THE EFFECTS OF VARYING LEVELS OF TRACE MINERAL SUPPLEMENTATION ON PERFORMANCE, CARCASS CHARACTERISTICS, MINERAL BALANCE, AND ANTIBODY CONCENTRATIONS IN FEEDLOT CATTLE

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Bachelors of Agricultural Science and Natural Resources

in Animal Science

Oklahoma State University

Stillwater, OK

2019

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of

MASTER OF SCIENCE
May, 2021

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ACKNOWLEDGEMENTS

I would like to thank Dr. Blake Wilson serving as my major advisor. Dr. Wilson first approached me during my junior year of undergraduate about being his graduate student. And from that day, he has been supported me and challenged me to succeed every step of the way. I cannot thank him enough for helping me grow, especially over the last two years. I would also like to thank the other members of my committee, Dr. Ryan Reuter and Dr. Paul Beck for all of the guidance and support they have provided me. Thank you all from the bottom of my heart for believing in me and investing your time in my education.

Thank you to my fellow lab mates and graduate students. I am so grateful to be surrounded by such hard working and intelligent individuals. I would like to especially thank Colton Robison and Andrea Warner for all their guidance and advice throughout my course work and research. I would also like to thank Jazmin Markey, Jocelyn Faisal, Jancy Jeffus, and Courtney McNeff for being my people. I love you ladies till the end of time. Thank you to all of the undergraduates and managers at WSBRC for all of hard work and making our research possible.

Thank you to my family and friends. Thank you to my mom and dad, my sisters, brother, brother in-laws, sister in-law, and nieces and nephews for their constant love and support. I am eternally grateful to have the best support system in the world, especially from 1,300 miles away. I would not be the person I am today without you all. Lastly to Collin. Thank you for being my rock for the last year. Your unwavering support and love mean the world to me. This paper is dedicated to my grandmother, Regina Richardson, who passed away during my research. I love you Mom-Mom.

Name: BRITTANY A. LIPPY

Date of Degree: MAY, 2021

Title of Study: THE EFFECTS OF VARYING LEVELS OF TRACE MINERAL SUPPLEMENTATION ON PERFORMANCE, CARCASS CHARACTERISTICS, MINERAL BALANCE, AND ANTIBODY CONCENTRATIONS IN FEEDLOT CATTLE

Major Field: ANIMAL SCIENCE

Abstract: The objective of this experiment was to determine the effects of increasing concentrations of trace minerals (TM) on finishing cattle performance, carcass characteristics, mineral balance, and antibody concentrations. Angus crossbred steers (n = 240; BW = 291 kg ± 27.4) were stratified by arrival BW and source and randomly assigned to 1 of 4 experimental treatments in a randomized complete block design (12 pens/treatment; 5 steers/pen). Treatments included a negative control (CON) in which cattle received no additional TM supplementation, a requirement treatment (1X) in which cattle received added Co (cobalt carbonate), Cu (cupric sulfate), Fe (ferrous sulfate), I (ethylenediamine dihydriodide), Mn (manganese oxide), Se (sodium selenite), or Zn (zinc oxide) at 2016 Nutrient Requirements of Beef Cattle required levels, a 2 times requirements (2X), and a 4 times requirements (4X) treatment. Selenium was included at 0.1, 0.2, and 0.3 mg/kg for 1X, 2X, and 4X respectively. There was no difference in overall BW, ADG, DMI and G:F due to supplementation (CON vs SUPP $P \geq 0.47$). There was no difference in marbling score, USDA Yield Grade, back fat, REA, HCW, or dressing percentage due to supplementation (CON vs SUPP $P \geq 0.30$). One steer was chosen at random from each pen to be evaluated for serum and liver TM status and antibody concentrations to respiratory viruses. There was treatment × day interaction for serum Co, and liver Cu and Se ($P < 0.0001$). Serum Co was greatest for the 4X treatment from d 28 through harvest. Liver Cu was greatest for the 2X and 4X treatments from d 56 through harvest. Liver Se was greatest for 2X and 4X from d 28 through harvest. There was an effect of day on liver Co, Fe, Mn, Mo, and Zn ($P \leq 0.0001$) and serum Cu, Mn, Mo, Se, and Zn ($P \leq 0.002$). Concentrations for individual TM had different trends over time, however, all reported values were within normal ranges. Serum Zn was greater at harvest ($P = 0.02$). There was an effect of time on Bovine Viral Diarrhea Virus Type 1A, Bovine Herpesvirus Type 1, Bovine Parainfluenza 3 virus antibody titer concentrations ($P \leq 0.0001$). Overall, TM supplementation above requirements had no effect on cattle performance, carcass characteristics, or immune response but does affect the storage of Cu and Se in the liver as well circulating Co levels.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

REVIEW OF LITERATURE

Introduction

Beef cattle require approximately 17 minerals for growth, muscle and fat development, immunity, and normal cell function (NASEM, 2016). Of these 17 minerals, there are 10 commonly recognized required trace (or micro) minerals (TM); chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium, and zinc. Micro minerals are required in concentrations of milligrams or micrograms per day (NASEM, 2016). Feeding insufficient amounts or a superabundance of TM can have detrimental effects on animal performance and health.

Trace minerals are of interest for those involved in feeding beef cattle because of the elements' vital role in production, carcass characteristics, immune function, enzyme activity, and therefore overall health. Many essential TM are present in adequate concentrations in common feedstuffs. Nevertheless, several TM are insufficient in common cattle diets, requiring supplementation. Understanding the complexities of mineral interactions whether synergistic or antagonistic, and the effects on animal production is likewise important. Many factors can affect an animal's response to supplementation including but not limited to source, concentration, duration of

intake, physiological status of the animal, the environment, or presence or absence of dietary antagonists (NASEM, 2016). It is vital that TM supplementation options and concentrations be explored more thoroughly to better understand the effects on performance, health, and overall wellbeing.

There are many factors that influence the bioavailability of minerals, especially the TM. A few include level of mineral intake, chemical form, overall diet digestibility, particle size, interactions with other minerals and nutrients, chelators, inhibitors, physiological state of the animal, water quality, processing conditions to which the individual ingredients or complete diet have been exposed and, of course, the age and species of the animal (Miles et al. 2003). There has been substantial variation in the concentration and source of trace mineral supplementation used in different studies, making interpretation of data and ability to reach a concise conclusion regarding TM supplementation challenging.

Sources of Trace Minerals

Trace mineral supplementation is often required to meet the requirements of growing and finishing beef cattle (Samuelson et al., 2016). Trace minerals can be provided through inorganic, organic, and hydroxy sources. Inorganic mineral compounds have weak ionic bonds. Inorganic trace mineral sources (primarily sulfate and oxide forms) were first supplemented to cattle to a limited extent in the 1930's and are the most widely used source of TM today (Spears, 2013). Inorganic compounds are comparatively inexpensive and readily available. Supplementation of inorganic trace minerals has been effective in correcting as well as preventing TM deficiencies in cattle (Spears, 2013).

However, in the presence of certain antagonists such as Mo, bioavailability of inorganic trace mineral sources can be low (Spears, 2013). Various feedgrade sources of a particular metal (oxide, sulfate, etc.) can also differ in purity and other factors that can affect bioavailability of the TM (Spears, 2013).

Organic sources of trace mineral are bound to an organic molecule. Organic trace mineral supplements commercially available vary regarding the type of ligand or ligands used to form the metal complex or chelate (Spears 1996). Most of the organic minerals marketed are classified as complexes, chelates, or proteinates (Spears, 1996). These structures decrease the compounds solubility in the rumen thus increasing their bioavailability for absorption in the small intestine. A limitation of organic sources would be the availability and increased cost.

Hydroxy trace minerals belong to a group of inorganic compounds but have similar properties to organic compounds (Spears, 2013). In terms of cattle nutrition, hydroxy trace minerals are a relatively new category in trace mineral supplementation. Zinc hydroxychloride ($Zn_5(OH)_8Cl_2$) and manganese hydroxychloride ($Mn_2(OH)_3Cl$) were introduced to the market in 2012 (Spears, 2013). In contrast to sulfates where the metal is bound to sulfate via weak ionic bonds, the metals in hydroxy trace minerals are covalently bonded to multiple hydroxy groups (Spears, 2013). One advantage of hydroxy minerals is the ability to by-pass the rumen, thus minimizing interactions that normally occur in the rumen (Spears, 2013). The ability to avoid interactions in the rumen can at least partially explain the higher bioavailability of hydroxy minerals compared with sulfate sources that have been reported in cattle (Spears, 2013).

Requirements and Industry Standards

According to the 2016 Nutrient Requirements of Beef Cattle, 7 of the 10 recognized essential TM have quantifiable requirements. Cattle have different requirements of cobalt, copper, iodine, iron, magnesium, manganese, selenium, and zinc based on stage and type of production (NASEM, 2016). Growing and finishing beef cattle require diets that, on a dry matter (DM) basis, have 0.15 mg/kg Co, 10.00 mg/kg of Cu, 50.00 mg/kg of Fe, 0.50 mg/kg of I, 20.00 mg/kg of Mn, 0.10 mg/kg of Se, and 30.00 mg/kg of Zn (NASEM, 2016).

While these requirement levels have been established and are widely accepted, nutritionists still deviate from these published requirements. In 2015, New Mexico State and Texas Tech University conducted a survey of feedlot nutritionists (Samuleson et al., 2016). The survey focused on summarizing the nutritional recommendations and professional practices of consulting feedlot nutritionists in the midwestern United States. With regards to TM supplementation, 45.5% of respondents considered only the partial value of trace mineral concentrations in the basal diet, whereas 36.4% of respondents did not consider trace minerals in the basal diet at all when formulating diets for feedlot cattle (Samuleson et al., 2016). Thus, the levels of recommended TM in the survey are expressed as the concentration of added TM via supplementation rather than the total presence in the diet (Samuleson et al., 2016). The average concentrations of trace minerals were as follows: 0.82 mg/kg of Co, 17 mg/kg of Cu, 13.8 mg/kg of Fe, 0.73 mg/kg of I, 47.9 mg/kg of Mn, 0.24 mg/kg of Se, and 87.3 mg/kg of Zn (DM basis; Samuleson et al., 2016). Recommended trace mineral concentrations for finishing cattle diets were 70 to 500% greater than those recommended by the NRC (1996) except for Fe,

which is a known antagonist to many TM. The lower values for Fe most likely occurred because many the respondents did not recommend that additional Fe be added to the diet above what was provided by the basal diet (Samuleson et al., 2016). Trace mineral supplementation is believed to play a vital role in optimizing the efficacy and efficiency of feedlot cattle production. It is important to understand the role each trace mineral plays in overall cattle performance and health.

Cobalt

Prior to the discovery of a Co requirement in ruminants, grazing animal could not be grazed effectively in many regions of the world due to insufficient levels of Co in the forages consumed (McDowell, 2003). Cobalt is widely distributed in the animal body, with high concentrations in kidney, liver, and bone (Suttle, 2010; McDowell, 2003; Suttle, 2010; Underwood and Suttle, 1999). Cobalt is an essential component of vitamin B_{12}. Vitamin B_{12} contains about 4.5% Co and is also referred to as cobalamin (McDowell, 2003). In normal situations, beef cattle do not have a requirement for dietary vitamin B_{12} because ruminal microorganisms are capable of synthesizing vitamin B_{12} from Co (NASEM, 2016). Measurements of the amount of dietary Co converted to vitamin B_{12} in the rumen range from 3 to 13% of Co intake, making the efficiency of Co utilization by vitamin B_{12}-producing rumen bacteria low (Paterson and Engle, 2005; Smith, 1987). Efficiency with which dietary Co is converted to into vitamin B_{12} is inversely proportional to Co intake (McDowell, 2003). Smith and Marston (1970) reported a higher conversion rate in sheep fed a Co-deficient diet than those fed a diet adequate in Co. Poor absorption in the rumen may be related to the rapid binding of Co by ruminal microorganisms (McDowell, 2003). Absorption is reduced due to rumen

microorganisms diverting Co to inactive forms of vitamin B_{12}-like compounds that are unavailable to the animal (Gawthorne, 1970; NASEM, 2016, Paterson and Engle, 2005).

Cobalamins are an essential part of enzymes involved in metabolic processes. A major metabolic process that is reliant upon a vitamin B_{12}-dependent enzyme is converting propionate to succinate. Succinate is a 4-carbon compound that requires the introduction of one carbon unit alongside the 3-carbon compound propionate (McDowell, 2003). Methylmalonyl CoA mutase is a vitamin B_{12}-dependent enzyme that catalyzes the conversion of methylmalonyl-CoA to succinyl CoA (McDowell, 2003; Smith, 1987; NASEM, 2016). From there, succinyl CoA can enter the tricarboxylic acid cycle and be converted to succinate. Due to the large quantities of propionates produced during carbohydrate fermentation in the rumen, the metabolism of propionate is of interest to ruminant nutritionists. In Co or vitamin B_{12} deficiency, the rate of propionate clearance from the blood is depressed and methylmalonyl-CoA accumulates (McDowell, 2003). This results in an increased urinary excretion of methylmalonic acid and loss of appetite because impaired propionate metabolism leads to higher blood propionate levels which are inversely correlated with voluntary feed intake (MacPherson, 1982; McDowell, 2003). The second vitamin B_{12} dependent enzyme which occurs in mammalian tissues is 5-methyltetrahydrofolate homocysteine methyltransferase which is heavily involved in methionine metabolism (Paterson and Engle, 2005).

A cobalt deficiency can manifest in many ways. Acute clinical signs of Co deficiency include decreased appetite and failure to grow or moderate weight loss (Smith, 1987). These symptoms are early signs of a Co deficiency. If Co is not supplied in the diet and the deficiency becomes more severe, animals demonstrate signs of fatty

degeneration of the liver, rapid weight loss, unthriftiness, and pale skin and mucous membranes as a result of anemia (NASEM, 2016).

Published literature regarding the effects of solitary Co supplementation on finishing cattle performance and carcass characteristics is limited. Tiffany et al. (2003) investigated the effect of Co concentration and source in growing and finishing cattle. treatments. Treatments consisted of a negative control, 0.05 mg, 0.10 mg, or 1.00 mg of supplemental Co/kg DM from Co carbonate ($CoCO_3$) and 0.05 mg or 10.0 mg of supplemental Co/kg DM from Co propionate. Steer performance during the 56-d growing phase was not affected by Co source or concentration. Cobalt supplemented cattle tended to have a greater final body weight (BW; Tiffany et al. 2003). Average daily gain (ADG) was improved in Co supplemented cattle during the first 56 d of the finishing phase. However, there was no difference in ADG between d 56 and 112 of the finishing phase from Co supplementation. Cattle supplemented with 0.05 mg Co/kg DM, from $CoCO_3$, tended to gain faster than cattle supplemented with 0.05 mg Co/kg DM from Co propionate (Tiffany et al. 2003). Average daily gain was increased by Co supplementation, regardless of source over the entire finishing phase. Cobalt addition to the control diet resulted in a linear increase in average daily feed intake (ADFI) during the first 56 d and a quadratic increase over the total finishing phase. Average daily feed intake was not affected by Co source during the finishing phase (Tiffany et al., 2003). There was a tendency for increased gain:feed (G:F) in steers supplemented with Co during the first 56 d. Gain:feed responded quadratically to Co supplementation from d 56 to 112 of the finishing phase. Gain:feed differed between Co sources supplemented at

0.05 mg Co/kg during the second 56-d period and over the entire finishing phase (Tiffany et al. 2003).

Schwartz et al. (2000) reported that cattle with 0.07 mg Co/kg DM had decreased hot carcass weights (HCW) in comparison to those fed diets with 0.11 mg Co/kg DM or greater. Tiffany et al. (2003) reported steers that received supplemental Co tended to have greater HCW. As Co supplemental level increased, there was a tendency for quadratic response in marbling score (Tiffany et al., 2003). Supplemental Co did not affect backfat thickness, dressing percentage, rib eye area (REA), or kidney pelvic and heart fat percentage (KPH%; Tiffany et al., 2003). Because of the limited breadth of research regarding Co supplementation in cattle, it is difficult to draw a definitive conclusion. The research that has been completed indicates that Co supplementation can have a positive effect on cattle performance, and possibly carcass characteristics however, the level of optimal supplementation during different stages of growing and finishing has not been quantified. More research in regard to concentrations and source of Co supplementation is needed to define Co supplementation recommendations.

Copper

Copper was not discovered in animal and plant tissues until the early 1800's (McDowell, 2003). Early investigators believed that plant and animal Cu concentrations represented accidental contamination from soil (McDowell, 2003). Copper was first determined to be essential for growth and hemoglobin formation in laboratory rats in 1928 (Suttle, 2010). Following this discovery, evidence accumulated that Cu was

essential for growth and for the prevention of a wide range of clinical and pathological disorders in several livestock species (McDowell, 2003; Paterson and Engle, 2005).

Copper absorption is extremely low in ruminants compared to nonruminants (Spears, 2003). In addition, absorption can vary greatly depending on concentrations of other dietary minerals such as Mo, S, and Zn. Copper functions as an essential component of several enzymes including lysyl oxidase, cytochrome oxidase, superoxide dismutase, ceruloplasmin, and tyrosinase (McDowell, 2003). Of these enzymes, only 2 control processes that have unequivocal dependency on a specific enzyme: pigmentation (tyrosinase) and connective tissue development (lysyl oxidases; Prohaska, 2006; Suttle, 1987; Suttle 2010).

Copper deficiency can result in poor growth, rough hair coat, fragile bones, diarrhea, impaired reproduction, and cardiac failure (Spears, 1995). In breeds of cattle with highly pigmented coats, loss of coat color (achromotrichia) is usually the earliest and sometimes the only visual clinical sign of copper deprivation. Greying of black or bleaching of brown hair is sometimes seen, especially around the eyes (Suttle, 2010). Copper has important an important role in immune function. The inability of neutrophils to kill yeast organisms has also been found as a result of copper deficiency (Boyne and Arthur, 1981). Foreign materials such as infectious organisms enter the body and are subjected to phagocytic cells (neutrophils in blood and macrophages in tissues) which serve to bind, ingest, and destroy foreign materials (Spears, 1995). The inability of neutrophils to destroy infectious organisms is a serious implication of a Cu deficiency.

The involvement of Cu and cattle performance has been of interest to researchers for several decades. Ward and Spears (1997) conducted a 284-d experiment examining

the effects of Cu supplementation against a negative control through the receiving, growing, and finishing phases of beef production. During the receiving phase, cattle received 6.89 mg/kg of Cu in the basal diet with 0 or 5 mg/kg of supplemented Cu from $CuSO_4$ daily. Experimental treatments and assignments remained the same through the growing and finishing phases, however, the basal diet contained 5.2 mg/kg Cu and 2.85 mg/kg of Cu in the growing and finishing diets, respectively. Cattle that did not receive Cu supplementation had decreased dry matter intake (DMI) during the receiving phase and tended to have decreased DMI during growing phases but ADG was not affected by Cu supplementation. During the growing phase, due to tendency for greater DMI and similar ADG, G:F tended to be lower for Cu-supplemented steers (Ward and Spears, 1997). However, researchers concluded that DMI was not influenced by Cu supplementation due to Cu status during the growing phase. During the finishing phase, Cu supplemented cattle had tended to have greater ADG and G:F. Engle and Spears (2000) reported no difference in BW, ADG, and ADFI between cattle receiving 4.9 mg/kg Cu in the basal diet with additional 0, 10, 20 mg/kg of Cu from $CuSO_4$. In 2001, Engle and Spears evaluated the effects of Cu supplementation on Simmental cattle for 140 d. Treatments consisted of: 0 mg/kg Cu, 10 mg/kg Cu, or 40 mg/kg Cu supplementation from Cu SO_4. The basal diet contained 9.8 mg/kg Cu in the growing diet and 5.1 mg/kg Cu in the finishing diet. There was no difference in BW, ADG, ADFI, or G:F during either the growing or finishing phases (Engle and Spears, 2001). Overall, Cu supplementation has seemed to have limited and variable impact on cattle performance during growing and finishing phases.

Similarly, to Cu effects on performance, Cu supplementation has had limited effects on carcass characteristics. Copper supplementation tended to decrease fat deposition at the 12th rib and tended to increase REA. Copper-supplemented cattle had lower USDA Yield Grades but similar HCW, marbling, and KPH% (Ward and Spears, 1997). Additional research has also reported no difference in HCW, KPH%, and USDA Quality Grade supplemented in cattle 0, 10, 20, or 40 mg per day of Cu from $CuSO_4$ (Engle and Spears, 2000; Engle and Spears, 2001). Cattle supplemented with 10 and 20 mg/kg Cu had decreased back fat but did not have improved USDA Yield Grade (Engle and Spears, 2000). Engle and Spears (2001) also reported a difference in back fat thickness in Simmental cattle. Cattle supplemented with 40 mg of Cu tended to have less back fat than cattle supplemented with 10 mg of Cu. However, there was no difference in back fat in cattle fed the control diet and those supplemented with Cu.

