THESIS

EFFECTS OF TRACE MINERAL SOURCE AND CONCENTRATION ON PRODUCTION PARAMETERS THROUGHOUT ONE COW-CALF PRODUCTION CYCLE.

Submitted by

Harrison Hallmark

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Master of Sciences

Colorado State University

Fort Collins, Colorado

Spring 2023

Master's Committee:

Advisor: Terry Engle
Co-Advisor: Jason Ahola

Franklyn Garry

ABSTRACT

EFFECTS OF TRACE MINERAL SOURCE AND CONCENTRATION ON PRODUCTION PARAMETERS THROUGHOUT ONE COW-CALF PRODUCTION CYCLE.

An experiment was conducted to evaluate the effects of Cu, Zn, and Mn supplementation on mineral status, production parameters, and cattle performance in a rangeland environment throughout a one -year period in eastern Colorado.

One hundred and eighty multiparous crossbred (Angus and Angus x Hereford) beef cows were blocked by body weight, age, and gestational status and randomly assigned to 1 of 3 free-choice mineral treatments (n = 60 cows per treatment). Treatments were then assigned to 1 of 9 replicates (n=20 cows per replicate), resulting in 3 replicates per treatment. Treatments consisted of, 1) 1X NASEM (2016) sulfate base source, 2) 1X NASEM (2016) Intellibond source, or 3) 0.5X NASEM (2016) Intellibond source. Treatments 1 (1X Sulfate) and 2 (1X Intellibond) contained 1,000, 2,000, and 3,000 mg/kg DM of Cu, Mn, and Zn, respectively. While treatment 3 (0.5X Intellibond) contained 500, 1,000, and 1,500 mg/kg DM of Cu, Mn, and Zn. All free-choice mineral supplements were formulated to provide 0.15% supplemental S, 15 mg/kg Co from Co carbonate, and 55 mg/kg I from Ca iodate (Hubbard Feeds; Mankato, MN). Supplement consumption was formulated for 113 $g \cdot animal^{-1} \cdot d^{-1}$. Supplement intakes were determined every 28 d. Liver biopsies and blood samples were obtained before the experiment was initiated (d -45), after calving (d 158 and 159), and after weaning (d 294) at the end of the first production year. Each replicate was rotated to a different pasture every 2 to 4 weeks to minimize pasture effects. Cows were weighed during each liver biopsy event and at each scheduled handling events. A two-day calf weaning weight was collected during weaning (d 260 and 261). Over the first year of the experiment, cow BW, BCS, mineral status, mineral intake, and calf weaning weight were collected. There was no impact of treatment on any of the response variables measured during the first year of the experiment.

ACKNOWLEDGEMENTS

I am truly humbled at the amazing support of so many of my friends, mentors, and family members during the journey of completing this degree. I would not have gotten this far without any of their unselfish investment of time they devoted to help and guide me.

Dr. Terry Engle, thank you for being the greatest advisor I could have ever asked for. You have taught me so much about research, the cattle industry, and servant leadership. I appreciate your patience with me and speaking words of wisdom and encouragement during challenges and self-discovery. Thank you for making this adventure a reality.

Dr. Jason Ahola, thank you for providing so many opportunities and resources to me, and so many others, to become more involved in animal agriculture during our time at CSU. I appreciate all of the time and knowledge you have contributed to help me find my place and build a foundation within the cattle industry. You have been an amazing mentor to me.

Dr. Frank Garry, your contagious enthusiasm and passion for teaching and the agricultural industry has been imprinted on so many of your students, including myself. I appreciate all of the wisdom and stories you have shared with me to enhance my curiosity and eagerness to understand more of the agricultural industry.

Doug and Austin Couch and the Couch family, thank you so much for all the arduous work you have done behind the scenes to make this project and so many other projects in the past possible. You all are truly an amazing family, and it has been a pleasure for me to get to know you all.

Thank you to my parents, brothers and sisters, and family members that have molded me into the individual I am today. You all have given me one of the greatest initiations in life and prepared me for future opportunities life has to offer.

Thank you to my friends, Tyler Thomas, Allison Vautier, Briana Tangredi, Meghan Thorndyke-Barlow, Octávio Guimarães, Huey Yi Loh, Matt Mcquagge, Paxton Sullivan and so many others for the adventures, support, challenges, and amazing moments we have shared.

Finally, Dr. Maria Brock, thank you for all the support, encouragement, and love you have contributed to me over the past couple of years. Your unwavering commitment continues to make me a better individual every day. I love you.

TABLE OF CONTENTS

CHAPTER 1 – REVIEW OF LITERATURE

INTRODUCTION

Living systems require macro and microminerals to perform essential biological processes. Macrominerals are classified by the relatively large quantities necessary in a diet and are typically expressed on a percentage basis(McDowell, 2003). These include calcium (Ca), chlorine (Cl), potassium (K), phosphorus (P), magnesium (Mg), sodium (Na), and sulfur (S; NASEM, 2016). Microminerals are required in much smaller quantities (typically expressed on a mg of mineral per kg diet DM basis). These include chromium (Cr), cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn). The appropriate dietary concentrations of both macro and microminerals are necessary to support carbohydrate, lipid, vitamin metabolism, as well as immune function and hormone production. Excessive or deficient amounts of dietary minerals can result in metabolic abnormalities in the animals that can only be treated with adjustments to dietary quantities (Spears, 1994). In cases regarding ruminant nutrition, imbalances in dietary macro and microminerals can also impact ruminal fermentation of fiber (McDowell, 2003). Therefore, the focus of this literature review will be to provide an in-depth review of the chemical characteristics of Cu, Mn, S, and Zn, natural and supplemental - sources of each element, and the metabolic functions, and homeostatic mechanisms associated with Cu, Mn, S, and Zn in beef cattle.

CHEMICAL CHARACTERISTICS

Elemental minerals are elements that are found in nature in a variety of chemical forms (metals, metalloids, nonmetals, and naturally occurring alloys) and concentrations, which vary by geological location. In this literature review, we will primarily focus on the elemental minerals and minerals compounds consisting of Cu, Zn, Mn, and S.

Copper:

Copper is a malleable and ductile metal that is an excellent conductor of heat and electricity, which makes it particularly important to society (Adriano, 1986). Reddish in color, Cu occurs in the I and II

oxidative states (Adriano, 1986). Existing primarily in nature associated with sulfides, sulfates, sulfosalts, carbonates, and other various anions, Cu can also be present in raw oxidative states (Alina Kabata-Pendias, 1992, Adriano, 1986).

Zinc:

Elemental Zn is a blueish white soft metal that naturally occurs almost exclusively in a II oxidation state (Adriano, 1986). Additionally, Zn tends to form a multitude of salts, both soluble (chlorates, chlorides, sulfates, nitrate) and insoluble (oxides, carbonates, phosphates, silicates, and sulfides (Adriano, 1986). Zinc is primarily found in ores compounded as zinc sulfide (ZnS), as well as associated with other metals such as, lead (Pb), copper (Cu), cadmium (Cd), and iron (Fe) (McDowell, 2003).

Manganese:

Representing approximately 0.10% of the earth's crust, Mn is the 12th most abundant element on the planet (McDowell, 2003). This whitish gray metal can be relatively brittle (Adriano, 1986). Additionally, it exists in multiple oxidative states of I, II, III, IV, VI, and VII (Adriano, 1986). However, it tends to be most stable in the II, IV, VI, and VII states (Adriano, 1986). The most important oxidative state for biological stability tends to be the II and III (McDowell, 2003). Manganese minerals are commonly found in oxide, carbonate, and silicate compounds (Adriano, 1986).

Sulfur:

Sulfur is a nonmetal that tends to be a brittle solid (McDowell, 2003). Substantial quantities of S are found in both a concentrated and blended form within the earth's crust (McDowell, 2003). The famous "rotten egg" odor if present when it is fermented or melted. (McDowell, 2003) Additionally, S can strongly influence the interaction and bioavailability of certain micronutrients such as Cu and Mo (Adriano, 1986). Sulfur is used in medicines to inhibit the growth of bacteria and other organisms, as well as many commercial uses for acids, salts, and dioxides (McDowell, 2003).

MINERAL SOURCES

Soil

For this literature review, the focus on soil minerals will be limited to the geographic location (eastern Colorado) of the research project described in the subsequent chapter. There are several influential factors that impact plant uptake of minerals in soils, including: 1) soil acidity; 2) soil moisture or drainage condition; 3) soil temperature and seasons; 4) plant genus, species, and variety; 5) fertilization (Reid & Horvath, 1980).

Copper:

In soils, Cu is considered an important mineral regarding agronomic practices (Alina Kabata-Pendias, 1992). Soil types such as sandy loam, valent sand, and complex soils make up the majority of the Eastern Colorado Research Center (ECRC) property (NRCS- Web Soil Survey, 2005). Copper concentrations within loamy and clay soils tend to average between 29-30 ppm with a range of 7-70 ppm (Alina Kabata-Pendias, 1992, Adriano, 1986).

Copper is an exceptional multipurpose cation that exhibits unique characteristics, which allows it to interact chemically with various anions, minerals, and organic compounds within soils (Alina Kabata-Pendias, 1992). This results in Cu being relatively immobilized within soil (Alina Kabata-Pendias, 1992). Furthermore, the solubility of Cu is directly related to the soil pH and moisture content (Alina Kabata-Pendias, 1992). A soil pH range of 5.0 – 7.0 is optimal for absorption of Cu by most plant root systems (Reid & Horvath, 1980). Furthermore, soil temperatures can affect the uptake of macro- and microminerals depending on the soil and plant type. For example, studies have shown that brome grass and timothy grass grown at warm temperatures tend to have lower plant tissue Cu concentrations those grown at lower temperatures; where Cu concentrations were greater (Reid & Horvath, 1980). Additionally, alfalfa that is grown at higher temperatures tends to contain greaater concentrations of Cu that when grown at lower temperatures (Reid & Horvath, 1980).

Zinc:

Zinc concentration in soils in the eastern Colorado region generally ranges from 17- 125 ppm. In loamy and clay soils, Zn concentrations range from 20-220 ppm. Examining the impact of soil type on Zn uptake by plants, data indicates that adsorption and retention of Zn in plants grown in clay type soils is relative unavailable to the plants due to strong binding coefficients of the soil organic matter to Zn (Alina Kabata-Pendias, 1992). In other soil types, Zn tends to be readily soluble and mobile compared to other heavy metals. This is especially evident within acidic soils (Alina Kabata-Pendias, 1992). Similar, to Cu, Zn tends to have a pH range of 5.0 – 7.0 for optimal root surface interaction (Reid & Horvath, 1980).

Manganese:

Alina Kabata-Pendias, (1992) reported that Mn is one of the most abundant minerals within the crust and upper mantle (lithosphere) of earth. The concentrations vary among a variety of rock types but typically range from 350-2000 ppm (Alina Kabata-Pendias, 1992). Manganese is present in multiple oxidation state (Mn 2+, Mn 3+, and Mn 4+), however, it is most commonly found in the Mn 2+ form frequently in rock forming silicate minerals (Alina Kabata-Pendias, 1992). Manganese hydroxides ($MnOH_2$) are frequently found in soils as dark brown and black nodules (Dixon et al., 1990). However, Alina Kabata-Pendias (1992) reported that the behavior of Mn in surficial deposits is very complex, which is determined by environmental factors such as, pH conditions.

Manganese is an essential element for both plant and animal nutrition due to the rapid oxidation and reduction characteristic which in turn impacts the amount of the element available for uptake (McKenzie, 2018). If excessive oxidation occurs, Mn is not available in adequate amounts for absorption, however, if reduction Mn is reduced, Mn is typically more available for plant and animal uptake (McKenzie, 2018). Manganese compounds are crucial to soil constituents because the negative charges of $Mn(OH)_4$ and $Mn(OH)_2$ which greatly influence the association of Mn with other heavy metals, specifically, Co, Ni, Cu, Zn, Pb, Ba, TI, and Mo (Alina Kabata-Pendias, 1992). Additionally, the availability of Mn to plants is highly dependent on the pH of the soils, as well as the loss or gain of electrons from external interactions or sources (redox potential) (Alina Kabata-Pendias, 1992). Manganese typically has an optimal pH root

surface interaction that ranges between 5.0 – 6.5 (Reid & Horvath, 1980). This results in the reactions that occur most frequently being oxidation-reduction and hydrolysis reactions (Alina Kabata-Pendias, 1992). The solubility of Mn in soils is significant to plant absorption depending on the oxidative state Mn is found in the soil (Alina Kabata-Pendias, 1992). In highly drained soils, the Mn solubility increases, as soil acidity increases, however, in alkalotic soils Mn has the ability to form complexes to decrease Mn solubility.

Sulfur:

In recent decades, a gradual increase of S emission has resulted in an increase of hydrogen ions in topsoil, which can cause an imbalance of all nutrients and impact the natural buffering properties of soils (Alina Kabata-Pendias, 1992). A common effect of these alterations is an increase of mobility of trace metals. This increase of mobility of trace elements can affect the bioavailability and leaching profiles of soil (Alina Kabata-Pendias, 1992). Loamy soils have been reported to accumulate a high concentration of elements with much less environmental risks, however, overall chemical imbalance can result in decreased biological activity, fluctuations in pH, degradation of organic and mineral adsorption-type complexes (Alina Kabata-Pendias, 1992).

Metallic ions, such as Cu^{2+} and Mn^{2+}, may form relatively stable sulfides of acidic or neutral reducing potential in flooded soils (Alina Kabata-Pendias, 1992). Sulfates of heavy metals are readily available to plants and are important in agricultural production and practices (Alina Kabata-Pendias, 1992).

