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Effects of supplementing sources of zinc on digestibility parameters of beef steers

Arminda Spikes James
Mississippi State University, aspikes1822@gmail.com

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Effects of supplementing sources of zinc on digestibility parameters of beef steers

By

Arminda Spikes James

Approved by:

Brian J. Rude (Major Professor)
Trent Smith
Angela Boyer
Jamie Larson (Graduate Coordinator)
Scott T. Willard (Dean, College of Agriculture and Life Sciences)

A Thesis
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Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agriculture with a Concentration in Animal Science
in the Department of Animal and Dairy Sciences

Mississippi State, Mississippi

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2023
Zinc (Zn) has numerous functions and life sustaining processes depend on the presence of Zn within the body. Zinc sulfate is a common form of Zn supplemented in the beef industry. Inorganic sulfate based minerals have been associated with negative rumen effects, mainly, decreased rumen fermentation and protozoa numbers. Our studies concluded no difference in digestibility of three different forms of Zn (zinc glycinate, zinc sulfate, and zinc hydroxychloride). Although overall digestibility did not change, there were specific mineral concentration differences after steers consumed concentrate treatment for 10 days. These differences implied that the mineral fed was consumed and absorbed in post-treatment sample collection of rumen fluid, plasma, and liver. Overall liver Zn levels were within normal ranges and implies the zn fed was absorbed. Based on the results of this study bioavailability of, zinc glycinate, zinc sulfate, or zinc hydroxychloride were similar.
DEDICATION

I would like to dedicate this thesis to my husband, Tyson James, who has given me constant support throughout my graduate studies. He has played the most significant role in my success in life and at this university. From Idaho to Mississippi, he has followed me in the fulfillment of whatever goal I set. He has always pushed me to persevere and be the best wife, student, mother, sister, daughter, and friend possible. Having a child in the middle of graduate school was one of the hardest things I have ever done, and he helped in every way possible. Every sleepless night, every doctor’s appointment, every daily challenge of parenting, he has been the best partner. He has made dinners, fed animals, helped halter break calves, taken care of our kids, and always been my shoulder to lean on. He has made me laugh in the middle of the lows and celebrated the incredible highs on this journey. Tyson, you have had the largest role in my completion of this degree, and we can take credit for this achievement together. I love you so much and cannot wait to see what else we achieve on this wild ride of life.
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# TABLE OF CONTENTS

DEDICATION................................................................................................................... ii

ACKNOWLEDGEMENTS.................................................................................................. iii

LIST OF TABLES.............................................................................................................. vii

CHAPTER

I. REVIEW OF LITERATURE.............................................................................................. 1

- Introduction .................................................................................................................. 1
- Digestion and Absorption ............................................................................................ 2
- Movement, Distribution, Storage ............................................................................... 5
- Deficiency .................................................................................................................... 6
- Toxicity ......................................................................................................................... 9
- Interaction With Other Minerals ................................................................................. 10
- Zn in Common Livestock Feeds .................................................................................. 10
- Conclusion ................................................................................................................... 14
- Tables ......................................................................................................................... 15

II. MATERIALS AND METHODS .................................................................................... 16

- Metabolism Trial ......................................................................................................... 16
  - Trial Procedures ....................................................................................................... 16
  - Laboratory Analysis ................................................................................................. 19
  - Statistical Analysis .................................................................................................. 20
- Tables ......................................................................................................................... 22

III. RESULTS AND DISCUSSION.................................................................................... 24

- Nutrient Digestibility .................................................................................................. 24
- Rumen Fluid Mineral Concentration .......................................................................... 25
- Plasma Mineral Concentration ................................................................................... 26
- Liver Mineral Concentration ....................................................................................... 27
- Conclusion and Implications ....................................................................................... 28
- Tables ......................................................................................................................... 29

LITERATURE CITED........................................................................................................... 40
LIST OF TABLES

Table 1.1  Percentage of Zinc (Zn) and Bioavailability of different Zinc forms in traditional supplements..........................................................15

Table 2.1  Nutrient composition of feedstuffs and concentrate treatments consumed by Angus steers to evaluate three different forms of dietary Zinc (Zn) .........................22

Table 2.2  Mineral composition of feedstuffs and concentrate treatments consumed by Angus steers to evaluate three different forms of dietary Zinc (Zn) .........................23

Table 3.1  Average body weight and dry matter intake of growing Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride supplemented diets ...................29

Table 3.2  Apparent nutrient digestibilities (%) of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride supplemented diets ........................................30

Table 3.3  Nitrogen retention of angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride supplemented diets .................................................................31

Table 3.4  Specific mineral digestibilities (%) of growing Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride supplemented diets ................................32

Table 3.5  Rumen fluid mineral pre-trial concentrations compared to post-trial concentrations of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride..................33

Table 3.6  Rumen fluid mineral concentration LSmeans of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride .........................................................34

Table 3.7  Plasma mineral pre-trial concentrations compared to post-trial concentrations of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride ..........35

Table 3.8  Plasma mineral concentration LSmeans of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride .................................................................36

Table 3.9  Liver Sodium concentration LSmeans of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride .................................................................37

Table 3.10 Liver mineral pre-trial concentrations compared to post-trial concentrations of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride .............38
Table 3.11  Liver mineral concentration Least square means of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride..........................................................39
CHAPTER I

REVIEW OF LITERATURE

Introduction

Vitamins and minerals are nutrients that must be consumed by the animal and are required in small but varying quantities. These ingredients are vital for rumen digestive systems to function properly, as well as many other metabolic functions. Minerals are separated into two distinct categories: microminerals and macrominerals. Macrominerals are normally present at greater amounts in the body and (or) are required in relatively greater amounts in the diet. Microminerals are normally present in less amounts in the body and are required in small amounts of the diet.

Zinc has been recognized as an indispensable micromineral for decades and is essential for proper growth and health (NRC, 2016). A study conducted by Todd et al., (1934) demonstrated that Zn is necessary for animal development and survivability. Zinc is a vital micromineral fed to ruminants and plays an important role in bone development and several enzyme systems. According to Vallee and Falchuk (1993), Zn is required for over 300 enzymes and far many more functional Zn proteins (Coleman, 2002). The essentiality of Zn makes identifying the rate limiting factor for Zn-responsive disorders challenging (Suttle, 2010). Additionally, Zn is required for structural and functional integrity of transcription factors and almost every signaling and metabolic pathway is dependent on one or more Zn-dependent proteins (Beattie and Kwun, 2004; Cousins et al., 2006). Zinc is a component of all six enzyme
classes established by the International Union of Biochemistry and is the only metal present in each enzyme class (Vallee and Falchuk, 1993). Examples of Zn in all six enzyme classes are: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Vallee and Falchuk, 1993). The frequent use of Zn as the foremost functional metal of biological molecules showcases how critical Zn is to biological processes (Vallee and Falchuk, 1993).

Recommendations for beef cattle are 30 mg/kg DM of Zn in their diet (NRC, 2016). Zinc requirements are not specified because little is known about what influences Zn requirements (NRC, 2016). Zinc is the most deficient micromineral in legume forages, as a result, cattle grazing pasture are often deficient of Zn (Greene, 2000). Greene, (2000) reported an average of 27.2 μg/g Zn in warm-season grasses and an average of 25.0 μg/g in cool season grasses. Spears (1989) evaluated the effect Zn had on growth and performance of growing heifers. Heifers were supplemented 25 mg/kg of Zn Oxide or Zn Methionine in addition to a basal diet of corn silage containing 24 mg Zn/kg DM, greater feed efficiency and average daily gain were observed during the first 56d of the study, no advantage was seen after that (Spears, 1989).