Iodine

The only known role of I is the synthesis of the thyroid hormones: thyroxine (T4) and triiodothyronine (T3; McDowell, 2003). More than 95% of iodine in the body is accumulated in the thyroid gland (Flachowsky, 2007). Thyroid hormones are active in thermoregulation, intermediary metabolism, cellular respiration, energy production, reproduction, growth and development, immune function, circulation, and muscle function (McDowell, 2003; Suttle, 2010). An increase in thyroid hormone levels results in an increase in the basal metabolic rate (BMR; NASEM, 2016). As a determinant of metabolic rate, T3 interacts with hormones such as insulin, growth hormone and corticosterone (Ingar, 1985), and regulatory proteins of exocrine origin. For example, leptin production by adipose tissue is induced by thyrotrophic hormones and controls

appetite (Menedez *et al*., 2003; Suttle, 2010). Furthermore, the rate of gene transcription is controlled by T3 (Bassett et al., 2003), which thus influences protein synthesis in all cells (Erenberg et al., 1974; Hopkins, 1975; Suttle, 2010).

Iodine has very little published research regarding supplementation and finishing cattle performance and carcass characteristics. There have been a few studies examining the effects of I supplementation on cattle performance or carcass characteristics. Downer et al. (1981) examined the effects of dietary iodine in the form of ethylenediamine dihydriodide (EDDI). Dietary treatments included a negative control, 50 mg of EDDI, and 400 mg of EDDI. There were no differences between treatments for ADG. However, iodine concentrations of the basal diet were not reported. It is possible that the basal diet contained sufficient iodine and additional supplementation would not affect weight gain. Meyer et al. (2008) also reported no difference in performance due to I supplementation. Due to the limited published studies regarding finishing cattle and I supplementation, a conclusion as to the effects of additional I above NASEM (2016) requirements has on feedlot cattle performance and carcass characteristics cannot be made.

Manganese

Manganese has been written about since Roman times and is derived from the Greek word for magic (McDowell, 2003). Manganese was first recognized as an essential mineral for growth and reproduction in rats and humans in 1931 (McDowell, 2003). Since this discovery, studies in several species have been conducted to determine the functions of Mn (McDowell, 2003). Ruminants have an effective homeostatic control for Mn levels in blood and tissues, and Mn retention by the animal depends on the amount of Mn excreted from the bile into the intestine (Hidroglou, 1979). The liver, bones,

pancreas, and kidneys have relatively high concentrations of Mn while muscles have very low Mn concentrations. However, Mn concentrations of most tissues is quite characteristic to the individual tissue and not very responsive to changes in intake (Miller, 1979; McDowell, 2003).

Like other essential trace minerals, Mn can function as both an enzyme activator and a constituent of metalloenzymes. (McDowell, 2003). Manganese functions as a component of the enzymes pyruvate carboxylase and superoxide dismutase and as an activator for a number of enzymes (Hurley and Keen, 1987; NASEM, 2016). Pyruvate carboxylase is responsible for lipid and glucose metabolism (Underwood and Suttle, 1999; McDowell, 2003). Gregory and Fridovich (1974) discovered superoxide dismutase contains 2 mg Mn per mol. Located primarily in the mitochondria, superoxide dismutase protects cells from damage by reactive oxygen species (Suttle, 2010). Manganese deprivation lowers superoxide dismutase activity in the heart (Davis et al., 1992) and increases the peroxidative damage caused by high dietary levels of polyunsaturated fatty acids (Malecki and Greger, 1996; Suttle, 2010). Manganese also plays a role in immunological function (Hurley and Keen 1987). Manganese interacts with the plasma membrane of cells employed in the immune response, specifically neutrophils and macrophages (Rabinovitch and Destefano, 1973; McDowell, 2003)

A deficiency in Mn has been experimentally produced in cattle, but the practicality of Mn deficiency in a standard production setting has been questioned (McDowell, 2003). It has been reported that some ruminants experience a Mn deficiency while grazing. Deficiency symptoms include excess accumulation of body fat, reduced tissue storage of Mn in bone, liver, hair, and ovary, decreased bone strength, abnormal

bone shape, and muscular weakness (Thomas, 1970; Hidiroglou, 1980; McDowell, 2003).

While manganese has important functions as a component of important enzymes and metabolic reactions, very little research has been conducted evaluating the impact supplemental Mn has on cattle performance and carcass characteristics. One study evaluated the effects of supplemental Mn from $MnSO_4$ at 6 levels of supplementation: 0, 10, 20, 30, 120, 240 mg/kg of Mn (Legleiter et al., 2005). The experiment reported no effect of supplementation on BW, ADG, DMI, or G:F through the growing or finishing phases. However, the basal diet of the growing phase contained sufficient levels of Mn (29.2 mg Mn/kg). In theory, additional supplementation to a diet already providing adequate levels of Mn should not affect performance. However, the basal finishing diet contained less than half (8.1 mg Mn/kg) of the required level of Mn. Cattle receiving no additional Mn supplementation did not experience detrimental effects from a low Mn concentration in the basal diet (Legleiter et al., 2005). Additionally, Mn supplementation did not affect HCW, REA, fat thickness, USDA Yield Grade or marbling score (Legleiter et al., 2005). The limited research on finishing cattle and Mn supplementation suggest that cattle performance and carcass characteristics are not affected by Mn supplementation. Additionally, it is quite possible that the Mn requirement of 20 mg/kg (NASEM, 2016) could be called into question since no detrimental effects on performance or carcass merit occurred with a lower concentration of Mn in the basal diet.

Selenium

The fundamental role of Se in nutrition was misinterpreted for 2 decades, where in the 1930's selenium was believed to be a toxic element in some forages that caused

animals to lose hair and hooves (McDowell, 2003). However, in 1957, the essential nature of Se was discovered and the body of information regarding Se soon began to change (McDowell, 2003). Absorbed Se is carried in the plasma until it enters tissues. In individuals consuming deficient or adequate levels of Se, the majority of the Se in plasma is associated with a selenoprotein referred to as selenoprotein P(Xia et al., 2000; McDowell, 2003). In individuals consuming excess Se, most of the plasma Se is associated with albumin (Xia et al., 2000; McDowell, 2003). With required dietary intake of Se, the kidneys contain the highest concentration of Se, followed by the liver, spleen, and pancreas (McDowell, 2003).

In 1973, glutathione peroxidase was identified as the first known Se metalloenzyme (Rotruck et al. 1973; NASEM, 2016). This enzyme aids in protection of cellular and subcellular membranes from oxidative damage. If peroxidation is uncontrolled, there is a chain reaction of free-radical generation and tissue damage (McDowell 2003). This antioxidant function of Se is closely linked to Vitamin E. Vitamin E in cellular and subcellular membranes has been determined to be a potent antioxidant against peroxidation of phospholipids. The Se-containing metalloenzyme glutathione peroxidase likewise destroys peroxides before they have an opportunity to cause damage (McDowell, 2003). Arthur et al. (1990) identified a second selenometalloenzyme, iodothyronine 5'-deiodinase. This enzyme catalyzes the deiodination of T4 to the more metabolically active T3 in tissues (NASEM, 2016).

Levels of Se and vitamin E above the generally accepted requirements have been shown to enhance the immune response in several species. Studies have indicated that selenium deficiency can affect the ability of neutrophils to kill microorganisms (Boyne

and Arthur 1981; Spears 1995). Spears (1995) reported that a selenium-vitamin E injection reduced calf death losses from birth to weaning. Most of the deaths on the study were attributed to diarrhea and subsequent unthriftiness. It is evident from this study that increased death due to selenium deficiency could easily go unnoticed under practical conditions (Spears, 1995). The classical clinical sign of selenium deficiency is white muscle disease in young calves. Affected animals may show stiffness, lameness, or even cardiac failure. Other signs of selenium deficiency that have been observed include unthriftiness (associated with weight loss and diarrhea), and anemia (Spears, 1995).

As with several other trace minerals, specific and targeted research on the effects of Se supplementation on performance and carcass characteristics in cattle is limited. However, of the research conducted, it appears that Se supplementation has inconsistent effects on performance. Perry et al. (1976) conducted 2 experiments involving supplementation through sodium selenite. In the first experiment, a negative control was investigated against a supplement containing 0.1 mg/kg of Se. Cattle fed 0.1 mg/kg of supplemental Se in addition to 0.08 mg/kg of Se in the basal diet exhibited a greater ADG than those receiving no supplemental Se (Perry et al. 1976). In a second experiment, 0, 0.1, 0.2, and 0.4 mg/kg of supplemental Se were evaluated. Overall, no level of supplementation had any beneficial effect on ADG. Supplemental levels of 0.2 or 0.4 mg/kg resulted in depressed ADG. Based on the data in this paper, it was concluded that the range between suboptimal and possible growth depressing levels of selenium in the diet of finishing cattle are quite narrow (Perry et al., 1976). Based on this research, supplemental selenium does not appear to have quantifiable and replicable results in improving cattle performance.

Zinc

Zinc has been used for a multitude of purposes for 2000 years. Nutritional interest in zinc was increased when the element was discovered to be deficient in swine diets in the mid 1950's (McDowell, 2003). Shortly following a discovery of zinc deficiency causing parakeratosis in poultry, Legg and Sears discovered that the same occurred in cattle (Nielson, 2012). The ability of Zn to be absorbed and used for various bodily functions has become increasingly evident since Zn's nutritional role discovery. The percentage of dietary Zn that is absorbed deceases as dietary Zn increases in ruminants (Miller, 1970; Spears, 2003) Zinc absorption was directly reflective of the physiological demand for Zn for both calves and lactating cows (Miller, 1969; Stake, 1975).

Zinc is widely stored throughout the body; however, animals have a limited capacity for storing Zn in a form that can be mobilized rapidly to prevent deficiency (Underwood 1977; McDowell, 2003). Although Zn is present in most tissues, Zn is particularly stored in the liver, kidney, pancreas, and intestine (McDowell; 2003). Zinc is an essential component of several important metalloenzymes such as Cu-Zn superoxide dismutase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase, and RNA polymerase (Hambidge et al. 1986; NASEM, 2016).

Blackmon et al. (1967) summarized the clinical signs of Zn deficiency in ruminants to include inflammation of the nose and mouth, unthrifty appearance accompanied by rough hair coat, stiff joints, and cracked skin, especially on the hooves and ears, and bowing of the back legs. Growth retardation and depressed feed intake and feed efficiency are universally observed in Zn deficiency(McDowell, 2003). Loss of

appetite is one of the first signs of Zn deficiency. Part of the explanation for reduced feed intake may relate to the role of Zn in taste sensation (McDowell, 2003). Experiments involving livestock reported that with the onset of Zn deficiency and anorexia, livestock experiences a loss of taste acuity (Komai et al. 2000).

Zinc supplementation in beef cattle has been researched extensively. Researchers have explored various concentrations of Zn from the same source and similar concentrations from varying sources, with inconsistent results. Malcom-Callis et al. (2000) evaluated supplemental Zn concentration with 3 treatments: 20 mg/kg, 100 mg/kg, and 200 mg/kg of Zn from $ZnSO_4$. Basal diet contained 70 mg Zn/kg (DM basis). Initial and final BW were not affected by zinc concentration. From d 28 to d 56, a linear decrease in ADG was reported among cattle receiving increasing concentration of zinc. Overall ADG was not affected by zinc concentration (Malcom-Callis et al. 2000). There was an overall linear decrease in daily feed consumption with increased Zn. Increasing zinc concentration appeared to have a negative effect on feed consumption suggesting that a higher concentration of $ZnSO_4$ may negatively influence palatability (Malcom-Callis et al. 2000). Overall G:F was not affected by zinc concentration. (Malcom-Callis et al. 2000). In a study evaluating zinc sources against a negative control, Greene et al. (1988) reported that cattle supplemented with 360 mg/kg ZnO tended to have a greater ADG during the first 28 of treatment than cattle fed no additional zinc. Nevertheless, there was no difference in ADG through d 112. Over the course of 112 d study, DMI did not differ due to source or supplementation. However, it is important to note that the basal diet contained 82 mg/kg of Zn (Greene et al., 1988), which is higher than growing

finishing cattle requirements. Perry et al. (1968) reported that Zn supplementation to finishing diets contain 18 to 29 mg/kg DM of Zn, improved ADG in 2 of 4 experiments.

Studies involving cattle supplemented with various sources of Zn have reported variable results. In a study conducted by Malcom-Challis et al. (2000) zinc supplementation was evaluated from three sources, $ZnSO_4$, a Zn amino acid complex, and a Zn polysaccharide complex. Overall BW, ADG, DMI, and G:F were not affected by source of Zn supplementation. Spears and Kegley (2002) evaluated zinc supplementation from different sources and levels against no zinc supplementation. The basal diet contained 33 mg Zn/kg DM during the growing phase and 26 mg Zn/kg DM during the finishing phase. Zinc sources included ZnO, and two different zinc proteinates. Zinc supplementation regardless of source, increased ADG but did not affect DMI or G:F during the 84-d growing phase. Average daily gain and G:F tended to be greater in cattle fed an organic source of Zn than cattle fed an inorganic source of Zn during the finishing phase. Zinc supplementation did not affect performance in the finishing phase (Spears and Kegley, 2002)

Studies involving cattle and Zn supplementation have reported inconsistent results on carcass characteristics. In other studies, cattle supplemented with various concentrations of Zn did not exhibit a difference in HCW, dressing percentage, REA, KPH%, or marbling score (Malcom-Callis et al. 2000; Nunnery et al. 1996). In a study evaluating zinc sources against a control, Greene et al. (1988) reported differences in several carcass characteristics. Zinc methionine increased USDA Quality Grade and marbling score compared to ZnO or no supplemental Zn, with no difference between the control and ZnO (Greene et al. 1988). Spears and Kegley (2002) reported no difference in

USDA Quality and Yield Grade, marbling score, or fat thickness due to Zn supplementation. However, HCW and dressing percentage were affected by Zn source (Spears and Kegley; 2002). Inconsistent results from Zn supplementation research can be attributed to several factors including sufficient or insufficient amounts of Zn in the basal diet, length of trials, and initial mineral status at the beginning of an experiment.

SUMMARY OF LITERATURE REVIEW

Trace minerals are vital for normal metabolic, enzymatic, and immune response in the body. Current TM requirements in growing and finishing beef cattle recommended by NASEM (2016) are 0.15 mg/kg of Co, 10.00 mg/kg, 0.50 mg/kg of Cu, 50.00 mg/kg of I, 20.00 mg/kg of Mn, 0.10 mg/kg of Se, and 30.00 mg/kg of Zn on a DM basis. However, according to the consulting feedlot nutritionist survey, most nutritionists recommend supplemental TM levels that are 2 or more times greater than NASEM (2016) requirements (Samuleson et al., 2016).

The current literature suggests that there are inconsistency in response in beef cattle performance and carcass characteristics from individual supplementation of cobalt, copper, iodine, manganese, selenium, or zinc. Studies evaluating Co supplementation report increased ADG and no effect on ADFI due to Co supplementation in the finishing phase (Tiffany et al. 2003). Schwartz et al. (2000) reported increased HCW with increased Co concentrations. In previous research, Cu supplementation had no significant effect on BW, ADG, G:F, HCW, marbling, or KPH% (Engle and Spears, 2000; 2001). Copper supplementation decreased fat deposition at the 12th rib; however, resulted in inconsistent effects on USDA Yield Grades (Engle and Spears, 2000; Engle and Spears,

2001;Ward and Spears, 1997). Limited research has been conducted on I supplementation in finishing cattle. However, published research reports indicate that supplementary I has no effect on growth (Downer et al. 1981; Meyer et al., 2008). A study evaluating Mn supplementation observed no effect of supplementation on BW, ADG, DMI, G:F, HCW, REA, fat thickness, USDA Yield Grade, or marbling score (Legleiter et al. 2005). Supplemental Se has shown inconsistent results in 2 experiments conducted by Perry et al. (1976). Zinc supplementation at varying concentrations did not affect ADG, HCW, dressing percentage, REA, KPH%, or marbling score (Greene et al., 1988; Malcom-Callis et al. 2000; Nunnery et al., 1996).

It is evident from the literature that the effects of individual TM supplementation on beef cattle performance and carcass characteristics are limited. However, is it also evident that when TM are restricted or not provided in the basal diet or via supplementation, detrimental deficiencies can occur. These deficiencies can lead to depressed growth and decreased carcass value. Although, individual supplementation has not produced significant improvements, it is possible that TM mixtures have beneficial effects on cattle production. However, more research regarding current industry practices compared to published requirement levels is necessary to determine if elevated levels of TM supplementation is advantageous to finishing beef cattle production.

CHAPTER II

THE EFFECTS OF VARYING LEVELS OF TRACE MINERAL SUPPLEMENTATION ON PERFORMANCE, CARCASS CHARACTERISTICS, MINERAL BALANCE, AND ANTIBODY CONCENTRATIONS IN FEEDLOT CATTLE

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ABSTRACT : The objective of this experiment was to determine the effects of increasing concentrations of trace minerals (TM) on finishing cattle performance, carcass characteristics, mineral balance, and antibody concentrations. Angus crossbred steers (*n* = 240; BW = 291 kg ± 27.4) were stratified by arrival BW and source and randomly assigned to 1 of 4 experimental treatments in a randomized complete block design (12 pens/treatment; 5 steers/pen). Treatments included a negative control (CON) in which cattle received no additional TM supplementation, a requirement treatment (1X) in which cattle received added Co (cobalt carbonate), Cu (cupric sulfate), Fe (ferrous sulfate), I (ethylenediamine dihydriodide), Mn (manganese oxide), Se (sodium selenite), or Zn (zinc oxide) at 2016 Nutrient Requirements of Beef Cattle required levels, a 2 times requirements (2X), and a 4 times requirements (4X) treatment. Selenium was included at

0.1, 0.2, and 0.3 mg/kg for 1X, 2X, and 4X respectively. There was no difference in overall BW, ADG, DMI and G:F due to supplementation (CON vs SUPP $P \geq 0.47$). There was no difference in marbling score, USDA Yield Grade, back fat, REA, HCW, or dressing percentage due to supplementation (CON vs SUPP $P \geq 0.30$). One steer was chosen at random from each pen to be evaluated for serum and liver TM status and antibody concentrations to respiratory viruses. There was treatment × day interaction for serum Co, and liver Cu and Se ($P < 0.0001$). Serum Co was greatest for the 4X treatment from d 28 through harvest. Liver Cu was greatest for the 2X and 4X treatments from d 56 through harvest. Liver Se was greatest for 2X and 4X from d 28 through harvest. There was an effect of day on liver Co, Fe, Mn, Mo, and Zn ($P \leq 0.0001$) and serum Cu, Mn, Mo, Se, and Zn ($P \leq 0.002$). Concentrations for individual TM had different trends over time, however, all reported values were within normal ranges. Serum Zn was greater at harvest ($P = 0.02$). There was an effect of time on Bovine Viral Diarrhea Virus Type 1A, Bovine Herpesvirus Type 1, Bovine Parainfluenza 3 virus antibody titer concentrations ($P \leq 0.0001$). Overall, TM supplementation above requirements had no effect on cattle performance, carcass characteristics, or immune response but does affect the storage of Cu and Se in the liver as well circulating Co levels.

INTRODUCTION

Individual trace mineral supplementation of Cobalt (Co), Copper (Cu), Iron (Fe), Iodine (I), Manganese (Mn), Selenium (Se), and Zinc (Zn) has produced variable results. Published research indicates that supplying the 7 TM with quantifiable requirements do not consistently enhance performance or carcass characteristics (Downer et al., 1981; Engle and Spears, 2000, 2001; Legleiter et al., 2005; Malcolm-Callis et al., 2000; Tiffany

et al., 2003). Typically, feedlot diets are fortified with TM supplements due to perceptions of insufficient and variable mineral content of common feedstuffs and presence of antagonistic compounds. The current recommendations of the NASEM (2016) are for TM diet concentrations of 15 mg Co/kg , 10.00 mg Cu/kg, 50.00 mg Fe/kg, 0.50 mg I/kg, 20.00 mg Mn/kg, 0.10 mg Se/kg, and 30.00 mg Zn/kg (DM basis) to meet the dietary requirements of growing and finishing cattle.

