Implications of trace mineral supplementation strategies to overcome the effects of high antagonist diets in feedlot cattle

Sarah Jeanette Hartman
Iowa State University

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Implications of trace mineral supplementation strategies to overcome the effects of high antagonist diets in feedlot cattle

by

Sarah Jeanette Hartman

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Animal Science

Program of Study Committee:
Stephanie Hansen, Major Professor
Steve Ensley
Kristin Hales

Iowa State University

Ames, Iowa

2017

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>NOMENCLATURE</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>vii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ix</td>
</tr>
<tr>
<td>CHAPTER I  GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER II  REVIEW OF THE LITERATURE</td>
<td>3</td>
</tr>
</tbody>
</table>

- Chemistry of Trace Minerals ........................................ 3
  - Inorganic ........................................................................ 3
  - Organic .......................................................................... 4
  - Hydroxy .......................................................................... 6
  - Injectable ........................................................................ 7
  - Source bioavailability of trace minerals .......................... 8
  - Summary - trace mineral sources .................................... 9
- Review of the Trace Minerals Copper, Selenium, Manganese, and Zinc ........................................ 11
  - Copper ............................................................................. 11
    - Dietary sources of copper ........................................... 12
    - Copper supplementation .............................................. 13
    - Copper absorption ...................................................... 17
    - Copper status biomarkers .......................................... 18
    - Copper excretion ........................................................ 20
  - Selenium .......................................................................... 20
    - Dietary sources of selenium ....................................... 22
    - Selenium supplementation .......................................... 22
    - Selenium absorption .................................................. 25
    - Selenium status biomarkers ...................................... 25
    - Selenium excretion ..................................................... 27
  - Manganese ........................................................................ 27
    - Dietary sources of manganese ..................................... 29
    - Manganese supplementation ....................................... 30
    - Manganese absorption ................................................ 31
    - Manganese status biomarkers ...................................... 32
    - Manganese excretion .................................................... 33
  - Zinc ................................................................................. 33
    - Dietary sources of zinc .............................................. 34
    - Zinc supplementation ................................................ 35
    - Zinc absorption .......................................................... 36
Zinc status biomarkers .................................................................................. 37
Zinc excretion .................................................................................................. 38
Trace Mineral Conclusions ........................................................................... 38
Trace Mineral Interactions ........................................................................... 39
Review of Trace Mineral Antagonisms ......................................................... 40
Sulfur ................................................................................................................ 40
  Dietary sources of sulfur ............................................................................. 41
  Sulfur metabolism in ruminants ................................................................. 43
  Sulfur toxicity – H₂S production ................................................................. 45
  Ruminal availability of sulfur .................................................................... 46
  Effects of sulfur on performance ............................................................... 47
  Sulfur excretion ........................................................................................... 47
  Summary - sulfur ........................................................................................ 48
Molybdenum ..................................................................................................... 49
  Dietary sources of molybdenum ............................................................... 49
  Thiomolybdate formation ........................................................................... 50
  Thiomolybdate chemistry .......................................................................... 52
  Thiomolybdate antagonism on copper ....................................................... 53
  Summary - thiomolybdates ....................................................................... 56
Literature cited ................................................................................................ 56

CHAPTER III  EFFECT OF TRACE MINERAL SOURCE ON MINERAL
STATUS AND PERFORMANCE OF BEEF STEERS FED LOW OR HIGH
SULFUR DIETS ................................................................................................. 74
  Abstract ....................................................................................................... 75
  Introduction ................................................................................................. 76
  Materials and Methods ............................................................................. 77
    Experimental design and sampling procedures ..................................... 77
    Tissue and diet analysis ......................................................................... 79
    Carcass data collection ......................................................................... 80
    Statistical analysis .................................................................................. 81
  Results .......................................................................................................... 81
    Trace mineral concentrations ............................................................... 81
    Antioxidant analysis .............................................................................. 82
    Live performance and carcass characteristics ...................................... 83
  Discussion ................................................................................................... 83
  Literature cited ........................................................................................... 90
CHAPTER IV  COMPARISON OF TRACE MINERAL REPLETION STRATEGIES IN BEEF CATTLE TO OVERCOME A HIGH ANTAGONIST DIET

Abstract .................................................................................................................................. 101
Introduction ............................................................................................................................ 102
Materials and Methods ........................................................................................................ 103
  Experimental design and sampling procedures ................................................................. 104
  Tissue analysis ..................................................................................................................... 106
  Statistical analysis ............................................................................................................... 107
  Removed samples .................................................................................................................. 108
Results ..................................................................................................................................... 109
  Depletion period liver and plasma TM ............................................................................. 109
  Repletion period liver TM .................................................................................................. 109
  Repletion period plasma TM ............................................................................................. 111
  Repletion period antioxidant activity ................................................................................. 112
  Steer growth performance .................................................................................................. 112
Discussion .............................................................................................................................. 113
Literature cited ....................................................................................................................... 120