**Digestion and Absorption**

The digestive tract of ruminant animals is unique in function, as well as components of the system. Ruminant saliva contains sodium and potassium that help to buffer feed against acid. The reticulum and rumen are the first two portions of the ruminant stomach and is the site of esophageal expulsion of ingested feed. Ingesta flows between the two portions freely until particle size is small enough to exit. The rumen is the largest compartment of the ruminant digestive system and is lined with tongue like projections (papillae). The rumen itself has muscular tissue that divides it into several sacs. These muscles contract to mix and break down ingesta further. Fibrous feeds are regurgitated and rechewed. The next compartment (the
omasum) prevents large particles from leaving the rumen and entering the abomasum. The abomasum is the last compartment before the small intestine and functions similarly to a nonruminant glandular stomach (Kellums and Church, 2010). The ingesta then proceeds to the small intestine. The process of rumination helps to increase surface area of feed particles, making it easier for microbes to access the nutrients and perform degradation processes.

The rumen is an anaerobic environment, its optimal temperature is 39°C, and optimal pH ranges from 6 to 7 (Millen et al., 2016). It is essential that the rumen maintain the correct pH. Carbon dioxide along with bicarbonate help to achieve this. The rumen contains microorganisms that aid in the breakdown of cellulolytic feeds. Some examples of rumen microbes are archaea, fungi, bacteria, and protozoa (Mathews et al., 2019). The specific contents of the rumen environment are almost entirely responsible for providing nutrients to the animal and vary largely based on contents in the diet. The contents of the rumen are a mixture of solids, liquid, and microbes. The liquid portion accounts for about 25 % mass, solid portion about 70 % mass, and epithelial cells and protozoa 5 % mass (Mathews et al., 2019). The rumen, reticulum, and omasum are lined with stratified squamous cells that regulate absorption. Similarly, the rumen does not allow large particles to exit into the omasum until they have been broken down in particle size thus, making it a feed regulator as well (Moran, 2005).

As feed is consumed and fermented, the microbes produce volatile fatty acids (VFA’s). Volatile fatty acids are the main energy source for the ruminant animal (Thibodeaux et al., 2020). The main VFA’s produced are acetate, propionate, and butyrate and are necessary for fatty acid synthesis. Volatile fatty acids are absorbed through the rumen wall and delivered to the liver and then partitioned to adipose tissue, mammary gland tissue, and muscles. Nutrients in the diet are absorbed through epithelial cells in the gastrointestinal (GI) tract. Most mineral absorption in
ruminants occurs in the small intestine via epithelial cells (Goff, 2018). The balance between the host ruminant animal and the microbes within them is essential for the survival of the animal.

Zinc absorption is regulated, according to need, by an active process if fed at correct amounts (Suttle, 2010). This process primarily occurs in the duodenum, which is the first section of the small intestine (Davies, 1980). Enterocytes line the small intestine and contain organelles (i.e. mitochondria, lysosomes, and endoplasmic reticulum) that are essential for absorption of minerals. Tight junctions connect enterocytes and function in regulating mineral and water absorption. Paracellular absorption allows for Zn to pass through tight junctions and through the basolateral membrane by an electrochemical gradient (Goff, 2018). When mineral concentration is increased in the interstitial space minerals move into the lumen of the cell and as a result are secreted to other cells (Goff, 2018). To specify Zn absorbability, absolute values are needed from specific sources. Very few have been published due to difficulty in the ability to measure (Underwood and Suttle, 1999). Three challenges are associated with achieving an accurate Zn absorption value. First, since it is regulated according to need, measurement tests that are performed use excess amounts of Zn; thus, underestimating the potential of the Zn source (Underwood and Suttle, 1999). Secondly, a major source of phosphorus in grains is phytate ligands, phytate forms complexes with Zn that render it unabsorbant (Underwood and Suttle, 1999). Thirdly, amount of calcium (Ca) in the diet affects the interaction between phytate and Zn (Underwood and Suttle, 1999). Therefore, absorbability is measured from the total diet, instead of a specific Zn source supplement (Underwood and Suttle, 1999). Wedekind et al. (1994) conducted a study to measure the effect of dietary Ca on inorganic Zn in chicks. Results showed that increasing dietary Ca from 6.0 to 7.4 Ca/kg DM, reduced availability of Zn 3.8 fold (Wedekind et al., 1994).
Movement, Distribution, Storage

Bioavailability is a measurement of what fraction of a specific feedstuff the animal can use and can be an overall measurement of efficacy (Rein et al., 2013). The bioavailability of Zn varies based on how the mineral is fed to ruminants. Based on the compound form, the percentage of bioavailable Zn varies (Thibodeaux et al., 2020). Multiple factors influence absorption such as: age of the animal, form of the element, pH of the intestinal tract, and interaction with other minerals and compounds. The absorption of a mineral may not be attributed to the amount given, but more the form of compound (Spears, 1996). Zinc has the potential to bind to chelates and is most often fed in a chelated form. Chelating agents provide Zn stability and increased water-solubility. Chelating agents normally contain amino acids or small peptides (Thibodeaux et al., 2020). Table 1-1 shows the most common forms of inorganic Zn used. This table exemplifies when comparing Zn sources, the amount of Zn within varies. Zinc chelated with an amide or hydroxyl group allows for a ring structure to form, providing protection for Zn throughout the digestive process (Thibodeaux et al., 2020).

Zinc is released in the form of free ions from feedstuffs, the ions can bind to ligands before movement into the small intestine (Thibodeaux et al., 2020; Roohani et al., 2013). Researchers have concluded a potential electrostatic effect, binding Zn to plant fibers, allowing Zn to pass through the rumen or to be utilized in the digestive tract (Goff, 2018). Zinc transportation takes place in the portal bloodstream while loosely bound to plasma albumin (Underwood and Suttle, 1999).

Once Zn is absorbed by the cell, it is then distributed to the cytoplasm (50 %), the nucleus (30 % to 40 %), and cell membrane (10 %, Kimura and Kambe, 2016). Zinc transporters are in the form of proteins and are categorized by metallothioneins and two groups of soluble
ligand carriers (Thibodeaux et al., 2020). The soluble ligand carriers are referred to as the Znt group, which is part of the cation diffusion facilitator division of proteins, and the ZIP group which is classified as Zrt and Irt like proteins (Kimura and Kambe, 2016). The Znt group moves Zn out of the cell, while ZIP moves Zn into the cell (Kimura and Kambe, 2016). Zinc present in plasma is present in the forms of \( \alpha_2 \)-macroglobulin and traces of metallothioneins (Underwood and Suttle, 1999). Hepatic metallothioneins synthesis occurs when Zn arrives at the liver and is critical in Zn partitioning (Bremner, 1993). Kelly et al. (1996) found that mice lacking metallothioneins experienced increased signs of both Zn deficiency and toxicity.

Zinc requirements are fulfilled by consuming forages and feeds. Different forages vary with the amount of Zn available, and many factors influence the amount of Zn available. These factors are species, soil, and maturity (Mir et al., 2018). Each feedstuffs fed should always be evaluated for mineral content to ensure enough mineral is available and being consumed by the animal. Grace (1983) conducted a Zn study with sheep specifying the distribution and outcome of Zn through body systems. The majority of Zn absorbed was distributed to wool, muscle, bone, GI tract, skin, lungs, and red blood cells, respectively (Grace, 1983).

**Deficiency**

An important function of Zn in livestock species is the halting of processes that contribute to health and production when deprived of Zn. When a cell is deprived of Zn, the sequence of events is depletion, deficiency, dysfunction, and disorder (O’Dell, 2000). Once the cell is depleted of Zn, deficiency of alkaline phosphatase changes protein content, dysfunction changes calcium ions, and disorder is due to impaired platelet aggregation and increased erythrocyte fragility (O’Dell, 2000). In any system deprived of Zn, one concept is consistent,
cells fail to divide and differentiate with consequent growth impairment in animals, plants and phytids (Vallee and Falchuk, 1993).

