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Effect of Source of Trace Minerals on Nutrient Digestibility and Rumen Fermentation of Dairy Cows

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EFFECT OF SOURCE OF TRACE MINERALS ON NUTRIENT DIGESTIBILITY AND RUMEN FERMENTATION OF DAIRY COWS

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by
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ABSTRACT

The main objective of this study was to evaluate the effects of trace mineral source supplementation on apparent nutrient digestibility, apparent retention and absorption and rumen fermentation of lactating dairy cows. A second objective was to determine the effects trace mineral sources on cow performance. Nine multiparous Holstein cows (eight ruminally fistulated) were averaging approximately 90 DIM (days in milk) at the beginning of the trial, were blocked by DIM and randomly assigned to 1 of 3 supplemental mineral treatments in three 3 x 3 Latin squares. The cows were fed once a day with a TMR (DM basis: 15.2% CP, 36.5% NDF, 9.7 mg Cu/kg, and 44.3 mg Zn/kg) for 21 days. Treatments consisted of 10 mg Cu/kg DM and 35 mg Zn/kg DM from one of the three treatments sulfate (STM), Mono-glycinates (MGTM), or Bis-glycinate (BGTM) into a split-plot, 3 × 3 Latin square design. Following a 14-d adaptation period, milk was collected during the next 3 d during morning and night milking. Before starting urine and feces samples, cows were moved to a tight stall barn. Spot urine and spot feces were collected during days 17 to 20 in a sampling every four hours until completing a 24 h sampling. Rumen fluid was collected at 0, 2, 4, 8, 12, 18, and 24 h post-feeding to analyze pH, NH_3N, and rumen solubility. Treatment did not affect dry matter intake or milk production. Dry matter digestibility was similar (61.8 ± 1.99), and NDF digestibility was not affected by STM vs. MGTM vs. BGTM supplemented lactating cows. The apparent absorption and retention of Cu and Zn were similar between treatments. Apparent Cu absorption and retention had similar effects between treatments. Rumen fluid collection used for measuring pH and NH_3N showed no treatment x time or treatment interactions for any measured response

variables. Ruminally soluble Cu and Zn concentrations were similar across trace mineral sources. Serum Cu and Zn concentrations were similar across treatments. Under the conditions of this study, supplementing trace minerals such as STM, MGTM, and BGTM did not affect the milk performance or DMI. Rumen fluid concentration did not affect the amount of Cu and Zn in the rumen. The rumen solubility had similar effects with Cu and Zn. Overall, source of Cu and Zn supplementation did not influence animal performance, nutrient digestibility, or mineral partitioning. Furthermore, the results of this study do not support our hypothesis that mono and bis-glycinate sources of Cu and Zn, with lower rumen solubility compared with sulfate sources, will improve apparent fiber digestibility compared with sulfate sources.

DEDICATION

I dedicate this thesis to my parents, Cesar and Norma, my siblings, Armandito and Normita, and my girlfriend, Madeline, for their endless love, advice, and patience for never letting me surrender even when times get rough.

ACKNOWLEDGMENTS

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My family, this is for you, have always been the center of my life, and every day far from you makes me miss you more. However, as you always tell me, "This is for the best" All of you have a big part in the person I have become over the years. For cheering me, whenever I decided to pursue higher education at Clemson, my family cheered me, gave me the support I needed, and mainly supported me in the following educational steps I would take. With no more to say, I love you so much.

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CHAPTER ONE

Literature Review

Introduction

A mineral is an inorganic element required for maintenance and production. They can be classified by the amount required in their daily diet. Macromineral (Ca, Cl, K, Mg, Na, P, S) > 100 mg and microminerals (Co, Cu, F, Fe, I, Mn, Mo, Ni, Se, Zn) < 100 mg where our interest is on Cu and Zn. Minerals are usually classified into four big groups according to their physiological functions. Structural minerals, physiological minerals, catalytic minerals, and regulatory minerals. However, this classification is common since the same minerals can fulfill not only one function. In most cases, they can complete multiple functions (Underwood & Suttle, 1999). The minerals industry has been developing an organic trace mineral centered around the theory that they are more bioavailable or similar to inorganic sources to forms that occur in the body. For example, the metal used in the premix of the trace minerals chelates or complex is stable in the digestive tract. In that case, this metal will be protected from forming complexes with other dietary components that inhibit absorption. With this, we can say that it will let the animal absorb a higher amount. The utilization of inorganic trace minerals depends on the animal's ability to convert them to organic, biologically active forms. Trace minerals naturally occurring in feeds also exist primarily as organic chelates or complexes (Spears, 1996a).

Inorganic trace mineral supplements are naturally of less value to livestock than the natural forms in which elements are present in feeds, and usually are less available than synthetic organic complexes containing those elements. Trace metal–amino acid complexes can mimic the process by which trace elements are absorbed (as metal-peptides) and might thus be more available to livestock than inorganic mineral sources (Suttle, 2010). Trace mineral solubility can significantly affect the total concentration available to rumen microbes because only soluble minerals are available for use or interactions (Torre et al., 1996). Organic Zn sources are often soluble in the rumen than in inorganic sources (Spears, 2003), whereas hydroxy-trace minerals (HTM) sources may be less soluble (Spears et al., 2004).

The rumen presents a single challenge to the absorption of trace minerals because the formation of insoluble complexes can decrease the later availability of specific trace minerals. Sulfide is generated by reducing sulfate in the rumen (Drewnoski et al., 2014). It can combine with dietary Mo to form thiomolybdates, complex with Cu, and bind directly to Cu to form insoluble Cu sulfide (Suttle, 1991). However, Cu must be in a soluble form, and not attached to particulate matter, to be available for interaction with Mo and S (Suttle, 1991).

Ruminal soluble zinc concentrations were much higher in steers that were fed zinc methionine than in steers supplemented with a similar zinc concentration from zinc sulfate or zinc oxide (Spears, 1996b). These studies suggest that the zinc methionine complex will be intact in the rumen with the feed particles and microorganisms to make them an insoluble form of insoluble complexes shorter extent than inorganic forms of zinc.

Less ruminal solubility of these sources may prevent decreased D.M. digestibility from T.M. supplementation while remaining available to the animal for absorption later in the intestine (Genther & Hansen, 2015). Increasing the bonding of metals in the rumen limits potential antagonism with other digesta, such as fiber fractions, and could increase the bioavailability of trace minerals. Hydroxy Cu and Mn are less soluble in the rumen compared with sulfate sources (Genther & Hansen, 2015). However, data is inconsistent regarding differences in Zn solubility between sulfate and hydroxy sources (Cao et al., 2000; Genther & Hansen, 2015).

Importance of Cu and Zn in cattle diets

Dietary Cu and Zn are critical in animal growth, production, and reproduction. They are vital for the functioning of several immune system components. As a result, they contribute to maintaining proper health and immunity. Copper is a component of several proteins, including cytochromes oxidase, used for aerobic respiration; lysyl oxidase, required for collagen and elastin; and tyrosinase, necessary for pigmentation (Torre et al., 1996). Additionally, copper is required for hemoglobin synthesis and is involved with iron metabolism (NRC, 2021).

Zinc is a component in more than 200 enzymes, including oxidoreductases, transferases, hydrolases, lyases, and ligases (Kidd et al., 1996). Zinc is also involved with metabolism, the immune system, gene regulation, hormonal regulation, and neurotransmission (Fraker & King, 2004). The first evidence that zinc is necessary for growth and health was obtained in laboratory rats (Todd et al., 1933)). Zinc is also a component of cytosolic superoxide dismutase, which will protect the cells from the toxic effect of reactive oxygen species (NRC, 2021) and is vital for synthesizing proteins, keratin formation, immune function, and insulin function.

Zinc is needed for the structural and functional development of over 2000 transcription factors, and almost every signaling and metabolic pathway depends on at least one zinc-requiring protein (Beattie & Kwun, 2004; Cousins et al., 2006). Fetal growth is mainly affected by a lack of zinc, which reflects Zn roles in DNA synthesis, nucleic acid, and protein metabolism (Hurley, 1981). The pancreas secretes zinc-dependent phospholipase A2 (Kim et al., 1998). The liver concentration of vitamin E and the fat-soluble vitamin A are reported in zinc-deprived animals, interest became possible because of the role of zinc in fat absorption.

Copper was first shown to be essential for growth and hemoglobin formation in a laboratory study with rats in 1928 by Hart and Elvejhem. A well-known interaction between copper and molybdenum was observed when cattle with severe diarrhea consumed forage with high molybdenum concentrations(W. S. Ferguson et al., 1943). Moreover, both copper and molybdenum can further interact with a third element, sulfur (Dick, 1956; Suttle, 2010). It is usually difficult to justify the term "requirements" for trace minerals in the same way it will be used for energy, protein, or amino acids. Requirements for minerals are much more challenging to establish, and most estimates are based on the lower level required to control a deficiency symptom and not necessarily to promote productivity (NRC, 2000). Usually, mineral recommendations should include a safety margin of error because of the antagonism between each micromineral. For example, in ruminants, copper uptake is inhibited remarkably by Mo, but also by S and, to a lesser extent, by Fe, and high Ca levels in the feed impede the uptake of Zn (Suttle, 2010). In cattle, higher levels of Cu are needed in the presence of high levels of Zn. Additionally, animals under stress require higher levels of Cu and Zn. Moreover, the following list shows some factors that may affect the mineral levels: the quantity, the type of ingredients, the mineral content, the processing of the diet, the storage and environmental conditions, and the inclusion and range of other minerals (NRC, 2000).

In combination with sulfur and iron, molybdenum antagonizes copper absorption of ruminants. As one of the antagonists, zinc must have a higher concentration than the original requirements by more than ten times (W. J. Miller et al., 1989). To be considered an antagonism of copper absorption, iron needs to be at least higher than 250 mg Fe/Kg of the dry matter of the diet (Chase et al., 2000; Mullis et al., 2003).

There are no studies on how bovines absorb zinc from their diets, but they have models of how they are absorbed in rodents and poultry. In rats and poultry, Zn absorption occurs throughout the small intestine and perhaps in the large intestine in two ways: saturable and non-saturable diffusion. In poultry, saturable absorption occurs in the duodenum and jejunum and is non-saturable in the ileum (Yu et al., 2008).

Effect of Cu and Zn deficiency in ruminant diets

Copper Deficiency

Copper deficiency in cattle can result in several health and performance complications from very early in the animal's productive life. Some of those problems include poor hair coat, reduced weight gain, impaired immune system, broken bones, or reduced reproduction rates (Thomas, 2009). Two processes have an unequivocal dependency on a specific enzyme: pigmentation on tyrosinase and connective tissue development on lysyl oxidase (Prohaska et al., 2006; Suttle, 1987).

In cattle with highly pigmented coats, loss of coat color is usually the earliest and sometimes the only clinical sign of copper deprivation. Grayish black or bleaching brown hair is occasionally seen around the eyes. In Aberdeen Angus cattle, a brownish tinge can be given to the coat, and the skin becomes mottled (Hansen et al., 2009). In addition, diarrhea events can also be related to a Cu deficiency, but several studies (Hogan et al., 1971; Suttle & Field, 1968) have indicated that this problem might also be related to high dietary levels of molybdenum. In other deficiency situations, Cu is the cause of anemia in animals (Underwood, 1981). Hypocuprosis in cattle and sheep is related to female reproductive disorders such as preventing embryo implantation and high prenatal mortality, particularly early embryonic loss (Howes & Dyer, 1971).