Requirements consider TM provided by the entire diet. However, professionals within the industry commonly do not take basal diet TM concentrations into consideration. From a 2015 survey, consulting nutritionists recommend, on average, 0.82 mg Co/kg, 17 mg/kg of Cu, 13.8 mg Fe/kg, 0.73 mg I/kg, 47.9 mg Mn/kg, 0.24 mg Se/kg, and 87.3 mg Zn/kg (DM basis) of supplemental TM (Samuleson et al., 2016) added to the basal diet. The average recommendations of added TM are between 2 and 4 times the published requirements for total diet. Much of the published TM research lacks a negative control, dietary concentrations similar to industry diets, and a depletion period to ensure TM status is equivalent before treatment allocation. Therefore, the objectives of this study were to (1) determine the effects of varying levels TM supplementation from inorganic sources on feedlot cattle performance and carcass characteristics, (2) serum and liver tissue TM status, and (3) respiratory virus antibody titers.

MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee at Oklahoma State University (Animal Care and Use Protocol number: AG-19-8)

Cattle and Processing

In mid-February 2020, 164 crossbred Angus steers (initial body weight; BW = 297 ± 31.2 kg) were transported approximately 948 km from Burke, SD to the Willard

Sparks Beef Research Center (WSBRC) in Stillwater, OK on d -41. The initial processing procedures of the 140 steers included collection of individual BW and application of individual identification tags. Cattle were held in a pen overnight with ad libitum access to prairie hay and water. The initial 140 processed cattle were deemed Group 1 (G1).

The remaining twenty-four head were placed in a feedlot pen and were fed ad libitum prairie hay and water until the arrival of the second load of cattle. On d -43, 81 steers (initial BW = 280 ± 19.1 kg) from Mobridge, South Dakota traveled approximately 1,328 km to the WSBRC. The 81 steers were combined with the 24 cattle from the first load for processing and deemed Group 2 (G2). On d -43, 105 cattle from Group 2 (G2) were individually weighed, tagged, and held in a pen overnight with ad libitum access to prairie hay and water. While cattle were received 5 d apart, treatment allocations (d 0) were staggered by 7 d to accommodate for logistics due to the time required to obtain liver.

Cattle from G1 and G2 were processed the day following arrival. Processing procedures were similar to that described by Warner et al. (2020). Steers were individually weighed, implanted (Component TE-IS; Elanco Animal Health, Greenfield, IN), vaccinated against clostridial (Vision with SPUR; Merck Animal Health, Madison, NJ) and viral and bacterial respiratory pathogens (Nuplura PH and Titanium 5 + PH-M; Elanco Animal Health), administered an anthelmintic (Safeguard; Merck Animal Health), and a pour-on insecticide (StandGuard; Elanco Animal Health). Administration of all products was done in accordance with label instructions and BQA guidelines. During processing, 13 intact bulls were castrated by banding and monitored daily following castration. Cattle were revaccinated for viral and bacterial respiratory pathogens on d 0

(Nuplura PH and Titanium 5 + PH-M; Elanco Animal Health). Cattle were reimplanted on d 28 (Component TE-S; Elanco Animal Health).

Steers were blocked by body weight (BW) and group and randomly allocated to pens within block. Steers were housed in forty-eight 4.57 × 13.24-m partially covered dirt feedlot pens. Cattle housed in 2 adjacent pens shared a 76-L concrete water fountain (model J 360-F; Johnson Concrete, Hastings, NE).

Steers were monitored daily for health status as described by Wilson et al. (2015) and were treated according to standard WSBRC protocol, if necessary. No cattle were treated for suspected bovine respiratory disease (BRD). Several cattle were treated for lameness over the last 60 d of the trial. Further investigation did not reveal any correlation due to dietary treatment.

A total of 8 cattle died or were removed from the study for reasons unrelated to treatments. One steer, on the CON treatment, was found deceased on d -5 from apparent bloat. On d 28, prior to data collection, a steer on the 4X treatments was found with its head stuck in a fence line gate. The gate was cut to remove the calf, but too much time had passed, and neurological damage had occurred. The calf was euthanized 2 d later. One steer from the CON treatment and 2 steers from the 1X treatment, were removed from study due to apparent neurological issues. Cattle demonstrated signs of lameness and were uncoordinated. Two cattle from the 2X treatment, were found dead in the pen with no further cause found after necropsy. One steer on the CON treatment was found dead in the pen and an enlarged heart was discovered after necropsy.

Treatments

Dietary treatments were modelled from the NASEM (2016) requirements and the recommendations from the 2015 consulting feedlot nutritionist survey (Samuelson et al.

2016). The survey reported mean levels of TM as follows: 0.82 mg Co/kg, 17 mg/kg of Cu, 13.8 mg Fe/kg, 0.73 mg I/kg, 47.9 mg Mn/kg, 0.24 mg Se/kg, and 87.3 mg Zn/kg (DM basis). Treatment TM supplements included a negative control (**CON**) in which cattle received the same supplement from the depletion period (no supplemental TM), a treatment designed to meet NASEM(2016) requirements (**1X**;15 mg Co/kg, 10.00 mg Cu/kg, 50.00 mg Fe/kg, 0.50 mg I/kg, 20.00 mg Mn/kg, 0.10 mg Se/kg, and 30.00 mg Zn/kg; DM basis), 2 times the NASEM (2016) requirements (**2X**), and 4 times the NASEM (2016) requirements (**4X**) levels recommended by the NASEM (2016). Iron was kept constant at 50 mg/kg (DM basis) for 1X, 2X, and 4X treatments. Selenium was included at 0.1, 0.2, and 0.3 mg/kg (DM basis) for 1X, 2X, and 4X treatments respectively. Pelleted supplements were formulated with ground corn and wheat midds. Mineral sources included cobalt carbonate ($CoCO_3$), cupric sulfate ($CuSO_4$), ferrous sulfate ($FeSO_4$), ethylenediamine dihydriodide (EDDI), manganese oxide (MnO), sodium selenite (Na_2SeO_3), and zinc sulfate ($ZnSO_4$).

Diets and Feed Management

Within block, 5 cattle were randomly assigned to each pen and treatment was randomly assigned to pens within block. Bulls were evenly distributed between treatments. A total of 48 pens were used for this experiment, with 12 pens per treatment. All steers were fed a receiving diet for 13-15 d to allow cattle to acclimate to the feedlot environment (Table 2.1). After completion of a receiving diet, cattle were acclimated to a high grain finishing diet through a series of 4 step-up diets during a 28 d transitioning period. Diets were gradually transitioned every 7 d, prior to treatment administration (Table 2.1).

During the 28 d transitioning period, cattle did not receive any TM supplementation but did receive the **CON** supplement that was formulated to meet macro mineral deficiencies present from the basal finishing diet. Cattle did not receive TM supplementation for 42 d prior to experimental treatment administration The **CON** supplement and all other treatments included 0.65% Ca, 0.30% NaCl, 4,715 IU/kg of Vitamin A, 25.1 IU/kg of Vitamin E, 142 IU/kg of Vitamin D, 30 g/ton of monensin (Rumensin 90, Elanco Animal Health), and 9 g/ton of tylosin (Tylan, Elanco Animal Health).

On a DM basis, finishing diets consisted of 7% hay, 15% Sweet Bran, 65% rolled corn, 5% liquid supplement, 1% urea and 5% vitamin and supplement (Table 2.2). Twenty-eight days prior to harvest, ractopamine hydrochloride (Optaflexx 45; Elanco Animal Health) was included in the diet (calcualted average ractopamine hydrochloride intake = 390 $mg \cdot steer^{-1} \cdot d^{-1}$).

Feeding procedures were similar to those described by Warner et al. (2020). At 0500 h each morning, feed bunks were visually evaluated to determine the amount of feed remaining from the previous day. The amount of feed to be delivered that day was adjusted based on this evaluation. Cattle were fed once daily at 1000 h. Feed was mixed and delivered using a trailer-mounted feed mixer (274-12B feed mixer; Roto-mix, Dodge City, KS). Treatment supplements and urea were weighed by hand separately from other ingredients, individually added to the mixer, and mixed into the complete ration. Supplements were bagged and color coded to ensure proper addition of experimental treatments. Mixers were cleaned of all remaining ration prior to mixing the CON ration and to mixing subsequent experiment rations. Feeding and mixing of rations were

completed in order of increasing supplementation levels to decrease contamination. Pens were individually marked to guarantee proper feeding.

Diet samples were collected twice weekly and DM was calculated after samples were dried in a forced air oven at 60°C for 48 h. A monthly composite was created after DM was calculated and stored in a freezer until nutrient analysis could be completed. Feed refusals were weighed back before feeding on d 0, 14, 28, 56, 84, 112, 140, and 168 or if excessive orts remained in the bunk and refusal samples were dried to determine DM content and were subtracted from DM delivered in order to calculate dry matter intake (DMI).

Data Collection

Individual BW were recorded for all steers on d 0,14, 28, 56, 84, 112 and prior to shipping for harvest. Body weights were measured before feeding at approximately 0400 h with no withdrawal from feed or water. All BW were adjusted using a 4% shrink (BW × 0.96). Average daily gain was calculated by dividing shrunk BW gain in kg by days on feed (DOF) for each period. Pen average daily gain (ADG) was calculated as the average of individual ADG for each steer in the pen for that period. Dry matter intake was calculated from total DMI for the pen for that period divided by DOF and number of steers for each period. Gain to feed ratio was calculated by dividing pen ADG by average daily DMI for the pen for each period.

The data from the 8 steers that died or were removed from the experiment were excluded from all analyses (deads out data). Since feed intake was not measured on an individual animal basis, intake data were corrected by removing the average daily DMI for each steer removed from the pen until the respective steer ceased gaining BW. From the time the steer ceased gaining BW until the steer was physically removed from the pen

and the experiment, DMI maintenance were estimated and removed using the NASEM (2016) equation where $NE_m = 0.077\ (SBW)^{0.75}$.

Serum and Liver Sampling

A subset of steers (48 animals; 1 animal/pen; 12 animals/treatment) was used to obtain blood and liver biopsy samples prior to the administration of TM treatments and subsequent to TM supplementation to examine the impact of the TM treatments. All blood samples were collected via the jugular venipuncture and stored on ice prior to harvesting serum. On d 0, 28, 56, 84 and 7 d prior to shipping to harvest, two 6-mL blood samples were collected into a tube containing silicone (BD Vacutainer; Franklin Lakes NJ) for TM analysis. On d 0, 14, 28, and 56, two 6 mL blood samples were collected into a tube containing a positive gel barrier and silicone glass powder (Medtronic; Fridley, MN) for antibody titer analysis. Blood was allowed to clot for an average of 2 h before centrifuging. All blood tubes were centrifuged at 1,294 × g for 30 min at 4° C (Sorvall RC6; Thermo Scientific, Waltham, MA). Serum collected for TM analysis was collected and shipped overnight to the Texas A & M Veterinary Medical Diagnostic Laboratory (TVMDL) for further analysis. Serum was collected and stored in a -80° C freezer until analysis for Bovine Viral Diarrhea Virus (BVDV) Types 1A, 1B, and 2, Bovine Herpesvirus Type 1 (BHV-1), and Bovine Parainfluenza 3 (PI-3) Virus. All serum samples for antibody titer analysis were sent to TVMDL for analysis after the completion of the experiment.

On d 0, 14, 28, 56, and 7 d prior to harvest, a 100-mg liver sample was collected using the procedure described by Sexten et al. (2012) and Wilson et al. (2016) with slight modifications. To obtain the biopsies, steers were briefly restrained in a hydraulic squeeze chute, hair was removed from the biopsy site (area between the 11th and 12th

ribs on the right side of the animal) using surgical clipper blades, and a local anesthetic (lidocaine HCl, 20 mg/mL, 5 mL/biopsy site) was administered after a preliminary scrub with iodine. Lidocaine was injected first intramuscularly and then subcutaneously.

The biopsy site was surgically scrubbed 3 times with a commercially-available iodine scrub (Betadine; Avrio Health L.P., Stamford, CT) followed by rinsing with a 70% isopropyl alcohol solution. After the third scrub and rinse, a commercial iodine solution was sprayed on the injection sites prior to obtaining a biopsy. After ensuring the biopsy sites were thoroughly anesthetized (an additional 5 mL of lidocaine HCl was used in some instances if the biopsy site was not thoroughly anesthetized), a scalpel was utilized to make an approximately 1-cm stab incision for the insertion of the biopsy needle. The incision was made between the 11th and 12th ribs approximately 25 cm lateral to the vertebrae, and a 2.1 mm × 15.2 cm 14-gauge Tru-cut biopsy needle was inserted and then directed cranially and ventrally toward the opposite elbow. The biopsy needle was advanced through the peritoneum, through the diaphragm, and then into the liver to obtain an approximately 100-mg sample of liver tissue. The incision site was closed with veterinary glue to prevent infection and sprayed again with iodine. Animals were observed twice daily for 7 d after the procedure. The 100-mg liver sample was transferred to a 2 mL centrifuge tube and stored on ice. Liver samples were shipped overnight to TVMDL for TM analysis.

Harvest and Carcass Evaluation

Cattle were shipped approximately 116 km to Creekstone Farms in Arkansas City, Kansas in 3 harvest groups. The 5 heaviest blocks were shipped on d 126 of the experiment, the 4 middle blocks were shipped on d 140, and the 3 lightest blocks were

shipped on d 156. Carcass data was collected by Creekstone Farms trained personnel via carcass camera imaging because of COVID-19.

Feed Analysis

For all rations and supplements, a single 400-g sample from the middle of the feed batch was collected from the mixer twice weekly. A 400-g sample of the dietary TM supplements were collected twice weekly. Within each month, the twice weekly finishing diet and supplement samples were individually composited and stored until analysis. Monthly composite samples of finishing diets and supplements, as well as composites of receiving and transition diets, were ground through a 2mm screen (Pulverisette 19, Fritsch; Pittsboro, NC), and were composited for each diet and supplement. Approximately 200 g of each diet and supplement composite were sent in duplicate to a commercial laboratory for trace mineral and proximate analysis (Table 2.2; Servi-Tech, Dodge City, KS).

Trace Mineral Tissue and Serum Analysis

Liver tissue samples and serum samples were analyzed for Co, Cu, Fe, Mn, Mo, Se, and Zn concentrations at TVMDL (College Station, TX). In brief, a portion of the liver samples were dried in a 95°C oven overnight to determine DM. The remaining portion of the sample was prepped for analysis. Sample was weighed and combined with 5 mL HNO_3, 0.25 mL HCl, and a glass encased stir bar. One mL of H_2O_2 was added. The sample was stirred for approximately 10 min and transferred to an automated microwave digester (Discover SP-D 80; CEM Corporation, Matthews, NC). The sample was transferred to a tube and brought to 50 mL with tissue sample diluent (2% HNO_3 and 1 µg/mL ISTD), inverted, and assayed. Aliquots of serum were diluted 1:10 with a serum

diluent (2% methanol, 0.5 % HNO_3, 0.1 % HCl, 0.05 % Triton X-100, 50 ng/mL ISTD). Samples were then vortexed and assayed.

Antibody Titer Analysis

Serum samples were analyzed for antibody titers specific to BRD, specifically BVDV Types 1A, 1B, and 2, infectious bovine rhinotracheitis (IBR) specifically BVH-1 titers, and PI-3 using the serum neutralization test in the virology section at the TVMDL (Canyon, TX). In brief, a 2-fold serial dilution was performed for all serum. Diluted samples were challenged with aliquots of specific stock virus. Samples are agitated and incubated at 37 ± 1°C, in a humidified 5 ± 1% CO_2 atmosphere. After 72-96 h of incubation depending on virus; samples were analyzed. A serum dilution was considered positive if there is a 100% reduction in the amount of virus in the serum test wells compared to that present in the positive control wells. The highest dilution of serum resulting in complete neutralization of virus is the end point titer for that serum. All titers are reported as the final serum dilution prior to the addition of the cell culture suspension. Titers are reported as the reciprocal of the dilution.

Statistical Analysis

The experimental design of the study was a randomized complete block design with BW and source as blocking factors. For all performance measurements, pen served as the experiential unit (n = 48). All performance and carcass characteristic data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc.; Cary, NC) with treatment as a fixed effect and block as a random effect. Pre-ordained contrasts were used to separate treatment means including CON vs TM supplement and the linear and

quadratic effects of TM supplementation. Due to unequal spacing in dietary treatments, contrast coefficients were found using the proc IML of SAS.

Trace mineral serum and liver concentrations and antibody titer data were analyzed using the GLIMMIX procedure of SAS as a repeated measure with treatment, day, and treatment × day as fixed effects and block as a random effect. Covariance structures within the model were compared. The appropriate covariance structure was chosen based off the lowest Akaike Information Criterion (AIC). Analysis for Co liver, Cu serum and liver, Se serum concentrations and BVDV Type 1B, 2, and BHV-1 antibody titers utilized an unstructured covariance structure. Analysis for Co serum, Fe liver, Mn serum, and Se liver concentrations utilized a heterogeneous autoregressive (1) covariance structure. Analysis for Fe serum, Mo serum concentrations, BVDV Type 1A and PI-3 antibody titers utilized a heterogenous compound symmetry covariance structure. Analysis for Mn, Mo, and Zn liver concentrations utilized a heterogeneous Toeplitz covariance structure. Analysis for Zn serum concentrations utilized a variance components covariance structure. Day was included as a repeated measure with pen as the subject. All data from steers removed from the experiment were excluded from statistical analysis. Significance was determined when $P \leq 0.05$ and tendencies were considered when $P > 0.05$ and $P \leq 0.10$.

RESULTS AND DISCUSSION

Experimental Diets

Experimental diets were formulated to be identical except for the TM composition of the supplement. Proximate analysis of dietary treatments is presented in Table 2.2. The consulting feedlot nutritionist survey reported that most feedlots do not take TM in the basal diet into consideration when formulating (Samuelson et al. 2016). Therefore, the

objective of this experiment was to create supplements that reflected nutritional practices in the feedlot industry. Based off a 5% supplement inclusion rate and the mineral analysis of TM supplements included in dietary treatments, presented in Table 2.3, the TM inclusion in the diet was calculated (Table 2.4). The 1X supplement was calculated to include 0.09 mg/kg of Co, 7.60 mg/kg of Cu, 42.6 mg/kg of Fe, 14.0 mg/kg of Mn, 0.09 mg/kg of Se, and 23.7 mg/kg of Zn when supplemented to the diet. The 2X supplement was calculated to include 0.20 mg/kg of Co, 18.6 mg/kg of Cu, 64.9 mg/kg of Fe, 34.9 mg/kg of Mn, 0.13 mg/kg of Se, and 66.8 mg/kg of Zn when supplemented to the diet. The 4X supplement was calculated to include 0.26 mg/kg of Co, 36.2 mg/kg of Cu, 68.1 mg/kg of Fe, 70.9 mg/kg of Mn, 0.49 mg/kg of Se, and 107.5 mg/kg of Zn when supplemented to the diet. Trace mineral analysis of supplements yielded results within the targeted range of the experimental design. Total dietary TM inclusion from the basal diet and the supplement inclusion is presented in Table 2.4. It should be noted, the CON diet provided sufficient levels of almost all TM, with the exception of Cu, to meet NASEM (2016) requirements. Copper levels (6.5 mg/kg) in the CON diet were below the NASEM (2016) requirements.

Performance

The effects if supplemental TM on steer performance and DMI are presented in Table 2.6. There was no difference in BW (CON vs SUPP $P \geq 0.18$) or ADG (CON vs SUPP $P \geq 0.11$) due to TM supplementation. Furthermore, there was no linear or quadratic trend in BW (Linear $P \geq 0.17$; Quadratic $P \geq 0.17$) or ADG among treatments (Linear $P \geq 0.12$; Quadratic $P \geq 0.38$). The lack of performance response to supplemental TM was unexpected. There was no difference in DMI during the first 13 d of the experiment due to TM supplementation (CON vs SUPP $P = 0.39$). However, from d 14 to

d 27, DMI for cattle consuming the CON treatment was less than (P = 0.04) cattle fed 1X, 2X, and 4X. However, over the next 28 d, cattle on the CON diet had average DMI that did not differ from that of the other 3 TM treatments. During the last 28 d of the experiment, cattle consuming the CON diet tended (P = 0.08) to have decreased DMI than cattle consuming the 1X, 2X, or 4X diets. However, there was no difference in overall DMI between cattle consuming the CON diet and cattle consuming the 1X, 2X, or 4X diets (P = 0.99). While supplemented cattle were more efficient during the final 28 d (P = 0.03) compared with CON cattle, there was no difference in overall G:F for cattle offered the CON diet compared with cattle offered the 1X, 2X, or 4X diets (P = 0.48).