There are four specific areas where systems devoid of Zn fail, those are: Gene expression, fat absorption, appetite control, and antioxidant defense (Suttle, 2010). Zinc aids in gene regulation which is involved in various bodily processes. Signal transduction, responses to stress, reduction/oxidation reactions, and growth and energy utilization all rely on gene regulation to function properly (Cousins et al., 2003). O’Dell and Reeves (1989) conducted research on the effect of Zn deprivation in laboratory rats. Acute Zn deprivation caused rats to nibble instead of ‘meal eat’, causing abnormal growth and development. Acute Zn deprivation resulted in a reduction of appetite selectivity, where proteins and fats are preferred over carbohydrates (Kennedy et al., 1998). Studies have shown that the transition from ‘meal eating’ to nibbling is linked to Zn deprivation in ruminants as well (Droke et al., 1993).

Miller et al. (1967), discovered that many of the results of Zn deprivation happened after loss of appetite. Phospholipase $\alpha_2$ is secreted by the pancreas and is dependent on Zn (Kim et al., 1998). Phospholipase $\alpha_2$ hydrolyses phosphatidylcholine, aiding in its absorption and forming chylomicrons. Chylomicrons are necessary for the absorption of fat (Noh and Koo, 2001). Zinc may provide protection from iron induced lipid peroxidation. This is achieved by Zn and vitamin E blocking iron binding sites at cell surfaces (Zago and Oteiza, 2001). According to Beattie and Kwun (2004), deprivation of Zn can make endothelial cells more prone to oxidant stress.

Mineral deficiency usually involves more than one mineral; however, the deficiency of one mineral may supersede another (Hidiroglou, 1979). Like all minerals, deficiency of Zn can have lasting and detrimental effects. Specifically, Zn deficiency has been correlated with reproductive failure. According to Molefe and Mwanza (2020), dairy cows fed grass with less
amounts of Zn experienced more reproductive complications. Cows that did not receive supplementation experienced dystocia and retained placenta, while cows that received supplementation experienced no complications. In early studies performed with pigs, lack of Zn reduced litter size (Hoekstra et al., 1967). Bull calves (*Bos Taurus*) deprived of Zn did not produce adequate amounts of testosterone resulting in hypogonadism (Pitts et al., 1966). Miller et al. (1967) supported this as well, finding that deficiency of Zn in kids (*Capra Aegagrus Hircus*), resulted in underdeveloped testes. Underwood and Somers (1969) reported similar findings in lambs deficient of Zn. Spermatogenesis ceased within 20 days of the absence of Zn in the diet, however it recovered completely after supplementation was restored (Martin and White, 1992).

Along with reproduction, Zn deficiency can affect growth, and many other bodily functions. Zinc deficiency can negatively impact hematological and immunological processes. Ibrahim et al. (2016) supported this by finding that sheep with Zn deficiency experienced alopecia, and skin abnormalities. Evidence of Zn deficiency has been found by the presence of thickening, hardening, and fissuring of the skin on different areas of the animal, with areas varying based on the species. It displayed by young pigs on the extremities (Tucker and Salmon, 1955), in chicks, on the feet and feathers (Sunde, 1972; 1978), around the muzzle, eyes, ears, neck and scrotum of calves (Miller et al., 1965), and on the scrotum and above the hoof of lambs (Ott et al., 1964).

A critical process that Zn contributes to is cell division. Zinc deficiency can inhibit cell division of which would cause a large effect on tissues that grow rapidly. One example of this process is fur growth. Cui et al., (2017) reported increased fur growth in mink supplemented with Zn compared to no supplementation. In a study that evaluated the effect of Zn concentrations on
the immune system of white-tailed deer (*Odocoileus Virginianus*), it was determined that deer which received Zn supplementation showed greater ($P < 0.05$) immune response to an antigen challenge and less immune inhibition from stress hormones (Bartoskewitz et al., 2007). In piglets deprived of Zn, the size and strength of the femur was reduced (Miller et al., 1968). Absence or lack of Zn significantly impacts embryonic skeletal growth of chick embryos, this is evident with abnormalities of the head, limbs, and vertebrae (Kienholz et al., 1961). Specific to ruminants, an early sign of Zn deprivation is excess salivation and may be coupled with difficulty swallowing (Mills et al., 1967; Apgar et al., 1993). Hind limbs will bow, stiffness of the joints, and swelling of the hocks can be present in calves devoid of Zn (Miller and Miller, 1962).

**Toxicity**

Zinc toxicity is possible, but not very common. Unlike other metals, the processes that regulate distribution and excretion of Zn are efficient to the point that toxicity is rare (Vallee and Falchuk, 1993). Most signs of toxicity occur when 1000 ppm or more is consumed (NRC, 1980). Sheep, poultry, pigs, and cattle tolerate different amounts of Zn. This concept is largely relative to other minerals in the diet, specifically: Ca, Cu, Fe, and Cd. The interactions of these elements with Zn during the process of absorption and utilization is the primary factor (Underwood, 1981). Allen et al., (1983) induced Zn toxicity in sheep and cattle. They observed varying degrees of severity in toxic effects in both sheep and cattle. The animals that displayed the mildest form of toxicity experienced inappetence and gradual loss of body condition. More severely affected animals experienced diarrhea, with dehydration and subcutaneous edema. Diarrhea resulted in severe weakness, body condition deterioration, and death. Both calves and sheep showed ulceration of the abomasal mucosa, increased mucus in the abomasum, and catarrhal enteritis. Interestingly, affected kidneys were shrunken and pale or swollen, soft and
orange or brown. Results also showed that both cattle and sheep were anemic, of which they could not locate the cause (Allen et al., 1983). Zinc toxicity in rats also results in anemia, it has been suggested that this is due to copper and iron deficiencies instead (Underwood, 1977). Allen et al. (1983), reported finding lesions on the kidney and abomasum of all affected animals, naming this a large contributor to health deterioration.

**Interaction With Other Minerals**

Minerals are interrelated to one another and can affect the absorbability of other minerals present in the diet. Of the minerals that interact, Zn, Fe, Ca, and Cu are the most influential. Standish et al. (1971) showed that steers supplemented with 1000 mg Fe/kg DM had reduced liver Zn concentrations. This was attributed to greater Fe intake affecting bioavailability of Zn, due to coordinate covalent bonding between metal and organic functional groups (Mullis et al., 2003). Zinc and Cu form compounds (chelate, complex, sulfate, oxide, etc.) and their interactions are not fully understood (Hatfield et al., 2001). Zinc and Cu compete for binding sites and thus can be considered antagonistic to an extent (Hatfield et al., 2001). Wellington et al. (1998) cautioned over supplementing Zn and Cu to avoid compromising the status of either mineral. Scientists have concluded that feeding Cu along with Zn could increase absorption and retention of Zn (Hatfield et al., 2001).

**Zn in Common Livestock Feeds**

Adequate supply of microminerals is vital to the performance of any animal. According to Minson (1990) all pastures worldwide contain Zn, ranging from 25 to 50 mg/kg DM. The main factors that determine Zn status of forage is Zn soil status, maturity of the plant (Minson, 1990), and in small amounts, Zn fertilizer (Masters and Somers, 1980). If a pasture experiences
numerous cuts, Zn content can decrease by almost 50% (Underwood and Suttle, 1999). Thus, hay normally contains less amounts of Zn while silage contains slightly greater amounts of Zn (Suttle, 2010). However, Zn content among grass species has little variation (Suttle, 2010). According to Minson (1990) the Zn content varies greatly in legumes and is primarily dependent on soil Zn content.

Like forages, Zn content in feed concentrates also varies greatly and is dependent on soil Zn content (Suttle, 2010). Wheat, oats, barley, and millet range from 26 to 35 mg Zn/kg, while corn contains less Zn (Suttle, 2010). According to Cakmac (2008) cereal grains contain reduced amounts of Zn but if treated with a Zn fertilizer, Zn values can triple. Zinc is most abundantly present in the outer layers of cereals (Underwood and Suttle, 1999). Protein sources from vegetables and animals contain drastically greater amounts of Zn than other feedstuffs, specifically feather meal (Suttle, 2010).