However, limited data supports the effect of Cu supplementation on cattle fertility. Moreover, the positive effects of Cu supplementation on fertility have mainly been attributed to reducing the impact of excessive Mo intake (Phillippo et al., 1987) and the formation of thiomolybdates (Kendall et al., 2006). Growth retardation and infertility are associated with impaired increased release of luteinizing hormone (L.H.), which was induced in molybdenum-supplemented infertile heifers. However, it is not present in those with an equally severe hypocupremia induced by iron, and previous growth was only delayed in groups given Molybdenum (Phillippo et al., 1987). There are three possible explanations:

- Infertility and growth are impaired by molybdenum exposure per se.
- Extreme hypocupremia is predisposed to molybdenum toxicity.

- Molybdenum induced a localized copper deprivation previously reaching a lower point, not reflected by the liver and plasma copper.

Despite rations with lower copper-to-molybdenum ratios, they failed to impair L.H. release or fertility with molybdenum supplementation (0.8 versus 1.9) (Xin et al., 1993).

Zinc Deficiency

Zinc deficiency increases the risk of mastitis and other infectious diseases. For example, dairy cows fed a diet with 41 mg Zn/per kg of D.M. were more susceptible to having a higher somatic cell count (SCC) than cows fed a diet with 63 mg Zn/ per kg of D.M. (Cope et al., 2009). Interestingly, A genetic defect that significantly reduces zinc absorption has been found in Dutch-Friesian cattle, and they become severely deficient in zinc unless the diet has a high concentration of (Flagstad, 1976).

Newborns of all species can obtain the minerals by the process of endocytosis. These small amounts of copper ingested are very well absorbed. Colostrum has at least two to three times as much copper concentration and 14 mg of zinc compared to milk. Interestingly, ruminants secrete milk with low copper levels than sows (Suttle, 2010). The swine's higher Cu levels reflect the need to sustain large, rapidly growing litters during a short lactation. Milk is the most crucial source of minerals for animals in the suckling stage. The mineral composition of milk varies with parity, lactation stage, nutrition, and disease presence (Suttle, 2010). Although Cu secretion in milk is reduced in dams deprived of copper, it cannot increase above normal levels by dietary copper supplements (Underwood & Suttle, 1999). Zinc supplementation to the dam will only increase the amount of milk zn if they have a deficiency in their diet (Kirchgessner & Schwarz, 1976).

Published data on the impact of Cu and Zn supplementation on milk production and composition needs to be more consistent. Several studies reported that milk composition is not affected by the level or source of trace mineral supplementation (Ballantine et al., 2002; Kellogg et al., 2003; Uchida et al., 2001). However, other studies reported higher milk protein content for cows supplemented with complex trace minerals (J. D. Ferguson et al., 2004; Kincaid et al., 2003). Interestingly, organic Zn supplementation increases milk yield (Cope et al., 2009), reduces somatic cell count in lactating dairy cows (Kellogg et al., 2004), and improves the hoof's health (Siciliano-Jones et al., 2008). Similarly, a meta-analysis study observed increased fat and protein, milk yield, and improved reproductive performance with an organic source of trace minerals (Rabiee et al., 2010). On the other hand, several studies found that milk yield does not increase using sources of organic trace minerals (DeFrain et al., 2009; Hackbart et al., 2010; Nemec et al., 2012).

Rumen metabolism of Cu and Zn

Solubility

Trace mineral (Cu and Zn) rumen solubility can significantly affect the ability of these minerals to interact with feed particles, minerals antagonists, or the rumen microbial population (Torre et al., 1996). Furthermore, the rumen presents a challenge to the intestinal absorption of trace minerals because of the formation of insoluble complexes in the rumen that later decrease the bioavailability of specific trace minerals. For example, sulfides are generated when sulfate is reduced in the rumen (Drewnoski et al., 2014) and can then combine with dietary Mo to form thiomolybdates. This complex can then bind directly to Cu to form insoluble Cu sulfide (Suttle, 1991), which reduces

Cu bioavailability in the intestine. The theory that organic trace minerals are more available than inorganic trace minerals is based on the thought that organic forms are more like the physiological ones in the body. Less ruminal solubility of these sources may prevent decreased D.M. digestibility from T.M. supplementation while remaining available to the animal for absorption later in the intestine (Genther & Hansen, 2015). Increasing the bonding of metals in the rumen limits potential antagonism with other digesta, such as fiber fractions, and could increase the bioavailability of trace minerals. Hydroxy Cu and Mn are less soluble in the rumen compared with sulfate sources (Genther & Hansen, 2015). However, data is inconsistent regarding differences in Zn solubility between sulfate and hydroxy sources (Cao et al., 2000; Genther & Hansen, 2015). It is hypothesized that the differences in availability may be due to the variability of absorption of organic trace minerals and that they can remain intact until the absorption site is reached (Spears, 1996a). The differences in availability may be due to the variability of absorption of organic trace minerals and the fact that they can remain intact until the absorption site is reached. Recently, there has been an increase in the use of organic, organically complex, and hydroxy sources of trace minerals (Overton & Yasui, 2014).

Organic Zn and Cu sources are often more soluble in the rumen than inorganic sources (Spears, 2003). In contrast, hydroxy-trace minerals (HTM) have been shown to have very low ruminal solubility (Spears et al., 2004). Excess soluble Cu and Zn in the rumen can negatively impact microbial populations and reduce dietary fiber digestibility. Several in vitro studies reported that excess Cu and Zn supplementation negatively impacted rumen fermentation (Arelovich et al., 2000; Eryavuz & Dehority, 2009). Furthermore, the negative effect of Cu and Zn supplementation on microbial fermentation and the concomitant reduction in fiber digestibility are likely associated with the ruminal solubility of the supplemented trace minerals. Thus, supplemental sources of Cu and Zn with low ruminal solubility that would not affect ruminal microorganisms or interact with Mo and S are a practical approach to supplement Cu and Zn, not to penalize dietary fiber digestibility. In addition, this mineral source will have to simultaneously maintain or increase the bioavailability of Zn and Cu after exposure to the acid environment of the abomasum.

Ruminal soluble Zn concentrations were much higher in steers fed Zn- methionine than in steers supplemented with similar Zn from zinc sulfate or zinc oxide (Spears, 1996a). These studies suggest that the zinc methionine complex will be intact in the rumen and will bind with the feed particles or microorganisms to form an insoluble complex to a lesser extent than inorganic forms of zinc.

Copper and Zn hydroxychloride is relatively insoluble (0.6%) in water, whereas Cu and Zn sulfates are almost completely soluble (Spears et al., 2004). Caldera et al., 2019) observed that NDF digestibility tended to be greater in hydroxy trace minerals vs. sulfate trace minerals supplemented steers (41.2 vs. 37.8% of NDF, respectively). Similarly, (Faulkner et al., 2017) reported that feeding hydroxy Cu, Zn, and Mn to dairy cows increased NDF digestibility compared with cows fed sulfate trace mineral sources (48.5 vs. 46.4% of NDF, respectively). In a recent meta-analysis of beef and dairy cattle studies, (Ibraheem et al., 2023) evaluated the impact of sulfate versus hydroxy trace mineral source

(Cu, Zn, and Mn) supplementation on nutrient digestibility. The authors observed a 2.7 and 1.1 units increase in NDF digestibility for studies using total collection or undigested NDF as digestibility markers, respectively, when cows were supplemented with hydroxy vs. sulfates trace minerals.

Chelation

Chelated Cu and Zn are bound to a chelating agent, typically an organic compound or amino acid that helps prevent the minerals from interacting with other compounds, theoretically improving intestinal absorption (Ashmead, 1992). Chelation is a particular complex formed between a ligand and a metal ion. To be classified as a chelate, the ligand or chelating agent must: 1) contain a minimum of two functional groups (oxygen, nitrogen, amino, hydroxyl), each capable of donating a pair of electrons to combine (via coordinate covalent bonding) with metal and 2) form a heterocyclic ring structure with the metal (Kratzer & Vohra, 1986; Spears, 1996a). While amino acid chelates have been shown to strengthen Cu bioavailability relative to $CuSO_4$ (Hansen et al., 2008), other authors have reported no difference in bioavailability between inorganic and organic Cu sources (VanValin et al., 2019). Not all metal complexes are chelated. While the effectiveness of organic minerals for ruminants has been firmly established for chelation to be effective, the chelating agent should have more robust stability for the metal than the metal-binding substances in feed. However, reduced strength is constant in the tissue system where the metal is required (Roy & Misger, 2008; Suttle, 2010). In addition, other factors, including metal ion equilibria, kinetic elements, pH gradients, and redox equilibrium (in the case of redox-active metals such as Cu^{2+}), may also affect the uptake mechanism of metal ions (Hynes & Kelly, 1995).

Mono and bis-glycinates result from a mechanochemical reaction between the amino acid glycine and Zn or Cu minerals. This amino acid chelates consist of one (mono-glycinates Figure 1. 3) or two amino acids (bis-glycinates Figure 1. 2) molecules and are bound to the mineral compared to the sulfate sources Figure 1. 4. Due to their ring structure, they are highly stable over a wide pH range. However, research utilizing mono and bis-glycinate as Cu and Zn supplemental sources for lactating dairy cows is still being determined. Copper glycinate is a chelated Cu source which, to our knowledge, has not been evaluated as a source of dietary Cu for cattle (Hansen et al., 2008). Supplementation of glycinate minerals has been shown to alter milk fatty acid profiles in lactating dairy cattle, which may reflect changes in rumen microbial populations (Faulkner & Weiss, 2017b).

Traditional supplementation of Cu has been in the form of inorganic Cu sulfate (CuSO4), which disassociates in the rumen, leaving Cu to form insoluble complexes with thiomolybdates (Suttle, 1991). Chelation prevents mineral solubility and unfavorable interactions in the gastrointestinal tract, theoretically improving intestinal absorption (Ashmead, 1992). While amino acid chelates have been shown to improve Cu bioavailability relative to CuSO4 (Hansen et al., 2008), others have reported no difference in bioavailability between inorganic and organic Cu sources (VanValin et al., 2019). Thiomolybdates can form insoluble complexes with Cu, resulting in reduced Cu absorption. Independent of dietary Mo, S can decrease Cu absorption by forming insoluble

Cu sulfide in the rumen (Spears, 2003). Hansen et al., (2008) indicate that the copper Glycinate source used in this study is more bioavailable than CuSO4 in cattle-fed diets high in Mo and S.

Potentially digestible fiber

Trace minerals concentrations in feed are often taken for granted that can cause an effect on the animal's performance and most of the time producers underestimate this important source information (López-Alonso, 2012). By not giving the right amount of importance to these concentrations on trace minerals, it can affect the availability that they can give to the animal. Furthermore, the bioavailability can be affected by the source of the mineral and the chemical composition that might be in the diet (M. D. Miller et al., 2020). Also, it is affected in the digestion of the fiber from the diet and have a problem with some antagonism that we can find on the diets as form of feed (Kabaija & Smith, 1988; Overton & Yasui, 2014).