Previous research involving individual TM supplementation has been variable (Engle and Spears, 2000, 2001; Legleiter et al., 2005; Malcolm-Callis et al., 2000; Perry et al., 1976; Tiffany et al., 2003). The variation in experimental design and objectives of TM studies differ as well. There have been a few published experiments with similar dietary treatments to the current study; however, these experiments involved other factors such as TM source and implant status. Berrett et al. (2015) evaluated no supplemental TM, TM offered at NRC (1996) requirements from inorganic sources, or TM offered based on consulting nutritionist averages (Samuleson et al., 2016) from organic and inorganic sources. Final BW, ADG, DMI, and G:F were not different across treatment, regardless of supplementation, concentration, or source (Berrett et al., 2015).

Rhoads et al. (2003) investigated mineral source and concentrations within source for Zn, Cu, Mn, and Co. While there was not a negative control and fewer minerals were investigated, there was no difference in BW regardless of dietary treatment or a difference between inorganic sources of trace minerals at 1.5 or 3 times the NRC (1996)

requirements. There was also no difference in ADG, DMI, or G:F regardless of source or concentration of TM (Rhoads et al. 2003).

Niedermayer et al. (2018) evaluated varying levels of TM supplementation with steer implant status. There was no implant × TM interaction for cattle during the last 68 d of the finishing phase. Data were then evaluated for the main effect of TM supplementation. There was no difference in overall BW due to dietary treatment. Furthermore, there was no effect of TM concentration on DMI in TM supplemented steers. However, there was an effect of supplementation on DMI. Steers fed a TM supplement had a higher DMI than non-supplemented steers. Overall ADG tended to be affected by TM supplementation while overall G:F was not affected by supplementation (Niedermayer et al., 2018).

Carcass characteristics

There was no difference in hot carcass weight (HCW), rib eye area (REA), fat thickness (FT), marbling score, USDA Yield Grade or dressing percentage in cattle fed the CON treatment and those fed a TM supplement treatment ($P \geq 0.30$). There were also no linear or quadratic trends for any carcass characteristics (Linear $P \geq 0.24$; Quadratic $P \geq 0.23$).

These results are similar to those published by Berrett et al. (2015) who reported no difference in HCW, dressing percentage, FT, REA, USDA Yield Grade or marbling score. Rhoads et al. (2003) reported no difference between cattle fed inorganic sources at 1.5- or 3-times NRC requirements for HCW, USDA Yield Grade, FT, and marbling score. However, dressing percentage was lower for cattle fed either the organic or inorganic mineral source at 1.5 times the NRC recommendation than for cattle fed either the organic NRC recommended level or at 3 times NRC recommendations through

inorganic sources (Rhoads et al., 2003). Niedermayer et al. (2018) reported no effect of TM supplementation on dressing percentage, FT, REA, or marbling score.

Trace Mineral Liver and Serum Concentrations

Trace mineral concentrations were observed from a subset of 48 steers (1 steer per pen/12 steers per treatment).

Cobalt concentrations in the liver are displayed in Fig 2.1a. There was no treatment × day interaction ($P = 0.31$) or effect of treatment on Co concentrations ($P = 0.11$). There was an effect of day on Co concentrations in the liver ($P < 0.0001$). Concentrations for all treatments decreased on d 0 to d 28, increased on d 56, and remained relatively constant until harvest. Kincaid et al (2003) reported that Co supplementation in nonpregnant, nonlactating Holstein cows did not affect Co concentrations in the liver. Liver samples were only taken at harvest, so no time effect was evident. In contrast to the present study, Niedermayer et al. (2018) reported an effect of TM supplementation on Co liver concentrations on d 70. Cattle receiving NASEM (2016) requirements had greater liver Co concentrations than cattle receiving the consulting nutritionist mode (Samuleson et al., 2016) and negative control steers. On d 124, supplemented cattle had greater Co liver concentrations than the control. (Niedermayer et al., 2018).

There was a treatment × day interaction for Co concentrations in serum ($P < 0.0001$) as shown in Fig. 2.1b. There was no difference in serum Co concentration on d 0 or d 14. Serum Co concentrations for all treatments increased on d 28, decreased on d 56, and stabilized though harvest. Cattle on the 4X treatment had greater ($P < 0.0001$) serum Co concentrations on d 28 through harvest compared to cattle on the CON, 1X, and 2X treatments, which did not differ ($P \geq 0.17$) from d 28 through harvest. Kincaid et al.

(2002) reported Co concentrations in serum of nonpregnant Holstein cows were not affected by supplemental Co. However, serum Co concentrations, regardless of Co supplementation increased with time.

There was a treatment × day interaction ($P < 0.0001$) of copper concentrations in the liver as shown in Fig. 2.2a. Liver Cu concentrations did not differ on d 0 between treatments ($P = 0.57$). However, concentrations for cattle on the 1X, 2X, and 4X treatments increased steadily over the course of the experiment while cattle on the CON treatment maintained baseline liver Cu concentrations through harvest. Liver Cu concentrations differed ($P \leq 0.04$) between all 4 treatments on d 28 with 4X having the greatest concentrations, followed by 2X, 1X, and then the CON. Liver Cu concentrations were not different for cattle on the 4X and 2X treatments from d 56 through harvest ($P \geq 0.20$). However, liver Cu concentrations were greater for 4X and 2X than the 1X or CON treatments and liver Cu concentrations were greater for 1X treatment than CON from d 56 through harvest ($P \leq 0.001$). Cattle on the 4X treatment exhibited concentrations on d 56 and at slaughter that were outside of the range considered normal for beef cattle (12.5 – 150 µg/g); however, levels were not considered toxic (Herdt and Hoff, 2011). Previous research regarding concentrations of Cu from $CuSO_4$ reported similar findings. Engle and Spears (2000) evaluated 0, 10, and 20 mg/kg of supplemental Cu from $CuSO_4$ fed to Angus steers and reported a treatment × time interaction in liver Cu concentrations. Liver Cu concentrations were similar across treatments on d 0 and increased over time. Liver Cu concentrations were greater at harvest for steers fed 20 mg/kg of Cu than cattle consuming 10 mg/kg of Cu (Engle and Spears, 2000). Other research conducted on Simmental cattle reported a similar treatment × time interaction that indicated Cu supplementation increased liver Cu concentrations (Engle and Spears, 2001). Liver Cu

concentrations of cattle supplemented with 40 mg of Cu/kg DM were greater than cattle fed 10 mg of Cu/kg DM over the finishing phase (Engle and Spears, 2001).

There was no treatment × day interaction ($P = 0.35$) or effect of treatment ($P = 0.11$) for serum Cu concentrations as shown in Fig. 2.2b. However, there was an effect of day on serum Cu concentrations ($P < 0.0001$). Copper concentrations remained stable from d 0 to d 14. From d 14 to 28 serum Cu concentrations increased, followed by a decrease in serum concentrations from d 28 to d 56. Serum Cu concentrations increased for all treatments from d 56 through harvest. Although serum Cu concentrations fluctuated over time, cattle maintained concentrations within a narrow margin. Published research regarding Cu supplementation and blood biomarkers typically evaluated plasma rather than serum. Several studies investigated plasma Cu concentrations, in regard to supplementation levels. Published research indicates that steers supplemented with Cu had greater plasma Cu concentrations than non-supplemented cattle at several sampling times (Engle and Spears; 2000, 2001).

Previous research has reported that Cu levels in serum are less than that in plasma making the use of serum Cu levels not equivalent to plasma Cu levels as an indicator of Cu status (Paynter,1981). Laven et al. (2007) compared the use of serum and plasma when determining Cu status in cattle. They determined that serum Cu concentration was not a suitable substitute for plasma Cu concentration for the detection of 'marginal' blood Cu status in cattle as there is variability in the amount of Cu sequestered during clotting (Laven et al. 2007). This variability and loss of Cu may cause serum to be an ineffective measure of Cu status. Based on Cu liver data and Cu serum data from the present study, liver Cu concentrations are more indicative the effects of Cu supplementation levels. Serum Cu concentrations remained within a tight margin as the body has a tight

homeostatic control on circulating Cu. Changes in serum Cu concentrations over time are more indicative of Cu use within the body.

As expected, there was not a treatment × day interaction ($P = 0.69$) or effect of treatment ($P = 0.59$) for concentrations for Fe in the liver, as shown in Fig. 2.3a, due to Fe being supplemented at the same rate in all supplemented treatments. There was an effect of day on liver Fe concentrations ($P < 0.0001$). Concentrations for all for treatments were similar on d 0. On d 28, liver Fe concentrations were stable. However, on d 56 liver Fe concentrations increased and continued to increase through harvest. Standish et al. (1969) evaluated various levels of dietary Fe on tissue mineral composition in cattle. Liver Fe reflected the level of dietary Fe. The change in liver Fe due to dietary Fe was almost totally linear for the steers fed 0, 400, and 1,600 ppm Fe (Standish et al. 1969). If dietary levels of Fe are accurately reflected in the liver, then the response in the present study is reasonable.

There was no treatment × day interaction or effect of day or treatment on Fe serum concentrations ($P \geq 0.13$) as shown in Fig. 2.3b. Due to no variations in supplemental Fe among supplemented treatments, this result was also expected. Koong et al. (1970) reported that a dietary increase in Fe increased serum Fe concentrations in calves. These results would support the present study reporting no differences in serum Fe concentrations.

There was no treatment × day interaction ($P = 0.74$) or effect of treatment ($P = 0.39$) for Mn concentrations in the liver as shown in Fig. 2.4a. There was an effect of day on liver Mn concentrations ($P < 0.0001$). Liver Mn concentrations did not differ on d 0. On d 28, liver Mn concentrations increased, then slightly decreased on d 56. Liver Mn concentrations then increased through harvest. All liver Mn concentrations were within

normal ranges supporting that Mn was neither deficient in the basal diet nor toxic in the 4X diet (Herdt and Hoff, 2011). In an experiment by Legleiter et al. (2005), liver samples collected directly after harvest displayed a linear increase in Mn with increasing levels of dietary Mn from $MnSO_4$. Ahola et al. (2004) evaluated the effects of source of TM against a negative control in grazing beef cows over a 2-yr period. Manganese concentration in the liver had year × treatment interaction where liver Mn concentrations were greater in supplemented cattle at the end of yr 1, but, lower in cattle supplemented with Mn at the end of yr 2 compared to control cattle. Liver Mn concentrations were not affected by TM source (Ahola et al., 2004). Due to varying results from the current study and previous studies, it is possible that the liver is not the most reliable biomarker of Mn status. According to Kincaid (2000), Mn taken up by the liver is excreted endogenously via the bile, and accumulation of Mn in the liver often do not reflect dietary intakes of Mn (2000). Underwood and Suttle (1999) reported liver Mn concentration does not respond to Mn supplementation, even at potentially toxic concentrations.

There was no treatment × day interaction ($P = 0.45$) or effect of day ($P = 0.82$) for serum concentrations of Mn as shown in Fig. 2.4b. There was an effect of day on Mn serum concentrations ($P < 0.0001$). Serum Mn concentrations decreased from d 0 through d 28 and then stabilized through harvest. Legleiter et al. (2005) reported that plasma Mn concentrations were not affected at harvest by supplemental Mn concentration (Legleiter et al., 2005)

There was no treatment × day interaction ($P = 0.19$) or effect of treatment ($P = 0.12$) on Mo concentrations in the liver as shown in Fig. 2.5a. There was an effect of day on liver Mo concentrations ($P < 0.0001$). Liver Mo concentrations did not differ on d 0. Mn liver concentrations varied on d 28, tended to increase on d 56, and then plateaued

through harvest. The present study did not supplement with Mo; it is plausible based on previous research that regardless of treatment, Mo liver concentration would not differ as changes in liver Mo concentrations could reflect use of Mo within the body. Clawson et al. (1972) reported that the addition of supplemental Mo increased liver Mo concentrations.

There was no treatment × day interaction ($P = 0.13$) or effect of treatment ($P = 0.75$) for serum concentrations of Mo as shown in Fig. 2.5b. There was an effect of day on Mo serum concentrations ($P = 0.0019$). Serum Mo concentrations decreased from d 0 through d 14. Serum Mo concentrations slightly increased on d 28 and then stabilized through harvest. To the authors knowledge, there are no published studies evaluating TM supplementation on Mo serum. However, Clawson et al. (1972) evaluated various Mo supplementation strategies on Mo plasma concentrations. Molybdenum plasma concentrations increased with the presence of supplemental Mo. Since Mo was not supplemented in this study, it is not expected that there would be an effect of treatment on Mo serum concentrations.

There was a treatment × day interaction for Se concentrations in the liver ($P < 0.0001$) as shown in Fig. 2.6a. There was no difference in concentrations on d 0 among treatments. By d 28, all treatments displayed an increase in Se liver concentrations. Liver Se concentrations of 2X and 4X treatments did not differ on d 28 through harvest ($P \geq 0.11$). However, liver Se concentrations of cattle on the 2X and 4X treatments were greater ($P \leq 0.05$) on d 28 than cattle on the CON and 1X treatments, which did not differ ($P = 0.37$). Liver Se concentrations of 2X cattle did not differ from 1X cattle on d 56 ($P = 0.19$). Cattle offered the 1X, 2X, and 4X diets had greater liver Se concentrations than CON cattle on d 56 and at harvest ($P \leq 0.05$). Arthington (2008) investigated source of Se

against a negative control. Liver concentrations were greater in Se supplemented steers over the course of the experiment compared with non-supplemented steers regardless of Se source. Niedermayer et al. (2018) reported TM supplementation increased liver Se concentrations. Steers supplemented with TM at the surveyed consulting nutritionist mode (Samuelson et al., 2016) had greater liver Se than control steers on d 70. Cattle supplemented with TM had greater Se liver concentrations at harvest (Niedermayer et al., 2016).

There was no treatment × day interaction ($P = 0.99$) or effect of treatment ($P = 0.32$) for Se concentrations in serum as shown in Fig. 2.6b. There was an effect of day on serum Se concentrations ($P < 0.0001$). Serum Se concentrations exhibited a decrease from d 0 through d 14 and an increased on d 28. Serum Se concentrations remained stable through harvest. In sheep, serum Se increased as dietary Se increased (Henry et al., 1988). In general, Perry et al. (1976) reported that Se concentrations in serum increased with supplementation and over time; however, no statistical analysis was performed due to the nature of sampling. Based on liver and serum Se concentrations in this study, liver Se concentrations appear to be more indicative of the effects Se supplementation on Se status in cattle.

There was no treatment × day interaction ($P = 0.91$) or effect of treatment ($P = 0.47$) for Zn concentrations in the liver as shown in Fig. 2.7a. However, there was an effect of day on Zn concentrations ($P < 0.0001$). Liver Zn concentrations decreased from d 0 through d 28. Liver Zn concentrations for all treatments increased through d 56 and continued to increase through harvest. Berrett et al. (2015) reported that TM supplementation had no effect on Zn liver concentrations at harvest. Niedermayer et al. (2018) reported Zn liver concentrations were not affected by TM supplementation on d

70. However, steers receiving requirements had greater liver Zn concentrations than control cattle and steers supplemented at consulting nutritionist modes at harvest (Niedermayer et al., 2018).

There was no treatment × day interaction for serum Zn concentrations ($P = 0.33$). However, there was an effect of treatment ($P = 0.02$) and day ($P < 0.0001$) on serum Zn concentrations as shown in Fig. 2.7b. Serum Zn concentrations did not differ on d 0 through d 14. On d 28, all cattle displayed a slight increase in concentration followed by a slight decrease in Zn concentrations on d 56. Serum concentrations for cattle on the 4X treatment were greater at harvest than cattle on the CON, 1X, and 2X treatments ($P = 0.001$). Greene et al. (1988) reported Zn supplementation and source did not affect Zn serum concentrations. It is well accepted that plasma Zn is not a reliable indicator of Zn status unless animals are severely deficient in Zn (Underwood and Suttle, 1999). It is likely that serum Zn concentrations are also not the most accurate measure Zn status in the animal.

Antibody concentrations

There was no treatment × day interaction ($P = 0.34$) or effect of treatment ($P = 0.89$) for BVDV Type 1A antibody titers as shown in Fig. 2.8a. However, there was an effect of day on antibody titer concentrations ($P = 0.03$). Antibody titer concentrations increased at d 14 for BVDV Type 1A; however, titer concentrations decreased over the rest of the sampling period. There was no treatment × day interaction, effect of treatment or effect of day on BVDV Type 1B or BVDV Type 2 antibody concentrations ($P \geq 0.44$) as shown in Fig. 2.8b and 2.8c. Previous research reported no treatment ×day interaction for neutralizing antibody concentrations for BVDV Type 1 and BVDV Type 2 for cattle injected with a TM supplement (Arthington and Havenga; 2012). However, antibody

titers increased following vaccination and were greater than baseline on d 30 for BVDV-1 and BVDV-2 titers and remained greater on each of the subsequent sampling days.

There was no treatment × day interaction for BVH-1 ($P = 0.34$) or effect of treatment ($P = 0.77$). However, there was an effect of day ($P < 0.0001$) as shown in Fig. 2.9. Antibody titers increased on d 14 after revaccination. However, after d 14 antibody concentrations decreased for the remainder of the sampling period. Rhoads et al. (2003) reported that TM supplementation had no effect on antibody titers specific to IBR. However, Arthington and Havenga (2012) reported that an injectable TM supplement containing Cu, Mn, Zn, and Se increased neutralizing antibody titers against BHV-1 on d 14, 30, and 60 post-vaccination compared with a control. It should be noted that cattle used by Arthington and Havenga (2012) had no previous vaccination exposure. It is plausible that a increase was not elicited in the current study due to previous vaccination at arrival and unknown vaccination status prior to arrival.

There was no treatment × day interaction ($P = 0.93$) or effect of treatment ($P = 0.22$) for PI-3 antibody titers as shown in Fig. 3.0. However, there was an effect of day ($P < 0.0001$). Antibody concentrations were similar on d 0 . Concentrations increased on d 14 but decreased throughout the remainder of the sampling days. George et al. (1997) evaluated heifers that had been vaccinated only with clostridial bacterins prior to arrival at a research feedlot. All heifers were vaccinated for viral respiratory pathogens upon arrival. Treatments consisted of a basal 55% concentrate receiving diet with supplemental inorganic or organic sources of Co, Cu, Mn, and Zn. Although calves had titers to PI-3 on arrival, the secondary PI-3 antibody titer response on d 14 and 28 after arrival vaccination was increased by organically complexed minerals. However, TM supplementation was not evaluated against a negative control (George et al., 1997).

Antibody concentration data from the current study and previous research report varying results. Cattle in the current study were considered low to moderate risk. (Wilson et al., 2017) Vaccination status prior to arrival was unknown but cattle received respiratory vaccination at arrival, prior to allocation of treatment. Cattle also had the opportunity to acclimate to the feedlot environment. Many previous studies have investigated the effect of TM supplementation on high-risk receiving calves.

CONCLUSION

Trace mineral supplementation had no effect on cattle performance or carcass characteristics. The supplementation of TM did have an impact on serum Co concentrations, liver Cu concentrations and liver Se concentrations where increased dietary inclusion of TM increased the previously mentioned TM concentrations over time. Antibody titers for common respiratory viruses were not affected by supplemental TM inclusion. There are several reasons why TM supplementation may not have effected cattle performance, carcass characteristics, and health in the current experiment. Cattle were receiving sufficient TM concentrations from the basal diet, with the exception of Cu, during the transition period and without additional TM supplementation. It is probable that due to sufficient TM levels in the basal diet, additional supplementation would not enhance performance or carcass characteristics. Cattle were considered to be low to moderately stressed upon arrival and were acclimated to the feedlot environment prior to dietary treatments were allocated. Based on the results from this experiment, supplementation beyond the NASEM (2016) recommended requirements does not appear to be warranted.

ACKNOWLEDGEMENTS

The authors would like to thank the employees of the Willard Sparks Beef

Research Center for assisting with this experiment. This experiment was funded in part by the USDA National Institute of Food and Agriculture Hatch project and the Oklahoma Agricultural Experiment Station of the Division of Agricultural Sciences and Natural Resources at Oklahoma State University.