In animal production, Zn is often supplemented in mineral mixes and fed in various forms. Traditionally, organic microminerals have been fed as inorganic salts, however, recently there has been some interest in feeding microminerals as different compound types (Spears, 1996). The interest is attributed to studies reporting that feeding minerals in compounds results in improved growth, reproduction, and health in ruminants (Spears, 1996). Based on some of these studies, it is not possible to conclude if the results reported were due to the mineral supplemented or specifically due to the other components of the compound (Spears, 1996). The compound types are referred to as complexes, amino acid chelates and proteinates (Spears, 1996). The Association of American Feed Control Officials (AAFCO) define each compound differently. Metal amino acid complexes are defined as the complexing of a soluble metal salt with an amino acid (AAFCO, 2021). Metal amino acid chelates are defined as the result of the
reaction of a metal ion from a soluble metal salt with amino acids with a mole ratio of one to three (preferable two) moles of metal to one to three (preferably two) moles of amino acids to form coordinate covalent bonds (AAFCO, 2021). Metal proteinates are defined as the result of the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein (AAFCO, 2021).

Zinc glycinate metal amino acid complex structure is a metal ion, in this case Zn, bonded to the amino and carboxyl groups, in this case glycine, to form two five-membered rings (Yin et al., 2017). Zinc glycinate and Zn methionine are organic forms of zinc complexes used in production. Heinrichs and Conrad (1983) reported the methionine portion of Zn methionine was not degraded by rumen microbes. Ward et al., (1992) reported greater concentrations of Zn in rumen fluid of steers fed Zn methionine compared to Zn from Zn sulfate or Zn oxide. These findings suggested Zn methionine remains intact in the rumen and possibly binds with feed or microorganisms rendering it less insoluble compared to inorganic forms (Spears, 1996). Spears et al. (2004) supplemented zinc to angus steers in three forms: Zn sulfate, Zn methionine and Zn glycinate. Spears et al. (2004) reported greater liver Zn concentrations in the Zn glycinate group compared to the other forms of Zn supplemented, suggesting a greater bioavailability. Glycine is an improved stabilizer for Zn compared to Zn methionine and could yield Zn glycinate more available (Spears et al., 2004). Greater bioavailability has been reported for organic forms of Zn, due to this, organic forms of Zn are commonly used (Spears, 1989; Wedekind et al., 1992; Cao et al., 2000). However, over time, researchers have reported varying bioavailability values with trace mineral complexes and chelates, suggesting no advantage of using organic compared to inorganic forms (Ammerman et al., 1995).
Inorganic chelated forms of Zn commonly used are Zn sulfate and Zn oxide. Researchers have reported similar results when supplementing Zn oxide compared with Zn methionine. Spears (1989) reported similar bioavailability of Zn from Zn methionine and Zn oxide in lambs fed a semi-purified diet deficient in Zn. Spears (1989) reported similar average daily gain and feed efficiency in growing heifers supplemented with Zn methionine compared with Zn oxide. However, Green et al. (1988) reported higher quality grades, marbling scores, and percent kidney pelvic and heart fat in steers supplemented with Zn methionine compared with a basal diet or Zn oxide.

Sulfate forms, which are acid salts, are extremely soluble and increase free radical formation (Shaeffer et al., 2006). The reaction of sulfate and other feedstuffs provides the possibility of breaking down vitamins and degrading fats (Shaeffer et al., 2006). Zinc sulfate has posed a concern for many producers because of the effect it has on decreasing the nutritive value of other feeds present. It has also been reported that Zn sulfate decreased rumen fermentation and protozoa numbers (Froetschel et al., 1990).

Opposite of sulfate compounds, hydroxychloride trace minerals are less soluble in the rumen because they are not soluble at pH above 3 to 4 (Van Kuijk et al., 2022). Zinc hydroxychloride is produced by covalent bonding that results in a crystalline structure and optimum molecular stability. In steers supplemented with Zn sulfate and Zn hydroxychloride, Zn hydroxychloride produced greater absorption and retention, as well as, greater plasma Zn concentrations (Schaeffer et al., 2017). Daniel et al. (2020) reported a neutral detergent fiber digestibility increase when dairy cows were supplemented with hydroxychloride microminerals compared with sulfate microminerals.
Conclusion

Zinc utilizes multiple complex pathways and plays an essential role in cellular transcription and metabolism. Therefore, it directly effects cell replication and growth. As previously described, Zn deficiency has produced slowed growth, decreased bone growth and reproductive failures. These deficiencies can prove costly and detrimental to beef cattle producers. Feed costs can constitute up to 50 % variation in profit or loss (Rankins, 2002). Therefore, efficient feeding programs are beneficial financially, as well as essential to overall health of the herd. By increasing digestibility and utilizing feedstuffs correctly, the rumen can function properly, and maximization of growth and production can be achieved. If Zn is to be supplemented understanding which specific form is most bioavailable, especially compared to Zn sulfate, needs to be further examined. Therefore, the objective of this study was to evaluate the apparent digestibility and mineral status in liver and plasma of calves fed different forms of Zn.
Table 1.1  Percentage of Zinc (Zn) and Bioavailability of different Zinc forms in traditional supplements

<table>
<thead>
<tr>
<th>Source Compound</th>
<th>% Zn in Compound</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn carbonate</td>
<td>52.0</td>
<td>High</td>
</tr>
<tr>
<td>Zn chloride</td>
<td>48.0</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Zn sulfate</td>
<td>22.0 - 36.0</td>
<td>High</td>
</tr>
<tr>
<td>Zn oxide</td>
<td>46.0 – 73.0</td>
<td>High</td>
</tr>
</tbody>
</table>

Adapted from McDowell, (1996).
CHAPTER II
MATERIALS AND METHODS

Metabolism Trial

Trial Procedures

The Mississippi State University Institutional Animal Care and Use Committee (IACUC) (protocol #21-414) approved all procedures conducted in this study. This trial was conducted at the Mississippi State University Leveck Animal Research Center beef unit near Starkville, MS. Prior to the trial, all feed was mixed at the MSU beef unit. The concentrate treatments consisted of a CPC developer pellet (CPC 16 % developer, CPC Commodities, Fountain Run, KY) 0.63 g/d, cracked corn 0.63 g/d, molasses 0.2 g/d, and a specific isozinc compound 0.63 g/d. The three isozinc treatments were: Organic Zn glycinate, inorganic Zn sulfate (ZnSO$_4$), and Zn hydroxychloride (Phibro Animal Health, Teaneck, NJ). Twelve Angus steers (226 ± 16.17 kg, 4/treatment) were randomly assigned to one of three Zn treatments and housed in individual metabolism crates for a 10-day period. Steers had ad libitum access to hay (80 % cynodon dactylon, 20 % paspalum) and water and were fed treatment diets once daily (Table II-1). All feedstuffs used in this study were analyzed for mineral content and are shown in Table II-2.

Initial body weight (BW) was obtained the day before trial began and day 1 of the trial. The average of these two BW was used as the initial BW. Steers were weighed once at the end of the trial (d 10) and the average of this weight and initial BW were used to calculate total intake as a % of BW. On days 1 and 10, rumen fluid, liver biopsies performed, and blood was collected
from each steer. Rumen fluid was collected via oral lavage, using a backwards flow electric pump. Solids and liquid were separated using a 4 layered cheesecloth straining method and liquid was stored in a glass screw-top container in a -20°C freezer until analysis.