Common forms of trace minerals used to supplement cattle can be categorized between sulfates, oxides, carbonates, chlorides, hydroxy, and organic complexes (Daniel et al., 2020). In the past, cattle producers used oxide and later on they decided that the sulfates will have a higher benefit for animals, until recently the most common ones are the organic and inorganic forms of trace minerals (Overton & Yasui, 2014). Organic trace minerals had no effect on nutrients digestibility by heifers in most cases it will increase the volatile fatty acids production compared to the sulfates forms of the trace minerals (Pino & Heinrichs, 2016).

Ruminal solubility of trace minerals is most likely a factor that influences fermentation and microbial populations in which can have an effect on the nutrient digestibility (Faulkner & Weiss, 2017a). Reducing the concentration of soluble trace minerals such as copper, and by feeding hydroxy minerals it will help increase the ruminal digestibility in the rumen (Faulkner & Weiss, 2017a). There is a tendency of NDF digestibility, it will be greater on steers fed hydroxy trace minerals compared to the fed with sulfate trace minerals, this can be related due to the low solubility concentration of soluble minerals dosed with the hydroxy trace mineral (Caldera et al., 2019).

Organic Zn sources are more bioavailable than inorganic Zn sources when supplemented with ruminants (Ma et al., 2020; Millen et al., 2009; Pal et al., 2010), but little research has been conducted utilizing bis-glycinate bound Zn. Unlike other trace minerals, such as Cu, tissue concentrations of Zn do not readily change in response to dietary supplementation. Because Zn metabolism is highly conserved across species, small ruminants, such as sheep, serve as a useful experimental model for larger ruminants, such as beef cattle (Deters et al., 2021).

Copper and Zinc toxicity

Copper toxicosis can occur in cattle that consume excessive amounts of supplemental copper or feed contaminated with other products or from industries that may release it (Underwood & Suttle, 1999) and can affect cattle performance (Thomas, 2009). However, cattle are not susceptible to copper poisoning as some small ruminants. When fed at higher recommended amounts and in combination with an antagonist, copper accumulates in the liver when (Balemi et al., 2010). Whenever cattle consume high amounts of copper in their diets, they usually store it in the liver before toxicosis becomes evident. If the animal starts suffering from a stress crisis, this will cause the sudden liberation of it, causing hemolytic trouble. Such concerns were characterized by significant hemolysis, jaundice, methemoglobinemia, hemoglobinuria, icterus, necrosis, and death in animals (Johnston et al., 2014; Steffen et al., 1997; Underwood & Suttle, 1999).

Cattle usually tolerate high concentrations of dietary Zn levels. Clinically toxicity was observed whenever the diet contained more than 900 mg Zn/kg (Ott et al., 1966). Some parameters such as feed intake, milk production, and copper status were minimized when cows were fed diets with 2,000 mg Zn/Kg from zinc sulfate (ZnSO4), but not when they were given a diet with 1,000 mg Zn/Kg (W. J. Miller et al., 1989).

The growth rate in pre-ruminant calves is compromised when the mil replacer contains > 50mg kg-1 dry matter, usually added as zinc oxide. Zinc is produced in the digesta on highly digestible diets (Jenkins & Hidiroglou, 1991). Weaned ruminants are slightly more susceptible due to the vulnerability of the rumen microflora (Suttle, 2010). A high zinc level in the diet will reduce the rumen's volatile fatty acids (VFAs) and the ratio of acetic and propionic (Ott et al., 1966).

Summary

Hydroxy Cu and Mn are less soluble in the rumen when compared to sulfate trace mineral sources, whereas differences in solubility of hydroxy Zn and Zn sulfate are inconsistent (Caldera et al., 2019; Cao et al., 2000; Genther & Hansen, 2015). Furthermore, it is difficult to understand data from different studies since they have used various types of sources, cattle, and highly variable experimental designs. Moreover, the breed of cattle, diet minerals, and animal physiological stage must be considered when comparing the results from the different trace mineral source studies. However, only some studies have been done with glycinate as a chelated A.A. trace mineral. Therefore, the objectives of the current study were to be compared with Cu and Zn sulfate trace minerals, supplementation of mono and bis-Glycinates of Zn and Cu will reduce the rumen concentration of soluble trace minerals and increase apparent total tract digestibility of fiber without penalizing animal performance. Hence, the objectives of the current study were to evaluate the effects of 3 different sources of Cu and Zn supplementation on animal performance, apparent nutrient digestibility, and apparent absorption and retention of trace minerals when fed to dairy cows.

Figure 1. 1. Overview of published studies reporting the effect of sulfate Cu and Zn with other sources of Cu and Zn on apparent digestibility (Adapted from Daniel et al., 2017).

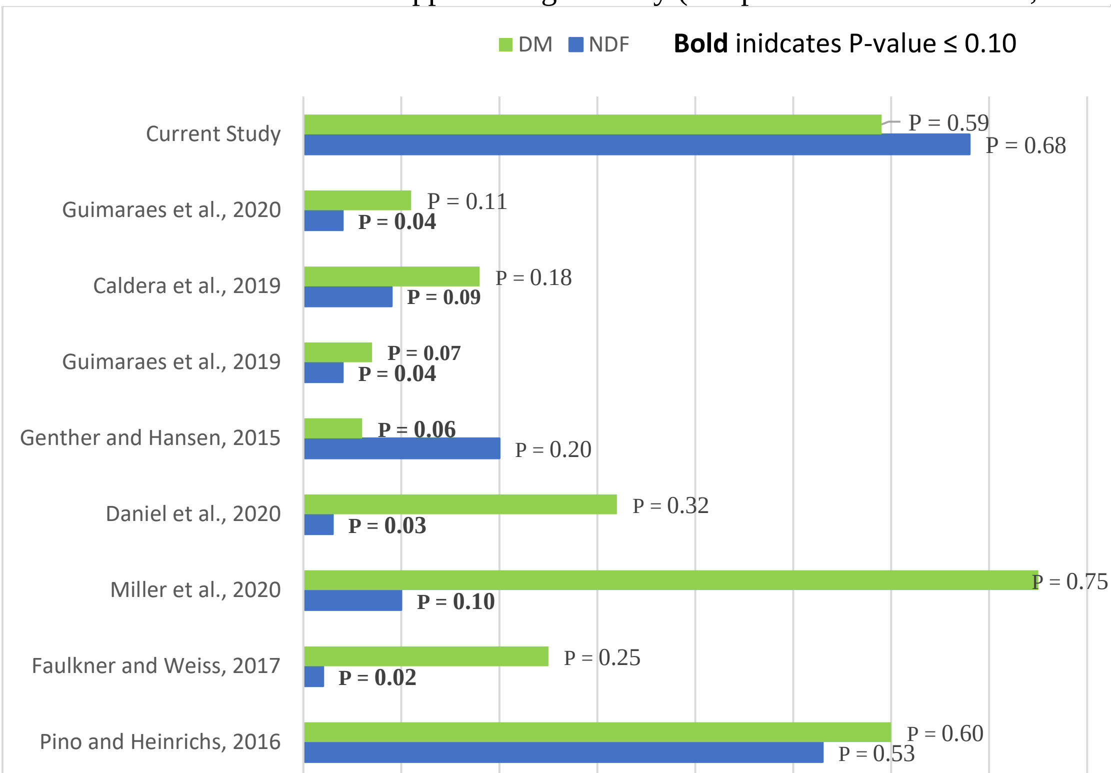


Figure 1. 2. Plexomin® Cu: Due to the ringing structure, the copper ion is protected (Adapted from Phytobiotics).

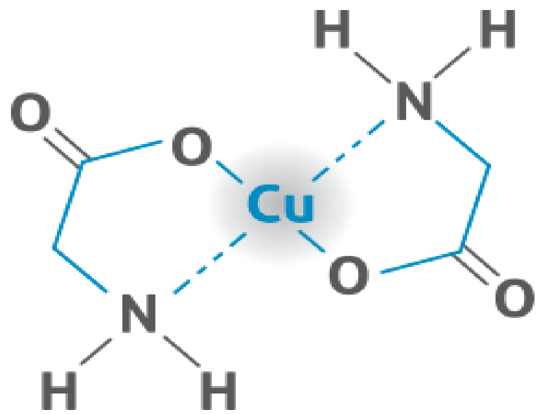


Figure 1. 3. Copper Glycinate: The central atom (mineral ion) and a surrounding array of bound molecules or ions, (Adapted information from phytobiotics and structure from Molview).

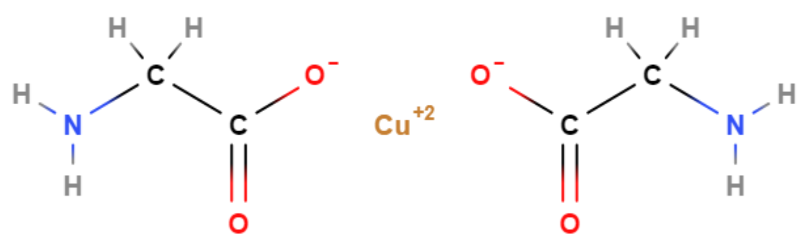
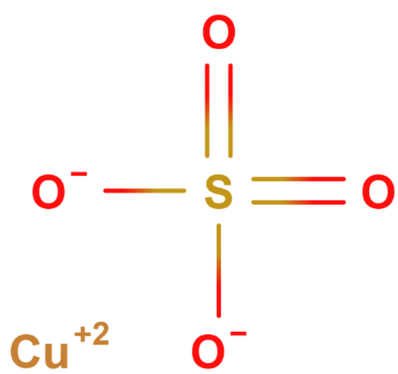


Figure 1. 4. Copper Sulfate ($CuSO_4$) chemical structure (Adapted from Molview).



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CHAPTER TWO

Effect of source of trace minerals on nutrient digestibility and rumen fermentation of dairy cows

Abstract

The main objective of this study was to evaluate the effects of trace mineral source supplementation on apparent nutrient digestibility, apparent retention and absorption and rumen fermentation of lactating dairy cows. A second objective was to determine the effects trace mineral sources on cow performance. Nine multiparous Holstein cows (eight ruminally fistulated) were averaging approximately 90 DIM (days in milk) at the beginning of the trial, were blocked by DIM and randomly assigned to 1 of 3 supplemental mineral treatments in three 3 x 3 Latin squares. The cows were fed once a day with a TMR (DM basis: 15.2% CP, 36.5% NDF, 9.7 mg Cu/kg, and 44.3 mg Zn/kg) for 21 days. Treatments consisted of 10 mg Cu/kg DM and 35 mg Zn/kg DM from one of the three treatments sulfate (STM), Mono-glycinates (MGTM), or Bis-glycinate (BGTM) into a split-plot, 3 × 3 Latin square design. Treatment did not affect dry matter intake or milk production. Dry matter digestibility was similar, and NDF digestibility was not affected by STM vs. MGTM vs. BGTM supplemented lactating cows. The apparent absorption and retention of Cu and Zn were similar between treatments. Apparent Cu absorption and retention had similar effects between treatments. Rumen fluid collection used for measuring pH and NH_3N showed no treatment x time or treatment interactions for any measured response variables. Ruminally soluble Cu and Zn concentrations were similar across trace mineral sources. Serum Cu and Zn concentrations were similar across treatments. Furthermore, the results

of this study do not support our hypothesis that mono and bis-glycinate sources of Cu and Zn, with lower rumen solubility compared with sulfate sources, will improve apparent fiber digestibility compared with sulfate sources.