Microorganisms contribute greatly to a soils ecosystem by producing, consuming, and transporting nutrients (Alina Kabata-Pendias, 1992). Microorganisms can adapt to high concentrations of elements in their environment; however, they can be relatively sensitive to both deficiencies and excesses of elements (Alina Kabata-Pendias, 1992). Microbial contributions of soil ecosystems can vary and can include activities, such as transport of elements into or out of a cell, charge alteration of an element, mobilization of elements, and organic compounds and elemental interactions and matching (Alina Kabata-Pendias, 1992). However, the most important microbial function is the degradation of plant and animal residues.

Forages

Forage has been defined by *The Forage and Grazing Terminology Committee*, as 'edible parts of plants, other than separated grain, which can provide feed for grazing animals or that can be harvested for feeding' (Allen et al., 2011). According to the Food and Agriculture Organization, agriculture utilizes approximately five billion hectares, or 38 percent, of the total land surface in the world (FAO, 2020). From that total, approximately two thirds consist of meadows and pastures for grazing livestock. This indicates that multiple species of grazing livestock rely mostly or completely on natural or enhance forms of forages, browse, or woody plants to meet their nutritional requirements (MacPherson, 2000). Allen et al., 2011 defines the differences between two grazing types: rangeland and pastureland. Rangeland is defined as "Land on which indigenous vegetation is predominantly grasses, grass-like plant, forb or shrubs that are grazed or have the potential to be grazed, and which is used as a natural ecosystem for the production of grazing livestock and wildlife."(Allen et al., 2011). Pastureland is "Land (and the vegetation growing on it) devoted to the production of introduced or indigenous forage for harvest by grazing, cutting, or both. Usually managed to arrest successional processes" (Allen et al., 2011). These two definitions are significant to animal production because they differ depending on several factors such as, geographical location, nutritional composition, cultivated or indigenous processes. Livestock performance in a rangeland or pastureland environment will fluctuate depending on the quality and quantity of nutrients available.

Cattle have a large rumen and reticulum, which allows them to digest and metabolize forages that contain high concentrations of cellulose, hemicellulose, proteins, and lipids (Hofmann, 1989). Hofmann, 1989, categorizes ruminants based on their physiological and evolutionary characteristics into three separate categories: Concentrated selectors, Intermediate types, and Grass/Roughage easters. For this literature review, I will focus on beef cattle, which fall under the Grass/Roughage eater category.

Overall, the world's ruminant production systems are focused primarily on forages, especially regarding grassland feeds (Givens & Owen, 2000). Therefore, pasture forage quality must meet or exceed the animal's nutritional requirements to ensure the appropriate level of health and production performance

is being attained (Givens & Owen, 2000). Minerals are important nutrients that alter an animal's overall performance. According to Arthington & Ranches, (2021) and McDowell, (1996) forage is the leading source of trace minerals that grazing cattle will receive on an annual basis. However, this can sometimes be difficult and complicated to achieve due to multiple environmental and production management factors. Soil mineral concentrations can directly influence the forage diversity and quality of pasture and rangeland, which can result in either an incomplete or excessive amount of nutrients. Mineral supplementations such as mineral blocks, loose mineral, or other mineral alternatives are necessary to provide adequate minerals to grazing animals to prevent deficiencies from occurring. Proper supplement formulations can vary depending on availability of natural occurring minerals within a geological location or region. For example, general forage nutrient content for this project at ECRC can vary. Concentrations of Cu, Zn, and Mn can range from 5-8 mg/kg DM of Cu, 18-30 mg/kg DM of Zn, 48-68 mg/kg DM of Mn throughout a given year of production (Jalali et al., 2020).

Seasonal changes can also influence the availability of minerals to the plant and within the plant which can impact the overall nutrient quality and palatability of forages. Mineral uptake by plants can vary due complex factors including soil moisture and temperature, as well as intensity of grazing, utilization and maturation of herbage, and frequency of defoliation and fertilization (Reid & Horvath, 1980). These factors can influence the performance of forages and therefore directly impact the nutritional composition of the plants. Other factors that influence the uptake of minerals, include plant genus, species and variety, and fertilization (Reid & Horvath, 1980). Studies have concluded in Reid & Horvath (1980) that legume species usually contain higher concentrations of Co, Cu, Fe, Mo, and Zn compared to grasses, while Mn tended to have lower concentrations in legumes. Lastly, fertilization is primarily used to increase dry matter (DM) yield of crops to significant boost the output of crop production (Reid & Horvath, 1980). However, this can influence the mineral concentrations by decreasing desirable minerals or increasing undesirable minerals that plants have access to if the management of production is not balanced.

Trace elements metabolism has been extensively studied in plants where basic processes have been characterized regarding the roles of each trace element in plants. These roles include 1. Uptake/absorption and transport within a plant, 2. Enzymatic processes, 3. Concentrations and forms of occurrence, 4. Deficiency and toxicity, 5. Ion competition and interactions (Alina Kabata-Pendias, 1992). Plants able to take up metals above the established concentrations and more than other plant species from the same soils are called hyperaccumulators (Alina Kabata-Pendias, 1992). Prasad· & Hagemeyer, (1999) reported, based on a literature review, that hyperaccumulators should contain trace metals in leaves above the following levels for Cu, Mn, and Zn (ppm): >1000 – Cu, and >10,000 – Mn and Zn.

Copper:

In plants, Cu contributes to several physiological processes including photosynthesis, respiration, carbohydrate distribution, nitrogen reduction and fixation, cell wall metabolism, and protein metabolism (Alina Kabata-Pendias, 1992). Additionally, Cu is responsible for DNA and RNA production, which can affect and inhibit seed production or pollen sterility in plants during a deficit of Cu concentrations (Alina Kabata-Pendias, 1992).

Givens & Owen (2000) reports the lowest source of available Cu comes from fresh grass, as well as silage, hay, brassicas, and certain cereal grains.

Zinc:

In plants, Zn is responsible for essential metabolic processes such as activation of components of a variety of enzymes (dehydrogenases, proteinases, peptidases, and phosphohydrolases) (Alina Kabata-Pendias, 1992). Additionally, Zn tends to be readily available depending on the level of solubility, rate of absorption, and both plant species and growth media (Alina Kabata-Pendias, 1992). Although, there is general agreement of the main forms of uptake of both hydrated Zn and Zn^{2+} form there has not been an accurate definition of the form that is absorbed by the roots (Alina Kabata-Pendias, 1992). Basic physiological functions of Zn include metabolism of carbohydrate, proteins, and phosphate and also to auxins, RNA, and ribosome formation. Additionally, evidence has been shown that Zn influences and stabilizes cellular components, as well as microorganism systems(Alina Kabata-Pendias, 1992).

In general, Zn deficiencies is rare with ruminants grazing forages, however, supplement studies have found forages low in Zn has resulted in impaired reproductive performance and decreased weight gains (Fahey, 1994). Zinc requirements of ruminants fed forage diets are poorly defined, however, there is evidence that suggests that Zn is affected by dietary or physiological factors (Fahey, 1994).

Manganese:

Forages rarely lack inadequate amounts of Mn for grazing cattle. Therefore, there are very few reported accounts of Mn deficiencies in beef cattle (Arthington & Ranches, 2021). Manganese requirements for ruminant growth are relatively low, typically 10 $mg \cdot kg^{-1}$ DM, however, normal reproduction requirements are higher, 20 to 40 $mg \cdot kg^{-1}$ DM (Fahey, 1994). Concentrations of Mn vary significantly in forages but are generally around 20 $mg \cdot kg^{-1}$ (Fahey, 1994). Some studies reported by Fahey (1994) have mentioned that high dietary concentrations of Ca and P may increase the requirements of Mn.

Sulfur:

Sulfur, compared to other minerals, is difficult to analyze in biological materials, which results in a scarcity of S analysis (Givens & Owen, 2000). However, Givens & Owen (2000) reported that temperate forages are generally adequate in S compared to tropical forages. Forages, high in S combined with Mo will create thiomolybdates, which can bind up available Cu resulting in a Cu deficiency in forages (Givens & Owen, 2000). Sulfur fertilization of sulfur – deficient soils has been reported to increase forage yield of temperate forages, increase leaf percentage, and increase sulfur concentrations (Fahey, 1994; Givens & Owen, 2000). Additionally, Spears et al. (1985) reported that digestibility of fiber was higher in steers that were provided sulfur-fertilized hay (orchard grass) compared to the control hay. Although, S requirements that influence S availability to ruminants fed forage-based diets are poorly defined, S is required for protein formation through rumen microbial production (Givens & Owen, 2000; Spears et al., 1985). Therefore, adequate concentrations of S are necessary to maintain healthy soil and plant environments, which can directly influence livestock production performance.

Mineral Supplementation

There are a number of methods of delivering mineral supplements to the animal to ensure it is receiving the appropriate quantities of TM based on the studied requirements. A few of the most common methods include dietary inclusion, free-choice mineral supplementation, intramuscular (IM) or subcutaneous (SQ) injections, protein supplementation, and bolus and drenching administration.(Arthington & Ranches, 2021). Arthington & Ranches (2021) also suggests that injections should be utilized more as a complementary tool in situations where oral TM supplementation has been unsuccessful. In many grazing situations, free-choice mineral supplements or mineral blocks are the most common, which are typically blended or pressed with salt to regulate consumption (Arthington & Ranches, 2021). However, these methods generate challenging situations to monitor intakes of individual animals, especially as forage quality changes across seasons (Arthington & Ranches, 2021).

To manage the intake of minerals or forage, free-choice salt can be utilized as an important tool. During periods of drought, cattle can have an increased risk of mineral deficiency due to low mineral availability in forages, as well as decreased intakes of forages due to plant species being less palatable (McDowell, 1996). Mineral supplement intakes tend to increase over winter or dry seasons (McDowell, 1996). Furthermore, mineral supplement intakes tend to be lower during wet seasons or when forage quality is optimal (McDowell, 1996). Nutritive composition of forages is highly variable depending on the species of plant and seasonal status (Givens & Owen, 2000). This can significantly alter the contribution of nutrients available for livestock utilization in a production system (Givens & Owen, 2000).

Along with delivery and seasonal considerations of TM, the source of mineral can also influence the rate of consumption and bioavailability. Some of these sources include inorganic (sulfate, nitrate, and oxides), organic (amino acid and protein chelates, and metal propionates), and hydroxy compounds. Chapman & Bell (1963) reported that Cu nitrate ($CuNO_3$), and Cu sulfate ($CuSO_4$) had the highest absorption rates and Cu oxide (CuO) had one of the lowest absorption rates. In contrast, Springman et al., (2021) reports that hydroxy Cu chloride had a low water solubility compared to Cu sulfate. Additionally, Spears et al., (2004) discovered hydroxy Cu chloride to be more bioavailable than Cu sulfate when

supplemented with sulfur (S) or molybdenum (Mo) to beef cattle. The increase in bioavailability may be due to the lower solubility of hydroxy CuCl in neutral or slightly acidic environments such as the rumen environment of grazing beef cattle (Spears et al., 2004). Similar to Cu hydroxy chloride, Zn hydroxychloride, and Mn hydroxychloride are crystalline mineral sources connected by covalent bonds between the trace mineral and a hydroxy group (Ryan et al., 2015). Recently, Genther and Hansen (2015) confirmed that Cu, Mn, and Zn from these hydroxy sources were relatively insoluble in the rumen but increased once in the abomasum, which increases dietary levels compared to sulfate sources. However, according to a study by Vanvalin et al., (2019) they observed that steers receiving a Cu supplement from a variety of sources (organic, inorganic, and hydroxy treatments) and concentrations (5 or 10 mg Cu/kg DM) performed better compared to steers (Control treatment) that were not receiving any Cu supplement.

TRACE MINERAL METABOLISM

Functions

Trace mineral functions within mammals can be grouped into 4 categories based on function: 1) structural organization of organs, tissues, cells, and molecules, 2) conservation of physiological processes, 3) catalytic and endocrine reactions of enzymes and hormones, and 4) regulation of cellular replication (Suttle, 2010b). Arthington & Ranches (2021) mentions there are 10 trace minerals that are known to be essential to cattle health. These include Cr, Co, Cu, I, Fe, Mn, Mo, Ni, Se, and Zn. From these 10 essential minerals: Se, Cu, Zn, Mn, I, and Co, are the main minerals relevant to grazing cattle (Arthington & Ranches, 2021). The minerals focused on in this review: Cu, Zn, and Mn are predominantly responsible for enzymatic activation and cellular replication and regulation (McDowell, 2003).

When essential minerals are inadequately supplied, toxicities and deficiencies can develop that result in imbalances in overall homeostasis. According to Ahola et al (2004), trace mineral deficiencies can occur as either primary or secondary deficiencies. Primary trace mineral deficiencies result from insufficient intake of trace mineral. While secondary deficiencies occur when one or more mineral antagonists are present that impact the availability of specific trace minerals (Ahola et al., 2004). Mineral

deficiencies often arise due to the animal's inability to effectively absorb or metabolize antagonistic compounds that are bound to specific elements or render the element insoluble (Ahola et al., 2004).