Liver biopsy samples were collected according to the procedure performed by Burnett et al. (2021). Briefly, steers were restrained in a squeeze chute and a 10 cm x 10 cm square was clipped and scrubbed using aseptic technique at the 10th intercostal space identified on the right side of the abdomen. Chlorhexidine gauze sponges were used to clean the area, followed by 91 % isopropyl alcohol, and finally 95 % betadine surgical solution. The incision site was then injected with 5 ml of 2 % lidocaine solution followed by a 5-minute period to allow for lidocaine numbing properties to take effect. A puncturing needle was then used to break the skin and enter the intercostal space. A disposable core biopsy instrument (Bard Biopsy, Bard Peripheral Vascular, Inc., Tempe, AZ) was placed into the incision to collect the liver sample. The same biopsy instrument was used to collect multiple liver samples from the same steer until the desired amount (approximately 0.05 g) was obtained. Using sterile tweezers, the liver sample was then removed from the collection instrument and placed into a cryovial and immediately froze in liquid nitrogen. Once collection had concluded, cryovials were taken to Mississippi State University Animal and Dairy Science laboratory and stored in a -80 °C freezer until analysis.

Plasma samples were collected via jugular venipuncture using a 15 ml vacutainer. Samples were immediately placed on ice and after the conclusion of collection taken to Mississippi State University Animal and Dairy Science laboratory. Blood samples were then centrifuged at 2,000 x G for 20 minutes. Plasma was removed, placed in cryovials, and stored in a -80 °C freezer until analysis was performed.
Following collection on day 1, steers were relocated to the metabolism barn of the beef unit and housed in individual metabolism crates in a randomly assigned order. Crates provided orts, feces, and urine sample collection, as well as ad libitum access to hay and water. Upon entering crates, steers were allowed a four-day (d 1 through 4) adaption period where behavior and intake were monitored, in order to assess acclimation to crates. After the adaption period, sample collection began and continued for five days (d 5 to 10).

Orts, feces, and urine were collected daily at time of feeding (approximately 0600 h). Orts were quantified at 5% of the total and composited for the entirety of the trial and stored in sealed plastic bags. Hay bales were sampled in various sections each day and were then stored in sealed plastic bags and composited into one sample at the completion of the trial. Feces were measured and sampled daily at 5% of the total output, dried in a forced air oven at 65°C for 24 to 48 hours, and then composited by animal. Total urine output was collected daily and sampled at 10% of daily volume and were then composited by animal. Prior to compositing, each day approximately 1% of previous day urine volume of 2 N metaphosphoric acid was added to each urine collection bucket to collect the current day urine. When urine samples were collected, metaphosphoric acid was added to urine sample to equalize to 2% sample volume. Metaphosphoric acid served to acidify the urine and prevent ammonia volatilization. Urine was then stored in a screw-top container and placed in a -20°C freezer until analysis occurred. Excessive water contamination of concentrate treatment pans did not allow for accurate weight assessment of orts, therefore concentrate treatment consumption was visually assessed and recorded by a trained technician. Once feeding concluded, percentage consumed was determined by the amount of concentrate treatment that remained in the feed pan.
Laboratory Analysis

Prior to laboratory analysis, concentrate treatment, orts, hay, and feces were ground via a Thomas Wiley Mill® (Arthur H. Thomas, Philadelphia, PA) to pass a 2-mm particle screen. Concentrate treatment, orts, hay, and feces were analyzed according to AOAC, (2020) for dry matter (DM), ash, organic matter (OM), crude protein (CP; DT220 Digestor, Kjeltec 8460 Distiller and Auto Sampler System, Foss Tecator, Eden Prairie, MN), and ether extract (EE). Van Soest (1991) fiber analysis was used for determining neutral detergent fiber (NDF), and acid detergent fiber (ADF). Hemicellulose (HC) was calculated by subtracting ADF from NDF (NDF - ADF = HC).

Mineral analysis preparation was performed for concentrate treatment, orts, hay, and feces by wet ash digestion according to the procedure performed by Burnett et al., (2017). Briefly, approximately 1 ± 0.01 g of sample was weighed into a glass digestion tube and 15 ml micromineral grade nitric acid or fuming nitric acid based on availability, was added to each tube. The tubes were covered with a watch glass and placed into a heating block, at 80 °C for 15 minutes, or until no brown gas was present, or foaming had ceased. The temperature was then set at 115 °C for 1 hour. After 1 hour had elapsed, tubes were cooled, contents transferred to a 50 ml centrifuge tube, and filled with deionized water to a total of 45 ml volume. After which, each sample was capped and inverted. Samples were analyzed by ICP-OES (Varian 715-ES, Centennial, CO) at the state soil testing laboratory at Mississippi State University.

Urine was analyzed for CP content and mineral content. Urine, plasma, and rumen fluid were prepared for mineral analysis by the same procedure as feed, orts, and feces. Briefly, samples were thawed to room temperature and urine and rumen fluid vortexed to mix thoroughly. Sample (500 μl) was pipetted into a 15 ml centrifuge tube and diluted with 9.5 ml of
1N-nitric acid. Samples were allowed a 24-hour incubation period at room temperature. Following the incubation period, samples were centrifuged at 2000 x G at 21 °C for 20 minutes. The supernatant was separated from the pellet, placed in a 15 ml screw-top conical tube, and analyzed by ICP-OES (Varian 715-ES, Centennial, CO) at the state soil testing laboratory at Mississippi State University.

Prior to liver preparation, borosilicate tubes and glass marbles were washed with 1N-nitric acid and weighed. Liver preparation for mineral analysis began as samples were thawed, transferred to borosilicate tubes, re-weighed, and placed in an oven to ensure no moisture present. After 45 h elapsed, dried samples were weighed to achieve a dried sample weight. Borosilicate tubes were placed in a heating block and 2 ml of fuming nitric acid added to each tube. An acid-washed marble was placed on top of the test tubes and tubes heated at 80 °C for 15 minutes or until brown gases were not present and foaming had ceased. Temperature was reset to 115 °C for 1 hour. After 1 hour had elapsed, tubes were removed from the heating block, cooled, weighed, and then filled to 5 ml total volume with deionized water. Weight of total deionized water was recorded. Samples were transferred to a 15 ml screw-top conical tube and vortexed. The prepared samples were analyzed by ICP-OES (Varian 715-ES, Centennial, CO) at the state soil testing laboratory at Mississippi State University.

**Statistical Analysis**

Statistical analysis was performed using the GLM procedure of SAS 9.4. Response variables were mineral concentrations in plasma, liver, and rumen fluid and body weight, feed intake, and nutrient and mineral digestibility. The model included fixed effects of treatment, day,
and the treatment by day interaction. Means were separated using PDIFF and significance was determined at $P \leq 0.05$. 
### Tables

Table 2.1  Nutrient composition of feedstuffs and concentrate treatments consumed by Angus steers to evaluate three different forms of dietary Zinc (Zn)

Nutrient Parameters\(^1\)

| Ingredient       | Feedstuff Composition
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM</td>
</tr>
<tr>
<td>Hay</td>
<td>89.60</td>
</tr>
<tr>
<td>CPC Developer</td>
<td>90.23</td>
</tr>
<tr>
<td>Corn</td>
<td>88.48</td>
</tr>
</tbody>
</table>

Concentrate Composition by Treatment\(^2\)

<table>
<thead>
<tr>
<th></th>
<th>DM</th>
<th>Ash</th>
<th>OM</th>
<th>CP</th>
<th>NDF</th>
<th>ADF</th>
<th>HC</th>
<th>EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn G</td>
<td>91.67</td>
<td>30.94</td>
<td>69.06</td>
<td>8.95</td>
<td>16.87</td>
<td>7.33</td>
<td>9.54</td>
<td>0.83</td>
</tr>
<tr>
<td>Zn S</td>
<td>91.53</td>
<td>32.68</td>
<td>67.32</td>
<td>7.29</td>
<td>20.57</td>
<td>8.90</td>
<td>11.67</td>
<td>1.03</td>
</tr>
<tr>
<td>Zn H</td>
<td>91.57</td>
<td>35.47</td>
<td>64.53</td>
<td>6.94</td>
<td>16.70</td>
<td>7.45</td>
<td>9.25</td>
<td>1.07</td>
</tr>
</tbody>
</table>