Introduction

Copper and Zn are critical in cattle's physiological and biological processes, including immune function, protein synthesis, reproduction, and oxidative metabolism. In addition, antagonists, such as molybdenum, sulfur, and iron, can decrease the absorption of Cu (Underwood & Suttle, 1999). Common forms of trace minerals used to supplement cattle can be categorized between sulfates, oxides, carbonates, chlorides, hydroxy, and organic complexes (Daniel et al., 2020). In the past, cattle producers used oxide and later on they decided that the sulfates will have a higher benefit for animals, until recently the most common ones are the organic and inorganic forms of trace minerals (Overton & Yasui, 2014). Organic trace minerals had no effect on nutrients digestibility by heifers in most cases it will increase the volatile fatty acids production compared to the sulfates forms of the trace minerals (Pino & Heinrichs, 2016).

Therefore, dietary supplementation of Cu and Zn is a common nutritional management strategy to prevent deficiency of this mineral that will impact animal performance and health. However, excess soluble Cu and Zn in the rumen can negatively impact rumen microbial populations and reduce dietary fiber digestibility. Several *in vitro* studies reported that higher levels than the required of Cu and Zn supplementation negatively impacted rumen fermentation (Arelovich et al., 2000; Eryavuz & Dehority, 2009). However, Perrin et al., (1990) describes how does an error in the formulation can lead to a toxicity in the dairy herd, where there have a copper levels as 400-500 ppm. Furthermore, the negative effect of Cu and Zn supplementation on microbial fermentation and the concomitant reduction in fiber digestibility are likely associated with the ruminal solubility of the supplemented trace minerals. Furthermore, the rumen presents a challenge to the intestinal absorption of trace minerals because of the formation of insoluble complexes in the rumen that later decrease the bioavailability of specific trace minerals. For example, sulfides are generated when sulfate is reduced in the rumen (Drewnoski et al., 2014) and can then combine with dietary Mo to form thiomolybdates. This complex can then bind directly to Cu to form insoluble Cu sulfide (Suttle, 1991), which reduces Cu bioavailability in the intestine. In addition, Cu and Zn supplementation sources include inorganic sources (oxides, sulfates, and chlorides), and organic complexes have varying water solubility that may affect how much they impact the rumen microorganisms. For example, Cu and Zn hydroxy chloride is relatively insoluble (0.6%) in water, whereas Cu and Zn sulfates are almost completely soluble (Spears et al., 2004). Caldera et al., (2019) observed that NDF digestibility tended to be greater in hydroxy trace minerals vs. sulfate trace minerals supplemented steers (41.2 vs. 37.8% of NDF, respectively).

Similarly, Faulkner et al., (2017) reported that feeding hydroxy Cu, Zn, and Mn to dairy cows increased NDF digestibility compared with cows fed sulfate trace mineral sources (48.5 vs. 46.4% of NDF, respectively). In a recent meta-analysis of beef and dairy cattle studies, Ibraheem et al., (2023) evaluated the impact of sulfate versus hydroxy trace mineral source (Cu, Zn, and Mn) supplementation on nutrient digestibility. The authors observed a 2.7 and 1.1 units increase in NDF digestibility for studies using total collection or undigested NDF as digestibility markers, respectively, when cows were supplemented with hydroxy vs. sulfates trace minerals.

Thus, supplemental sources of Cu and Zn with low ruminal solubility would not affect ruminal microorganisms or interact with Mo and S, while simultaneously maintaining or increasing the bioavailability of Zn and Cu after exposure to the acid environment of the abomasum is a practical approach to supplement Cu and Zn. As a result, it would not penalize dietary fiber digestibility while reducing the environmental impact of livestock operations. In addition to the potential effects on nutrient digestibility, the source of Cu and Zn supplementation can also affect the excretion of this micromineral in manure, which could have adverse environmental impact (Castillo et al., 2013).

Mono and bis-glycinates result from a mechanochemical reaction between the amino acid glycine and Zn or Cu minerals. This amino acid chelates consist of one (mono-glycinates) or two amino acids (bis-glycinates) molecules and are bound to the mineral. Due to their ring structure, they are highly stable over a wide pH range. However, research utilizing mono and bis-glycinate as Cu and Zn supplemental sources for lactating dairy cows is still under investigation.

We hypothesized that compared with Cu and Zn sulfate trace minerals, supplementation of mono and bis-glycinates of Zn and Cu will reduce the rumen concentration of soluble trace minerals and increase apparent total tract digestibility of fiber without penalizing animal performance. Hence, the objectives of the current study were to evaluate the effects of 3 different sources of Cu and Zn supplementation on animal performance, apparent nutrient digestibility, and apparent absorption and retention of trace minerals when fed to dairy cows.

MATERIALS AND METHODS

Care and handling of animals used for the study were conducted as outlined in the guidelines of the Clemson University Committee on Animal Use (AUP #2022-0377-01). One cow was removed from the study before the start of period 1 because of health issues (toxic mastitis) and was replaced with a non-fistulated cow.

Experimental Design and Treatments

Nine multiparous Holstein cows (eight ruminal fistulated, 620 ± 25.0 kg of BW; 149 ± 48 DIM) were blocked by DIM, and within each block, cows were randomly assigned to one of three dietary treatments sequences in a replicated 3 x 3 Latin squares design with 21-d periods. Treatments consisted of a basal diet with the following Cu and Zn supplemental sources: 1) Sulfate (10 mg Cu/kg DM from $CuSO_4$; 35 mg Zn/kg DM from $ZnSO_4$); 2) Mono-Glycinates (10 mg Cu/kg DM from mono-glycinate of copper, 35 mg Zn/kg DM from a chelated form of zinc, Plexomin® Cu24 Plexomin® Zn26, respectively); and 3) Bis-Glycinates (10 mg Cu/kg DM from copper bis-glycinate; 35 mg Zn/kg DM from Zinc bis-glycinate, Plexomin® Cu and Plexomin® Zn, respectively). The chemical composition of dietary ingredients, relative proportion of dietary ingredients, and basal diet nutrient composition are reported in Table 2. 1 and, Table 2. 2 respectively. The use of two different types of corn silage was used due to the not enough of the corn silage 1 for the whole trial, during the second period this corn silage 1 was substitute into the corn silage 2. Supplemental trace minerals were top-dressed using a similar approach as reported by (Faulkner, et al., 2017). The top dress consisted of a mix of ground corn plus the appropriate amount of supplemental Cu and Zn sources fed at 0.2 kg/d. Basal diet concentration of Cu and Zn is showed in Table 2. 2 were is showed that values were near the requirements needed from the (NRC, 2021).This rate supplied dietary concentrations of 10 and 35mg/kg of diet DM of supplemental Cu and Zn based on average daily DMI. Treatment sequences within each square were balanced for carryover effects (i.e., each treatment followed each of the others once within each square). Each experimental period consisted of 14 d for adaptation and 7 d for sample and data collection, this days were similar as the experimental period of Daniel et al., (2020) and Faulkner, et al., (2017). Between d 1 to 16 of each period, cows were housed in a free stall pen fitted with Calan gates (American Calan, Inc). During d 17 to 21 of each period, cows were moved to a tie-stall barn bedded on rubber mats with chopped wheat straw for fecal, urine, and rumen sampling. Diets were offered as total mixed rations (TMR) once daily, allowing 5 to 10% refusals. In addition, 0.455kg of pellets were fed to cows in the automatic robot twice daily. The pellet nutritional composition was used to determine the amount of Cu and Zn the cow was consumed and to calculate the DMI. The ingredient mix was adjusted based on weekly forage DM. `

Sample collection and analysis

Total mixed rations (TMRs) and individual feed refusals were sampled daily during the last week of each period and stored at -20°C. Thawed TMR and refusal samples were composite by period, dried at 55°C (forced-air oven) for 48 h, and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA, USA). Ground TMR and refusal samples were analyzed for DM at 100°C for 24 h. Ash concentration was determined after combusting samples in a furnace for 3 h at 600 °C (Method 942.05, AOAC International, 2006). Neutral detergent fiber (NDF) concentrations were determined using an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY, USA) and corrected for ash concentration. Sodium sulfite and α-amylase (Sigma no. A3306: Sigma Chemical Co., St. Louis, MO, USA) were included in the NDF analysis (Van Soest et al., 1991). For each sample, a subsample was separated and submitted to Cumberland Valley Analytical Services (Waynesboro, PA, USA) to determine the concentrations of N (Method 990.03, AOAC International, 2000) and starch concentration as described by (Hall, 2009). Crude protein concentration was calculated as a percentage N × 6.25. Copper and zinc were analyzed were also analyzed Cumberland Valley Analytical Services Samples were ashed (1 h at 535°C), digested in 15% nitric acid, diluted, and assayed by inductively coupled plasma emission spectroscopy (Perkin Elmer 5300 DV ICP. Perkin Elmer, 710 Bridgeport Avenue, Shelton, CT 06484). Individual cow DMI was computed the last 7 days of each period based on daily records of TMR offered and re-fused and the 105 °C DM contents of the TMR and refusals.

Throughout the study, milk production was recorded on each cow at the 2 daily milkings (0600 and 1800 h). Milk samples were collected from six consecutive milking on d 14, 15, and 16 of each experimental period. Milk sampling collection containers were acid-washed (10% hydrochloric acid) before each collection period. One subsample was collected in a bottle with preservers and analyzed for fat, true protein, lactose, and MUN by infrared analysis (Lancaster Dairy Herd Association, Manheim, PA, USA) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN). A second subsample from each milking was collected in a bottle without a preserver, composited by cow and period for

Cu and Zn analyses by Michigan State University Veterinary Diagnostic Laboratory (East Lansing, MI) using inductively coupled plasma MS (Wahlen et al., 2005). The average daily concentration and yield of milk components were computed using morning and evening milk production as a weighting factor. Yield of fat- and protein-corrected milk (FPCM) was calculated as milk (kg/d) × [0.1226 × fat (%) + 0.0776 × true protein (%) + 0.2534] according to IDF (2010).

Spot fecal and urine samples were collected from each cow the last week of each period as described by (Lee et al., 2019), in which they stated 12-time points with 2-h intervals to cover the 24-h clock over 3 d (0800, 1400, 2000, and 0200 h on d 18; 1000, 1600, 2200, and 0400 h on d19; 1200, 1800, 2400, and 0600 h on d 20). Approximately 100 g of fresh feces were collected from the rectum at each sampling time and then frozen at −20°C for later analysis. Upon thawing, samples were composite by cow using a commercial mixer (Commercial Series 8, KitchenAid, Benton Harbor, MI, USA), dried at 55°C in a forced-draft oven for 48h, and ground through a 1-mm Wiley mill screen (Arthur H. Thomas Co.). As previously described, ground samples were analyzed for DM, ash, total N, NDF, and starch.