Table 2.1 Ingredient and nutrient composition of receiving and transition diets

	Diet[1]				
Ingredient, % of DM	RCV	Step 1	Step 2	Step 3	Step 4
Rolled corn	16.7	25.9	36.6	46.9	57.7
Prairie hay	30.2	24.4	20.7	16.9	12.9
Sweet Bran[2]	53.1	44.7	37.7	31.2	24.4
Pelleted supplement[3]	—	5.0	5.0	5.0	5.0
Nutrient Composition, DM basis[4]	RCV	Step 1	Step 2	Step 3	Step 4
Dry matter, %	67.8	71.0	73.5	74.4	77.7
Crude protein, %	19.2	15.8	14.4	14.0	12.9
Acid detergent fiber, %	23.0	19.9	19.8	20.3	15.8
TDN%[5]	69.8	73.0	73.1	72.6	77.3
NE_m[6] Mcal/kg	1.63	1.72	1.74	1.72	1.87
NE_g[7] Mcal/kg	1.01	1.10	1.12	1.10	1.23
Ca %	0.20	0.86	0.68	0.59	0.70
P %	0.91	0.66	0.68	0.55	0.48
Mg %	0.39	0.29	0.27	0.27	0.21
K%	1.31	1.30	1.23	1.20	0.98

[1] RCV = common receiving diet for all cattle, Step 1 = diet fed from d -28 through d -22 for all cattle, Step 2 = diet fed from - 21 d through d -15 for all cattle, Step 3 = diet fed from d -14 through d -8 for all cattle, Step 4 = diet fed from d -7 through d -1 for all cattle

[2] Cargill Inc., Dalhart, TX

[3] Pelleted supplement – CON supplement containing no added trace minerals but contained ground corn, wheat midds, 0.65% Ca, 0.30% NaCl, 4,715 IU/kg of Vitamin A, 25.1 IU/kg of Vitamin E, 142 IU/kg of Vitamin D, 30 g/ton of monensin (Rumensin 90; Elanco Animal Health, Greenfield, IN), and 9 g/ton of tylosin (Tylan; Elanco Animal Health, Greenfield, IN)

[4] Diets analyzed by Servi-Tech Laboratories: Dodge City, KS.

[5] Total digestible nutrients

[6] Net energy maintenance

[7] Net energy gain

Table 2.2 Nutrient composition of treatment finishing diets

	Treatment[1]			
Ingredient composition, % DM basis	CON	1X	2X	4X
Rolled corn	65.0	65.0	65.0	65.0
Prairie hay	7.0	7.0	7.0	7.0
Sweet Bran[2]	15.0	15.0	15.0	15.0
Liquid supplement[3]	5.0	5.0	5.0	5.0
Urea	1.0	1.0	1.0	1.0
Trace mineral supplement[4]	5.0	5.0	5.0	5.0
Nutrient composition[5] , % DM basis	CON	1X	2X	4X
Dry matter, %	79.6	79.8	79.4	79.9
Crude protein, %	14.0	14.7	14.6	14.4
Acid detergent fiber, %	10.5	11.3	10.8	10.4
TDN%[6]	87.7	86.4	87.6	88.0
NE_m[7] Mcal/lb	2.17	2.13	2.17	2.18
NE_g[8] Mcal/lb	1.49	1.46	1.49	1.50
Ca %	0.59	0.61	0.64	0.57
P %	0.42	0.46	0.45	0.44
Mg %	0.19	0.21	0.20	0.20
K%	0.88	0.93	0.91	0.89

[1] Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing approximately 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing approximately 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of 1Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing approximately 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn, on a DM basis)

[2] Cargill Inc., Dalhart, TX

[3] Liquid supplement was formulated to contain (% DM basis) 45.86% corn steep, 36.17% cane molasses, 6.00% hydrolyzed vegetable oil, 5.46% 80/20 vegetable oil blend, 5.20% water, 1.23% urea (55% solution), and 0.10% xanthan gum

[4] Trace mineral supplement contained ground corn, wheat midds, 0.65% Ca, 0.30% NaCl, 4,715 IU/kg of Vitamin A, 25.1 IU/kg of Vitamin E, 142 IU/kg of Vitamin D, 30 g/ton of monensin (Rumensin 90; Elanco Animal Health, Greenfield, IN), and 9 g/ton of tylosin (Tylan; Elanco Animal Health, Greenfield, IN)

[5] Diets analyzed by Servi-Tech Laboratories: Dodge City, KS

[6] Total digestible nutrients

[7] Net energy maintenance

[8] Net energy gain

Table 2.3 Analyzed mineral composition of treatment supplements

	Treatment[1]			
Mineral composition[2], DM	CON	1X	2X	4X
Calcium, %	14.1	14.0	14.5	15.4
Phosphorus, %	0.44	0.45	0.41	0.38
Magnesium, %	0.28	0.28	0.26	0.28
Potassium, %	0.52	0.53	0.49	0.48
Sulfur, %	0.16	0.23	0.32	0.38
Sodium, %	2.50	2.64	2.90	2.70
Cobalt, mg/kg	5.10	6.85	9.00	10.30
Copper, mg/kg	32.5	184.5	404	756
Iron, mg/kg	653	1505	1950	2015
Manganese, mg/kg	183	463	880	1600
Selenium mg/kg	1.10	2.90	3.60	10.9
Zinc, mg/kg	100	573	1435	2250

[1] Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing approximately 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing approximately 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing approximately 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn, on a DM basis)

[2] Diets analyzed by Servi-Tech Laboratories: Dodge City, KS

Table 2.4 Total trace mineral inclusion in the diet based on analyzed treatment supplements

	Treatment[1]			
Mineral composition[2], DM	CON[3]	1X	2X	4X
Cobalt mg/kg	0.15	0.24	0.35	0.41
Copper mg/kg	6.50	14.1	25.1	42.7
Iron mg/kg	119	161	183	187
Manganese mg/kg	30.5	44.5	65.4	101
Selenium mg/kg	0.25	0.34	0.38	0.74
Zinc mg/kg	44.5	68.2	111	152

[1] Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing approximately 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing approximately 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing approximately 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn, on a DM basis)

[2] Total trace mineral content in the treatment diets was calculated from the analyzed CON diet and analyzed supplements 1X, 2X, and 4X. See Table 2.3

[3] CON diet was analyzed for trace mineral content by Servi-Tech Laboratories: Dodge City, KS

Table 2.5 Effects of increasing levels of trace mineral supplementation on performance in feedlot steers.

Item	Treatment[1]					*P*-value		
	CON	1X	2X	4X	SEM[2]	Linear	Quad	CON vs SUPP
BW[3], kg								
Initial[4]	288	288	289	288	7.9	0.85	0.85	0.71
d 0	371	369	367	367	8.3	0.53	0.53	0.21
d 14	394	391	395	392	8.2	0.98	0.98	0.50
d 28	432	432	433	433	8.7	0.91	0.91	0.82
d 56	499	499	495	496	8.9	0.56	0.56	0.53
d 84	548	543	542	543	8.7	0.32	0.32	0.21
β-agonist[5]	574	568	567	570	7.5	0.17	0.17	0.18
Final[4]	592	592	590	596	7.4	0.58	0.58	0.90
ADG[6], kg								
d 0 – 13	1.67	1.54	1.98	1.81	0.189	0.23	0.44	0.50
d 14 – 27	2.71	2.93	2.77	2.95	0.149	0.31	0.91	0.25
d 28 – 55	2.37	2.40	2.18	2.26	0.070	0.12	0.34	0.25
d 56 – 83	1.76	1.59	1.70	1.68	0.075	0.72	0.38	0.20
d 84 – β-agonist	1.03	1.04	1.09	1.12	0.113	0.36	0.93	0.53
β-agonist – final	0.65	0.87	0.84	0.93	0.168	0.16	0.49	0.11
d 0 – final	1.61	1.62	1.63	1.67	0.045	0.20	0.75	0.47
DMI[8], kg/d								
d 0 – 13	10.7	10.8	11.1	10.8	0.31	0.68	0.23	0.39
d 14 – 27	12.3	12.8	12.8	12.8	0.30	0.17	0.11	0.04
d 28 – 55	13.7	14.1	13.7	13.6	0.24	0.40	0.39	0.58
d 56 – 84	13.2	13.1	13.3	13.0	0.25	0.68	0.65	0.79
d 84 – β-agonist	12.2	11.9	12.1	12.3	0.38	0.49	0.28	0.65
β-agonist – final	10.1	10.6	10.6	10.7	0.33	0.13	0.34	0.08
d 0 – final	12.2	12.1	12.2	12.3	0.24	0.51	0.55	0.99
G:F[9]								
d 0 – 13	0.133	0.138	0.162	0.156	0.0196	0.16	0.52	0.32
d 14 – 27	0.206	0.248	0.197	0.236	0.0174	0.48	0.79	0.31
d 28 – 55	0.164	0.166	0.163	0.164	0.0079	0.94	0.95	0.94
d 56 – 84	0.134	0.120	0.133	0.128	0.0098	0.92	0.78	0.56
d 84 – β-agonist	0.077	0.087	0.084	0.090	0.0110	0.39	0.82	0.43
β-agonist – final	0.055	0.087	0.086	0.085	0.0120	0.08	0.13	0.03
d 0 – final	0.132	0.134	0.134	0.136	0.0031	0.34	0.98	0.48

[1] Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn)

[2] n = 48; pens per treatment = 12

[3] Body weight adjusted by a 4% calculated pencil shrink

[4] Initial BW is an average of arrival BW and BW at processing
[5] Cattle were administered a β-agonist (Optaflexx; Elanco Animal Health, Greenfield, IN) 28 d prior to harvest
[6] Cattle were harvested in 3 groups; d 126 (*n* = 20 pens; 5 pens per treatment), d 140 (*n* = 16 pens; 4 pens per treatment), and d 154 (*n* = 12 pens; 3 pens per treatment).
[7] Pen average daily gain calculated from the average of individual shrunk BW gain; kg divided by days on feed for each period
[8] Pen dry matter intake calculated from total DMI for the pen for each period divided by the total steers and days on feed in each period
[9] Gain: feed calculated by dividing the ADG for the pen by the average daily DMI for the pen for each respective period.

Table 2.6 Effects of increased levels of trace mineral supplementation on carcass characteristics in feedlot steers.

Item	Treatment[1]					P-Value		
	CON	1X	2X	4X	SEM[2]	Linear	Quad	CON vs SUPP
HCW[3]	382	384	381	380	4.9	0.54	0.90	0.90
Dressing percent	63.7	63.8	63.7	63.5	0.26	0.46	0.51	0.95
Fat thickness[4],cm	3.84	3.51	3.73	3.73	0.076	0.99	0.36	0.30
Marbling score[5]	471	476	447	474	12.1	0.90	0.23	0.69
USDA Yield Grade	3.03	2.83	3.11	3.04	0.128	0.56	0.89	0.74

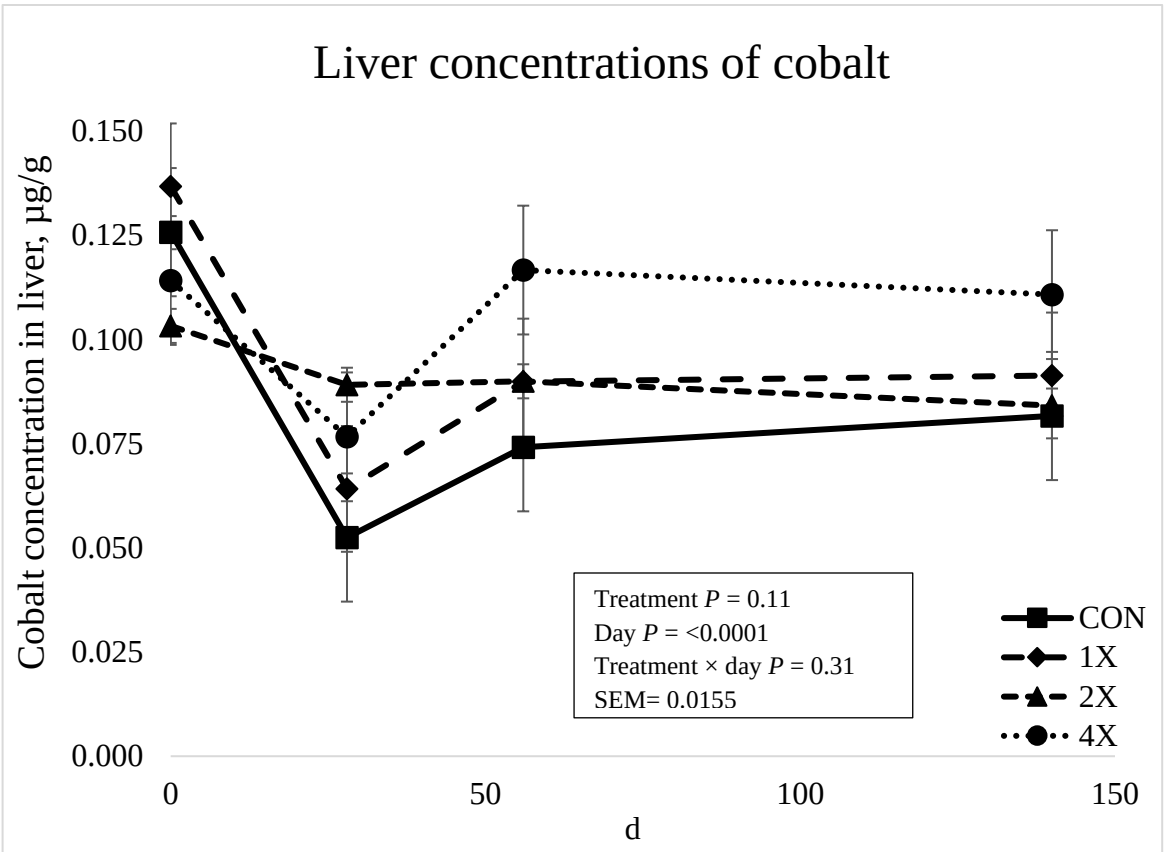
[1] Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn)

[2] n = 48, pens per treatment = 12

[3] Hot carcass weight

[4] Fat measurement was taken between the 12th and 13th rib

[5] Small^{00} = 400; Modest^{00} = 500; Moderate^{00} = 600


Liver concentrations of cobalt
Cobalt concentration in liver, µg/g
0.150
0.125
0.100
0.075
0.050
0.025
0.000
0
50
100
150
d
Treatment P = 0.11
Day P = <0.0001
Treatment × day P = 0.31
SEM= 0.0155
CON
1X
2X
4X

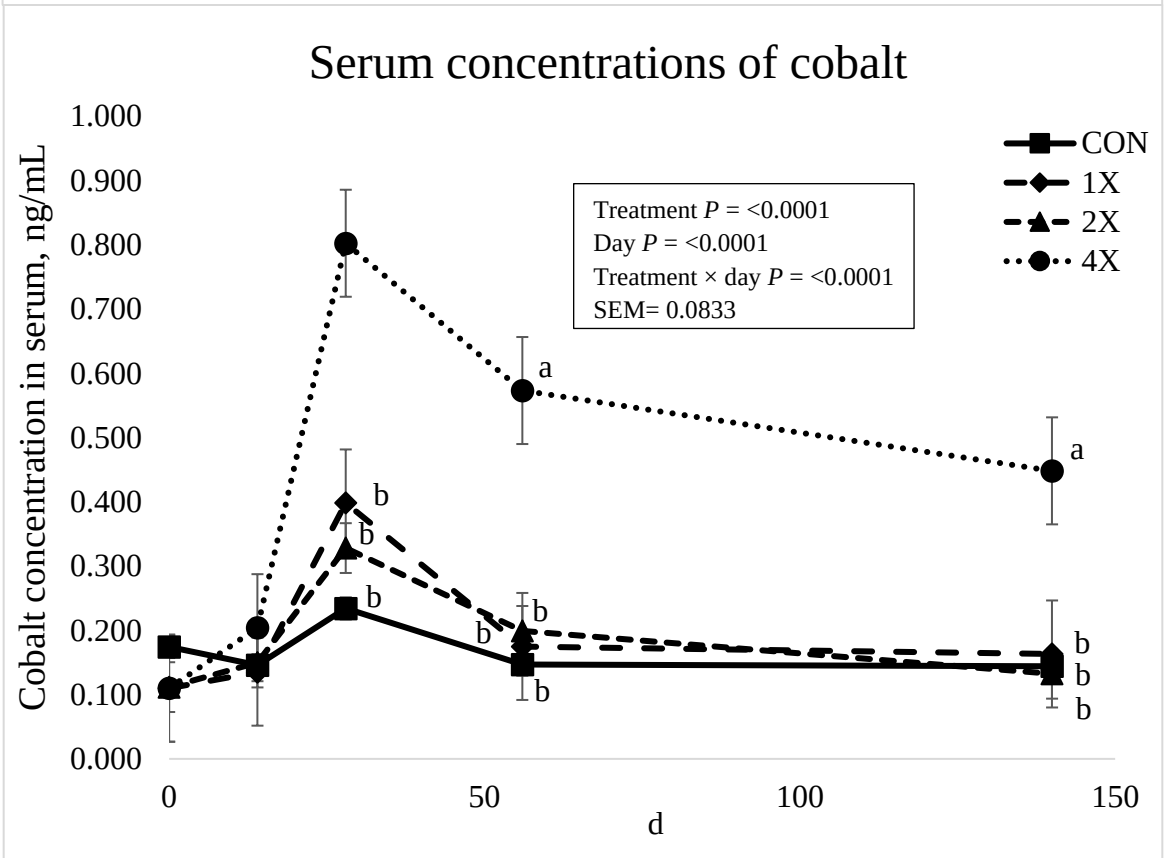
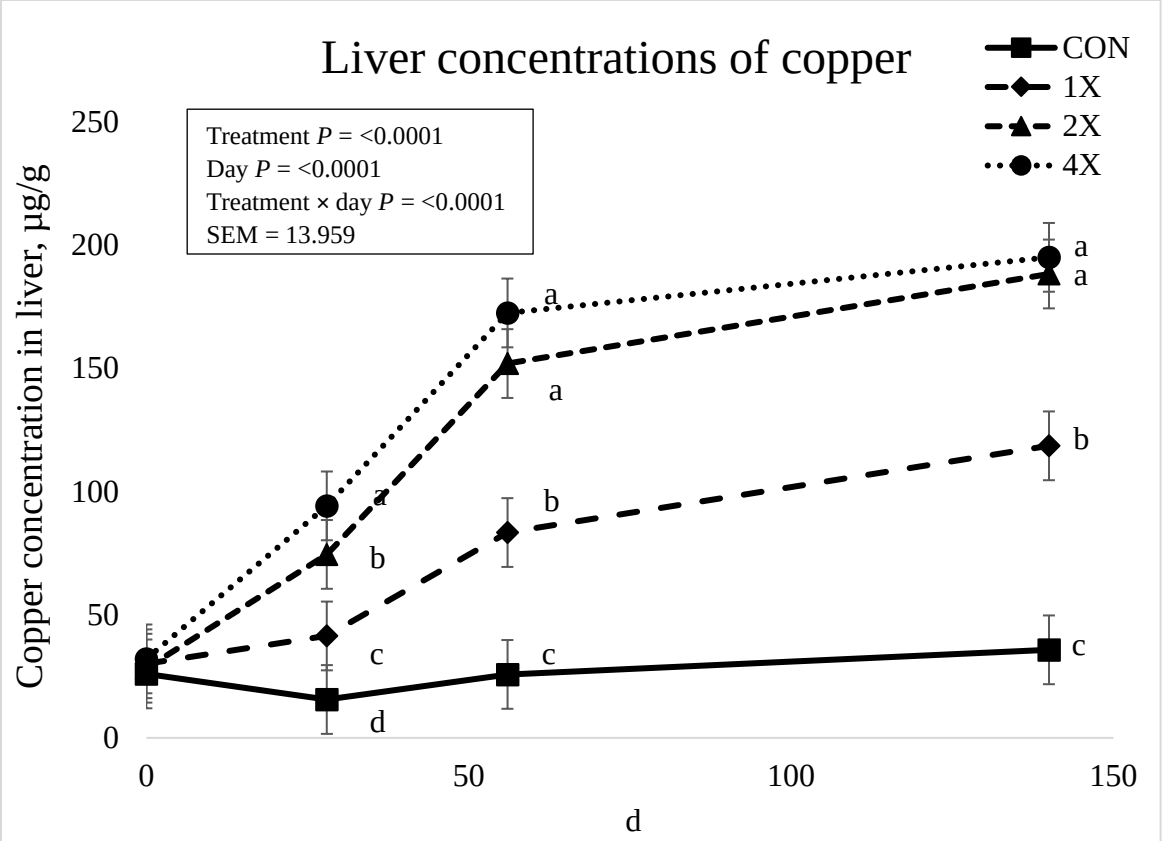

Serum concentrations of cobalt
Cobalt concentration in serum, ng/mL
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
50
100
150
d
Treatment P = <0.0001
Day P = <0.0001
Treatment × day P = <0.0001
SEM= 0.0833
CON
1X
2X
4X
a
a
b
b
b
b
b
b
b
b
b

Figure 2.1: Liver (**a**) and serum (**b**) concentrations of cobalt in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

[*] Cattle were harvested in 3 groups; d 126 (n =20 pens; 5 pens per treatment), d 140 (n = 16 pens; 4 pens per treatment), and d 154 (n = 12 pens; 3 pens per treatment). In this figure, 140 days on feed represents the final measurement, regardless of the actual harvest date.