Copper:

Copper is essential for major biological processes and functions that occur in beef cattle. Copper has been reported to be involved in enzyme activation and function, immune function, tissue and bone growth, hair pigmentation, and reproduction (Suttle, 2010, Arthington & Ranches, 2021). Copper influences the activation of multiple enzymes, cofactor, and protein, such as ceruloplasmin (assists with transfer), cytochrome c oxidase (an enzyme present in the electron transport chain), Cu-Zn superoxide dismutase (an enzyme that protects cells from oxidative stress), tyrosinase (an enzyme that converts tyrosine to melanin for hair pigmentation; (McDowell, 2003). These enzymes are present in a variety of concentrations and tissues depending on function and demand in tissues (McDowell, 2003).

Copper has been reported to be the second most common deficient mineral in beef cattle, second only to Se (Arthington & Ranches, 2021). The most common sign of Cu deficiency is abnormal hair pigmentation, however, decreased fertility in cows and bulls, slow growth, improper bone development, leg and hoof lameness, along with ataxia also have been observed in response to a Cu deficiency (Larson et al., 1992). The leading cause of Cu deficiency in beef cattle is typically due to a secondary Cu deficiency caused by elevated dietary Mo, S, Fe, and Zn (Larson et al., 1992). The interaction between Mo and S, which creates thiomolybdates, is one of the most well-known Cu antagonists that binds up with Cu within the rumen, as well as be absorbed through the intestinal wall to bind with Cu containing compounds, such as albumin (Gould & Kendall, 2011). Thiomolybdates are sometimes viewed as a Cu deficiency, however, they are actually a thiomolybdate toxicity, which can be treated with increased Cu supplementation (Gould & Kendall, 2011). Pogge et al., (2014) reports that for every 1 mg of Mo/kg present in a diet available Cu should increase to 8 mg Cu/kg to counter the toxicity. Other antagonistic interactions such as, iron sulfide (FeS) contribute to Cu becoming unavailable by inhibiting Cu from binding to the appropriate proteins or functioning improperly (CuS) (Arthington & Ranches, 2021; López-Alonso & Miranda, 2020).

Additionally, S can decrease Cu bioavailability by the formation of insoluble Cu sulfides (CuS, Cu2S) where the maximum tolerable level (MTL) is 4 g S/kg DM for steers (López-Alonso & Miranda, 2020).

Other factors such as cattle breed, frame size, growth rate, and sources of feed and water (Larson et al., 1992) can impact the Cu requirements for cattle. For example, Simmental cattle require approximately twice the amount of dietary Cu compared to Angus cattle (Larson et al., 1992). Ward et al., (1994) discovered that Angus heifers had greater Cu concentrations compared to, Simmental and Charolais heifers, when animals were not fed supplemental Cu for 140 d. Additionally, Ward et al. (1994) reported that Angus steers had greater Cu absorption and retention compared to Simmental steers.

Zinc:

Zinc influences multiple essential functions within the body associated with enzyme and protein activation, hormone development and storage, growth development, skin and wound healing, immune regulation, and central nervous system (CNS) function. (McDowell, 2003). Hormone receptor, responsiveness, production, storage, and secretion all depend on adequate amounts of Zn for proper function (McDowell, 2003). Furthermore, Zn is involved in over 200 enzymes activations that are essential for protein synthesis, carbohydrate metabolism, transcription and translation, and regulation of gene expression (Ahola et al., 2004, McDowell, 2003). Suttle (2010a) reported that Zn is responsible for the structural and functional integrity of over 2000 transcription factors and is essential for the formation of 'zinc-finger' domain in DNA binding proteins. Testosterone, insulin, and adrenal corticosteroids are hormones that most impacted during Zn deficiencies (McDowell, 2003). Abnormal functionality and development has been reported in male reproductive organs in calves that were deficient in Zn (McDowell, 2003). Suttle (2010a) reports hypogonadism was present in Zn-deprived bull calves, kids, and ram lambs.

Manganese:

Similar to Cu and Zn, Mn influences enzyme activity and reproduction, as well as bone development, lipid and carbohydrate metabolism, cell function and structure, immune health, and CNS function (McDowell, 2003). Much of the research that has been conducted on Mn functions are primarily

focused on poultry, since monogastric animals are more susceptible to deficiencies compared to ruminants (Ahola et al., 2004; McDowell, 2003).

Manganese can operate as both an enzyme activator and constituent of metalloenzymes (McDowell, 2003). Manganese metalloenzymes are limited to arginase, pyruvate carboxylase (lipid and carbohydrate metabolism), and manganese-superoxide dismutase (cell protection; Suttle, 2010a, McDowell, 2003). However, there are many enzymes dependent on Mn for activation, including: hydrolase, kinases, decarboxylases, and transferases (McDowell, 2003). Glycosyltransferase is one of the most recognized and studied transferases. Manganese is required for the activation of glycosyltransferase where its main responsibility is the synthesis of mucopolysaccharides for the development of the organic matrix in bone (McDowell, 2003).

Sulfur:

Metabolically, S is processed differently depending on the digestive tract of an animal (i.e., ruminant vs monogastric; McDowell, 2003). Sulfur is necessary to the microflora in the rumen to allow the production of essential amino acids to occur such as methionine, lysine, isoleucine, threonine, and leucine. However, regardless of digestive tract system, S provides support and functions to important physiological processes including, S amino acid formation, hormone and vitamin development, and creation of key enzyme and molecule components (McDowell, 2003). Hormones, such as insulin and oxytocin contain S, as well as the vitamins thiamin and biotin (Suttle, 2010a). Methionine, cystine, cysteine, homocysteine, cystathionine, and taurine are amino acids that share a dependency for S and play an essential role in protein structure (McDowell, 2003). Without the presence of adequate S in the rumen, rumen microbial populations cannot provide cattle, and other ruminants, the essential S-containing amino acids (McDowell, 2003; Suttle, 2010a). Bull & Vandersall, (1973) reports there is a noteworthy relationship with S and microbial cellulose digestion. The authors observed by increasing S levels from 0.20% to 0.34% it increased ADF digestibility from 33.7% to 42.3%. They concluded that the availability of supplemental S appears to be more conducive to metabolic response compared to S present within the diet. This suggests a rate limiting step at the rumen bacteria level for S (Bull & Vandersall, 1973). Signs of S deficiency are reported as loss of weight,

weakness, lacrimation, dullness, and death, as well as reduced microbial protein synthesis and signs of protein malnutrition (Givens & Owen, 2000).

Absorption

Copper:

In most mammals, and depending on species, absorption of Cu is limited to the small intestines (Evans, 1973). In small quantities Cu is transported from the intestinal mucosal cell wall to the serosal side of the enterocyte and within the cell cytoplasm of the enterocyte (Figure 1.1, Cater & Mercer, 2005; Evans, 1973; Cousins, 1985). Studies have shown that Cu absorption is facilitated by mineral specific proteins, such as albumin, ceruloplasmin, copper transporter protein (Crt1 & Crt2), cytochrome c oxidase (COX17) and non-specific divalent metal transport proteins (DMT; Suttle, 2010a). Although DMT is not considered to be involved in the regulation of Cu uptake when Cu is limited or other trace elements are absent, it is known to be involved with Cu, Zn, Fe, and Mn uptake (Hill & Link, 2009; Cater & Mercer, 2005). Copper transporter protein have three transmembrane regions, which creates a channel for Cu to be transported across the cytosol of the cell to an awaiting binding protein (Hill & Link, 2009). The rate of Cu absorption can vary with the source of Cu available. Much of the Cu released from ruminal digestion is typically unabsorbable once precipitated as copper sulfide (CuS) (Suttle, 2010a). Cu nitrate ($CuNO_3$), and Cu sulfate ($CuSO_4$) had the highest absorption rates (Chapman & Bell, 1963). Furthermore, the study discovered that Cu oxide (CuO) had one of the lowest absorption rates (Chapman & Bell, 1963).

Zinc:

A majority of studies that researched the mechanisms of Zn absorption have been focused primarily on species such as mice and rats, where little has been conducted on ruminants. Zinc is absorbed mainly from the small intestines, rumen, and reticulum. However, the majority of activity being focused on the duodenum or proximal portion of the small intestines (Figure 1.1, Miller, 1970). Studies have reported that Zn-specific transporters exist in two families. The first transporter ZnT1 protein (solute-linked carrier 30 (SLC30)) was discovered in 1995. This transporter mediates the influx of Zn into the cells by regulating the efflux or influx from intercellular vesicles (Lichten & Cousins, 2009). The Zrt- and Irt-like proteins

(SLC39A) (ZIP) is the second transporter that was discovered. The Zrt- and Irt-like proteins transport Zn from the extracellular fluid of from intracellular vesicles into the cytoplasm (Lichten & Cousins, 2009).

Zinc absorption occurs by first becoming soluble within the stomach where it is exposed to a pH of approximately 2-3. Once it proceeds to the lumen of the small intestine the majority soluble Zn binds to a ZIP-4 transporter that is located on the apical membrane of the enterocyte (Cousins et al., 2006). Zinc is transported to the cytosol of the enterocyte where it then is transported across the cytosol by a cysteine-rich intestinal binding protein (CRIP) to bind to a ZnT1 protein to transport Zn out of the cell and connect to albumin to distribute it throughout the body (Cousins et al., 2006; Guimaraes, 2020).

Absorption of dietary Zn ranges from approximately 15 - 60% of either free ions or complexes with amino acids (McDowell, 2003). However, Suttle (2010a) reported that cattle and sheep can reach a maximal efficiency of 75% absorption of Zn, primarily through the duodenal enterocytes. The rate of absorption can be affected by the source and amount of Zn available in the diet (Ahola et al., 2004). Additionally, the use of chelated agents such as ethylene diamine tetra-acetic acid (EDTA) can increase the absorption of Zn (Ahola et al., 2004).

Manganese:

Hidiroglou (1979) reports the majority of Mn is absorbed from the small and large intestines. Manganese is absorbed in a two-part process by taking it up in the gut and then transferred across the mucosal cells (Figure 1.1, McDowell, 2003). Manganese absorption is thought to be transported from the lumen by a divalent metal transporter 1 (DMT1) that is located on the apical membrane of the enterocyte (Underwood & Suttle, 1999). Once absorbed Mn can be distributed throughout the body by bound to a Fe-specific transporter protein, transferrin (Underwood & Suttle, 1999). Hidiroglou (1979) reports multiple experiments have been carried out with cattle that suggests Mn is only absorbed, in the intestines, at a fraction (approximately 0.5-1%) of dietary concentrations. However, Suttle (2010a) suggests that Mn absorbed by the same transporter proteins as Fe: ferritin, DMT1, and transferrin. Dietary Mn is poorly absorbed in nearly all species of livestock and some evidence suggest that high Ca and P may contribute to

the reduction in bioavailability (Spears, 2003). However, different sources of Mn can, such as Mn sulfate, Mn proteinate, and Mn methionine are absorbed to a greater extent than other sources (McDowell, 2003; Spears, 2003). Iron and Mg deficiencies can result in an increase of absorption of Mn. Although P is probably the true antagonist of Mn, Ca can still influence the absorption of Mn through the high levels of dietary calcium phosphate. Availability rates of Mn vary from 3 to 25% and cattle typically absorb approximately 1% of Mn that is ingested (McDowell, 2003). Young calves have greater absorption compared to other species (McDowell, 2003).

Sulfur:

Predominately, studies have shown that S is needed within the ruminant animal in the form of sulfur bearing amino acids. Whether that is in the provided form or through synthesis of the microflora of the rumen. Animal growth and nitrogen retention of the microflora in the rumen might be a key indicator for bioavailability and absorption of S (Henry & Ammerman, 1995). Sulfur absorption occurs mainly within the rumen via the rumen wall, as well as the small intestine (McDowell, 2003; Suttle, 2010a). A small proportion of S is also secreted in the saliva in both inorganic and organic forms, which is recycled in the rumen once ingested. Cattle tend to have higher levels of S in the saliva compared to sheep (McDowell, 2003). Due to the multiple varieties of organic and inorganic sources, S can be utilized that allows for many S-containing compounds that can be available for absorption (Goodrich & Garrett, 2015). Compounds such as SO_4^- and HS^- are absorbed through the rumen and intestines. Sulfate is reduced from SO_4^- to S_2^-, which is then implemented into bacterial protein (Suttle, 2010a). Additionally, S-containing amino acids and B-vitamins (thiamine and pantothenic acid) are absorbed from the small intestines (Goodrich & Garrett, 2015). Ruminal protozoa are important for producing essential S_{2-} for S-containing proteins within the rumen and post ruminal stages (Suttle, 2010a). However, excess amounts of S_{2-} can be toxic to cattle and introduce issues with enzyme activation, metabolic systems, and cell and tissue homeostasis once absorbed into the bloodstream through the ruminal wall (McDowell, 2003). Active transport is the main method of passage of inorganic sulfate that moves through the iliac walls of the small intestines where further digestion occurs to be incorporated into bacterial protein (McDowell, 2003; Suttle, 2010a).