1 = DM (dry matter intake), OM (organic matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber), HC (hemi-cellulose), EE (ether extract), N% (nitrogen retention)

2 = Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Hydroxychloride, 32.6% commodity pellet, 32.6% ground corn)
Table 2.2  Mineral composition of feedstuffs and concentrate treatments consumed by Angus steers to evaluate three different forms of dietary Zinc (Zn)

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>K %</th>
<th>Ca %</th>
<th>Mg %</th>
<th>Na %</th>
<th>Fe mg/kg</th>
<th>Mn mg/kg</th>
<th>Zn mg/kg</th>
<th>Cu mg/kg</th>
<th>Co mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>0.174095</td>
<td>0.0224785</td>
<td>0.0179305</td>
<td>0.006222</td>
<td>0.006403</td>
<td>0.0009195</td>
<td>0.002183</td>
<td>0.0052685</td>
<td>ND1</td>
</tr>
<tr>
<td>CPC Developer</td>
<td>0.176529</td>
<td>0.00235</td>
<td>0.0004365</td>
<td>0.0001615</td>
<td>0.000246</td>
<td>0.000011</td>
<td>ND</td>
<td>0.0006675</td>
<td>ND</td>
</tr>
<tr>
<td>Corn</td>
<td>0.194417</td>
<td>0.0037175</td>
<td>0.0025895</td>
<td>ND</td>
<td>0.0002765</td>
<td>0.0000265</td>
<td>ND</td>
<td>0.0006575</td>
<td>ND</td>
</tr>
<tr>
<td>Zn G²</td>
<td>0.2478</td>
<td>0.1762674</td>
<td>0.2078484</td>
<td>0.2003514</td>
<td>0.1599402</td>
<td>0.2323926</td>
<td>0.1461564</td>
<td>0.0562098</td>
<td>0.0007566</td>
</tr>
<tr>
<td>Zn S²</td>
<td>0.301631</td>
<td>0.1487232</td>
<td>0.1516686</td>
<td>0.2566002</td>
<td>0.1349928</td>
<td>0.1972986</td>
<td>0.0687864</td>
<td>0.0544842</td>
<td>0.0008514</td>
</tr>
<tr>
<td>Zn H²</td>
<td>0.202928</td>
<td>0.160167</td>
<td>0.1927662</td>
<td>0.3450438</td>
<td>0.1590918</td>
<td>0.2453928</td>
<td>0.1465716</td>
<td>0.060837</td>
<td>0.0008634</td>
</tr>
</tbody>
</table>

1 = ND (Not detectible)
2 = Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn
CHAPTER III
RESULTS AND DISCUSSION

**Nutrient Digestibility.**

There were no differences \( P = 0.3565 \) for BW of steers among the diets (Table III-1). Dry matter intake (DMI) did not differ among diets when evaluated as kg/d \( P = 0.8102 \) or % BW/d \( P = 0.404 \). There were no differences for apparent nutrient digestibility of DM \( P = 0.9252 \), ash \( P = 0.9082 \), OM \( P = 0.9313 \), CP \( P = 0.7731 \), NDF \( P = 0.9956 \), ADF \( P = 0.9463 \), HC \( P = 0.8264 \), and EE \( P = 0.8271 \) among diets (Table III-2). Mandal et al., (2007) supplemented Zn to 15 crossbred bulls. Bulls were separated into three groups of five and fed three Zn supplemented diets. The diets were: wheat straw with a concentrate mixture that contained 32.5 mg Zn/kg DM, 35 mg Zn sulfate/kg, and 35 mg Zn propionate/kg. All bulls were fed the treatment diets for 180 days. After 120 days, bulls were vaccinated with brucella abortus and cell mediated, and humoral immune responses were observed between d120 and d148. At day 150, a 6-day metabolism study was conducted and concluded no difference of nutrient digestibility. However, bulls supplemented with Zn propionate had greater cell mediated and humoral immune response. Jia et al., (2008) supported these findings by reporting no differences in digestibility of DM, CP, NDF, and ADF, by cashmere goats supplemented with Zn. Thirty-six cashmere goat wethers were divided into four groups and were fed a basal diet of 22.3 mg Zn/kg with 0, 15, 30, or 45 mg Zn/kg Zn sulfate additions. Goats were fed over 60-days and ended with
a 7-day metabolism trial. In the present study, there were no differences ($P = 0.9208$) in nitrogen retention among the diets (Table III-3).

There were no differences for apparent mineral digestibility of K ($P = 0.6343$), Ca ($P = 0.9869$), Mg ($P = 0.8331$), Na ($P = 0.0342$), Fe ($P = 0.6002$), Mn ($P = 0.1491$), Zn ($P = 0.4951$), and Cu ($P = 0.0412$) among diets (Table III-4). Cobalt was not present in amounts possible to detect by ICP-OES, thus Co digestibility could not be accurately calculated.

**Rumen Fluid Mineral Concentration.**

There was no treatment x day interactions for mineral content of rumen fluid after consuming concentrate treatment (Table III-5), and there were no differences of treatment for rumen fluid mineral concentration by Zn source (Table III-6). There was no difference of Ca ($P = 0.4576$) and Mg ($P = 0.0815$) concentration of rumen fluid. Khorasani et al., (1997) reported Ca disappearance in the rumen possibly by passive absorption. Therefore, it is possible the Ca fed to steers in this study disappeared in the rumen at a consistent rate pre- and post-treatment. Magnesium is largely absorbed by passive and active transport processes in the rumen (Suttle, 2010). Therefore, it is possible the Mg fed to steers in this study disappeared in the rumen at a consistent rate pre- and post-treatment. Potassium concentration of rumen fluid decreased ($P = 0.0091$) after consuming mineral treatments for 10 days. More than 50% of K entering the rumen is absorbed by passive absorption (Suttle, 2010). Rumen fluid Na concentration increased ($P = 0.0300$) post-treatment compared with pre-treatment. Rumen fluid Fe concentration decreased ($P = 0.0005$) pre-treatment compared to post-treatment. For rumen fluid collected in this study, decrease in rumen mineral concentrations could be attributed to rumination processes.
and collection times and practices. Manganese, Zn, Cu, and Co concentration was nondetectable by ICP-OES analysis.

**Plasma Mineral Concentration.**

Plasma mineral concentration after consuming mineral treatments for 10 days is shown in Table III-7. Potassium, Ca, Mg, Fe, Mn, Zn, and Cu plasma concentrations were greater ($P = <0.0001$) after consuming mineral treatments for 10 days. The effect of period indicates minerals were being absorbed for concentrate treatment in plasma after consuming mineral treatments for 10 days. According to concentrations reported by Kincaid (2000) adequate Cu and Zn plasma concentrations are 0.7 to 0.9 μg/ml and 0.8 to 1.4 μg/ml, respectively. Plasma Cu and Zn concentrations found in this study were within toxic ranges reported by Kincaid (2000; >1.2 μg/ml and 3 to 15 μg/ml, respectively). In contrast with these results, Ott et al., (1966); Kincaid et al., (1976); Kellogg et al., (1989), reported that large intakes of Zn reduced plasma Cu concentrations in cattle. Zinc plasma concentrations fluctuate with age, stress, feed restrictions, and infections (Kincaid and Hodgson, 1989). Plasma Zn is greater in newborn calves and decreases with age; however, stress has been reported to counteract this concept (Kincaid and Hodgson, 1989). Sodium plasma concentration was greater ($P = <0.0001$) at pre-treatment compared with post-treatment when all other mineral concentrations increased post-treatment. As weaning protocol at the beef unit dictates, calves were given access to a salt lick tub prior to metabolism trial beginning, and this could account for sodium decrease once calves were placed in metabolism crates. Cobalt concentration was nondetectable by ICP-OES analysis. There were no differences among treatment, for plasma mineral concentration (Table III-8).
Liver Mineral Concentration.