[a,b,c] means within grouping without a common superscript letter differ ($P < 0.05$)

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations


Liver concentrations of copper
CON
1X
2X
4X
Treatment P = <0.0001
Day P = <0.0001
Treatment × day P = <0.0001
SEM = 13.959
Copper concentration in liver, µg/g
250
200
150
100
50
0
0
50
100
150
d
a
a
a
a
b
b
b
c
c
c
d

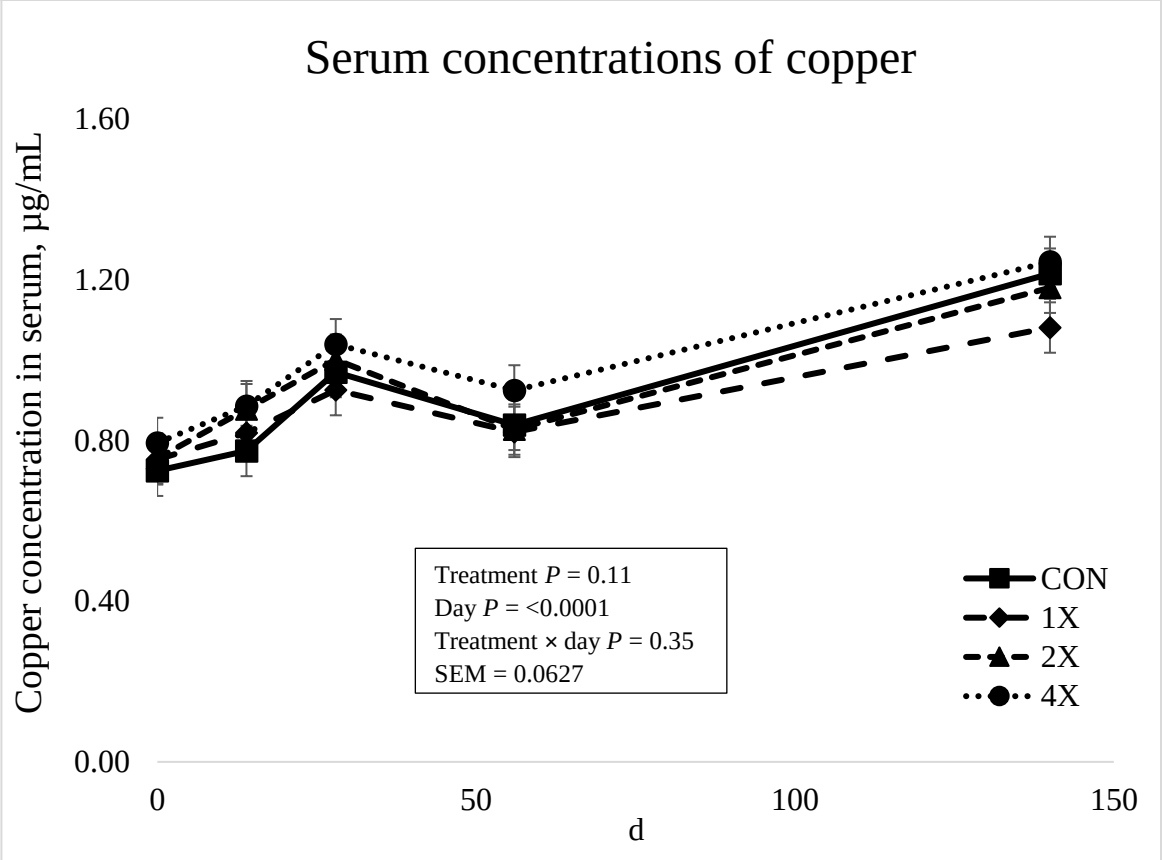
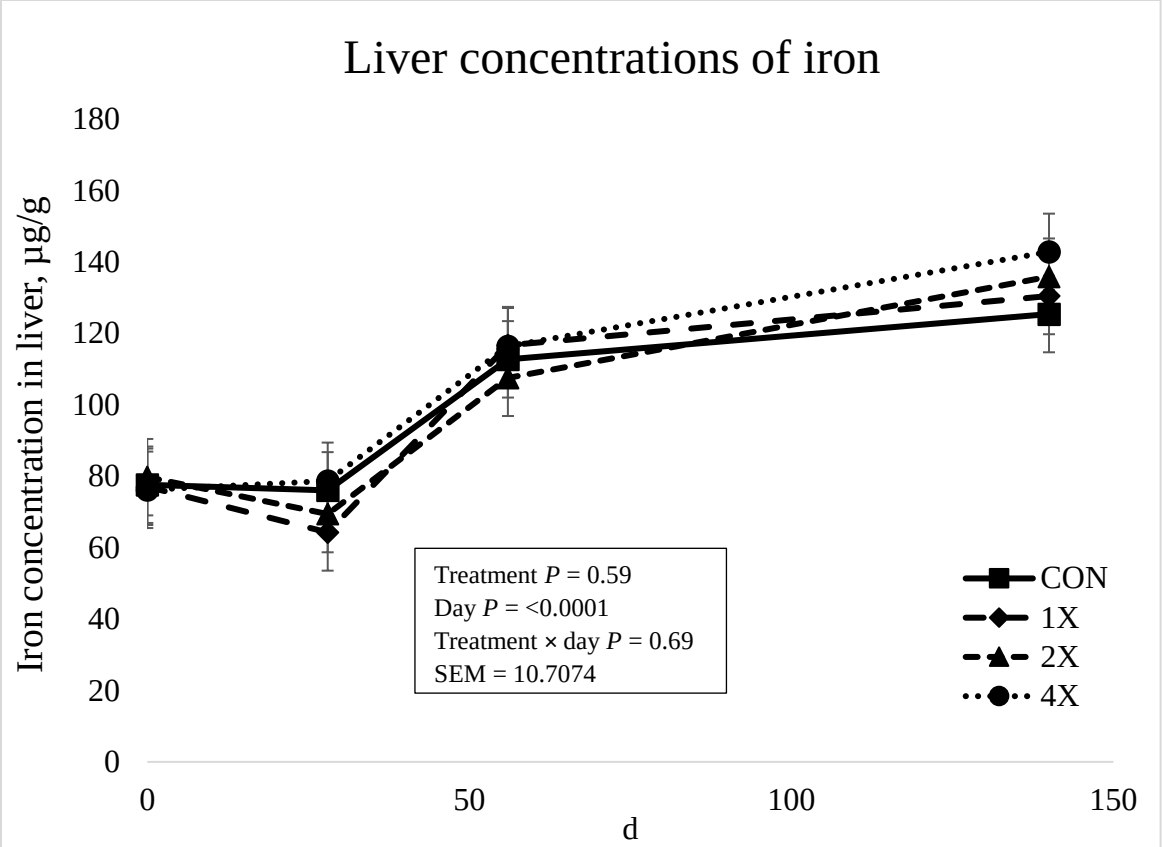

Serum concentrations of copper
Copper concentration in serum, µg/mL
1.60
1.20
0.80
0.40
0.00
0
50
100
150
d
Treatment P = 0.11
Day P = <0.0001
Treatment × day P = 0.35
SEM = 0.0627
CON
1X
2X
4X

Figure 2.2: Liver (**a**) and serum (**b**) concentrations of copper in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

[*] Cattle were harvested in 3 groups; d 126 (*n* =20 pens; 5 pens per treatment), d 140 (*n* = 16 pens; 4 pens per treatment), and d 154 (*n* = 12 pens; 3 pens per treatment). In this figure, 140 days on feed represents the final measurement, regardless of the actual harvest date.

[a,b,c] means within grouping without a common superscript letter differ ($P < 0.05$)

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations


Liver concentrations of iron
Iron concentration in liver, µg/g
180
160
140
120
100
80
60
40
20
0
0
50
100
150
d
Treatment P = 0.59
Day P = <0.0001
Treatment × day P = 0.69
SEM = 10.7074
CON
1X
2X
4X

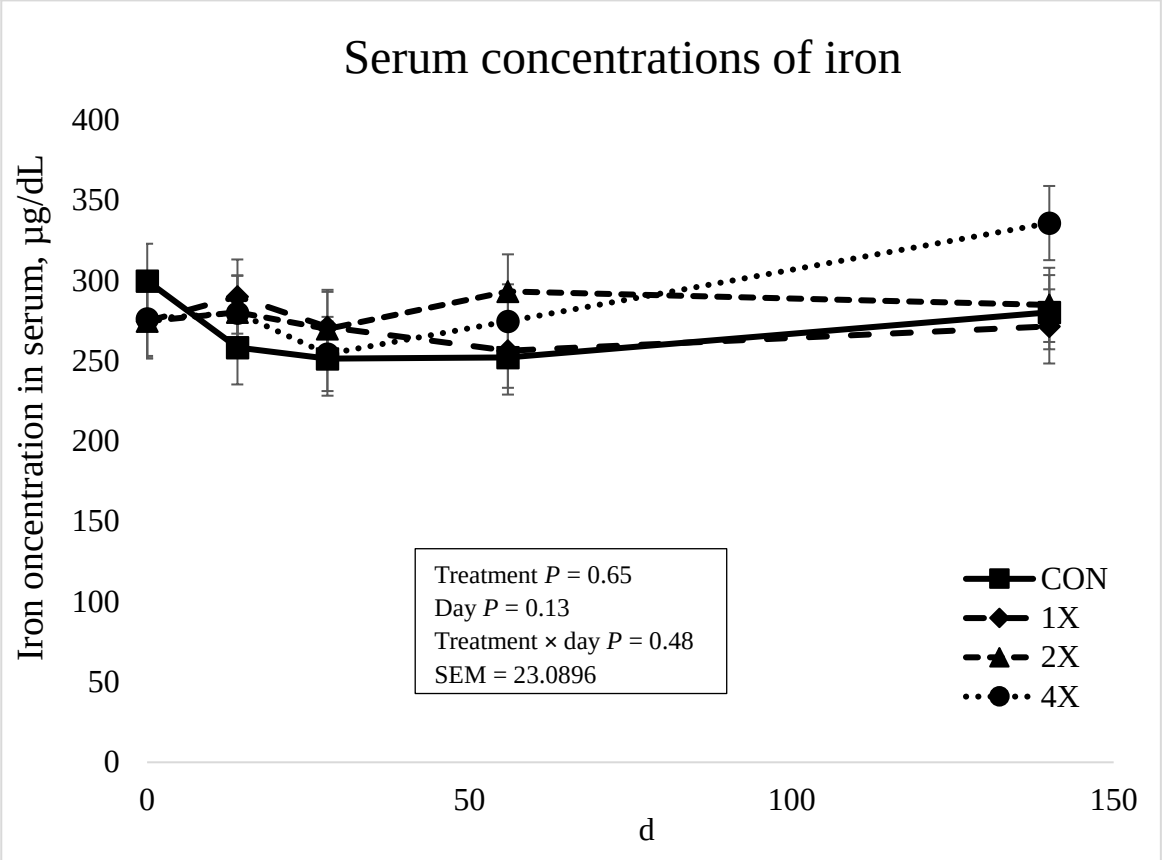
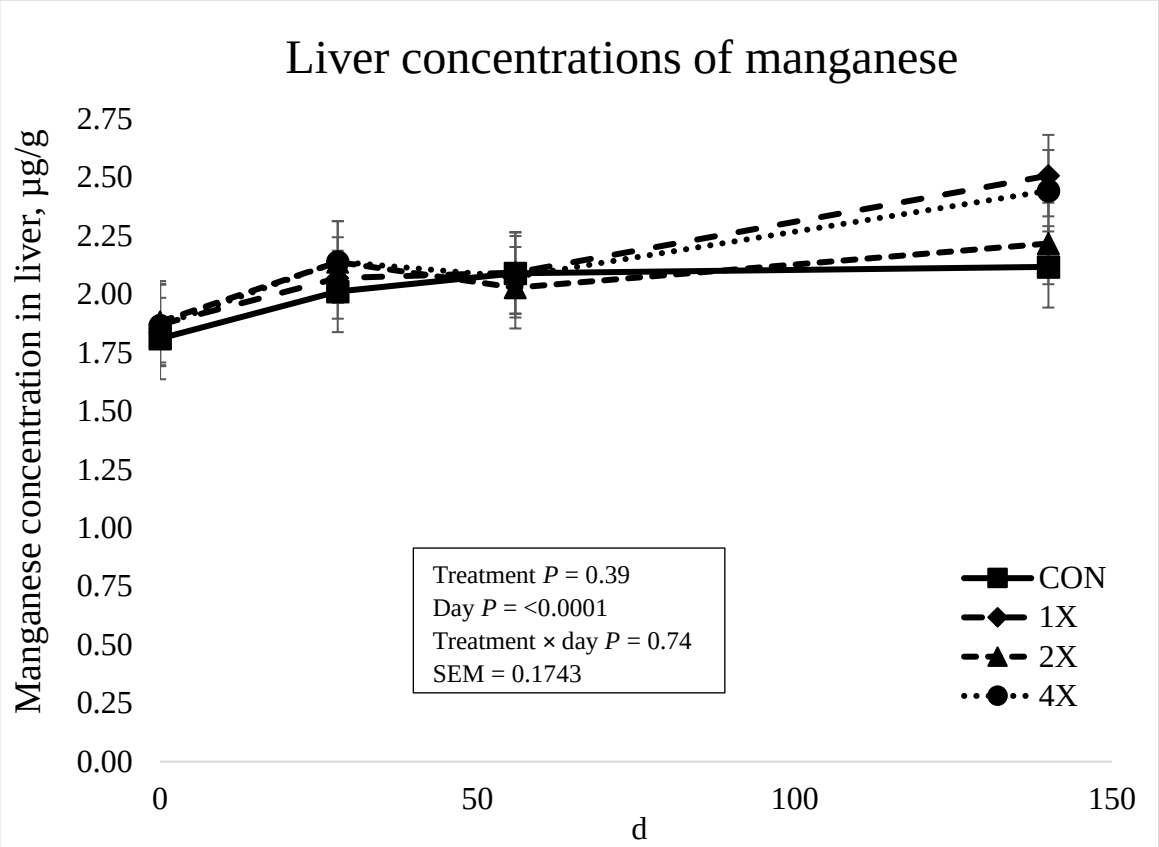

Serum concentrations of iron
Iron oncentration in serum, µg/dL
400
350
300
250
200
150
100
50
0
0
50
100
150
d
Treatment P = 0.65
Day P = 0.13
Treatment × day P = 0.48
SEM = 23.0896
CON
1X
2X
4X

Figure 2.3: Liver (**a**) and serum (**b**) concentrations of iron in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

* Cattle were harvested in 3 groups; d 126 (*n* =20 pens; 5 pens per treatment), d 140 (*n* = 16 pens; 4 pens per treatment), and d 154 (*n* = 12 pens; 3 pens per treatment). In this figure, 140 days on feed represents the final measurement, regardless of the actual harvest date.

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations


Liver concentrations of manganese
Manganese concentration in liver, µg/g
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
d
Treatment P = 0.39
Day P = <0.0001
Treatment × day P = 0.74
SEM = 0.1743
CON
1X
2X
4X

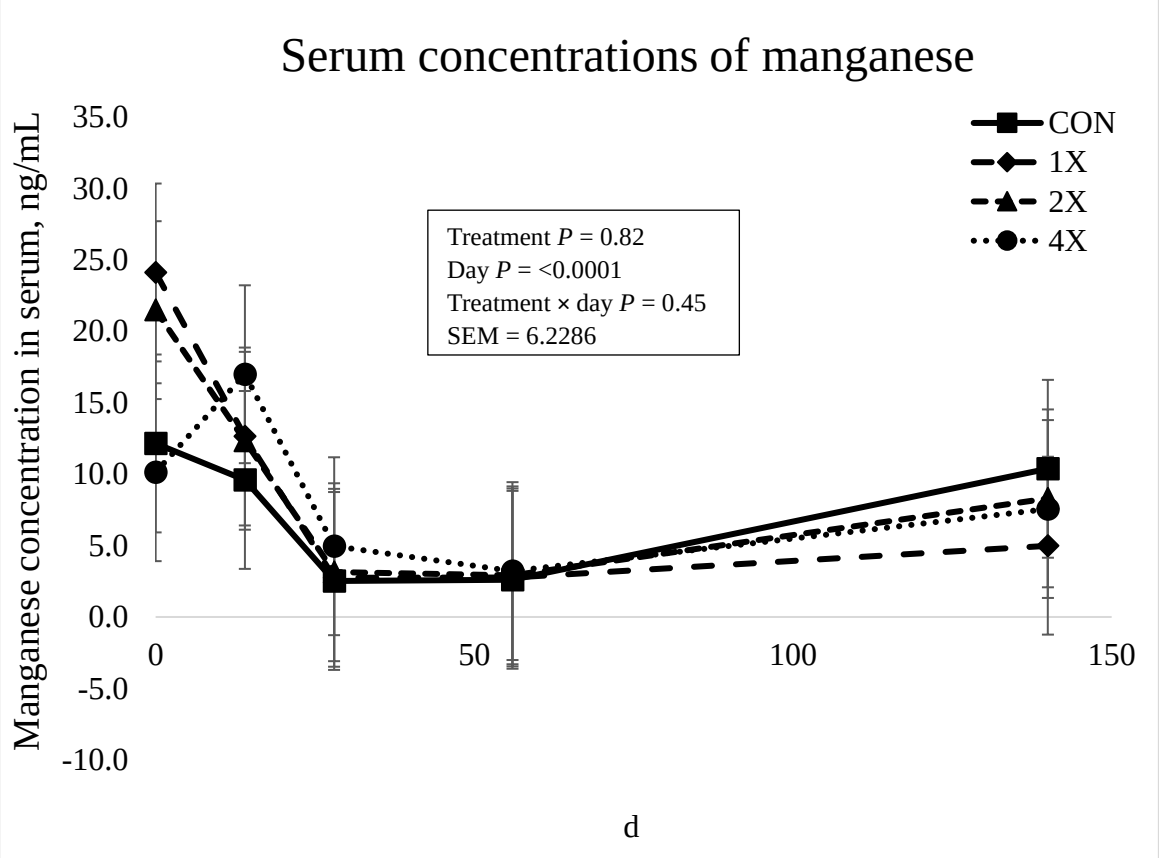
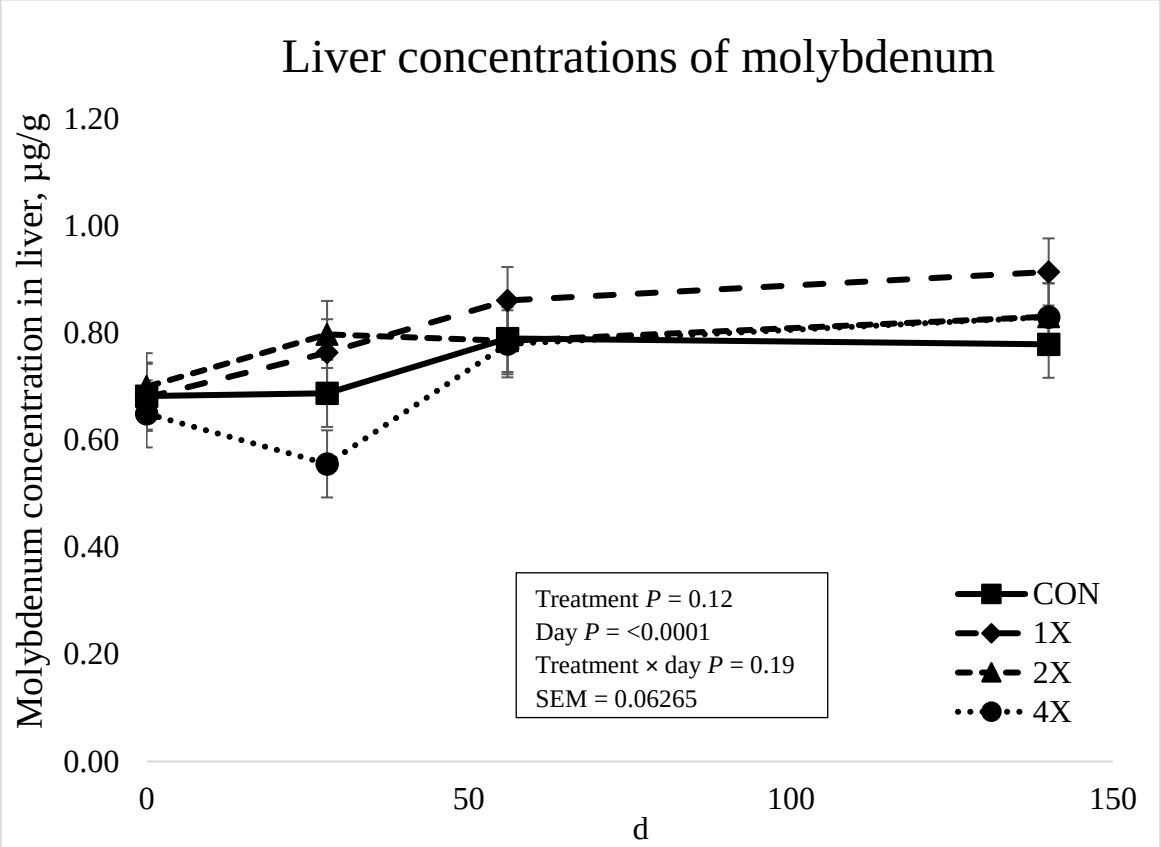

Serum concentrations of manganese
Manganese concentration in serum, ng/mL
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
0
50
100
150
d
Treatment P = 0.82
Day P = <0.0001
Treatment × day P = 0.45
SEM = 6.2286
CON
1X
2X
4X

Figure 2.4: Liver (**a**) and serum (**b**) concentrations of manganese in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

[*] Cattle were harvested in 3 groups; d 126 (*n* =20 pens; 5 pens per treatment), d 140 (*n* = 16 pens; 4 pens per treatment), and d 154 (*n* = 12 pens; 3 pens per treatment). In this figure, 140 days on feed represents the final measurement, regardless of the actual harvest date.