Transportation and Storage

Copper:

Copper naturally exists in the +2 redox state and can alter states by accepting and donating electrons. This redox state can result in free radicals causing damage to cells and tissues if Cu is not bound to a transporter protein (Hill & Link, 2009). Copper can be transported by erythrocyte superoxide dismutase (SOD), plasma albumin, plasma ceruloplasmin (ferroxidase), unidentified Cu complex in erythrocyte, and plasma amino acids (Evans, 1973). Superoxide dismutase is a protein that removes superoxide anions (free radical) that are damaging to the body (Evans, 1973). Evans (1973) reports that SOD is associated with 60% of the total Cu in the red blood cells. Albumin is one of the most abundant proteins involved in trace mineral transport (Cater & Mercer, 2005). However, Cu transport to tissues can proceed without the presence of albumin (Suttle, 2010a). Additionally, (Cater & Mercer, 2005) reports that albumin is not an essential component for Cu transport within the blood. Furthermore, Cater & Mercer (2005) states, only 12% of serum Cu is bound to albumin and small traces are connected to a variety of enzymes that are responsible for clotting factors and smaller proteins. Ceruloplasmin is synthesized in the liver and has been reported to transport 95% of plasma Cu for distribution throughout the peripheral tissues, however, the functions of ceruloplasmin are still unclear regarding Cu transport (Evans, 1973; Cater & Mercer, 2005; Fife et al., 1994). Evans (1973) states that ceruloplasmin constitutes to 60-99% of total plasma Cu and, similarly, Cater & Mercer (2005) reports that approximately 70% of the majority of serum Cu is contained in ceruloplasmin. It appears that Cu transportation and distribution to extrahepatic tissues involve multiple of molecules that are responsible for providing a balance of Cu throughout the body (Cater & Mercer, 2005). A small fraction of Cu can be filtered from plasma and the amino acid-bound fraction of Cu represents a small proportion of the total plasma Cu (Evans, 1973). These Cu complexes are important components of the Cu transport mechanism and can facilitate the transportation of Cu across cell membranes by being attached to an amino acid (Evans, 1973).

The liver is the initial repository for newly absorbed Cu, and most is either secreted into the blood (bound to ceruloplasmin) or excreted from the body in the bile (Cater & Mercer, 2005). In mature animals,

of most species, the liver contains approximately 10-50 mg of Cu/kg DM. However, in mature ruminants the liver can contain 100-400 mg Cu/kg (Ahola et al., 2004). This is possibly due to an improved capacity for cattle to bind Cu in the liver (Ahola et al., 2004). Although, Cu is present in high concentrations in the liver, over half of the total body Cu is located in the muscle and bone (Ahola et al., 2004).

Zinc:

Zinc is widely utilized and distributed throughout the body; however, the stockpiles of readily available Zn exist in small quantities (McDowell, 2003). Most concentrations of Zn within tissues range from 30 to 250 mg Zn/kg DM, compared to the rest of the body, which varies from 20 to 30 mg Zn/kg DM (Ahola et al., 2004). Zinc is present within most tissues; however, it is specifically concentrated within the liver, kidney, pancreas, and intestine (McDowell, 2003). In sheep, bone is the primary tissue responsible for Zn storage (Suttle, 2010a).

Manganese:

Absorbed Mn can remain as a free element or quickly bind to an α2-macroglobulin before circulating to the liver (McDowell, 2003). Similar to Fe, Mn is also transported by plasma transferrin receptors within the liver (McDowell, 2003). Davidson & Lonnerdal (1989) concluded that regardless of administration, transferrin is the main plasma carrier protein for Mn.

Manganese is one of the least abundant elements in tissues of all livestock species and ranges from 0.6 – 3.9 mg kg^{-1} of fresh weight in cattle and sheep (McDowell, 2003). Manganese is broadly distributed throughout the body in small quantities; however, higher concentrations of Mn are found in the skeletal system, liver, kidney, and pancreas compared with the skeletal muscle(McDowell, 2003). High concentrations of Mn have been identified in the liver of young calves following Mn supplementation to the dam prior to partuition (McDowell, 2003). Guimaraes (2020) mentions that fluctuations of Mn concentrations occur frequently within the liver and appears to resist changes when the liver reaches certain Mn concentrations.

Sulfur:

Sulfur leaves the rumen in several different forms specifically as intact dietary microbial and fungal protein (Suttle, 2010a). Due to these relatively stable forms, S can be transported and distributed throughout the body easily through the bloodstream. The primary organ responsible for storing S-containing compounds is the liver, however, high quantities can be present within the muscle (Rammell et al., 2011).

Excretion

Copper:

Liver Cu concentrations are maintained in the body by regulating the amount of Cu that is released into the bile (Cater & Mercer, 2005). Approximately 50% of dietary Cu is absorbed by the liver within 10 minutes upon entry of the hepatic-portal system (Cater & Mercer, 2005). Copper is typically excreted through the urine, bile, and sloughed intestinal cells (Guimaraes, 2020). However, urinary excretion tends to be in small quantities, while the majority of excretion of Cu is through the feces (Cater & Mercer, 2005; Guimaraes, 2020).

Zinc:

Whether injected or ingested, Zn is predominantly excreted through the feces, while smaller quantities are released in the urine (Suttle, 2010a; McDowell, 2003). Urinary excretion of Zn can increase if chelating agents, such as EDTA, are given in combination with the mineral element.

Manganese:

The amount of Mn that the body absorbs on a daily basis is typically between 25 – 50% of the Mn concentrations present in the diet (McDowell, 2003). Any excess Mn is excreted in the bile and then through the feces (95 – 98%). Urine excretion of Mn comprises a small percentage of the Mn excreted (0.1 – 0.3%; McDowell, 2003). However, pancreatic juices secreted into the intestine has also been identified as a mechanism to excrete Mn from the body (Guimaraes, 2020 ;McDowell, 2003). Biliary excretion is essential for homeostasis of Mn to help balance Mn concentration and prevent deficiencies or toxicities of Mn (McDowell, 2003). McDowell (2003) suggests that Mn can be reabsorbed once in the bile, as well as each individual atom can be recirculated multiple times before finally being expelled.

Sulfur:

Sulfur is primarily excreted in the urine in the form of sulfate (SO_4), which originates from the oxidation of sulfides, sulfur-containing amino acids (i.e., cystine, taurine, thiosulfates), ethereal S, and other organic molecules (Suttle, 2010a). Sulfates are filtered in the kidneys at the glomerulus where filtration rates exceed tubular reabsorption rates (Suttle, 2010a). However, when molybdate is present, SO_4 will compete for renal tubular reabsorption, which can impede molybdenum from being reabsorbed (Suttle, 2010a). Sulfur can also be excreted through the feces primarily in undegradable organic and inorganic forms (McDowell, 2003;Suttle, 2010a).

TABLE AND FIGURES

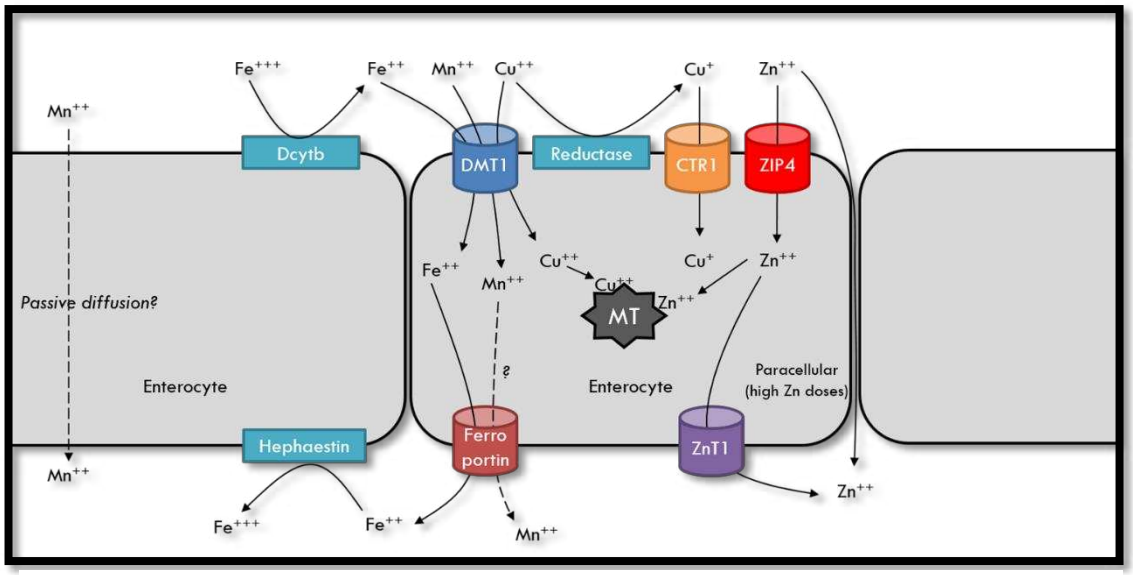


Figure 1.1. Mechanism of action of Cu, Zn, and Mn. [Adapted from *Micronutrients USA LLC (Indianapolis, IN)*]

LITERATURE CITED

Adriano, D. C. (2001). Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals (Vol. 860). New York: Springer.3:11

Ahola, J. K., Baker, D. S., Burns, P. D., Mortimer, R. G., Enns, R. M., Whittier, J. C., ... & Engle, T. E. (2004). Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period. Journal of animal science, 82(8), 2375-2383.

Allen, V. G., Batello, C., Berretta, E. J., Hodgson, J., Kothmann, M., Li, X., ... & Sanderson, M. (2011). An international terminology for grazing lands and grazing animals. Grass and forage science, 66(1), 2.

Arthington, J. D., & Ranches, J. (2021). Trace Mineral Nutrition of Grazing Beef Cattle. Animals, 11(10), 2767.

Bull, L., & Vandersall, J. (1973, January). Sulfur Source for in Vitro Cellulose Digestion and in Vivo Ration Utilization, Nitrogen Metabolism, and Sulfur Balance. Journal of Dairy Science, 56(1), 106–112. https://doi.org/10.3168/jds.s0022-0302(73)85122-7

Cater, M. A., & Mercer, J. F. (2005). Copper in mammals: mechanisms of homeostasis and pathophysiology. In Molecular Biology of Metal Homeostasis and Detoxification (pp. 101-129). Springer, Berlin, Heidelberg

Chapman Jr, H. L., & Bell, M. C. (1963). Relative absorption and excretion by beef cattle of copper from various sources. Journal of animal science, 22(1), 82-85.

Cousins, R.J. (1985) Absorption, transport and hepatic metabolism of copper and zinc with special reference to metallothionein and caeruloplasmin. Physiological Reviews 65, 238- 309.

Cousins, R. J., Liuzzi, J. P., & Lichten, L. A. (2006, August). Mammalian Zinc Transport, Trafficking, and Signals. Journal of Biological Chemistry, 281(34), 24085–24089. https://doi.org/10.1074/jbc.r600011200

Davidson, L. A., & Lonnerdal, B. (1989). Fe-saturation and proteolysis of human lactoferrin: effect on brush-border receptor-mediated uptake of Fe and Mn. American Journal of Physiology-Gastrointestinal and Liver Physiology, 257(6), G930-G934.

Dixon, J. B., Weed, S. B., & Parpitt, R. L. (1990). Minerals in soil environments. Soil Science, 150(2), 562.

Evans, G. W. (1973). Copper homeostasis in the mammalian system. Physiological reviews, 53(3), 535-570.

Land use in agriculture by the numbers. (n.d.). Food and Agriculture Organization of the United Nations. Retrieved September 28, 2022, from https://www.fao.org/sustainability/news/detail/en/c/1274219/

Fahey, G. C., & Spears, J. W. (1994, June). Forage Quality, Evaluation, and Utilization. Minerals in Forages (Pages: 281-317). https://doi.org/10.2134/1994.foragequality

Genther, O. N., & Hansen, S. L. (2015). The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. Journal of Dairy Science, 98(1), 566–573. https://doi.org/10.3168/jds.2014-8624

Givens, D. I., Owen, E., Omed, H. M., & Axford, R. F. E. (2000). Forage evaluation in ruminant nutrition. CABI.

Gould, L., & Kendall, N. R. (2011, June 1). Role of the rumen in copper and thiomolybdate absorption. Nutrition Research Reviews, 24(2), 176–182. https://doi.org/10.1017/s0954422411000059

Goodrich, R. D., & Garrett, J. E. (2015, October 26). Sulfur in Livestock Nutrition. Agronomy Monographs, 617–633. https://doi.org/10.2134/agronmonogr27.c23

Guimaraes, O., Jalali, S., Wagner, J. J., Spears, J. W., & Engle, T. E. (2021). Trace mineral source impacts rumen trace mineral metabolism and fiber digestion in steers fed a medium-quality grass hay diet. Journal of Animal Science, 99(9), skab220.

Henry, P. R., & Ammerman, C. B. (1995). Sulfur bioavailability. Bioavailability of Nutrients for Animals, 349–366. https://doi.org/10.1016/b978-012056250-3/50043-3

Hidiroglou, M. (1979). Manganese in ruminant nutrition. Canadian Journal of Animal Science, 59(2), 217-236.

Hill, G. M., & Link, J. E. (2009). Transporters in the absorption and utilization of zinc and copper. Journal of animal science, 87(suppl_14), E85-E89.

Hofmann, R. R. (1989). Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia, 78(4), 443-457.

Kabata-Pendias, A. (2000). Trace elements in soils and plants. CRC press.

Larson, Robert L.; Arthington, J.D.; and Corah, L.R. (1992) "Interrelationship between copper and bovine health," Kansas Agricultural Experiment Station Research Reports: Vol. 0: Iss. 1. https://doi.org/10.4148/ 2378-5977.2198

Lichten, L. A., & Cousins, R. J. (2009, August 1). Mammalian Zinc Transporters: Nutritional and Physiologic Regulation. Annual Review of Nutrition, 29(1), 153–176. https://doi.org/10.1146/annurev-nutr-033009-083312

López-Alonso, M., & Miranda, M. (2020). Copper supplementation, a challenge in cattle. Animals, 10(10), 1890.

MacPherson, A. (2000). 17 Trace-mineral Status of Forages. Forage evaluation in ruminant nutrition, 345.

Mc Kenzie, R. (2018, September 11). Manganese Oxides and Hydroxides. SSSA Book Series, 439–465. https://doi.org/10.2136/sssabookser1.2ed.c9

Mc Kenzie, R. M. (1989). Manganese oxides and hydroxides. Minerals in soil environments, 1, 439-465.