Indigestible NDF (iNDF; was determined in the TMR, refusals, and fecal samples after in situ ruminal incubation using 2 rumen-fistulated cows (1 Jersey and 1 Holstein). To determine iNDF, 0.25 g of sample was weighed into F57 Ankom bags (Ankom Technologies) that were inserted into a nylon laundry mesh bag (38.1 cm × 45.7 cm; Home Products International, Chicago, IL), which was inserted into the rumen via the cannula and incubated in the rumen for 240 h (Ferreira & Mertens, 2005). The cows were fed a diet containing 31.5% corn silage, 3.6% pearl millet silage, 3.3% bermudagrass hay, and 61.6% concentrate mix (DM basis). After the incubation, the mesh bags were removed from the rumen, submerged in cold water to stop bacterial fermentation, and rinsed five times (3-min washing + spinning cycles) using a washing machine (twin tub washing machine, super deal). After drying at 55°C for 48 h, NDF analyses were conducted in all bags as previously described.

In this study, iNDF was an internal marker for estimating fecal DM output and nutrient digestibility. The amount of nutrient intake (OM, NDF, CP, and starch) and iNDF intake during the 3 days of fecal collection in each period was calculated for each cow from nutrient concentration in feed ingredient multiplied by DMI minus respective refusal amounts. Feces output (DM basis) was estimated as iNDF intake divided by iNDF concentration in feces (Cochran et al., 1986). Total-tract apparent digestibility of nutrients was determined from the amount of nutrients in fecal excretion and daily nutrient intake.

Twelve spot urine samples were collected by stimulating the area below the vulva at the same time points used for fecal spot sampling. One subsample was acidified with 6N HCl with a 0.5:9.5-mL volumetric ratio of acid to urine, and the second subsample was not acidified. Both samples were stored at −20°C until further analyses. After thawing at room temperature, acidified samples were composited for each cow by period and analyzed colorimetrically for creatinine (assay kit no. 500701; Cayman Chemical Co.). Using the BW recorded at the end of each period (see below), total daily urine volume was estimated, assuming a constant creatinine excretion rate of 29 mg/ kg of BW, this procedure is showed below Equation 2. 3 (Valadares et al., 1999). As previously described, non-acidified urine samples were assayed for Cu and Zn by Michigan State University Veterinary Diagnostic Laboratory (East Lansing, MI).

On day 21 of each period, rumen samples were collected from multiple locations in the rumen to yield an 8-mL sample from 0 (pre-feeding), 2, 4, 8, 12, 18, and 24 h post-feeding. Rumen samples were strained through four cheesecloth layers (Grainger, Camden, SC), and rumen pH was determined in a subsample immediately after sample collection using a calibrated portable pH meter (VWR® sympHony Electrochemistry, USA). A second sub-sample of rumen fluid was acidified with 1 *M* H_2SO_4 and stored at −20°C until further analysis (Daniel et al. 2020). Samples were thawed and centrifuged at 5,000 x g for 15 min at 4ºC. Following centrifugation, 1 mL of the supernatant was transferred into a 1.5 mL Eppendorf microcentrifuge and analyzed for NH_3-N (Chaney & Marbach, 1962). Rumen solubility for Cu and Zn was determined as described by (Genther & Hansen, 2015), whole rumen-fluid samples were sub- sampled in duplicate per cow per period and were subjected to ultracentrifugation at 28,000 × g for 30 min at 4°C, and the supernatant was removed and considered the ruminally soluble fraction.

Individual cow BW (722 ± 62.68) was recorded for three consecutive days (d18, 19, and 20) after the afternoon milking on each experimental period. Blood samples were collected into 3 h after feeding from the coccygeal artery or vein of each cow on d 21 of each period into trace mineral-free vacutainers (BD Vacutainer, Franklin Lakes, NJ) for serum and into heparinized tubes for plasma collection. Blood was allowed to clot; serum was harvested (1,000 × g for 10 min) and stored at -80°C. As previously described, serum samples were assayed for Cu and Zn by Michigan State University Veterinary Diagnostic Laboratory (East Lansing, MI).

Values of Cu and Zn intake were used from the analysis of the TMR and refusals. Trace minerals intake and refusals from the analysis were multiplied by the intake and refusals of the feed and this was adjusted by the values from the pellet gave at the milking. After, the intake of TM was subtracted from the amount that was excreted in feces, milk, and urine to obtain the apparent absorption and apparent retention for copper and zinc; we used equation [1,2] for each cow during each of the three 7-d collection periods (Faulkner, et al., 2017).

Equation 2. 1 Apparent absorption.

$$Apparent\ absorption = \text{(Intake } - \text{ Fecal)/Intake)} * 100]\ X\ 100]$$

Equation 2. 2 Apparent retentions.

$$\text{Apparent Retention} = (Intake\ -\ Fecal\ -\ Milk\ -\ Urine)$$

For obtaining the total urine output creatinine method was used, in which we have used the values with (Valadares et al., 1999) constant shown:

Equation 2. 3 Creatine into urine volume.

$$Creatinine\ concentration = [Creatinine/(29mg/kg)\ X\ BW]$$

$$Urine\ volume = [\ Creatiner\ concentration\ /\ Creatine]$$

Statistical Analyses

Data were analyzed using JMP® Pro v16.0 software (SAS Institute Inc., Cary, NC), and figures were obtained using Excel (Office 365). One cow was removed from the study before the start of period 1 because of health issues (toxic mastitis) and was replaced with a non-fistulated cow. All the analysis were based in the nine cows that were involved in the research. For the ruminal fluid they were only eight cannulated animals used for this sampling. Dry matter intake, milk yield, milk composition, apparent nutrient digestibility, and Cu and Zn partitioning data were analyzed with a statistical model included the effect of square (1 to 3), the effect of the period (1 to 3), the effect of the cow within a square (1 to 3), the treatment effect (1 to 3), and the residual error. All terms were considered fixed except cow (within the square) and the residual error. The above model was expanded to include effect of time and the interaction between treatment and time to analyze rumen pH, NH_3-N, Cu and Zn concentration data as repeated measurements. Pre-planned orthogonal contrasts were used to test Organic vs inorganic Cu and Zn (Co1) and MGTM vs BGTM (Co2). In addition, protected least significant differences were used to detect differences among treatments Significance was declared for $P \leq 0.05$ and a tendency for $0.05 < P \leq 0.10$.

Results and Discussion

Animal Performance

The effect of supplemental Cu and Zn sources on animal performance is presented in Table 2.3. Under the condition of this study, DMI (average ± SD), milk yield (average ± SD), FPCM (average ± SD), feed efficiency (average ± SD), milk composition, fat (average ± SD) and protein yield (average ± SD) were similar among the treatments.

The lack of effect of supplemental sources of Cu and Zn on animal performance observed in the current study is consistent with previous studies comparing sulfates with hydroxy Cu and Zn supplements (Faulkner & Weiss, 2017). In contrast, Miller et al. (2020) reported that cows fed a hydroxy Cu, Zn, and Mn trace mineral mix had a higher DMI than sulfates. However, milk yield and concentration of milk fat and milk protein content were unaffected by trace mineral sources. Daniel et al. (2020) observed a higher milk yield on diets supplemented with a mix of organic and inorganic (hydroxychloride) trace mineral sources (Cu, Zn, and Mn) compared with supplemental trace minerals fed only as either sulfates or hydroxychloride to multiparous cows but not when the same treatments were offered to primiparous cows. Data from current and previous lactation studies suggest that Cu and Zn source has little impact on DMI, milk production, and composition.

Rumen pH, ammonia N, and trace mineral concentration

Averaged rumen pH values were not different between dietary treatments. As expected, there was a significant ($P < 0.01$) effect of pH over time, and the pattern of change was similar between all treatments (Figure 2.1). The rumen pH pre-feeding (time 0) was

6.26; it declined to a nadir on time 12 (5.57) and returned to similar pre-feeding levels by time 24 (6.18). The trace mineral supplements did not affect rumen NH_3-N (mg/dL) concentration. However, a significant ($P < 0.01$) effect of NH3-N over time followed a similar pattern to the one observed for rumen pH. The rumen NH_3-N concentration (mg/dL) at feeding (time 0) was 10.5; it declined to a nadir on time 12 (6.7) and returned to similar pre-feeding levels by time 24 (9.8). Satter & Slyter, (1974), suggested a minimal rumen NH3N of 5.0 mg/dl from their invitro trials. These results are consistent with previous studies feeding inorganic and organic sources of Cu and Zn to dairy (Daniel et al., 2020) and beef cattle (Guimaraes et al., 2021, 2022) that showed no effect of different sources of supplemental Cu and Zn on NH3-N concentration.

Copper and Zn sulfates supplements have been shown to have a very high solubility in the rumen. Spears et al. (2004) reported that 95% of Cu sulfate was soluble in water after 24 hours of incubation at 39 °C. Therefore, supplementation of Cu sulfate can increase the potential interaction of Cu with dietary antagonists (sulfide and molybdate) in the rumen, reducing its bioavailability. Also, increasing the rumen soluble Cu and Zn can affect fibrinolytic bacteria resulting in a concomitant reduction in fiber digestibility. Organic sources of Cu and Zn likely remain complex or chelated in the rumen, which minimizes the chances of interaction with dietary antagonists and rumen microorganisms. Interestingly, hydroxy Cu is almost insoluble (6%) in water after 24 hours of incubation at 39 °C (Spears et al., 2004), resulting in a consistently lower concentration of soluble Cu in the rumen in beef and dairy cattle, compared to sulfate sources. There is a tendency of NDF digestibility, it will be greater on steers fed hydroxy trace minerals compared to the fed with sulfate trace minerals, this can be related due to the low solubility concentration of soluble minerals dosed with the hydroxy trace mineral (Caldera et al., 2019).

Furthermore, **Error! Reference source not found.** showed the values for whole ruminal concentration (1.56 mg/dL) of Cu (1.56 mg/dL) and Zn (8.18 mg/dL). For instance, Genther & Hansen (2015) reported Cu concentrations in rumen fluid that ranged between 1.10 to 2.11 mg/dL when beef steers were supplemented with 5 to 25 mg/kg of DM of Cu from sulfates or hydroxychloride. In addition, the same authors reported Zn concentration in rumen fluid that ranged between 5.33 to 9.50 mg/dL when supplemental Zn in the diet from sulfates or hydroxychloride was 30 to 120 mg/kg of DM. The current study (Genther & Hansen, 2015) observed that supplementation with sulfates or hydroxides did not affect whole-rumen fluid concentration of Cu and Zn. However, the hydroxide Cu treatment had a lower rumen-soluble (supernatant of whole rumen fluid after ultracentrifugation) Cu concentration than sulfates.