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations


Liver concentrations of molybdenum
Molybdenum concentration in liver, µg/g
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
50
100
150
d
Treatment P = 0.12
Day P = <0.0001
Treatment × day P = 0.19
SEM = 0.06265
CON
1X
2X
4X

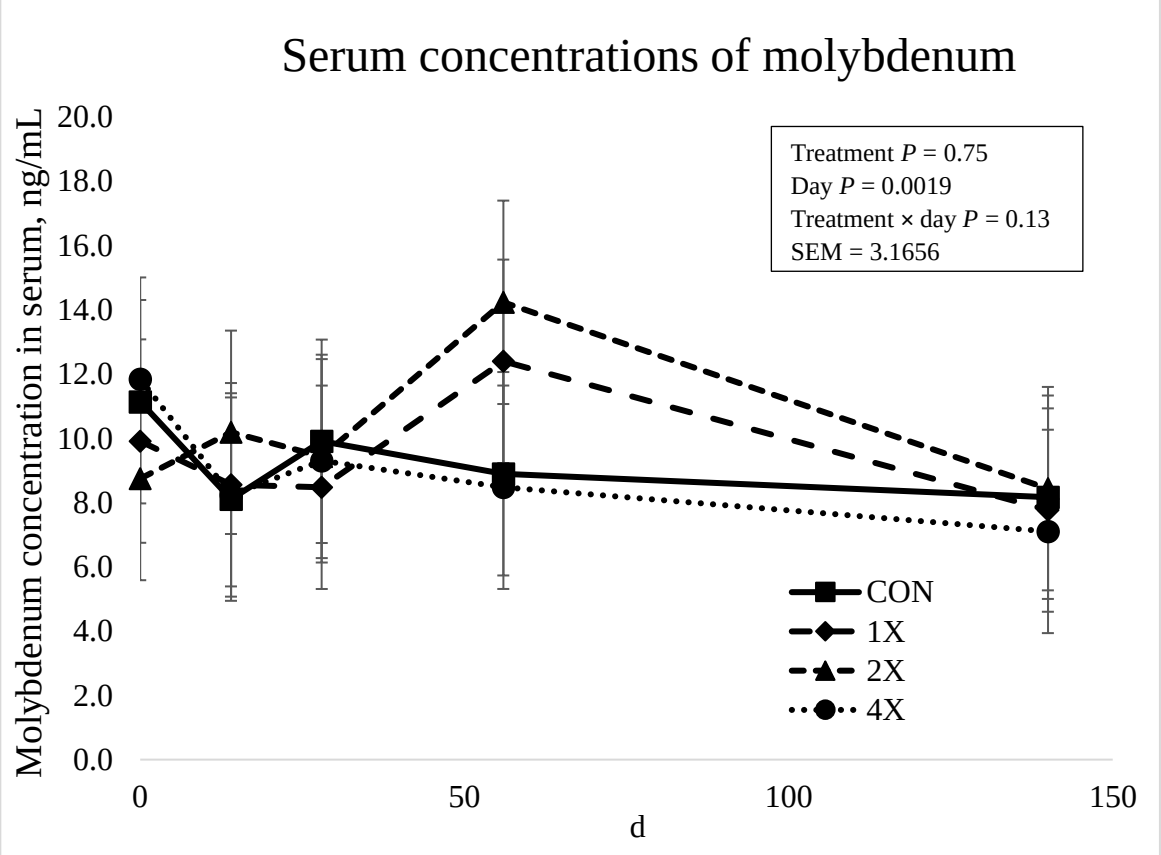
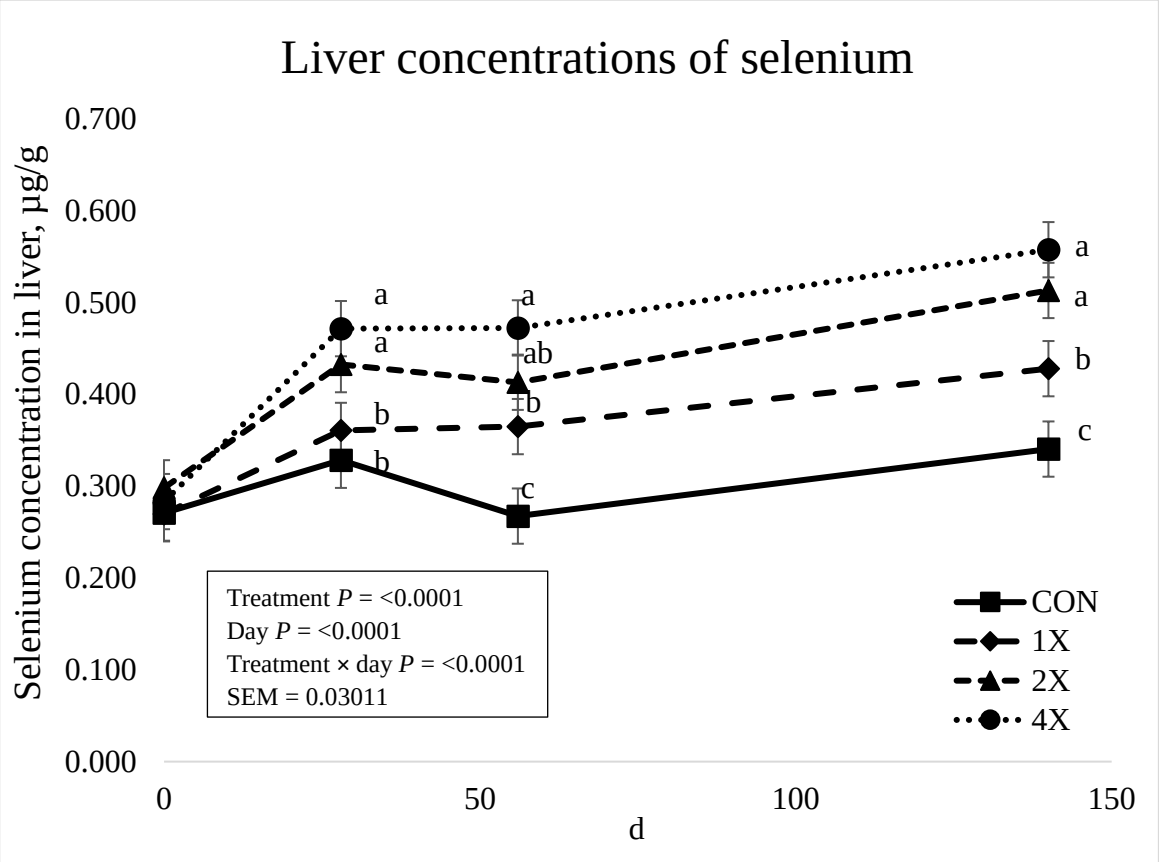

Serum concentrations of molybdenum
Molybdenum concentration in serum, ng/mL
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
50
100
150
d
Treatment P = 0.75
Day P = 0.0019
Treatment × day P = 0.13
SEM = 3.1656
CON
1X
2X
4X

Figure 2.5: Liver (**a**) and serum (**b**) concentrations of molybdenum in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

[1] Cattle were harvested in 3 groups; d 126 (*n* =20 pens; 5 pens per treatment), d 140 (*n* = 16 pens; 4 pens per treatment), and d 154 (*n* = 12 pens; 3 pens per treatment). In this figure, 140 days on feed represents the final measurement, regardless of the actual harvest date.

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations


Liver concentrations of selenium
Selenium concentration in liver, µg/g
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
50
100
150
d
a
a
a
a
ab
b
b
b
b
c
c
Treatment P = <0.0001
Day P = <0.0001
Treatment × day P = <0.0001
SEM = 0.03011
CON
1X
2X
4X

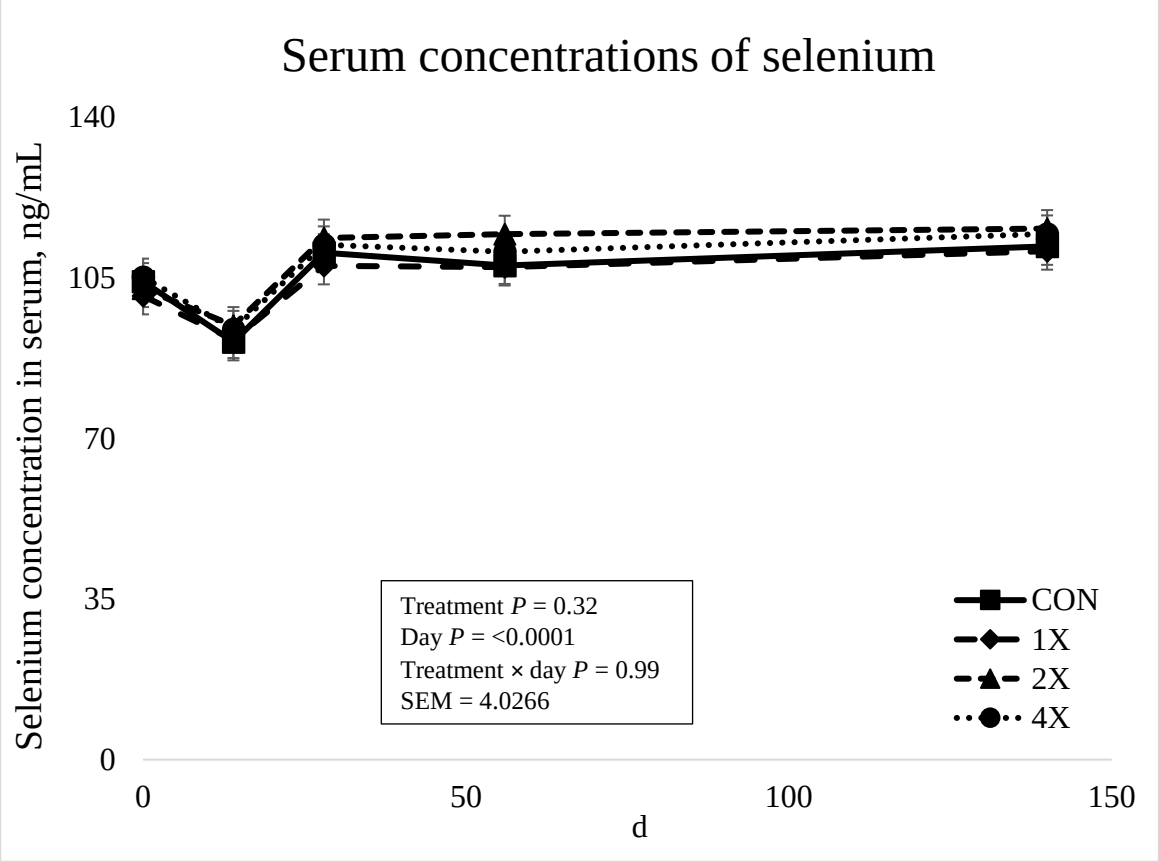

Serum concentrations of selenium
Selenium concentration in serum, ng/mL
140
105
70
35
0
0
50
100
150
d
Treatment P = 0.32
Day P = <0.0001
Treatment × day P = 0.99
SEM = 4.0266
CON
1X
2X
4X

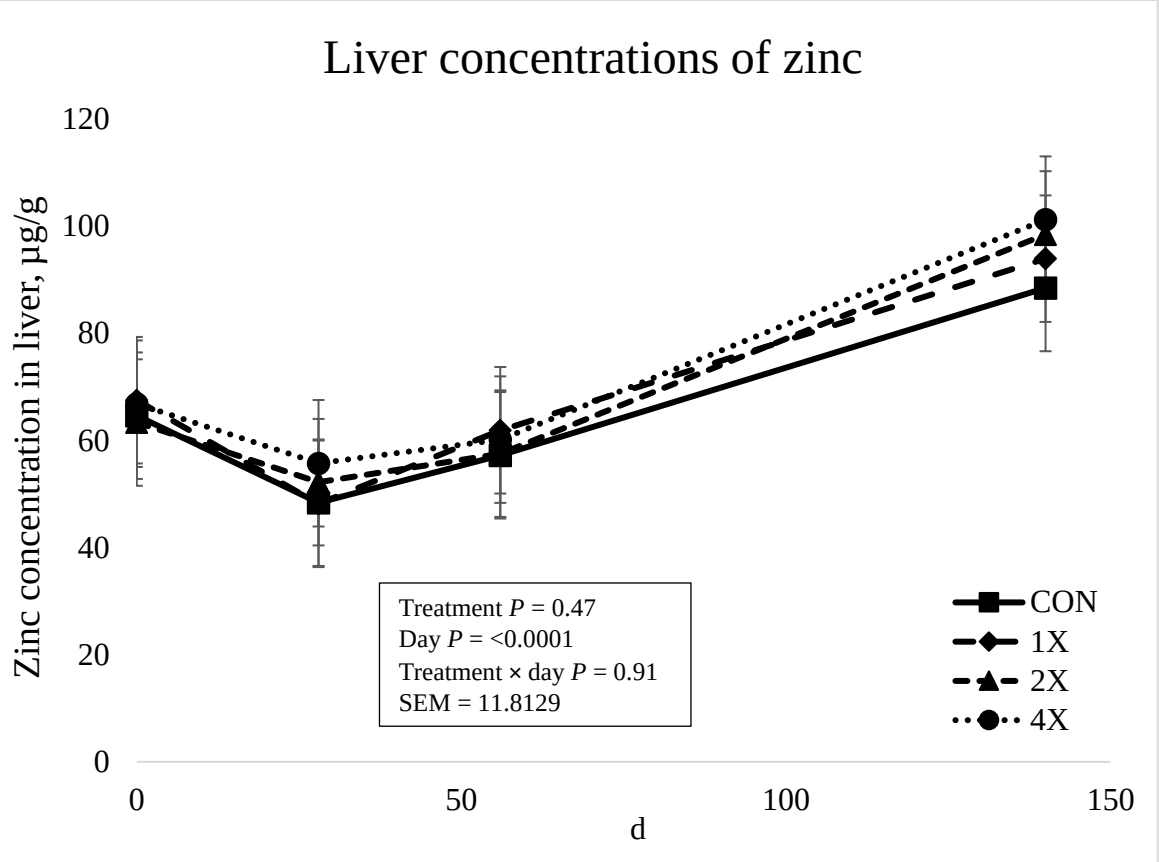
Figure 2.6: Liver (**a**) and serum (**b**) concentrations of selenium in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

a
a
b
b

[1] Cattle were harvested in 3 groups; d 126 (n =20 pens; 5 pens per treatment), d 140 (n = 16 pens; 4 pens per treatment), and d 154 (n = 12 pens; 3 pens per treatment). In this figure, 140 days on feed represents the final measurement, regardless of the actual harvest date.

[a,b,c] means within grouping without a common superscript letter differ ($P < 0.05$)

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations


Liver concentrations of zinc
Zinc concentration in liver, µg/g
120
100
80
60
40
20
0
0
50
100
150
d
Treatment P = 0.47
Day P = <0.0001
Treatment × day P = 0.91
SEM = 11.8129
CON
1X
2X
4X

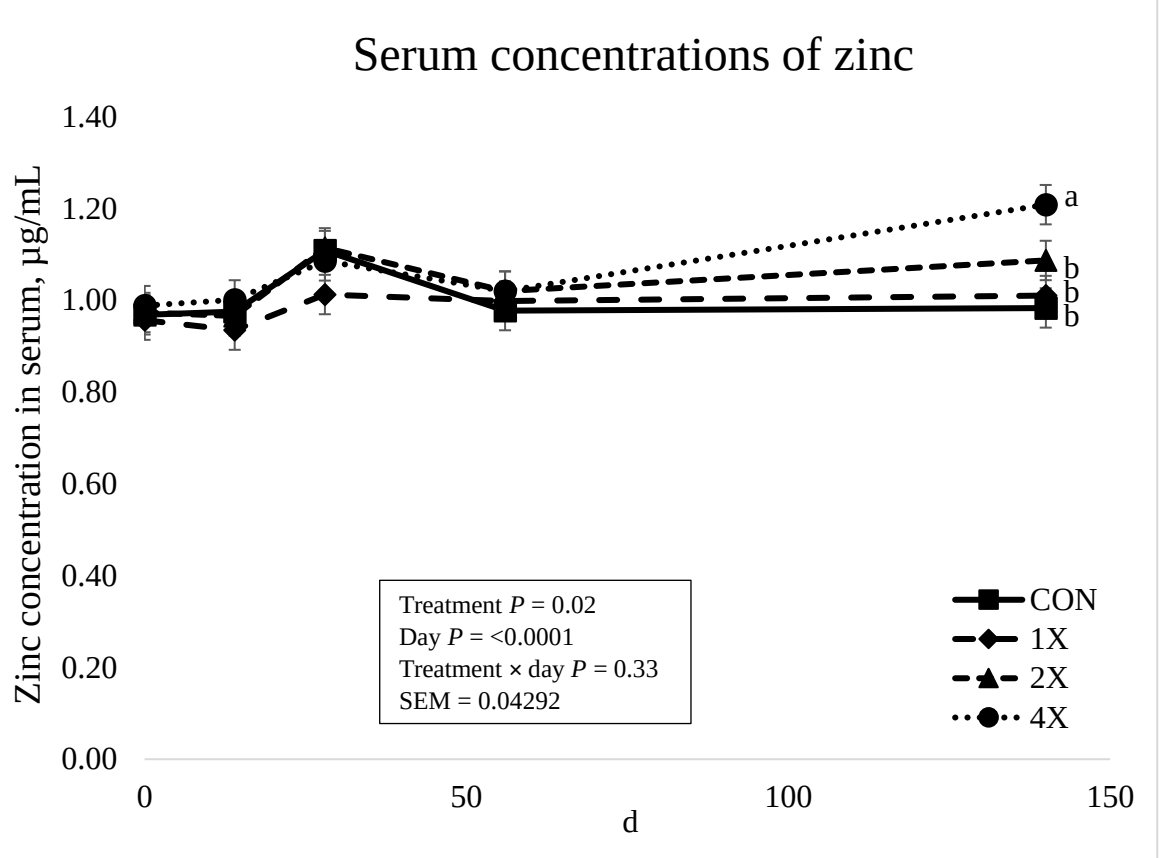

Serum concentrations of zinc
Zinc concentration in serum, µg/mL
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
50
100
150
d
a
b
b
b
Treatment P = 0.02
Day P = <0.0001
Treatment × day P = 0.33
SEM = 0.04292
CON
1X
2X
4X

Figure 2.7: Liver (**a**) and serum (**b**) concentrations of zinc in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

[1] Cattle were harvested in 3 groups; d 126 (n =20 pens; 5 pens per treatment), d 140 (n = 16 pens; 4 pens per treatment), and d 154 (n = 12 pens; 3 pens per treatment). In this figure, 140 days on feed represents the final measurement, regardless of the actual harvest date.