McDowell, L. R. (1996). Feeding minerals to cattle on pasture. Animal feed science and technology, 60(3-4), 247-271.

Miller, W. J. (1970). Zinc nutrition of cattle: a review. Journal of Dairy Science, 53(8), 1123-1135.

Minerals in Animal and Human Nutrition. (2003). Minerals in Animal and Human Nutrition: Second Edition. https://doi.org/10.1016/b978-0-444-51367-0.x5001-6

Nutrient Requirements of Beef Cattle, 8th Revised Edition. (2016). Nutrient Requirements of Beef Cattle, 8th Revised Edition. https://doi.org/10.17226/19014

Pogge, D. J., Drewnoski, M. E., & Hansen, S. L. (2014). High dietary sulfur decreases the retention of copper, manganese, and zinc in steers. Journal of Animal Science, 92(5), 2182–2191. https://doi.org/10.2527/jas.2013-7481

Prasad, & Hagemeyer. (2014, March 12). *Heavy Metal Stress in Plants: From Molecules to Ecosystems*. Springer.

Rammell, C., & Hill, J. (1986, December). A review of thiamine deficiency and its diagnosis, especially in ruminants. New Zealand Veterinary Journal, 34(12), 202–204. https://doi.org/10.1080/00480169.1986.35350

Reid, R. L., & Horvath, D. J. (1980). Soil chemistry and mineral problems in farm livestock. A review. Animal feed science and technology, 5(2), 95-167.

Ryan, A. W., Kegley, E. B., Hawley, J., Powell, J. G., Hornsby, J. A., Reynolds, J. L., & Laudert, S. B. (2015). Supplemental trace minerals (zinc, copper, and manganese) as sulfates, organic amino acid complexes, or hydroxy trace-mineral sources for shipping-stressed calves. The Professional Animal Scientist, 31(4), 333–341. https://doi.org/10.15232/pas.2014-01383

Spears, J. W., Kegley, E. B., & Mullis, L. A. (2004). Bioavailability of copper from tribasic copper chloride and copper sulfate in growing cattle. Animal Feed Science and Technology, 116(1-2), 1–13. https://doi.org/10.1016/j.anifeedsci.2004.06.002

Spears, J. W. (2003). Trace mineral bioavailability in ruminants. The Journal of nutrition, 133(5), 1506S-1509S.

Spears, J. W., Burns, J. C., & Hatch, P. A. (1985). Sulfur fertilization of cool season grasses and effect on utilization of minerals, nitrogen, and fiber by steers. Journal of Dairy Science, 68(2), 347-355.

Springman, S. A., Drewnoski, M. E., & Funston, R. N. (2021). Effects of hydroxy trace mineral supplementation on gain and reproductive performance in Beef Heifers. Livestock Science, 245, 104425. https://doi.org/10.1016/j.livsci.2021.104425

Suttle, N. F. (2010, October 19). *Mineral Nutrition of Livestock [OP] (CABI)* (4th ed.). CABI.

Ward, J. D., Spears, J. W., & Gengelbach, G. P. (1995). Differences in copper status and copper metabolism among Angus, Simmental, and Charolais cattle. Journal of Animal Science, 73(2), 571-577.

VanValin, K. R., Genther-Schroeder, O. N., Laudert, S. B., & Hansen, S. L. (2019). Relative bioavailability of organic and hydroxy copper sources in growing steers fed a high antagonist diet1. Journal of Animal Science, 97(3), 1375–1383. https://doi.org/10.1093/jas/sky487

CHAPTER 2 – EFFECTS OF TRACE MINERAL SOURCE AND CONCENTRATION ON PRODUCTION PARAMETERS THROUGHOUT ONE COW-CALF PRODUCTION CYCLE.

SUMMARY

An experiment was conducted to evaluate the effects of Cu, Zn, and Mn supplementation in the presence of elevated dietary sulfur on mineral status, production parameters, and cattle performance in a rangeland environment throughout a two-year period in eastern Colorado.

One hundred and eighty multiparous crossbred (Angus and Angus x Hereford) beef cows were blocked by body weight, age, and gestational status and randomly assigned to 1 of 3 free-choice mineral treatments (n=60 cows per treatment). Treatments were then assigned to 1 of 9 replicates (n=20 cows per replicate), resulting with 3 replicates per treatment. Supplement intakes were determined every 28 d. Liver biopsies and blood samples were obtained before the experiment was initiated (d -45), after calving (d 158 and 159), and after weaning (d 294) at the end of the first production year. Each replicate was rotated to a different pasture every 2-4 weeks to minimize overgrazing. Cows were weighed during each liver biopsy event and body condition scores were recorded. At weaning (d 260) all calves were weighed on two consecutive days. Over the first year of the experiment, cow BW, BCS, mineral status, mineral intake, and calf weaning weight were collected. There was no effect observed with these parameters during the first year of the experiment.

INTRODUCTION

Trace minerals (TM) supplementation is necessary for cattle operations to optimize key production parameters, such as growth, reproduction, and overall animal health, as well as the prevention of deficiencies (Suttle, 2010a; Arthington & Ranches, 2021). There are a variety of delivery systems to ensure the animal is receiving the appropriate quantities of TM based on the studied requirements. One of the most convenient methods of delivery is by either subcutaneous or intramuscular injection (Arthington & Ranches, 2021). However, the authors suggest that this method should be utilized more as a complementary tool in situations where oral TM supplementation has been unsuccessful. In most grazing situations, mineral supplements are blended with salt and offered as a free-choice mineral supplement (Arthington & Ranches, 2021). However, this method generates challenging situations to monitor and regulate intakes in grazing cattle, especially as forage quality changes across seasons (Arthington & Ranches, 2021).

Along with the delivery of TM, the source of mineral can influence TM bioavailability. Chapman and Bell (1963) reported that copper (Cu) nitrate ($CuNO_3$), and Cu sulfate ($CuSO_4$) had the highest absorption rates and Cu oxide (CuO) had one of the lowest absorption rates in steers. Additionally, Spears et al., (2004) reported hydroxy Cu chloride to be more bioavailable than Cu sulfate when supplemented with sulfur (S) or molybdenum (Mo) to beef cattle. The increase in bioavailability may be due to the lower solubility of hydroxy CuCl in neutral or slightly acidic environments such as the rumen environment of grazing beef cattle (Spears et al., 2004). Similar to Cu hydroxychloride, zinc (Zn) hydroxychloride, and manganese (Mn) hydroxychloride are crystalline mineral sources connected by covalent bonds between the trace mineral and a hydroxy group (Ryan et al., 2015). Recently, Genther and Hansen (2015) confirmed that Cu, Mn, and Zn from these hydroxy sources were relatively insoluble in the rumen but increased once in the abomasum, which increases dietary levels compared to sulfate sources.

Nutritional management during the breeding cycle of cows and for the lifetime of offspring is viewed as an essential management philosophy for optimizing the efficient production of beef. Several studies have either focused on the effects of trace mineral supplementation in cows or feedlot animals; however, little information is available on the effects of trace mineral supplementation through the entire cow-calf production cycle over multiple years. Furthermore, several experiments have shown that trace mineral sources can impact the bioavailability of certain trace elements. Therefore, the objective of this experiment was to determine the effects of trace mineral concentration and source on production parameters throughout the cow-calf production cycle while receiving elevated sulfur concentration in the free-choice mineral supplement. We hypothesized that beef cows supplemented with Cu, Zn, and Mn from hydroxy TM will have improved mineral status and production parameters compared to beef cows supplemented with concentrations of Cu, Zn, and Mn from sulfate mineral sources.

MATERIALS AND METHODS

Before initiation of this experiment, all animals use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee (Protocol # 2450).

Animals and Experimental Design

One hundred and eighty crossbred, multiparous (Angus and Angus x Hereford) beef cows were blocked by body weight, age, and gestational status and randomly assigned to 1 of 3 free-choice mineral treatments (n=60 cows per treatment; 3 replicates/treatment with n=20 cows per replicate). Treatments (n=3) consisted of free-choice mineral supplements containing Cu, Mn, and Zn from either IntelliBond or sulfate sources. Treatments 1 and 2 contained 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn, respectively. Supplement consumption was formulated for 113 $g \cdot animal^{-1} \cdot d^{-1}$ to provide approximately 1X NASEM (2016) requirements for Cu, Mn, and Z. A third treatment consisted of IntelliBond Cu, Mn, and Zn, supplied at approximately 0.5X NASEM (2016) requirements for Cu, Mn, and Zn. All free-choice mineral supplements contained 15 mg Co/kg DM from Co carbonate and 50 mg I kg DM from Ca iodate. All free-choice mineral supplements will be formulated to provide 0.15% supplemental S in the diet (DM

basis). The remainder of macro-and micro-minerals were formulated to meet or exceed NASEM (2016) requirements for beef cattle.

Free-choice mineral treatments were offered in amounts to allow ad libitum mineral intake throughout the course of the experiment. Each pasture replicate contained one cattle mineral feeder with a 50 kg capacity. Mineral feeders were checked approximately every two days throughout the experiment. When mineral was added the weight of the added mineral was recorded. Mineral intake for cows in each replicate pasture was determined every 28-d by summing the amount of mineral added to each mineral feeder over the 28-d period, subtracting the weight of the remaining mineral for that period, and dividing by the number of cows in that pasture replicate. When excess consumption was observed in all treatments, an additional 4.5 kg of salt was added to each 22.7 kg bag of mineral delivery to restrict excess intakes.

The project was initiated on January 21, 2021 (d 0), when cows began receiving mineral supplement treatments. Cows were maintained on native pastures that consisted primarily of blue grama (BG; *Bouteloua gracilis)*, needle-and-thread grass (NT; *Stipacomata*), and crested wheatgrass (CWG: *Agropyron cristatum*). Pasture replicates were rotated, and mineral weigh-backs were performed approximately every 28 d. Cows continued on their assigned treatments within their respective replicates for the remainder of the experiment. However, due to a series of unfortunate events treatment 2, replicate 2 was removed from the study (d 160). Cows calved between d 36 and d 96 in yr. 1 (Figure 2.1). Calves were weaned on d 260 of the experiment. Throughout the experiment, pasture forage, water, and basal stockpiled forage were sampled monthly for nutrient determination. Samples were collected and stored at -20°C until analysis could be performed.

Mineral Status

Liver biopsies and blood samples were obtained from 5 cows per replicate before the initiation of the experiment (d -45), after calving (d 158 and 159), and post weaning (d 294) for year one of the experiment (Figure 2.1). Liver biopsies were collected from 5 cows per replicate (n=45) – using the true-cut technique described by Pearson and Craig (1980) as modified by Engle and Spears (2000) to obtain a

50 mg sample of wet liver tissue (Figure 2.1). Liver biopsy sites were identified by locating the intercostal space between the 11th and 12th ribs on the right side of the animal. Biopsy sites were clipped of hair, scrubbed three times with Betadine, then scrubbed with 70% ethyl alcohol (alternating Betadine and &0% ethyl alcohol), and the area was locally anesthetized with 5 ml of lidocaine. A 1 cm incision was made with a scalpel blade between the 11th and 12th ribs on a line from the tubercoxae to the point of the shoulder. A core sample of liver was collected using a JamShedi bone marrow biopsy punch (0.7 cm in diameter to 14 cm in length) The biopsy probe was briefly inserted into the liver and negative pressure was applied with a 20-cc syringe to aspirate the sample into the biopsy probe. Instruments were rinsed in 50% Nolvasan and 50% deionized water solution and heat sterilized using a glass bead sterilizer after each biopsy procedure. Banamine (Flunixin Meglumine, 1.1 mg/kg BW, i.v.) and Oxytetracycline (19.8 mg/kg BW i.m.) was administered immediately post biopsy. Approximately 10 ml of blood was obtained via jugular venipuncture in trace mineral free vacutainer tubes (BD Vacutainer® SPC Plus metal free K_2EDTA 10.8 mg) at the time of liver biopsies to determine plasma concentrations focusing on Cu, Zn, and Mn. Blood samples were centrifuged at 1,000 x *g* for 20 minutes at room temperature before plasma was collected and stored separately. Plasma and liver samples were stored at -20ºC until analysis could be performed.

Liver and blood samples were processed by digesting samples in 70 % nitric acid (NO_3). Wet liver samples were weighed and then dried in an oven at 60°C for approximately 48 hours. Once dried DM weight was recorded and 1.5 ml of 70% NO_3 was added initially to dissolve samples. Additional NO_3 (1.5 ml) was added to all samples until samples were completely digested and in solution. Digested samples were then diluted (1:10) with deionized water. For plasma samples, 1 ml of plasma was taken and diluted with 1.0-1.5 ml of 70% NO_3 until sample was completely diluted. All samples were then analyzed via Flame Atomic Absorption Spectroscopy (AA Flame; PinAAcle 500 Instrument, PerkinElmer Inc. Waltham, MA). Pasture forage, water, and mineral samples were processed externally for nutrient composition (Dairy One Cooperative, Inc. Ithaca NY)

Chemical analysis of each supplement is shown in Table 1 and ingredient composition of free choice mineral supplements is presented in Table 2. Excluding Cu, Mn, and Zn, all other mineral and vitamin ingredients were added to the free-choice mineral supplement from the same sources for both treatments in concentrations to meet or exceed the NASEM (2016) recommendations requirements. Mineral treatments were provided in covered feeders located in a single location in each pasture and moved when replicates were rotated to a new pasture. Mineral was available in ad libitum quantities for all animals throughout the entirety of the experiment with a target intake of 113 $g \cdot cow^{-1} \cdot calf^{-1} \cdot d^{-1}$.

