additional research is warranted to further assess and potentially validate impacts of supplementing inorganic or organic complexed sources of zinc, copper, manganese, and cobalt to beef calves.

Following the receiving trial, a second experiment was conducted to determine the effects of inorganic or amino acid-complexed sources of trace minerals (zinc and copper) during a grazing period post receiving on mineral status, growth and feedlot performance, and carcass characteristics. In the grazing period, treatments consisted of supplemental zinc (540 mg/day) and copper (90 mg/day) from complexed (Availa, Zinpro Corp. Eden Prairie, MN) or inorganic sources (sulfates). Following grazing, calves were removed from treatments and sent to a Kansas feedlot where they were commingled and fed in a single pen for 141 days. Morbidity and mortality data were recorded, and after slaughter, carcass data were collected. During grazing, growth performance nor liver mineral comparisons were not different between dietary treatments. Following the grazing period, there were no differences in feedlot morbidity, mortality, or carcass characteristics.

In conclusion, although complexed sources of trace minerals (zinc, copper, manganese, and cobalt) improved body weight gain and decreased morbidity treatments during the receiving phase, there were no differences in growth performance when supplementing amino acid complexed in replace of inorganic sources of zinc and copper for the grazing period. Treatments did not reflect in liver mineral concentrations from receiving through grazing periods. There were no differences due to supplementation of complexed amino acid source nor inorganic sources of trace minerals during backgrounding on morbidity during the finishing phase or on carcass characteristics.