[a,b,c] means within grouping without a common superscript letter differ ($P < 0.05$)

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations

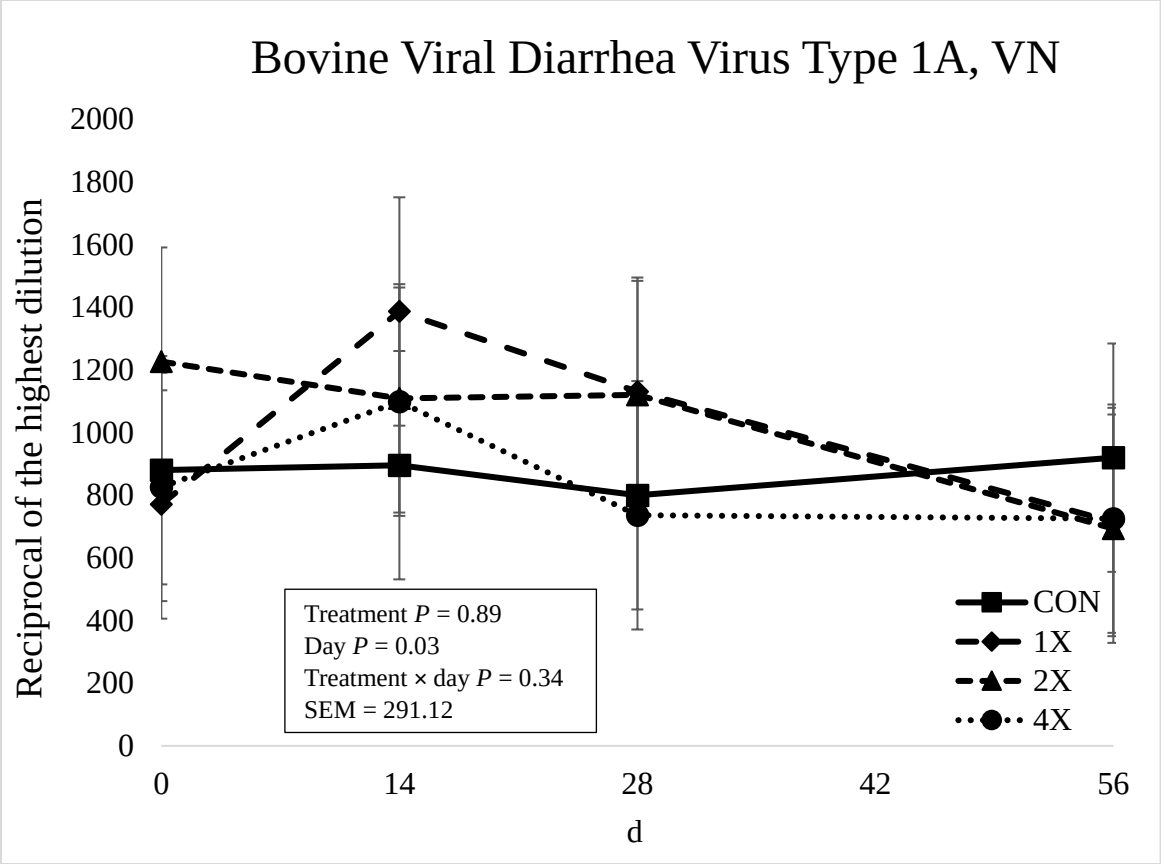


Figure 2.8a: Virus neutralization of Bovine Viral Diarrhea Virus Type 1A in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

The virus neutralization (VN; or serum neutralization, SN) antibody titer is reported as the reciprocal of the highest dilution of serum that neutralizes the infectivity of the virus (e.g. endpoint dilution 1:128 = antibody titer of 128). Values reported with a less than symbol (<) indicate no detectable antibody at the lowest readable dilution (e.g. <4 = no detectable antibody at a 1:4 dilution). Values reported with a greater than symbol (>) indicate titers that are greater than or equal to the highest dilution of the test (e.g. >16 = presence of antibody greater than or equal to a dilution of 1:16). Samples with a endpoint dilution greater than 4096 are reported as 4096.

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations.

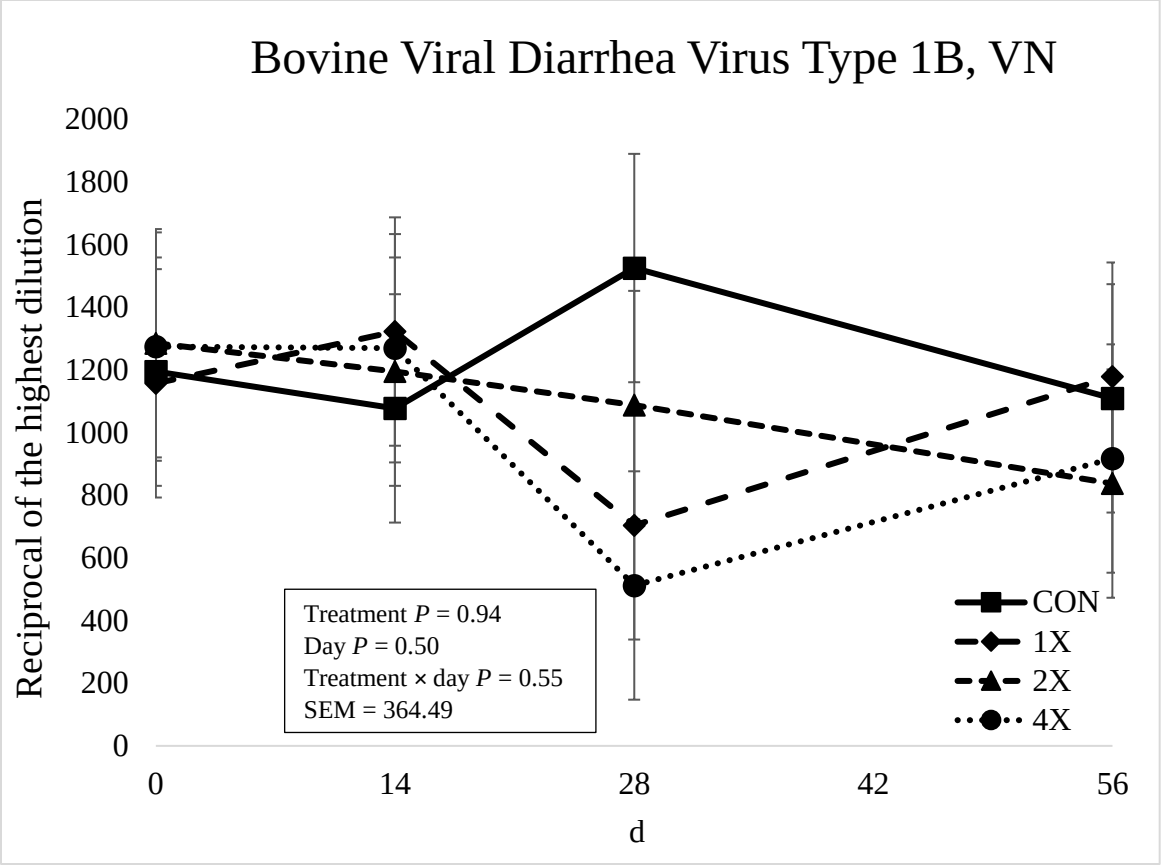


Figure 2.8b: Virus neutralization of Bovine Viral Diarrhea Virus Type 1B in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

The virus neutralization (VN; or serum neutralization, SN) antibody titer is reported as the reciprocal of the highest dilution of serum that neutralizes the infectivity of the virus (e.g. endpoint dilution 1:128 = antibody titer of 128). Values reported with a less than symbol (<) indicate no detectable antibody at the lowest readable dilution (e.g. <4 = no detectable antibody at a 1:4 dilution). Values reported with a greater than symbol (>) indicate titers that are greater than or equal to the highest dilution of the test (e.g. >16 = presence of antibody greater than or equal to a dilution of 1:16). Samples with a endpoint dilution greater than 4096 are reported as 4096.

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations.

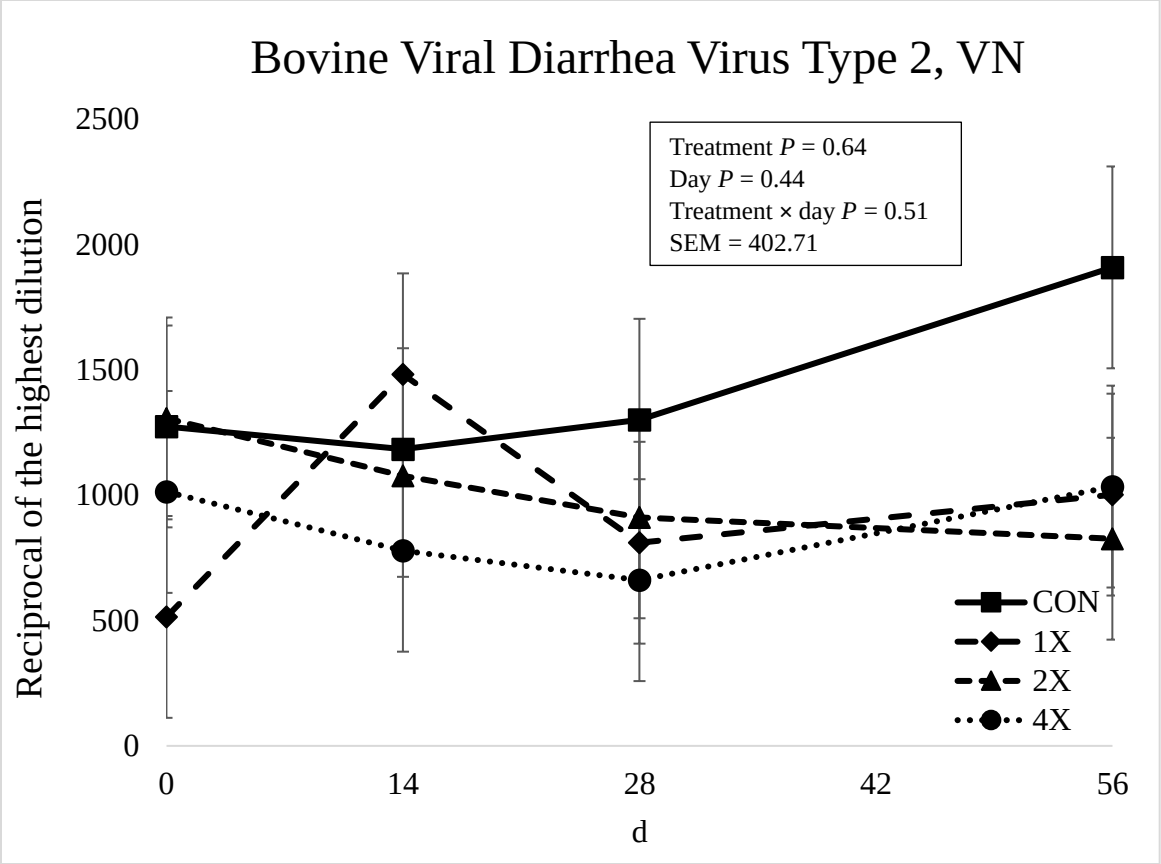


Figure 2.8c: Virus neutralization of Bovine Viral Diarrhea Virus Type 2 in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

The virus neutralization (VN; or serum neutralization, SN) antibody titer is reported as the reciprocal of the highest dilution of serum that neutralizes the infectivity of the virus (e.g. endpoint dilution 1:128 = antibody titer of 128). Values reported with a less than symbol (<) indicate no detectable antibody at the lowest readable dilution (e.g. <4 = no detectable antibody at a 1:4 dilution). Values reported with a greater than symbol (>) indicate titers that are greater than or equal to the highest dilution of the test (e.g. >16 = presence of antibody greater than or equal to a dilution of 1:16). Samples with a endpoint dilution greater than 4096 are reported as 4096.

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations.

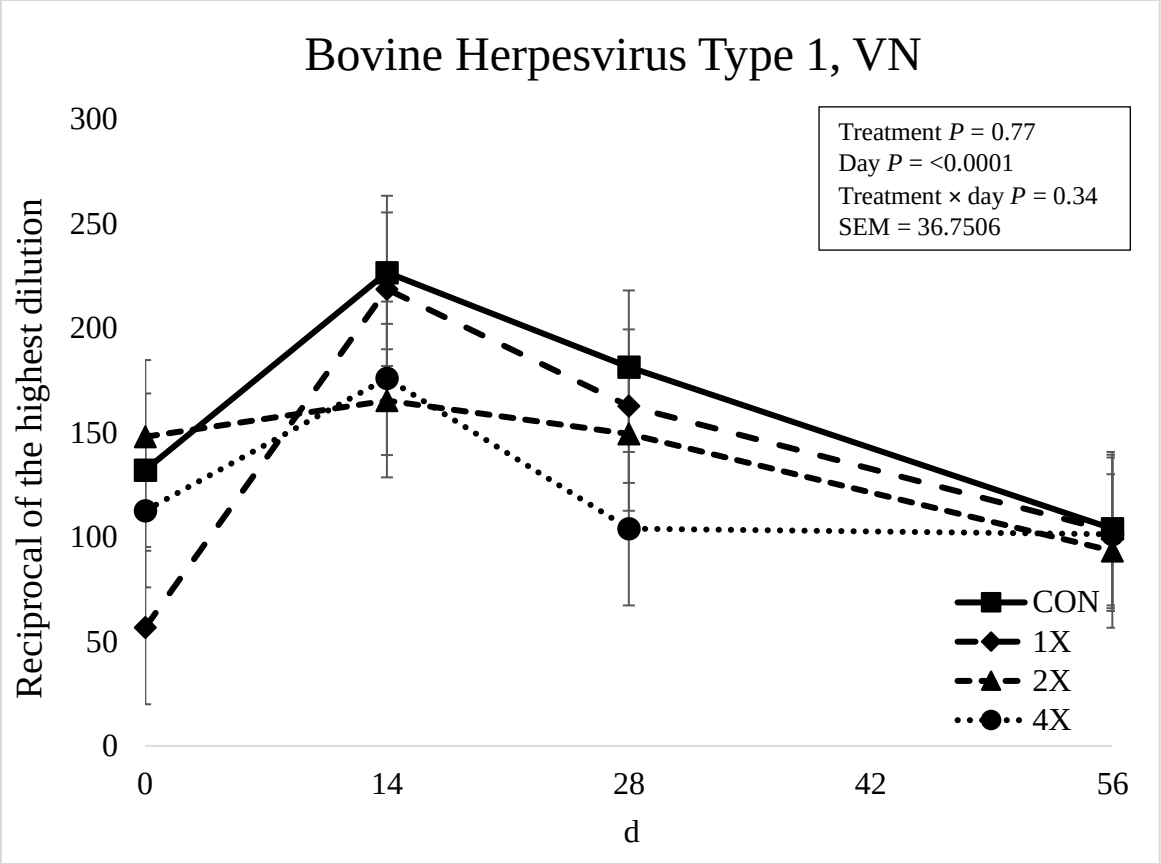


Figure 2.9: Virus neutralization of Bovine Herpesvirus Type 1 in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

The virus neutralization (VN; or serum neutralization, SN) antibody titer is reported as the reciprocal of the highest dilution of serum that neutralizes the infectivity of the virus (e.g. endpoint dilution 1:128 = antibody titer of 128). Values reported with a less than symbol (<) indicate no detectable antibody at the lowest readable dilution (e.g. <4 = no detectable antibody at a 1:4 dilution). Values reported with a greater than symbol (>) indicate titers that are greater than or equal to the highest dilution of the test (e.g. >16 = presence of antibody greater than or equal to a dilution of 1:16). Samples with a endpoint dilution greater than 4096 are reported as 4096.

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations.

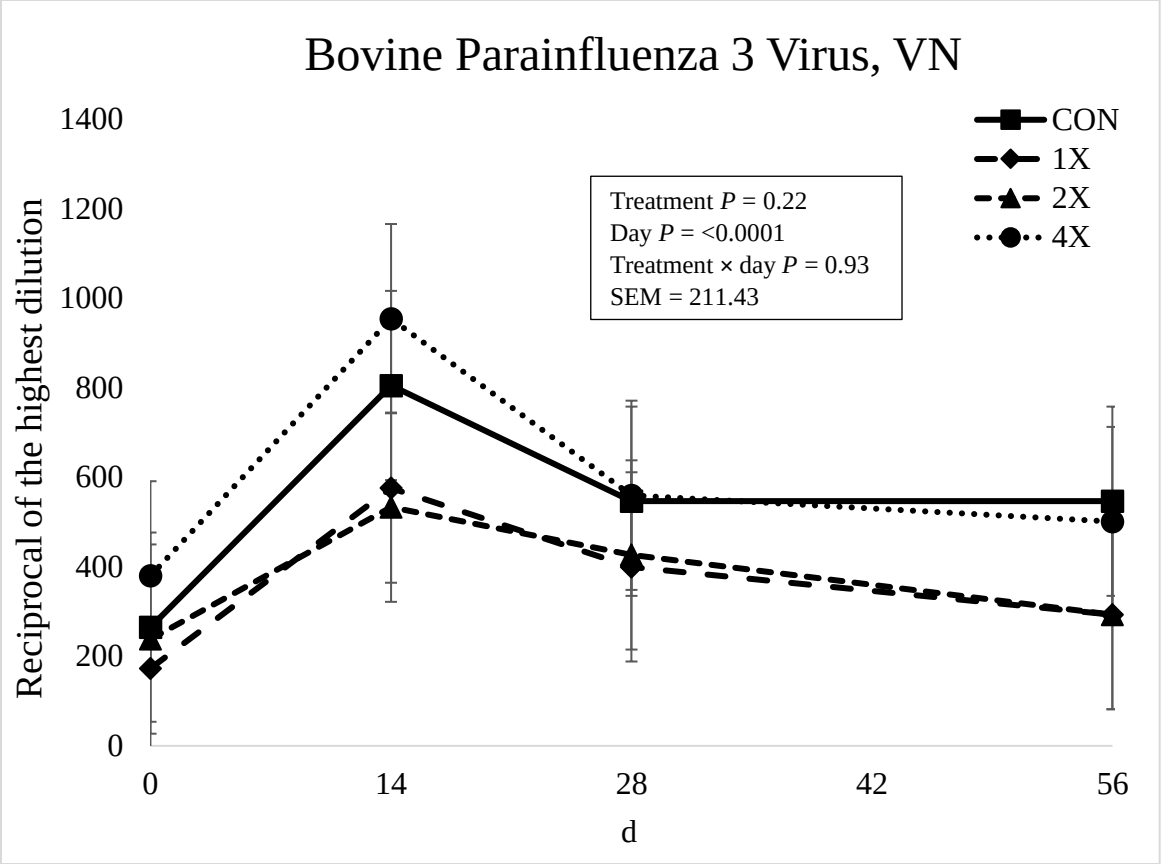


Figure 3.0:Virus neutralization of Bovine Parainfluenza 3 Virus in finishing steers consuming 1 of 4 trace mineral supplements. Treatments included a control (**CON**; no supplemental trace minerals), **1X** (supplement containing 0.15 mg/kg of Co, 10 mg/kg of Cu, 50 mg/kg of Fe, 0.5 mg/kg of I, 20 mg/kg of Mn, 0.1 mg/kg of Se, and 30 mg/kg of Zn, on a DM basis), **2X** (supplement containing 0.30 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Fe, 1.0 mg/kg of I, 40 mg/kg of Mn, 0.2 mg/kg of Se, and 60 mg/kg of Zn, on a DM basis), or **4X** (supplement containing 0.60 mg/kg of Co, 40 mg/kg of Cu, 50 mg/kg of Fe, 2.0 mg/kg of I, 80 mg/kg of Mn, 0.3 mg/kg of Se, and 120 mg/kg of Zn on a DM basis).

The virus neutralization (VN; or serum neutralization, SN) antibody titer is reported as the reciprocal of the highest dilution of serum that neutralizes the infectivity of the virus (e.g. endpoint dilution 1:128 = antibody titer of 128). Values reported with a less than symbol (<) indicate no detectable antibody at the lowest readable dilution (e.g. <4 = no detectable antibody at a 1:4 dilution). Values reported with a greater than symbol (>) indicate titers that are greater than or equal to the highest dilution of the test (e.g. >16 = presence of antibody greater than or equal to a dilution of 1:16). Samples with a endpoint dilution greater than 4096 are reported as 4096.

Values plotted represent least squares means ± SE of the mean, calculated for 12 animals per experimental group. Within time points, the slice output option of SAS (SAS Inst. Inc., Cary, NC) was used to perform mean separations.

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