Cow Breeding

Cows from each replicate received artificial insemination (A.I.) following a 7-d CO-Synch protocol as described by Ahola et al., (2004). Seven days before breeding all cows received GnRH injections and a vaginally inserted-controlled internal drug-release (CIDRs), which was removed seven days later. Prostaglandin (Lutalyse) was administered along with an estrus-detection patch when CIDRs were removed. Cows that had estrus-detection patches deployed received AI within 12 hours after visual detection of a deployed patch. All other cows received AI 2 days after prostaglandin injection.

Pregnancy rates were determined by rectal ultrasonography by a state-licensed veterinarian approximately 45 d after AI. Cow BW and BCS were collected from all cows when blood and liver biopsies were collected.

Statistical Analysis

Cow performance, mineral status, mineral intake, and forage nutrient and mineral composition were assessed using a mixed-effect model, repeated measures analysis (SAS Institute Inc., Cary, NC) for a completely randomized block design. Cow performance, mineral intake, and mineral status contained fixed effects of treatment, time, and the treatment x time interaction. Pasture replicate was used as the experimental unit. When a significant difference was detected, means were separated using the PDIFF option of the LSMEANS statement in SAS. Significance was determined at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

RESULTS AND DISCUSSION

Mineral and CP content of diets

The supplement's nutrient formulation and composition are shown in Tables 1 and 2, respectively. Table 1 shows the ingredient composition of each treatment based on NASEM (2016) requirements and experiment hypothesis. Table 2 shows the chemical analysis and composition of minerals for each treatment. Forage nutrient concentrations of pasture samples that were collected during yr.1 are shown in Table 3. Copper, Mn, and Zn concentrations of forage samples collected during the experiment are shown in Figure 2.2. Forage Cu concentrations remained relatively consistent ranging from 3 to 12 mg Cu/kg DM over the first year of the experiment. With the exception of a slight decrease during mid spring, (April; d 70). In general, pasture forage Mn and Zn concentrations decrease in early spring (March – April; d 39 and d 70) and then gradually increased in late spring (May; d 100). Both Mn and Zn concentrations began to decrease again in early to mid-summer (May – June; d 100 and d 131). Overall, for yr. 1, Mn concentrations ranged from 20 to 123 mg/kg of DM and Zn concentrations ranged from 9 to 76 mg/kg of DM. Monthly crude protein (CP) concentrations of pastures are presented in Figure 2.4 and 2.5. Over yr. 1 CP concentrations of pasture forage decrease from February (d 11) to March (d 39) and then increased in May (d 100). Following the month of May, CP concentrations gradually decreased until reaching dormant forage winter concentrations in November (d 284).

Normal nutritional values of native and non-native range forages can vary greatly during abnormal and harsh periods of low moisture accumulation, high soil temperature, and diversity of plant species. However, typical crude protein (CP) values of the most common forage species from Eastern Colorado Research Center range from 6 to 12% for CWG, 8 to 9% for BG, and 5-10% for NT (Adair et al., 2021; NASEM 2016; Uresk & Sims, 1975)

On d 300 of the experiment, cows were removed from pastures placed into their assigned treatments and housed in dry-lots by treatment where they were provided a millet, grass hay, and silage mixed ration during winter months. The winter diet's nutritional composition is shown in Table 3. Water

samples were collected monthly with forage samples. Analyzed water samples were <0.01mg of Zn/L, <0.003 mg of Mn/L, and had no evidence of Cu throughout yr.1.

Free choice mineral intakes

Free-choice mineral intake by treatment is shown in Figure 2.3. Mineral intakes were not affected ($P < 0.99$) by treatment and averaged 220.2 g·cow-calf pair $^{-1}$·d^{-1}. Average mineral intake exceeded the formulated target intake of 114 g·cow-calf pair $^{-1}$·d^{-1}. This is consistent with previous studies where Jalali et al., (2020) reported an increase in mineral intake that exceeded previously predicted concentrations of 114 g·cow-calf pair $^{-1}$·d^{-1} to 196.6 g·cow-calf pair $^{-1}$·d^{-1}. Additionally, the authors observed an increase in mineral intake during the late fall and winter months, which coincided with the cows at mid to late gestation where nutrient demands are greater for the cow. These data indicate that free-choice mineral intake can be highly variable and may be influenced by environmental conditions, forage quality, physiological status of the animal, and management methods (Patterson et al., 2013; Jalali et al., 2020).

Cow-calf performance

Cows BW ($P \geq 0.96$) and BCS ($P \geq 0.94$) were similar across all treatments at the end of yr. 1 (Table 5). Pregnancy rate to A.I., and overall pregnancy rate are also included in Table 5 There were no treatment x time interactions for reproductive performance for yr 1. Additionally, all data are presented as the overall main effects of treatments.

In other studies, Ahola et al., (2004) indicated that beef heifers receiving a supplement of 50% of sulfate trace minerals (STM) and 50% proteinate TM mixture tended to have a greater pregnancy rate following A.I. compared with 100% STM. However, these authors indicated that overall reproductive status was not influenced by TM sources. Overall, Ahola et al., (2004) reported there were no significant differences observed with supplementing cattle with organic TM (OTM) compared with sulfate TM (STM). Additionally, Jalali et al., (2020) reported supplementing beef cows and their calves with hydroxy trace minerals (HTM) does not differ from supplementing a combination of STM and OTM on reproductive performance or other production parameters.

Although these studies did not incorporate high levels of S within their treatments, there were similarities with the results of TM sources not having a strong impact on reproductive status. However, with the current experiment including elevated amounts of S within the design it can alter the availability of Cu, Mn, and Zn, which was not measured in this project.

Mineral Status

Initial mineral liver concentrations of Cu ($P \geq 0.52$), Mn ($P \geq 0.91$), and Zn ($P \geq 0.51$) were similar across all treatments (Table 4). Previous research has been conducted from Jalali et al., (2020) on free-choice mineral supplementation of inorganic-organic (IOC) and hydroxy trace mineral (HTM) sources. The researchers provided treatments to 261 crossbred (Angus and Angus x Hereford) beef cows over a two-year period and observed that cow BW ($P \geq 0.71$) and BCS ($P \geq 0.61$) were similar across treatments. Additionally, liver concentrations of Cu ($P \geq 0.91$), Mn ($P \geq 0.94$), Zn ($P \geq 0.79$) were also similar across treatments. However, Jalali et al., (2020) reported that liver Cu concentrations were greater ($P \geq 0.05$) in HTM compared with IOC at the end of yr 1 and 2. Liver Zn concentrations were also greater ($P \leq 0.05$) for HTM in yr 1 and tended ($P < 0.06$) to be greater in yr 2. Liver Mn concentrations were not affected by treatment at the end of yr 1 ($P \geq 0.64$) or 2 ($P \geq 0.72$). The greater liver Cu and Zn concentrations with HTM suggest that hydroxy forms of Cu and Zn were more bioavailable than the IOC. Furthermore, Cu hydroxy mineral has been shown to be more bioavailable when there is a high concentration of Mo (6.8-6.9 mg of Mo/kg of DM) and S (0.25-3.0% of S) present in plasma and liver concentrations compared to CuSO4 (Spears et al., 2004). Additionally, Ahola et al., (2004), reported a greater liver Cu concentration in cows supplemented with organic TM compared to an inorganic (ING) TM in yr. 1 of the experiment and did not see a difference in yr. 2. Studies have indicated the bioavailability of Cu from a hydroxychloride source is similar to Cu amino acids (AA) complexes (Jalali et al., 2020). However, liver Mn and Zn concentrations did not differ between ORG and ING treatments.

Plasma Cu ($P \geq 0.71$), Mn ($P \geq 0.61$), and Zn ($P \geq 0.81$) were similar across all treatments and there was no time x treatment interaction (Table 4). Plasma concentrations were within the appropriate levels according to Suttle, (2010a). Jalali et al., (2020), concluded plasma concentrations were not

affected by treatments. Furthermore, plasma concentrations from Ahola et al., (2004) were impacted by TM supplementation but not by source. The authors report that plasma Zn concentrations were greater in supplementation treatments compared to control treatments in the initial year of the experiment.

Sulfur concentrations (Table 3) for grazed forage and supplemental hay were below NASEM (2016) requirements of 0.15%. However, factoring in elemental S from the supplement, total dietary S concentration was approximately 0.35% (assuming 10 kg·cow $^{-1}$ d^{-1} of DM) which is over twice the NASEM (2016) recommendations. This could explain the similar liver mineral concentrations of all treatments. Jalali et al., (2020) reported pasture S concentrations reached approximately 0.14% and Mo concentrations ranged from 1.49 to 2.37 mg/kg creating an approximate Cu:Mo ratio of 3.9, which may have influenced the effects of treatment on cows. In a study by Ward & Spears (1997), they found that steers receiving supplemental Cu and Mo exhibited an antagonistic interaction, which possibly resulted in declining liver Cu concentrations. Additionally, Pogge et al., (2014) and Genther & Hansen, (2015) reported that excess dietary S had lesser absorption and retention of Cu, Mn, and Zn. Pogge et al., (2014) studied the effects of feeding high concentrations of S (0.68%) to beef steers and concluded that there was a negative retention of Cu, Mn, and Zn. This could have been due to antagonistic interactions caused by Mo and S binding to Cu, Zn, or Mn, which decreasd the bioavailability. Additionally, Pogge et al., (2014) reported that steers consuming a 0.65% S diet consumed less DM compared to steers consuming 0.42% S diet, which could have negatively impacted performance. 0.5X Intellibond was also similar by exhibiting no significant difference compared to both 1X Intellibond and 1X Sulfate.

In another study, Vanvalin et al., (2019) provided 84 steers with a corn silage-based total mixed ration (TMR) throughout a 90-d trial to study the effects of Cu availability of multiple sources of minerals when high antagonistic concentrations were present. The TMR was composed of micro and macro minerals, as well as 0.25% S and 6.8 mg Mo/kg DM in total. Treatments included: 1) Control (CON: no Cu supplement), 2) low inorganic (ING: 5 mg Cu/kg DM), 3) high inorganic (ING10: 10 mg Cu/kg DM), 4) low organic (ORG5: 5 mg Cu/kg DM), 5) high organic (ORG10: 10 mg Cu/kg DM), 6)

low hydroxy (HYD5: 5 mg Cu/kg DM), 7) high hydroxy (HYD10: 10 mg Cu/kg DM). Vanvalin et al., (2019) reported the hydroxy (HYD5 and HYD10) treatments were more available in steers that were fed high antagonist in the diets. Using the final liver Cu concentrations, the relative bioavailability (RBV) of HYD tended to be greater than ING ($P = 0.07$; HYD RBV = 112%). With such high concentrations of both Mo and S, trace minerals, such as Cu, can become unavailable very quickly. This can decrease bioavailability, which can result in mineral deficiencies. Hydroxy minerals have been shown (Vanvalin et al., (2019); Jalali et al., (2020a) to bypass the ruminal interaction with antagonistic minerals (Mo and S) to maintain available minerals in the small intestines. The impacts of elevated concentrations of S on mineral supplementation of grazing cattle can decrease production performance. Especially, if TM sources are exposed or considered susceptible to antagonistic influences.

APPLICATION

The first year of this three-year experiment compared the performance and mineral status of beef cows provided a free-choice mineral supplement focusing on Cu, Mn, and Zn from a sulfate or hydroxy base source. Liver mineral status, BW, and BCS for cows on either sulfate or hydroxy mineral treatment exhibited no difference in performance of mineral status throughout year 1 of this experiment.

TABLE AND FIGURES

Table 1. Free-choice mineral supplement ingredient composition on a DM basis (Year 1). *(Preliminary Data)*

	Free-choice mineral supplement treatment		
Item, %*	Sulfate[1]	IntelliBond 1X[2]	IntelliBond 0.5X[3]
Monocalcium Phosphate 21%	28.45	28.43	28.47
Salt, NaCl	25.55	25.55	25.45
Calcium Carbonate $CaCO_3$	20.75	21.45	22.00
Elemental sulfur, S	11.65	11.65	11.65
Corn Distillers Dried Grains	4.90	4.90	5.00
Magnesium Oxide, MgO	3.07	3.07	3.07
Selenium 0.16%, Se	1.88	1.88	1.88
Soybean Oil	1.00	1.00	1.00
Copper Sulfate (25%, Cu)	0.40	-	-
Zinc Sulfate (36%, Zn)	0.83	-	-
Manganese (32%, Mn)	0.63	-	-
Intellibond Cu	-	0.18	0.09
Intellibond Mn	-	0.46	0.23
Intellibond Zn	-	0.55	0.27
Cobalt Carbonate 2%, $CoCO_3$	0.08	0.08	0.08
Calcium Iodate 8%, CaI	0.06	0.06	0.06
Vitamin A		0.62	0.62
Vitamin D3	0.05	0.05	0.05
Vitamin E	0.11	0.11	0.11

[1] Sulfate= 1X NASEM (2016) requirements for Cu, Zn, and Mn – Sulfate source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn.
[2]Hydroxy 1X= 1X NASEM (2016) requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN).
[3]Hydroxy 0.5X= 0.5X NASEM (2016) requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 500, 1,000, and 1,500 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN).