Previous studies have also consistently shown that Cu and Zn hydroxy sources were less soluble in the ruminal environment than sulfate forms (Caldera et al., 2019; Guimaraes et al., 2021). The observations from the previous studies suggest that rumen-soluble Cu and Zn are a better indicator of how much of these trace minerals are readily available in the rumen than whole rumen fluid. Less rumen-soluble Cu and Zn in the rumen could decrease the impact of these microminerals on cellulolytic microorganisms and fiber digestibility. These sources may decreased D.M. digestibility from T.M. supplementation while remaining available to the animal for absorption later in the intestine (Genther &

Hansen, 2015). No time effect was observed for whole rumen Cu concentration. Apparent digestibility of nutrients

Error! Reference source not found. summarizes the effects of supplemental Cu and Zn sources on apparent nutrient digestibility. Dry matter, OM, NDF, N, and starch were similar among the treatments. Under *in vitro* conditions, excess Cu (Durand & Kawashima, 1980) and Zn (Arelovich et al., 2000) have been shown to impact rumen fermentation negatively. Thus, it has been proposed that Cu and Zn sulfate supplementation can reduce fiber digestibility due to their high rumen solubility. Organically bound Cu and Zn sources might mitigate potential interaction with antagonists in the rumen (Hansen et al., 2008). Supplementing organic trace minerals (Cu, Zn, Mn) in proteinates did not affect fiber digestibility when fed to dairy heifers, compared with sulfate minerals (Pino & Heinrichs, 2016). However, in a recent meta-analysis summarizing 8 cattle studies, Ibraheem et al. (2023) showed an overall increase of 0.5% and 1.51% in DM and NDF digestibility when comparing low (hydroxy) vs. high (sulfate) rumen soluble sources of Cu, Mn, and Zn fed to beef and dairy cattle. Results from the meta-analyses support the hypothesis that rumen solubility of supplemental Cu and Zn might play an essential role in NDFD.

In agreement with the current study, Deters et al. (2021) reported no treatment effect on DM, OM, and NDF digestibility in growing lambs fed 15 mg/kg DM of supplemental Zn as sulfate or as bis-glycinate. Because Zn metabolism is highly conserved across species. Small ruminants, such as sheep, serve as a useful experimental model for larger ruminants, such as beef cattle. Thus, despite the lower solubility of Zn- bis-glycinate in deionized water (Deters et al., 2021) compared to Zn-sulfate, results from the current and previous study suggest that mono and bis-glycinate bound Cu and Zn does not total tract apparent nutrient digestibility. However, it is also important to highlight that: 1) to our knowledge, there are no published data on the rumen solubility of Cu and Zn mono-glycinate or Cu bis-glycinate or their impact on apparent nutrient digestibility; 2) (Deters et al., 2021) observed that Zn bis-glycinate solubility was determined using deionized water with a pH of 5.2. This incubation media might not reflect the rumen conditions and the pH commonly expected in a dairy rumen cow; 3) It has been shown that the digestibility assessment method can affect the magnitude of the treatment response. For example, (Ibraheem et al., 2023) observed that studies used total collection had a higher increase in NDF digestibility (2.68% units) compared with with undigested NDF (1.08% units), respectively, as markers when cattle were fed hydroxy vs. sulfate mineral sources.

Total-tract Apparent Cu and Zn Absorption and Balance

Table 2. 6 summarizes the effects of the different supplemental sources of Cu and Zn on mineral partition in milk, feces, and urine. Milk production, DMI, and intake of Cu or Zn during the 4-d milk, urine, and fecal collection period were not affected by supplemental sources of Cu and Zn. Similarly, Cu and Zn secretions in milk were not significantly different between treatments. On average, milk Cu secretion was 0.3% of intake, while the amount of Zn secreted in milk was 6.5% of Zn intake. These results are similar to those reported by (Faulkner et al., 2017) in a study supplementing similar levels of Cu and Zn to those used in the current study.

Copper (2.93 mg/d) and Zn (16.5 mg/d) urinary excretion were unaffected by the supplemental mineral sources and averaged 0.5 and 0.67% of Cu and Zn intake, respectively. Similarly, the source of supplemental Cu and Zn did not impact the fecal excretion of Cu (572 mg/d) and Zn (2,050 mg/d). In agreement with our study, Faulkner et al. (2017) reported that supplemental sources of Cu and Zn (sulfate vs. hydroxy) did not affect Cu and Zn excreted in the urine or feces. However, compared to the values reported by Faulkner et al. (2017), fecal Zn excretion as a % of Zn intake was higher in the current study (85.0 vs. 74.5%, respectively). Deters et al. (2021) observed that urinary Zn excretion tended to be greater for growing lambs fed bis-glycinate bound Zn compared with Zn sulfate ($P = 0.09$); however, fecal Zn excretion was not affected by supplemental Zn sources. Pino & Heinrichs (2016) reported a 12% reduction in fecal Cu excretion when dairy heifers were supplemented with a proteinate vs. sulfate source of Cu. This can be related on the size of the rumen capacity and requirements that a heifer will have compared to a mature dairy cow.

The excretion of minerals in feces can negatively impact air, soil, and water quality (Powell & Broderick, 2011). Due to the low efficiency of utilizing micro minerals by livestock, most dietary Cu and Zn are excreted in the manure, particularly in the feces (Hristov et al., 2007). Thus, feeding strategies that reduce excess mineral excretion without penalizing animal performance and health are important tools to mitigate the negative impact of animal operations (Cerosaletti et al., 2004). However, results of the current and previous studies suggest that supplemental Cu and Zn source has a minimum impact on fecal and urinary excretion of this micromineral in cattle.

On average, Cu and Zn, apparent absorption (7.2 and 14.9%, respectively) and retention (36 and 166 g/d, respectively) did not differ between the supplemental sources of Cu and Zn evaluated in the current study. Deters et al. (2021) observed no effect on Zn apparent absorption (13.9%) for growing lambs fed bis-glycinate bound Zn compared with Zn sulfate. Faulkner et al. (2017) indicated Cu and Zn sources did not affect Zn absorption retention; however, dietary fiber sources (forage vs. by-product) affected apparent absorption and retention of Cu. When cows were fed a forage-based diet, apparent Cu absorption and retention were greater when hydroxy minerals were supplemented than sulfate. However, the opposite response was observed for cows fed a by-product-based diet. The authors hypothesized that the undigested fiber from the forage diet had an antagonistic effect on Cu absorption, whereas the undigested fiber from by-products does not.

Blood serum concentration

No treatment differences were observed for serum Cu and Zn concentrations (Figure 2.3). The observed serum Cu concentrations were adequate for lactating dairy cows (0.8 to 1.2 mg Cu/L; Underwood, 1977). Previous studies have shown that concentrations of Cu and Zn in serum are not a good indicator of adequacy when animals are not in a deficit status (Hansen et al., 2006; Mullis et al., 2003) and do not reflect changes in the dietary supply of these minerals unless supplemented at high concentrations (Wright & Spears, 2004). Faulkner et al. (2017) reported no effect on serum Cu when cows were fed sulfates or hydroxy minerals and a tendency for higher serum Zn for cows fed the hydroxy source. Similarly, Deters et al. (2021) observed no difference in Cu plasma concentration when growing lambs were supplemented with Cu sulfate or bis-glycinate bound Cu. However, plasma Zn concentrations were greater for lambs fed the bis-glycinate supplement than the Cu sulfate source. Hansen et al. (2008) observed no effect on Cu in plasma when beef steers were fed Cu sulfate, hydroxy Cu, or an organic source (Cu lysine). Shaeffer et al. (2017) observed a higher concentration of plasma Zn in steers supplemented with Zn from Zn hydroxychloride than in Zn sulfate ($ZnSO_4$). Another study by Pino & Heinrichs (2016) compared the level of starches and the two different sources of TM. They found no difference between treatment was found for Cu and Zn in the OTM and ITM. Serum is used to evaluate the health that the animal is having, many articles explained that serum is not a good method to understand the absorption that the animal will have. In this case, Cu and Zn did not show any difference between treatments and this is similar to recent publications that try to observe if serum was a good indicator for absorption.

Conclusion

In this study, different sources of supplemental Cu and Zn was added to the diets of lactating dairy to determine how they affected the apparent nutrient digestibility and animal performance. Overall, source of Cu and Zn supplementation did not influence animal performance, nutrient digestibility, or mineral partitioning. Furthermore, the results of this study do not support our hypothesis that mono and bis-glycinate sources of Cu and Zn, with lower rumen solubility compared with sulfate sources, will improve apparent fiber digestibility compared with sulfate sources. Future research should seek to understand how the digestibility and absorption of Cu and Zn does might be affected by sources available comparing the sulfates vs glycinate on beef cattle. The reason, the beef industry have showed some difference in digestibility and absorption using different types of trace mineral source.

Table 2. 1 Chemical Composition of dietary ingredients.[1]

	CS1	CS2	BB	BH	PMX	RP
DM, %	28.6	24.5	33.3	88.4	88.3	88.9
Ash, % DM	3.5	4.7	7.9	5.4	11.8	--
CP, % DM	7.6	8.4	14.6	12.2	24.2	19.8
NDF, % DM	39.9	51.2	50.3	69.2	--	28.1
Starch, % DM	34.0	12.9	0.6	2.5	22.2	--
Copper, mg/kg	10	6	10	10	18	10.7
Zinc, mg/kg	23	41	24	31	101	30

[1]CS1 = corn silage 1; CS2 = corn silage 2; BB = Barley Baleage; BH = Bermudagrass hay; PMX = premix Trial; RP = Robot pellet

Table 2. 2 Ingredient composition of the basal diet (% of DM).

Ingredient	Basal Diet	Pellet[3]
Corn silage	33.4	
Barley silage	5.40	
Bermuda hay	3.40	
Premix Grain[1]	57.8	
Chemical composition[2]		
DM,%	46.4	88.9
OM,%	90.6	--
NDF, %	36.5	28.12
CP,%	15.2	19.75
Starch,%	22.9	--
Ca,%	1.2	0.17
P,%	0.4	0.61
Mg,%	0.4	0.27
K,%	1.8	1.11
Na,%	0.8	0.34
Fe, mg/kg	492.3	109.9
Cu, mg/kg	9.7	10.7
Zn, mg/kg	44.3	30.5
Mn, mg/kg	47.1	15.6

[1] Premix grain contained Ground corn 33.72%, Soybean meal 14.98%, Soyplus 13.97%, Soybean hulls 11.51%, Whole Cotton 8.87%, Corn gluten 6.37%, Vitamins, and others 10.58%.
[2] Chemical composition of the basal diet without supplemental Cu and Zn.
[3] Nutrients components from the pellets used in the milking robot.

Table 2. 3 Effect of dietary treatments on animal performance.

Item	STM[3]	MGTM[4]	BGTM[5]	SEM[6]	P-value	Co1[7]	Co2[8]
Treatments							
DM Intake, kg/d	25.9	26.9	26.2	0.81	0.65	0.48	0.54
1Milk, kg/d	33.9	33.4	33.0	1.60	0.93	0.73	0.87
FPCM, kg/d[1]	34.7	34.4	34.3	1.45	0.98	0.85	0.97
Milk / DMI[2]	1.31	1.24	1.26	0.05	0.63	0.35	0.81
FPCM /DMI[2]	1.35	1.28	1.31	0.05	0.61	0.36	0.69
Milk composition							
Fat, %	4.2	4.2	4.3	0.12	0.73	0.81	0.69
True protein, %	3.3	3.4	3.4	0.06	0.52	0.59	0.97
Lactose, %	4.9	4.9	4.9	0.04	0.08	0.51	0.35
SNF, %	5.8	5.8	5.8	0.03	0.14	0.55	0.31
MUN, mg/dl	12.8	12.5	12.7	0.56	0.64	0.77	0.87
Milk components yield							
Fat, kg/d	1.4	1.4	1.4	0.07	0.60	0.68	0.61
True protein, kg/d	1.1	1.1	1.1	0.05	0.68	0.70	0.75
Lactose, kg/d	1.7	1.6	1.6	0.08	0.34	0.51	0.69

[1]Fat-and protein-corrected milk (FPCM) = Milk yield (kg/day) * [0.1226 * fat (%) + 0.0776 * protein (%) + 0.2534].
[2]Efficiencies calculated as milk (kg/d) or FPCM (kg/d) divided by DMI (kg/d).
[3]Sulfate Trace Minerals: 10 mg/kg from $CuSO_4$; 35 mg/kg from $ZnSO_4$.
[4]Mono-glycinates inorganic: 10 mg/kg from Mono-glycinate copper form; 35 mg/kg from Mono-glycinate from zinc form.
[5]Bis-glycinates organic: 10 mg/kg from Bis-glycinate copper form; 35 mg/kg from Bis-glycinate from zinc form.
[6]Standard error of the mean.
[7] Contrast between Inorganic vs Organic source.
[8] Contrast between MGTM and BGTM source.