There was a treatment X day interaction ($P = 0.0486$) for liver concentrations of sodium (Table III-9). Liver Na concentration was greatest ($P = 0.0486$) after consumption of concentration treatment for 10 days, by steers consuming Zn hydroxchloride, but all other concentrate treatments and days were not different from each other. Sodium and Cl are often discussed together because of their interactions with each other, related metabolism, functions, and requirements (Suttle, 2010). Studies have shown that the active transportation of Na and Cl are joined from one ion requiring the other (Martens and Blume, 1987). Thus, greater amounts of Na in the liver specifically with steers fed hydroxycholride was to be expected.

Other liver mineral concentrations after consuming concentrate treatment for 10 days are shown in Table III-10. Manganese, Cu, and Co were not present in quantities sufficient for detection by ICP-OES. Potassium concentrations were greater ($P = 0.0002$) after consuming mineral concentrate treatment for 10 days. There were no differences in liver concentration of Ca, Mg, Fe, and Zn. Liver Zn concentrations prior to and after consuming mineral concentrate treatment for 10 days (115.18 mg/kg and 130.88 mg/kg, respectively) were within normal range according to Kincaid (2000). Kincaid (2000) reported adequate Zn concentrations in the liver of 25 to 200 μg/g, which when translated to compare to present data of 25 to 200 mg/kg.

Other liver mineral concentration differences of Zn compounds are shown in Table III-11. There were no differences in concentrate treatment of mineral concentrations. Manganese, Cu, and Co concentration was nondetectable by ICP-OES analysis.
Conclusion and Implications

Feed costs are continually increasing and account for a main expense of cattle production. Achieving optimum digestibility of feedstuffs will allow producers to optimize profits. Mineral additives should be evaluated based upon feedstuff availability, other components of the diet, and specific animal requirements by each producer. Feeding minerals incorrectly could result in negative mineral association, as well as reduced growth and production. Depending upon the specific mineral being evaluated, different tissues (kidney, muscle, etc.) are superior indicators of overall mineral status. In this study, plasma concentrations of Zn were within toxic ranges, however liver Zn was not. Liver concentrations found in this study were within adequate ranges and indicate that all Zn forms used in this study were absorbed adequately. Based on collection parameters and laboratory analysis performed in this study, bioavailability was similar for glycinate, sulfate, and hydroxychloride Zn treatments. This suggests supplementing with glycinate, sulfate, or hydroxychloride sources of Zn should yield similar results. The information obtained in this study can be used to justify subsequent studies to determine any other possible differences in Zn bioavailability from these sources.
### Tables

Table 3.1  Average body weight and dry matter intake of growing Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride supplemented diets

<table>
<thead>
<tr>
<th>Diet 1</th>
<th>BW, kg</th>
<th>DMI kg/d</th>
<th>DMI % BW/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn G</td>
<td>233.7</td>
<td>11.71</td>
<td>5.0411</td>
</tr>
<tr>
<td>Zn S</td>
<td>216.7</td>
<td>12.66</td>
<td>5.8546</td>
</tr>
<tr>
<td>Zn H</td>
<td>227.1</td>
<td>12.38</td>
<td>5.4104</td>
</tr>
<tr>
<td>SEM</td>
<td>7.97</td>
<td>1.045</td>
<td>0.40700</td>
</tr>
<tr>
<td>P=</td>
<td>0.3565</td>
<td>0.8102</td>
<td>0.4040</td>
</tr>
</tbody>
</table>

1 = Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Hydroxychloride, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses); N=4/treatment
Table 3.2  Apparent nutrient digestibilities (%) of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride supplemented diets

<table>
<thead>
<tr>
<th>Nutrient Parameters¹</th>
<th>Concentrate Treatment²</th>
<th>DM</th>
<th>Ash</th>
<th>OM</th>
<th>CP</th>
<th>NDF</th>
<th>ADF</th>
<th>HC</th>
<th>EE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn G</td>
<td>75.8425</td>
<td>62.0160</td>
<td>77.1117</td>
<td>72.7657</td>
<td>81.5586</td>
<td>87.0393</td>
<td>66.3441</td>
<td>73.3498</td>
</tr>
<tr>
<td></td>
<td>Zn S</td>
<td>77.0629</td>
<td>63.5935</td>
<td>78.2255</td>
<td>75.0998</td>
<td>81.7923</td>
<td>86.6171</td>
<td>69.0169</td>
<td>75.8153</td>
</tr>
<tr>
<td></td>
<td>Zn H</td>
<td>76.1792</td>
<td>63.3896</td>
<td>77.3247</td>
<td>74.8165</td>
<td>81.7723</td>
<td>87.2628</td>
<td>66.4640</td>
<td>75.3378</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>2.2506</td>
<td>2.7503</td>
<td>2.2077</td>
<td>2.4751</td>
<td>1.9413</td>
<td>1.3910</td>
<td>3.4213</td>
<td>2.9693</td>
</tr>
<tr>
<td></td>
<td>P=</td>
<td>0.9252</td>
<td>0.9082</td>
<td>0.9313</td>
<td>0.7731</td>
<td>0.9956</td>
<td>0.9463</td>
<td>0.8264</td>
<td>0.8271</td>
</tr>
</tbody>
</table>

¹ = DM, OM (organic matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber), HC (hemi-cellulose), EE (ether extract)

² = Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Hydroxychloride, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses); N=4/treatment
Table 3.3  Nitrogen retention of angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride supplemented diets

<table>
<thead>
<tr>
<th>Concentrate Treatment&lt;sup&gt;1&lt;/sup&gt;</th>
<th>N,&lt;sup&gt;2&lt;/sup&gt; intake g/d</th>
<th>N, retained/N, intake</th>
<th>N, retained/DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn G</td>
<td>18.057</td>
<td>0.3491</td>
<td>0.001029</td>
</tr>
<tr>
<td>Zn S</td>
<td>24.329</td>
<td>0.3734</td>
<td>0.001138</td>
</tr>
<tr>
<td>Zn H</td>
<td>17.357</td>
<td>0.3174</td>
<td>0.000933</td>
</tr>
<tr>
<td>SEM</td>
<td>7.403</td>
<td>0.1220</td>
<td>0.000356</td>
</tr>
<tr>
<td>P=</td>
<td>0.7701</td>
<td>0.9486</td>
<td>0.9208</td>
</tr>
</tbody>
</table>

<sup>1</sup> = Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Hydroxychloride, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses); N=4/treatment
<sup>2</sup> = Nitrogen
Table 3.4  Specific mineral digestibilities (%) of growing Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride supplemented diets

<table>
<thead>
<tr>
<th>Concentrate Treatment&lt;sup&gt;1&lt;/sup&gt;</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn G</td>
<td>76.699</td>
<td>99.998</td>
<td>85.582</td>
<td>86.713</td>
<td>81.561</td>
<td>90.847</td>
<td>87.987</td>
<td>93.013</td>
</tr>
<tr>
<td>Zn S</td>
<td>78.496</td>
<td>99.998</td>
<td>85.270</td>
<td>95.127</td>
<td>88.662</td>
<td>98.332</td>
<td>93.965</td>
<td>98.648</td>
</tr>
<tr>
<td>Zn H</td>
<td>83.046</td>
<td>99.998</td>
<td>86.586</td>
<td>95.243</td>
<td>89.122</td>
<td>95.869</td>
<td>81.558</td>
<td>97.068</td>
</tr>
<tr>
<td>SEM</td>
<td>4.727</td>
<td>0.0002</td>
<td>1.593</td>
<td>2.181</td>
<td>5.765</td>
<td>2.479</td>
<td>7.113</td>
<td>1.349</td>
</tr>
<tr>
<td>P=</td>
<td>0.6343</td>
<td>0.9869</td>
<td>0.8331</td>
<td>0.0342</td>
<td>0.6002</td>
<td>0.1491</td>
<td>0.4951</td>
<td>0.0412</td>
</tr>
</tbody>
</table>