Table 2. Chemical analysis of free-choice mineral supplements (Year 1). (Preliminary Data)

Chemical analysis	Sulfate[1]	IntelliBond 1X[2]	IntelliBond 0.5X[3]
Dry Matter, %	97.2	97.2	97.2
Crude Protein, %	1.5	1.5	1.5
Crude Fat, %	1.4	1.4	1.4
Calcium, %	12.8	13.0	13.3
Phosphorus, %	6.1	6.1	6.1
Sulfur, %	12.5	12.2	12.1
Cobalt, mg/kg DM	15.2	15.3	15.2
Copper, mg/kg DM	1018.8	1023.9	513.7
Manganese, mg/kg DM	2162.7	2135.0	1138.3
Selenium, mg/kg DM	26.7	26.9	26.8
Zinc, mg/kg DM	3037.9	3034.0	1536.0

[1] Sulfate= 1X NASEM (2016) requirements for Cu, Zn, and Mn – Sulfate source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn.
[2]Hydroxy 1X= 1X NASEM (2016) requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN).
[3]Hydroxy 0.5X= 0.5X NASEM (2016) requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 500, 1,000, and 1,500 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN).

Table 3. Chemical analysis of feedstuffs (Year 1). *(Preliminary Data)*

Item	Range Forage	Grass Hay	Millet Hay	Silage	Feedlot Ration
Moisture (%)	4.7	5	3.4	4.2	5.1
DM (%)	48.4	83.8	91.7	36.8	94.9
CP (%)	8.7	6.8	6.3	6.6	8.8
ADF (%)	37.8	42.1	50.4	46.3	34.1
NDF (%)	64.2	67	72.3	69.7	48.7
TDN (%)	55.3	54	53	53.5	66.5
Cu ppm	6.2	3	5	4	10
Mn ppm	48.8	44	46	45	79.5
Mo, ppm	2.3	0.8	0.1	0.6	-
Zn ppm	18.5	16	17	16.5	19.5
S (%)	0.1	0.13	0.12	0.13	0.18

Average feedstuff analysis from monthly samples of range forage and dry lot ration from each replicate (n=9) for yr. 1 of experiment. Dry Matter (DM), Crude Protein (CP), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Total Digestible Nutrients (TDN),

Table 4. Influence of supplemental concentrations and source on plasma and liver mineral copper, manganese, and zinc of beef cows (Year 1). *(Preliminary Data)*

	Treatment				*P* <		
Item*	Sulfate[1] (mg/kg)	IntelliBond 1x[2] (mg/kg)	IntelliBond 0.5x[3] (mg/kg)	SEM	Treatment	Time	Time x Treatment
Plasma							
Cu, mg/L							
d 0	0.98	1.01	0.95	0.08	0.87	0.001	0.71
d 178	1.14	1.15	1.21	---	---	---	---
d 314	1.21	1.24	1.20	---	---	---	---
Zn, mg/L							
d 0	1.12	1.21	1.17	0.12	0.67	0.0002	0.81
d 178	1.24	1.28	1.22	---	---	---	---
d 314	1.20	1.27	1.29	---	---	---	---
Mn, µg/L							
d 0	9.27	9.34	10.11	1.22	0.85	0.0001	0.64
d 178	11.23	12.01	11.37	---	---	---	---
d 314	11.98	12.10	11.87	---	---	---	---
Liver							
Cu, mg/kg DM							
d 0	151.6	160.2	141.0		0.68	0.01	0.52
d 178	199.1	245.0	218.7		-	-	-
d 314	253.1	254.2	243.7		-	-	-
Zn, mg/kg DM							
d 0	173.5	178.4	180.9		0.47	0.002	0.51
d 178	258.6	277.6	243.8		-	-	-
d 314	301.3	306.6	329.5		-	-	-
Mn, mg/kg DM							
d 0	11.2	9.8	10.2		0.54	0.25	0.91
d 178	10.1	12.1	11.3		-	-	-
d 314	11.6	12.4	11.7		-	-	-

[1] Sulfate= 1X NASEM requirements for Cu, Zn, and Mn – Sulfate source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn.

[2]Hydroxy 1X= 1X NASEM requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN) .

[3]Hydroxy 0.5X= 0.5X NASEM requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 500, 1,000, and 1,500 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN).

Table 5. Effects of trace mineral supplement on cow BW, BCS, reproductive performance, and 205 day adjusted weaning weights. *(Preliminary Data)*

	Treatment				*P* <		
Item	Sulfate[1]	IntelliBond 1x[2]	IntelliBond 0.5x[3]	SEM	Treatment	Time	Time x Treatment
BW, kg							
d 0, yr. 1	562.6	568.0	569.2	11.3	0.81	<.0001	0.96
d 178, yr. 1	606.7	611.4	597.2	11.2	-	-	-
d 314, yr. 1	607.5	616.0	613.1	12.3	-	-	-
BCS[4]							
d 0, yr. 1	5.9	5.9	5.9	0.089	0.67	<.0001	0.94
d 178, yr. 1	6.1	6.0	6.2	0.09	-	-	-
d 314, yr. 1	5.7	5.8	5.8	0.10	-	-	-
Pregnancy rate to AI,[5] %	55.0	63.3	56.7	-	-	-	-
Overall Pregnancy Rate,[6] %	91.7	93.3	93.1	-	-	-	-
205d Adjusted weaning weight, kg	235.1	239.4	236.5	5.50	0.57	-	-

[1] Sulfate= 1X NASEM requirements for Cu, Zn, and Mn – Sulfate source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn.

[2]Hydroxy 1X= 1X NASEM requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN) .

[3]Hydroxy 0.5X= 0.5X NASEM requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 500, 1,000, and 1,500 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN).

4 1=emaciated, 9= obese; Richards eat al., 1986.

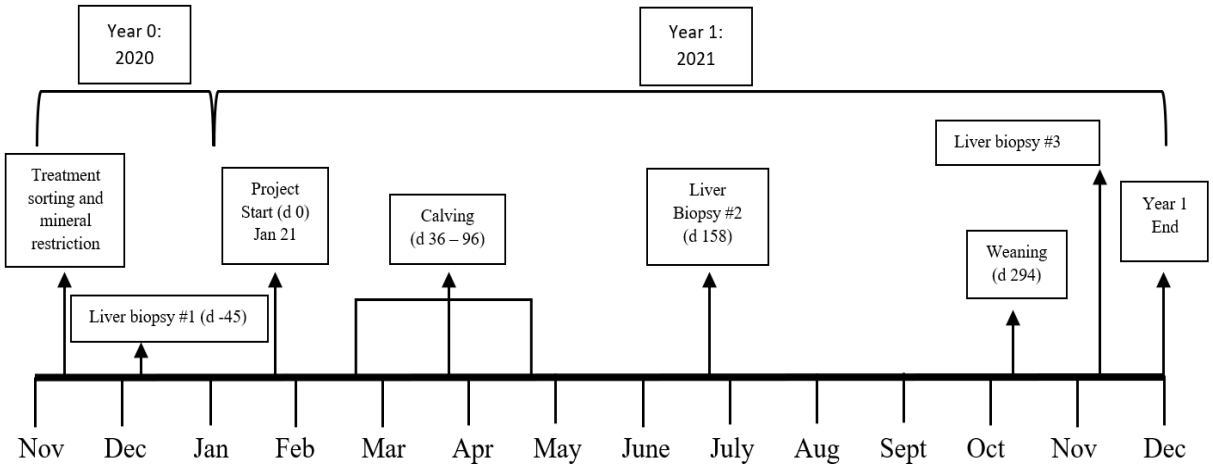


Figure 2.1: Project timeline of sample collection or major production events for year 1 .

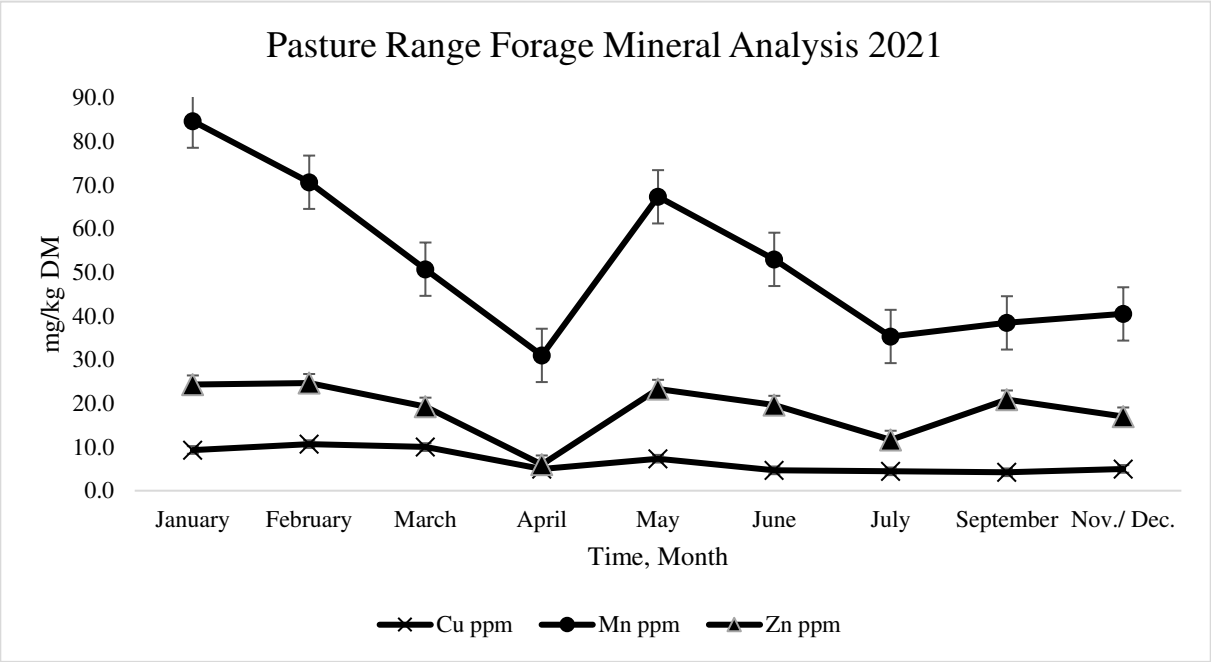


Figure 2.2. Monthly copper, manganese, and zinc concentrations of grazed, rangeland forage throughout yr. 1. Monthly pastures samples were collected from all replicates (n=9). Error bars represent SE.

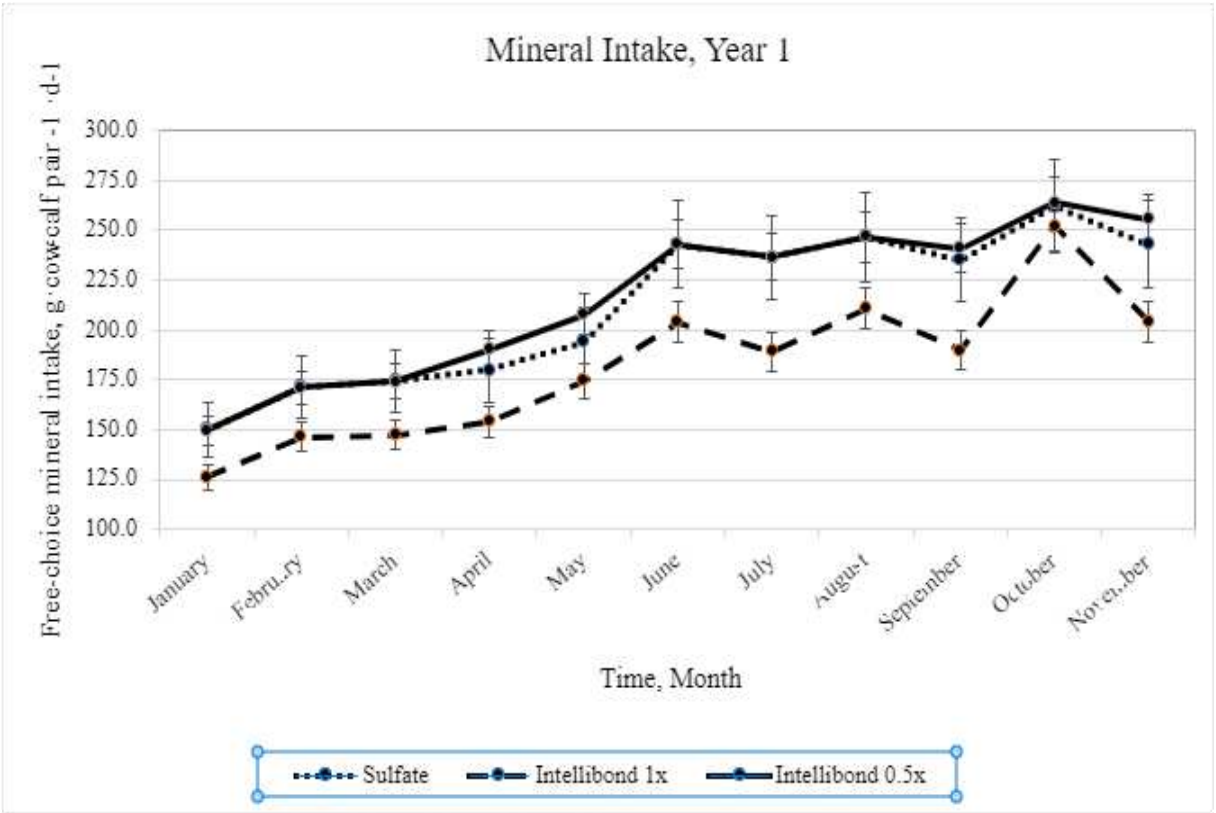
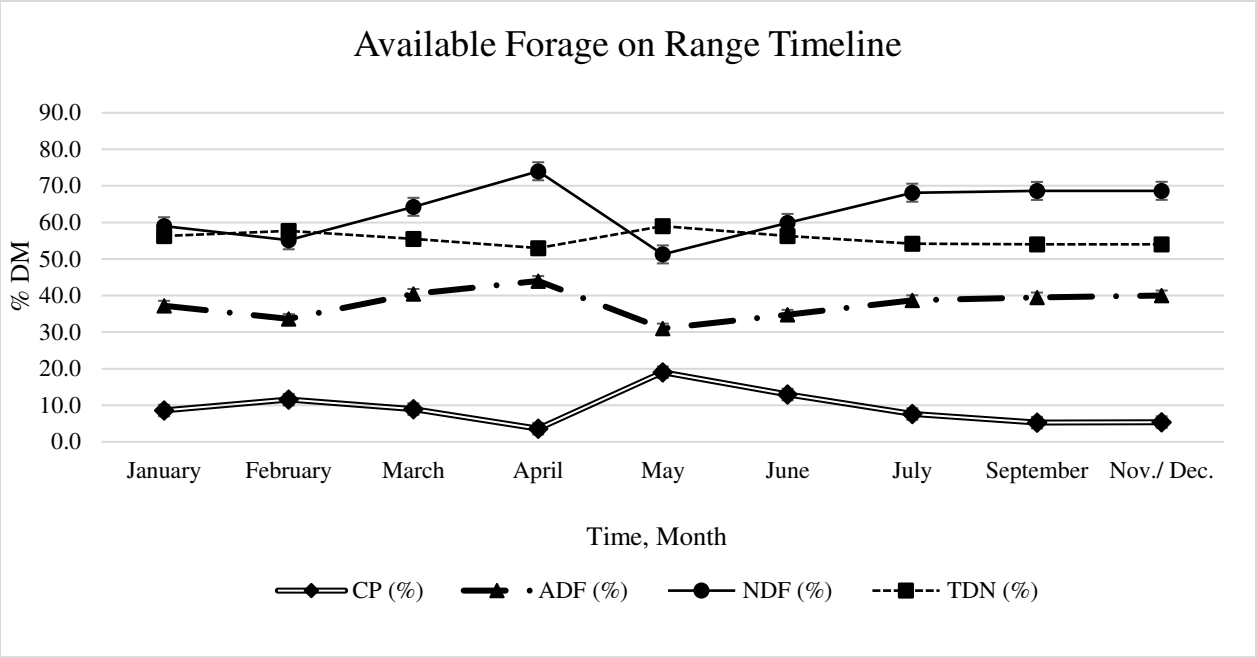