Table 2. 4 Effect of trace mineral (TM) concentration and source on ruminal-fluid TM concentrations and ruminal solubilities in cattle.

	Treatments						
Item	STM[1]	MGTM[2]	BGTM[3]	SEM[4]	P-Value	Co1[5]	Co2[6]
Whole ruminal fluid, mg/L							
Cu	1.7	1.5	1.6	0.05	0.22	0.87	0.85
Zn	8.4	8.0	8.3	0.23	0.54	0.32	0.76
Rumen-soluble mineral, mg/L							
Cu	0.44	0.41	0.53	0.05	0.24	0.82	0.55
Zn	1.88	1.78	1.42	0.24	0.40	0.62	0.32
Rumen soluble, % of whole rumen fluid							
Cu	24.2	27.9	27.9	4.69	0.82	0.99	0.77
Zn	22.9	25.6	15.1	5.68	0.43	0.47	0.50

[1]Sulfate Trace Minerals 10 mg/kg from $CuSO_4$; 35 mg/kg from $ZnSO_4$.
[2]Mono-glycinates inorganic 10 mg/kg from Mono-glycinate copper form; 35 mg/kg from Mono-glycinate from zinc form.
[3]Bis-glycinates organic 10 mg/kg from Bis-glycinate copper form; 35 mg/kg from Bis-glycinate from zinc form.
[4]Standard error of the mean.
[5]Contrast between Inorganic vs Organic source.
[6]Contrast between MGTM and BGTM source.

Table 2.5 Influence of trace minerals on dry matter (DM), organic matter (OM), neutral fiber detergent (NDF), nitrogen (N) digestibility in lactating cows, and starch.

	Treatments						
Item	STM[1]	MGTM[2]	BGTM[3]	SEM[4]	P-Value	Co1[5]	Co2[6]
DM, %	62.1	63.1	60.2	1.99	0.59	0.88	0.34
OM, %	66.1	63.1	63.5	2.14	0.55	0.27	0.97
NDF, %	44.8	47.0	47.9	2.59	0.68	0.39	0.81
N, %	58.8	61.4	55.6	3.46	0.50	0.95	0.30
Starch, %	99.1	99.3	98.8	0.27	0.42	0.84	0.26

[1]Sulfate Trace Minerals 10 mg/kg from $CuSO_4$; 35 mg/kg from $ZnSO_4$.
[2]Mono-glycinates inorganic 10 mg/kg from Mono-glycinate copper form; 35 mg/kg from Mono-glycinate from zinc form.
[3]Bis-glycinates organic 10 mg/kg from Bis-glycinate copper form; 35 mg/kg from Bis-glycinate from zinc form.
[4]Standard error of the mean.
[5]Contrast between Inorganic vs Organic source.
[6]Contrast between MGTM and BGTM source.

Table 2. 6 Effects of trace mineral source on the concentration of minerals apparent absorption and retention.

Item	STM[1]	MGTM[2]	BGTM[3]	SEM[4]	P-Value	Co1[5]	Co2[6]
Intake							
DM, kg/d	25.9	26.9	26.2	0.81	0.65	0.48	0.54
Cu, mg/d	612	621	616	15.8	0.93	0.76	0.86
Zn, mg/d	2,398	2,431	2,411	58.1	0.92	0.75	0.79
Milk, kg/d	33.9	33.4	33.0	1.60	0.93	0.73	0.87
Cu, mg/d	1.8	2.0	2.0	0.21	0.70	0.41	0.87
Zn, mg/d	163	150	153	9.96	0.65	0.36	0.82
Cu, % of intake	0.3	0.3	0.3	0.03	0.69	0.41	0.83
Zn, % of intake	6.8	6.2	6.4	0.39	0.54	0.28	0.78
Urine, kg/d	24.4	24.8	25.1	0.74	0.61	0.95	0.81
Cu, mg/d	2.5	1.6	4.7	1.26	0.21	0.70	0.15
Zn, mg/d	14.3	24.4	10.8	6.91	0.36	0.70	0.22
Cu, % of intake	0.4	0.3	0.8	0.21	0.21	0.68	0.15
Zn, % of intake	0.6	1.0	0.4	0.28	0.33	0.66	0.21
Feces, kg/d	9.8	9.9	10.3	0.47	0.72	0.61	0.52
Cu, mg/d	579	549	587	27.3	0.60	0.74	0.37
Zn, mg/d	2,005	2,057	2,086	91.3	0.82	0.55	0.83
Cu, % of intake	94.5	88.5	95.5	3.94	0.42	0.61	0.26
Zn, % of intake	83.6	84.6	87.0	3.72	0.81	0.62	0.70
Apparent absorption, %							
Cu	5.5	11.5	4.5	3.94	0.42	0.61	0.26
Zn	16.4	15.4	13.0	3.72	0.80	0.62	0.70
Apparent retention, mg/d							
Cu	29	68	23	24	0.38	0.59	0.25
Zn	216	200	160	86	0.89	0.73	0.78

[1]Sulfate Trace Minerals 10 mg/kg from $CuSO_4$; 35 mg/kg from $ZnSO_4$.

[2]Mono-glycinates inorganic 10 mg/kg from Mono-glycinate copper form; 35 mg/kg from Mono-glycinate from zinc form.

[3]Bis-glycinates organic 10 mg/kg from Bis-glycinate copper form; 35 mg/kg from Bis-glycinate from zinc form.

[4]Standard error of the mean.

[5]Contrast between Inorganic vs Organic source.

[6]Contrast between MGTM and BGTM source.

Figure 2. 1 Effect of time on pH with three different trace minerals sources.

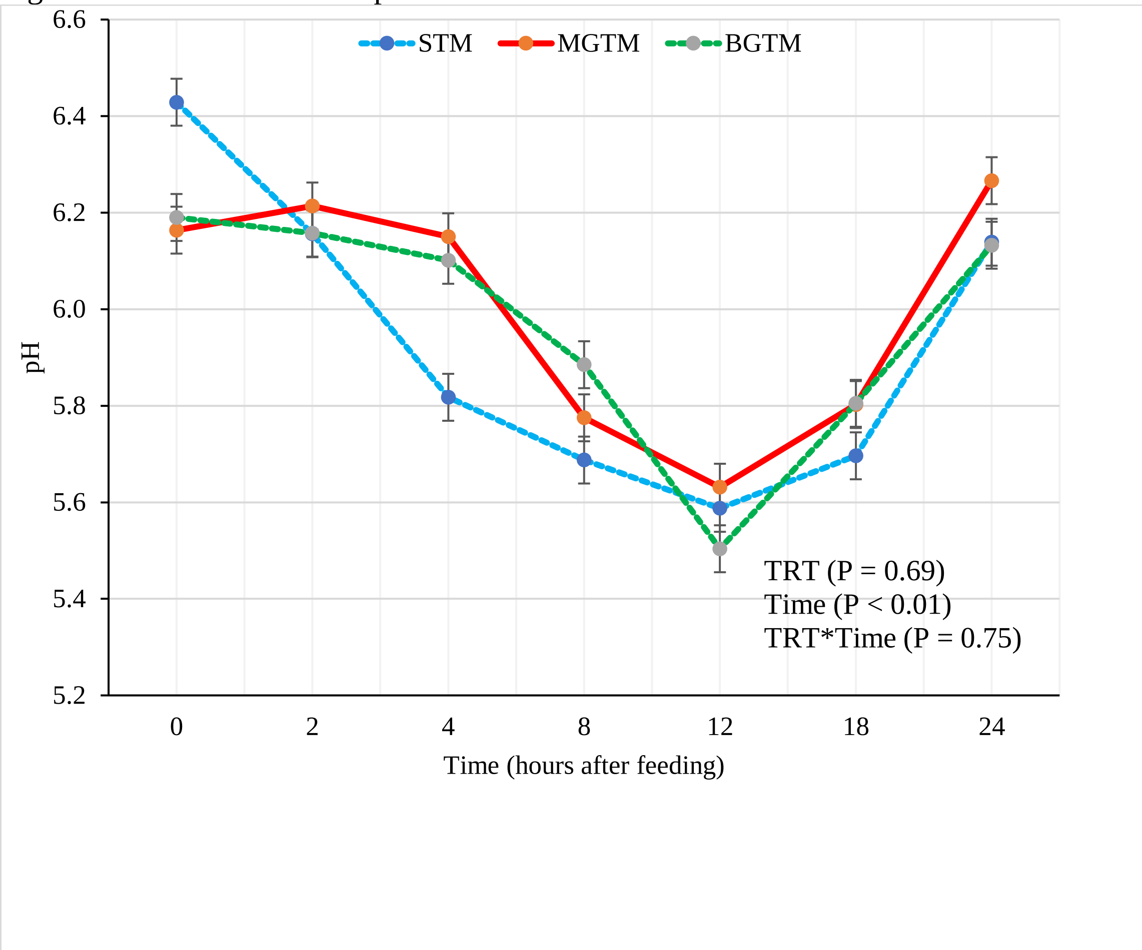


Figure 2. 2 Effect of time on NH3N with three different trace minerals sources.