<sup>1</sup> = Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Hydroxychloride, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses); N=4/treatment
Table 3.5  Rumen fluid mineral pre-trial concentrations compared to post-trial concentrations of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Pre- µg/ml</th>
<th>Post- µg/ml</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>76.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.114</td>
<td>0.0091</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.10</td>
<td>5.73</td>
<td>0.681</td>
<td>0.4576</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.47</td>
<td>1.66</td>
<td>0.311</td>
<td>0.0815</td>
</tr>
<tr>
<td>Sodium</td>
<td>120.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>137.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.232</td>
<td>0.0300</td>
</tr>
<tr>
<td>Iron</td>
<td>0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.063</td>
<td>0.0005</td>
</tr>
<tr>
<td>Manganese</td>
<td>ND&lt;sup&gt;1&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> ND (not detectible)  
<sup>ab</sup> differ within row (P<.05)
Table 3.6  Rumen fluid mineral concentration LSmeans of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Zn G µg/ml</th>
<th>Zn S µg/ml</th>
<th>Zn H µg/ml</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>64.96</td>
<td>77.84</td>
<td>62.48</td>
<td>5.853</td>
<td>0.1623</td>
</tr>
<tr>
<td>Calcium</td>
<td>5.05</td>
<td>5.83</td>
<td>5.21</td>
<td>0.826</td>
<td>0.7814</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.12</td>
<td>2.07</td>
<td>2.01</td>
<td>0.402</td>
<td>0.9796</td>
</tr>
<tr>
<td>Sodium</td>
<td>126.70</td>
<td>133.54</td>
<td>125.76</td>
<td>7.056</td>
<td>0.7002</td>
</tr>
<tr>
<td>Iron</td>
<td>0.32</td>
<td>0.33</td>
<td>0.27</td>
<td>0.102</td>
<td>0.8825</td>
</tr>
<tr>
<td>Manganese</td>
<td>ND¹</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ = ND (not detectible)

Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Hydroxychloride, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses);

N=4/treatment
Table 3.7  Plasma mineral pre-trial concentrations compared to post-trial concentrations of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Pre- µg/ml</th>
<th>Post- µg/ml</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>46.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>196.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.372</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Calcium</td>
<td>5.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.086</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.346</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sodium</td>
<td>159.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.358</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Iron</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.211</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Manganese</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.940</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Zinc</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.388</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Copper</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.505</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Cobalt</td>
<td>ND&lt;sup&gt;1&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> = ND (not detectible)  
<sup>ab</sup> = differ within row (P<.05)
Table 3.8  Plasma mineral concentration LSmeans of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Zn G µg/ml</th>
<th>Zn S µg/ml</th>
<th>Zn H µg/ml</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>120.18</td>
<td>115.42</td>
<td>128.77</td>
<td>33.875</td>
<td>0.9609</td>
</tr>
<tr>
<td>Calcium</td>
<td>29.52</td>
<td>29.62</td>
<td>29.29</td>
<td>9.153</td>
<td>0.9996</td>
</tr>
<tr>
<td>Magnesium</td>
<td>23.70</td>
<td>23.63</td>
<td>22.80</td>
<td>8.624</td>
<td>0.9966</td>
</tr>
<tr>
<td>Sodium</td>
<td>100.23</td>
<td>91.10</td>
<td>98.50</td>
<td>24.488</td>
<td>0.9609</td>
</tr>
<tr>
<td>Iron</td>
<td>11.10</td>
<td>10.86</td>
<td>9.07</td>
<td>4.140</td>
<td>0.9348</td>
</tr>
<tr>
<td>Manganese</td>
<td>6.28</td>
<td>6.99</td>
<td>5.49</td>
<td>2.616</td>
<td>0.9216</td>
</tr>
<tr>
<td>Zinc</td>
<td>7.07</td>
<td>7.38</td>
<td>6.24</td>
<td>3.081</td>
<td>0.9642</td>
</tr>
<tr>
<td>Copper</td>
<td>2.45</td>
<td>3.55</td>
<td>2.70</td>
<td>1.248</td>
<td>0.8111</td>
</tr>
<tr>
<td>Cobalt</td>
<td>ND¹</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ = ND (not detectible)

Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Hydroxychloride, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses);
N=4/treatment
Table 3.9: Liver Sodium concentration LSmeans of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Pre-Zn G mg/kg</th>
<th>Pre-Zn S mg/kg</th>
<th>Pre-Zn H mg/kg</th>
<th>Post-Zn G mg/kg</th>
<th>Post-Zn S mg/kg</th>
<th>Post-Zn H mg/kg</th>
<th>SEM</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>7395.85\textsuperscript{a}</td>
<td>7362.07\textsuperscript{a}</td>
<td>7050.28\textsuperscript{a}</td>
<td>6585.65\textsuperscript{a}</td>
<td>6478.39\textsuperscript{a}</td>
<td>9919.74\textsuperscript{b}</td>
<td>800.450</td>
<td>0.0486</td>
</tr>
</tbody>
</table>

Table III-10 abbreviations: Zn G (zinc glycinate), Zn S (zinc sulfate), Zn H (zinc hydroxychloride)

\textsuperscript{a}\textsuperscript{b} = differ within row (P<.05)
Table 3.10  Liver mineral pre-trial concentrations compared to post-trial concentrations of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Pre- mg/kg</th>
<th>Post- mg/kg</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.060</td>
<td>0.0002</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.03</td>
<td>0.06</td>
<td>0.011</td>
<td>0.1001</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.07</td>
<td>0.08</td>
<td>0.004</td>
<td>0.1074</td>
</tr>
<tr>
<td>Iron</td>
<td>565.28</td>
<td>619.11</td>
<td>52.180</td>
<td>0.4741</td>
</tr>
<tr>
<td>Manganese</td>
<td>ND&lt;sup&gt;1&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>115.18</td>
<td>130.88</td>
<td>13.285</td>
<td>0.4132</td>
</tr>
<tr>
<td>Copper</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> = ND (not detectible)
<sup>ab</sup> = differ within row (P<.05)
Table 3.11  Liver mineral concentration Least square means of Angus steers fed Zinc (Zn) glycinate, Zn sulfate, and Zn hydroxychloride

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Zn G mg/kg</th>
<th>Zn S mg/kg</th>
<th>Zn H mg/kg</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>1.18</td>
<td>1.09</td>
<td>1.12</td>
<td>0.100</td>
<td>0.8197</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
<td>0.014</td>
<td>0.7010</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.08</td>
<td>0.07</td>
<td>0.06</td>
<td>0.005</td>
<td>0.0965</td>
</tr>
<tr>
<td>Sodium</td>
<td>6990.70</td>
<td>6920.23</td>
<td>8485.01</td>
<td>624.280</td>
<td>0.1597</td>
</tr>
<tr>
<td>Iron</td>
<td>544.00</td>
<td>578.98</td>
<td>653.61</td>
<td>63.190</td>
<td>0.4691</td>
</tr>
<tr>
<td>Manganese</td>
<td>ND&lt;sup&gt;1&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>125.82</td>
<td>137.52</td>
<td>105.75</td>
<td>16.154</td>
<td>0.3883</td>
</tr>
<tr>
<td>Copper</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 = ND (not detectible)

Zn G (32.6% Zn Glycinate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn S (32.6% Zn Sulfate, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses), Zn H (32.6% Zn Hydroxychloride, 32.6% commodity pellet, 32.6% ground corn, 2.2% molasses);
N=4/treatment
LITERATURE CITED