Figure 2.3. *(Preliminary Data)* Free – choice mineral treatments (Trt) were as follows: 1) 1X NASEM requirements for Cu, Zn, and Mn – Sulfate source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn. 2) 1X NASEM requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 1,000, 2,000, and 3,000 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN). 3) 0.5 X NASEM requirements for Cu, Zn, and Mn – Hydroxychloride source mineral containing 500, 1,000, and 1,500 mg/kg of Cu, Mn, and Zn (Intellibond C, Z, M, Micronutrients USA LLC (Indianapolis, IN). With exception of Cu, Mn, and Zn, all macro- and microminerals and vitamins A, D, and E were added to free choice minerals supplements to meet or exceed NASEM (2016) recommended concentrations. Free – choice mineral treatments were provided in covered mineral feeders at a single location in each pasture. Cows and calves were allowed access to the same mineral feeders within a pasture. The targeted intake was approximately 113 g d^{-1} cow^{-1}. Error bars represent SE.



2.4. *(Preliminary Data)* Nutrient composition of grazed forage throughout the first year of the experiment. Monthly s samples were collected from all replicates (n=9). Error bars represent SE.

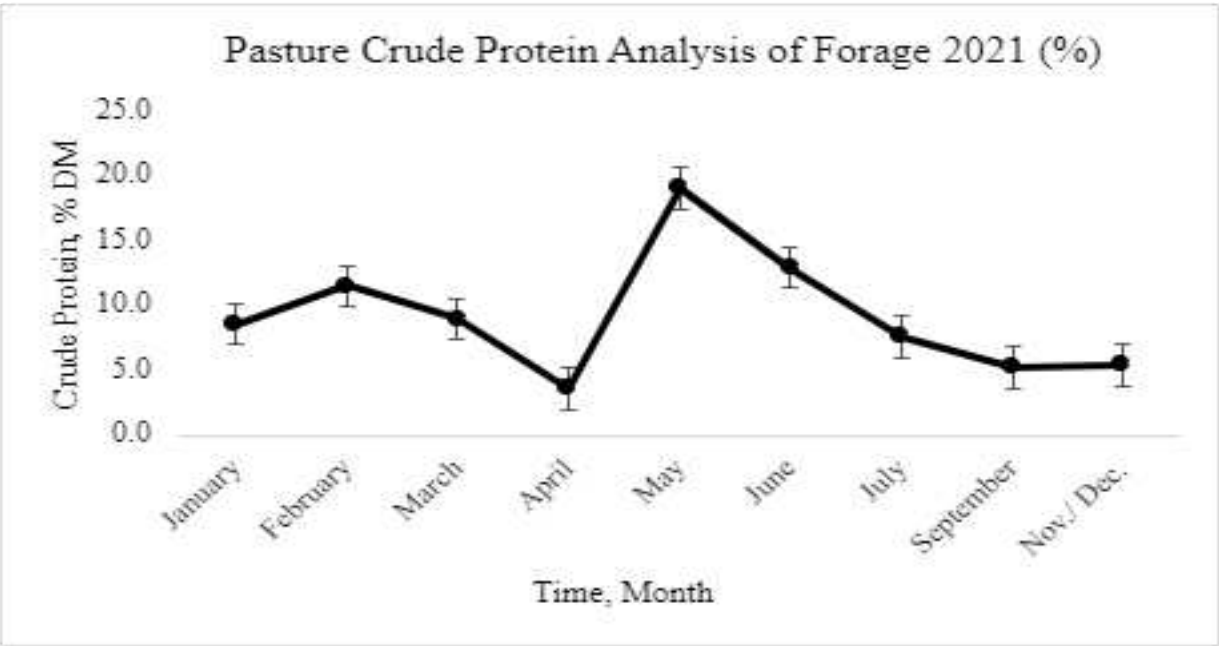


Figure 2.5. Monthly CP concentrations of grazed forages throughout yr. 1 of experiment. Monthly pastures samples were collected from all replicates (n=9). Error bars represent SE.

LITERATURE CITED

Adriano, D. C. (2001). Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals (Vol. 860). New York: Springer.3:11

Ahola, J. K., Baker, D. S., Burns, P. D., Mortimer, R. G., Enns, R. M., Whittier, J. C., ... & Engle, T. E. (2004). Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period. Journal of animal science, 82(8), 2375-2383.

Allen, V. G., Batello, C., Berretta, E. J., Hodgson, J., Kothmann, M., Li, X., ... & Sanderson, M. (2011). An international terminology for grazing lands and grazing animals. Grass and forage science, 66(1), 2.

Arthington, J. D., & Ranches, J. (2021). Trace Mineral Nutrition of Grazing Beef Cattle. Animals, 11(10), 2767.

Cater, M. A., & Mercer, J. F. (2005). Copper in mammals: mechanisms of homeostasis and pathophysiology. In Molecular Biology of Metal Homeostasis and Detoxification (pp. 101-129). Springer, Berlin, Heidelberg

Chapman Jr, H. L., & Bell, M. C. (1963). Relative absorption and excretion by beef cattle of copper from various sources. Journal of animal science, 22(1), 82-85.

Davidson, L. A., & Lonnerdal, B. (1989). Fe-saturation and proteolysis of human lactoferrin: effect on brush-border receptor-mediated uptake of Fe and Mn. American Journal of Physiology-Gastrointestinal and Liver Physiology, 257(6), G930-G934.

Dixon, J. B., Weed, S. B., & Parpitt, R. L. (1990). Minerals in soil environments. Soil Science, 150(2), 562.

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446–2451. https://doi.org/10.2527/2000.7892446x.

Evans, G. W. (1973). Copper homeostasis in the mammalian system. Physiological reviews, 53(3), 535-570.

Genther, O. N., & Hansen, S. L. (2015). The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. Journal of Dairy Science, 98(1), 566–573. https://doi.org/10.3168/jds.2014-8624

Givens, D. I., Owen, E., Omed, H. M., & Axford, R. F. E. (2000). Forage evaluation in ruminant nutrition. CABI.

Guimaraes, O., Jalali, S., Wagner, J. J., Spears, J. W., & Engle, T. E. (2021). Trace mineral source impacts rumen trace mineral metabolism and fiber digestion in steers fed a medium-quality grass hay diet. Journal of Animal Science, 99(9), skab220.

Hidiroglou, M. (1979). Manganese in ruminant nutrition. Canadian Journal of Animal Science, 59(2), 217-236.

Hill, G. M., & Link, J. E. (2009). Transporters in the absorption and utilization of zinc and copper. Journal of animal science, 87(suppl_14), E85-E89.

Hofmann, R. R. (1989). Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia, 78(4), 443-457.

Jalali, S., Lippolis, K. D., Ahola, J. K., Wagner, J. J., Spears, J. W., Couch, D., & Engle, T. E. (2020). Influence of supplemental copper, manganese, and zinc source on reproduction, mineral status, and performance in a grazing beef cow-calf herd over a 2-year period. Applied Animal Science, 36(5), 745–753. https://doi.org/10.15232/aas.2020-01982

Kabata-Pendias, A. (2000). Trace elements in soils and plants. CRC press.

Larson, Robert L.; Arthington, J.D.; and Corah, L.R. (1992) "Interrelationship between copper and bovine health," Kansas Agricultural Experiment Station Research Reports: Vol. 0: Iss. 1. https://doi.org/10.4148/2378-5977.2198

López-Alonso, M., & Miranda, M. (2020). Copper supplementation, a challenge in cattle. Animals, 10(10), 1890.

MacPherson, A. (2000). 17 Trace-mineral Status of Forages. Forage evaluation in ruminant nutrition, 345.

Mc Kenzie, R. M. (1989). Manganese oxides and hydroxides. Minerals in soil environments, 1, 439-465.

McDowell, L. R. (1996). Feeding minerals to cattle on pasture. Animal feed science and technology, 60(3-4), 247-271.

Miller, W. J. (1970). Zinc nutrition of cattle: a review. Journal of Dairy Science, 53(8), 1123-1135.

Minerals in Animal and Human Nutrition. (2003). Minerals in Animal and Human Nutrition: Second Edition. https://doi.org/10.1016/b978-0-444-51367-0.x5001-6

Nutrient Requirements of Beef Cattle, 8th Revised Edition. (2015). Nutrient Requirements of Beef Cattle, 8th Revised Edition. https://doi.org/10.17226/19014

Patterson, J.D., Burris, W.R., Boling, J.A. et al. Individual Intake of Free-Choice Mineral Mix by Grazing Beef Cows May Be Less Than Typical Formulation Assumptions and Form of Selenium in Mineral Mix Affects Blood Se Concentrations of Cows and Their Suckling Calves. Biol Trace Elem Res 155, 38–48 (2013). https://doi.org/10.1007/s12011-013-9768-7

Pearson, E. G., and A. M. Craig. 1980. The diagnosis of liver disease in equine and food animals. Mod. Vet. Pract. 61:233–237.

Pogge, D. J., Drewnoski, M. E., & Hansen, S. L. (2014). High dietary sulfur decreases the retention of copper, manganese, and zinc in steers. Journal of Animal Science, 92(5), 2182–2191. https://doi.org/10.2527/jas.2013-7481

Reid, R. L., & Horvath, D. J. (1980). Soil chemistry and mineral problems in farm livestock. A review. Animal feed science and technology, 5(2), 95-167.

Ryan, A. W., Kegley, E. B., Hawley, J., Powell, J. G., Hornsby, J. A., Reynolds, J. L., & Laudert, S. B. (2015). Supplemental trace minerals (zinc, copper, and manganese) as sulfates, organic amino acid complexes, or hydroxy trace-mineral sources for shipping-stressed calves. The Professional Animal Scientist, 31(4), 333–341. https://doi.org/10.15232/pas.2014-01383

Spears, J. W., Kegley, E. B., & Mullis, L. A. (2004). Bioavailability of copper from tribasic copper chloride and ranch equipmentcopper sulfate in growing cattle. Animal Feed Science and Technology, 116(1-2), 1–13. https://doi.org/10.1016/j.anifeedsci.2004.06.002

Spears, J. W. (2003). Trace mineral bioavailability in ruminants. The Journal of nutrition, 133(5), 1506S-1509S.

Spears, J. W., Burns, J. C., & Hatch, P. A. (1985). Sulfur fertilization of cool season grasses and effect on utilization of minerals, nitrogen, and fiber by steers. Journal of Dairy Science, 68(2), 347-355.

Suttle, N. F. (2010). Mineral nutrition of livestock. Cabi.

VanValin, K. R., Genther-Schroeder, O. N., Laudert, S. B., & Hansen, S. L. (2019). Relative bioavailability of organic and hydroxy copper sources in growing steers fed a high antagonist diet1. Journal of Animal Science, 97(3), 1375–1383. https://doi.org/10.1093/jas/sky487

Ward, J. D., Spears, J. W., & Gengelbach, G. P. (1995). Differences in copper status and copper metabolism among Angus, Simmental, and Charolais cattle. Journal of Animal Science, 73(2), 571-577.