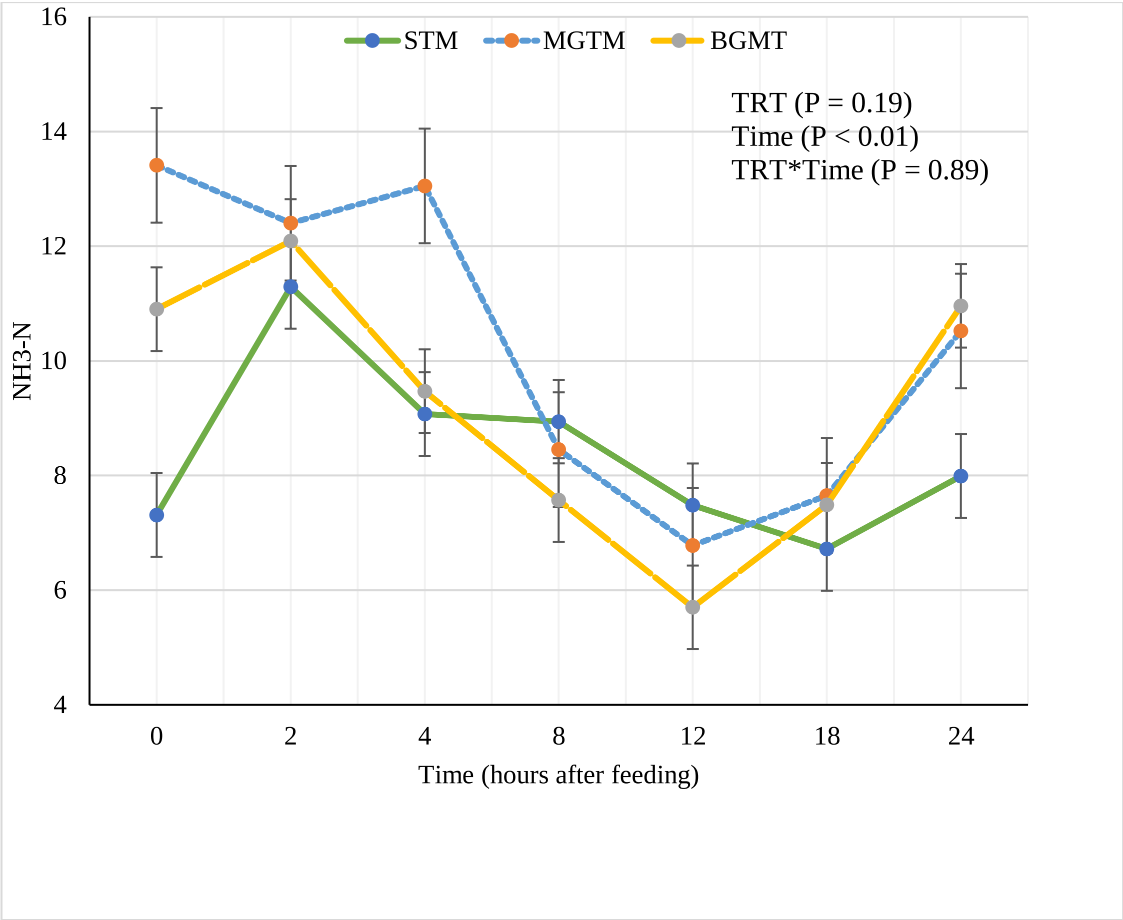
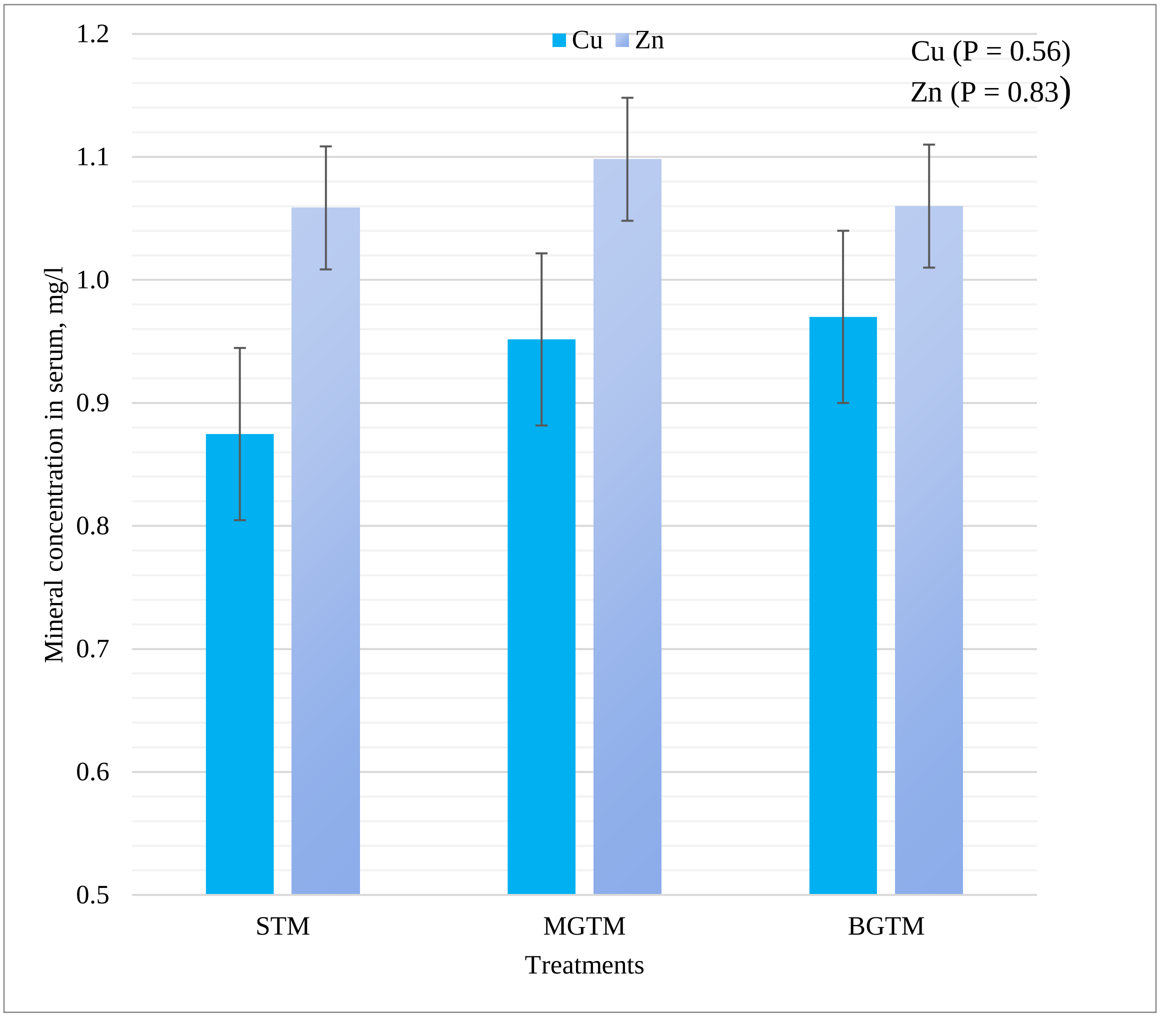


Figure 2. 3 Effect of different sources of trace minerals on serum concentration of Cu and Zn.



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CHAPTER THREE

OVERALL CONCLUSIONS AND FUTURE RESEARCH

This chapter summarizes the results of the experiments reported in this thesis and includes an overview of proposed future studies that will build on the findings from this work. One of the most difficult challenges for producers of the livestock industry is to produce more animal products to address the needs of a growing population. To combat this challenge, improvements in production efficiency are crucial. In the food chains, ruminants have a particular spot in which they can convert a low-quality feed into a high-quality product with their synergy with microorganisms in the rumen. Some of the problems we found with ruminants are that microorganisms can be sensitive to the interaction of metals in the rumen, which can be beneficial to increase the microbial population, in which they can help have higher digestibility and absorption of the nutrients fed in their diets. Trace minerals have been successfully used in livestock production to support this microbial population increase and digest fiber efficiently; however, public concern about becoming environment-friendly, researchers and producers are looking forward to the number of minerals being excreted from the animals via feces to understand the negative effect in soils and later crop productions.

Conclusions

This thesis mainly focused on investigating the effect of STM, MGTM, and MGTM sources of Cu and Zn to identify nutrient digestibility, animal performance, and rumen fermentation. Under the conditions of this study, the different sources of trace minerals, either STM, MGTM, or BGTM, had similar effects on them. For example, DMI showed no difference between the treatments. However, cows' intake slightly decreased when fed STM instead of MGTM and BGTM. Despite the intake reduction, there was no effect on the milk yield of this study. Nutrient digestibility showed no difference between the treatments. Despite no difference, MGTM had more digestibility in DM and NDF than STM and BGTM. When giving MGTM to the lactating cows, the absorption and retention showed a higher amount of Cu that will stay in the animal's body. However, we identified that the STM source had better body retention than the other sources for Zn retention. Although the mineral source did not affect pH, there was a linear decrease from the time fed to the animal until the last sampling showing how it started increasing after 12 h fed. Ammonium nitrogen was not affected by the treatments. Hence, the MGTM source showed a better digestibility of N, which can result in a higher NH3-N amount. Preliminary results from the solubility of the products show no difference under the circumstances of the study. However, these preliminary results show that the BGTM source is less soluble in Zn than the STM and MGTM. This might be related to the pH level found in the rumen fluid during the sampling. Serum concentrations of Cu and Zn were not affected by the source. Nevertheless, Cu concentrations were higher in the BGTM compared to the STM source.

Possible Future Directions

Evaluating three different sources of trace minerals in dairy cows, is an unclear result for comparing the sources of minerals. The reason to consider this is that the products containing Glycine as a bond are recently in the market, and there needs to be more information on cattle for this product. Before saying that a source of trace minerals is better than another, more research needs to be done to determine which has better nutrient digestibility and fermentation. So, in the future, it would be essential to continue evaluating the BGTM Cu and Zn response to nutrient digestibility and rumen fermentation, emphasizing solubility and retention.

Research done in the past with Cu and Zn trace mineral sources has been done most in beef cattle; this suggests that beef cattle are more susceptible to the impact of the head of trace minerals in digestibility. The BGTM has limited data in livestock; therefore, future studies should dedicate time to see the objective of the follow-up of supplementation of Cu and Zn BGTM sources on nutrient digestibility, rumen fermentation, and solubility. Twelve cannulated multiparous beef steers would be randomly assigned to a three-trace mineral source (STM, MGTM, and BGTM) for Cu and Zn and fed a corn silage-based TMR. Treatments will be provided in a 3 x 3 Latin square experimental design with three periods of 21 days each, 16 d for mineral adaptation, and five days for sample collection. Feed offers and refusals will be recorded daily to calculate dry matter intake (DMI).

Additionally, during days 17^{th} to 20^{th}, total feces and urine will be collected to determine the complete absorption and retention of Cu and Zn. Hence, this will help to understand the nutrient digestibility (DM, OM, NDF, TN, starch). Over the last 24 hours of the sample collection on each period, rumen fluid will be collected every two h. After feeding, this will be stored until VFA, Ammonia, and soluble content are analyzed. This recreation of the study will help us understand the benefits of the Cu and Zn Bis-glycinate in beef steers because beef steers have been shown to have a higher acceptance for studying different sources of trace minerals.

APPENDIX

Table A. 1 Effects of trace mineral source on the concentration of minerals excreted.

	Treatments				
Item	STM[1]	MGTM[2]	BGTM[3]	SEM[5]	P-Value
Milk					
Cu, mg/kg	0.05	0.06	0.06	0.16	0.76
Zn, mg/kg	4.9	4.6	4.8	6.23	0.33
Feces, kg/d of DM[4]					
Cu, mg/kg of DM	60.0	55.7	57.1	2.41	0.44
Zn, mg/kg of DM	206.7	209.0	202.2	5.52	0.68
Urine, kg/d[4]					
Cu, mg/kg	0.10	0.06	0.20	0.05	0.22
Zn, mg/kg	0.6	1.0	0.4	0.29	0.35

[1]Sulfate Trace Minerals 10 mg/kg from $CuSO_4$; 35 mg/kg from $ZnSO_4$.

[2]Mono-glycinates inorganic 10 mg/kg from Mono-glycinate copper form; 35 mg/kg from Mono-glycinate from zinc form.

[3]Bis-glycinates organic 10 mg/kg from Bis-glycinate copper form; 35 mg/kg from Bis-glycinate from zinc form.

[4] Data based on sampling days assigned.

[5]Standard error of the mean