CHAPTER V  GENERAL CONCLUSIONS ............................................................................ 130
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG</td>
<td>Average daily gain</td>
</tr>
<tr>
<td>BF</td>
<td>Back fat</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>Cl</td>
<td>Chloride</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>Cys</td>
<td>Cysteine</td>
</tr>
<tr>
<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>DGS</td>
<td>Distillers grains with solubles</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DMI</td>
<td>Dry matter intake</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>G:F</td>
<td>Gain-to-feed ratio</td>
</tr>
<tr>
<td>Gly</td>
<td>Glycinate</td>
</tr>
<tr>
<td>GSH</td>
<td>Reduced glutathione</td>
</tr>
<tr>
<td>GSH-px</td>
<td>Glutathione peroxidase</td>
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<tr>
<td>GSSG</td>
<td>Oxidized glutathione</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HCW</td>
<td>Hot carcass weight</td>
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<tr>
<td>TM</td>
<td>Trace minerals</td>
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<tr>
<td>Abbreviation</td>
<td>Term</td>
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<td>--------------</td>
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<tr>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>Mn</td>
<td>Manganese</td>
</tr>
<tr>
<td>Mo</td>
<td>Molybdenum</td>
</tr>
<tr>
<td>MS</td>
<td>Marbling store</td>
</tr>
<tr>
<td>NASEM</td>
<td>National Academy of Science, Engineering, and Medicine</td>
</tr>
<tr>
<td>NRC</td>
<td>National research council</td>
</tr>
<tr>
<td>O</td>
<td>Oxide</td>
</tr>
<tr>
<td>QG</td>
<td>Quality grade</td>
</tr>
<tr>
<td>PEM</td>
<td>Polioencephalomalacia</td>
</tr>
<tr>
<td>Prot</td>
<td>Proteinate</td>
</tr>
<tr>
<td>RAS</td>
<td>Ruminally available sulfur</td>
</tr>
<tr>
<td>REA</td>
<td>Ribeye area</td>
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<tr>
<td>S</td>
<td>Sulfur</td>
</tr>
<tr>
<td>Se</td>
<td>Selenium</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SO$_4$</td>
<td>Sulfate</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SRB</td>
<td>Sulfate reducing bacteria</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>TM</td>
<td>Trace minerals</td>
</tr>
<tr>
<td>YG</td>
<td>Yield grade</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
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“The future belongs to those who believe in the beauty of their dreams.”

-Eleanor Roosevelt

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“Mischief Managed.”
Trace minerals (TM) serve functions in multiple enzymes and proteins that are essential to growth, development, and antioxidant status. As a result, TM are typically supplemented in ruminant diets to ensure animals maintain adequate status; however, a number of interactions can occur in the rumen that may inhibit the absorption of TM. Sulfur decreases the absorption of TM and can decrease cattle performance. The addition of dietary Mo to a diet containing high concentrations of S can also irreversibly inhibit the absorption of Cu. Hydroxy TM and injectable TM are novel supplements and little is known about the effectiveness of these sources with diets containing antagonists. Therefore, research was undertaken to 1) determine the effect of hydroxy (HYD) or inorganic (ING) TM supplementation on TM status and performance of beef steers fed high S diets, 2) evaluate the effect of TM repletion strategies in high S diets when sourced from an injectable TM or from increased concentrations of dietary TM, and 3) determine the impact of dietary S concentrations on the length of time an injectable TM would maintain improved TM status. In experiment 1, steers were supplemented with either HYD or ING TM within low S (0.27% S) or high S (0.54% S) diets. High S decreased Cu status and tended to decrease Zn status during the growing period, but did not affect Zn status in the finishing period. Additionally, there were interactions between S and TM source on liver Mn status; this, combined with the lack of effect on finishing period Zn status, suggest there may be an influence of diet on the S by TM interactions occurring in the rumen that alter the availability of TM. Interestingly, liver Cu concentrations were also greater for ING than HYD at the end of experiment 1, which is contrast to previous research stating HYD Cu and Mn were less ruminally soluble and more bioavailable than ING TM.
the absorption of Cu, Mn, and Zn. All animals had adequate status at the start of the trial and maintained this status throughout the study, suggesting the addition of national recommended concentrations may be sufficient to maintain TM status when dietary S is high (0.54%). There is an extensive amount of literature reviewing the effects of dietary organic TM in ruminants, however, less is known about how an injectable TM (Multimin90) may improve TM when dietary antagonists are present. A second trial was conducted to explore the effect of alternative TM supplementation strategies on TM status and performance steers when additional dietary antagonists were included. Steers were fed either a control (CON) diet containing supplemental TM at national recommended concentrations, or an antagonist (ANT) diet containing no supplemental Cu, Se, Mn, or Zn, and supplemental 0.3% S and 2 mg Mo/kg DM. At the end of the 90 d depletion period, ANT had a 90% decrease in liver Cu concentrations, and decreased liver Mn and Se status when compared to CON. The addition of Mo to the diet in the second experiment resulted in greater antagonism of TM absorption in comparison to the first experiment, however both trials recorded the high antagonist diet to decrease Cu and Mn, and some markers of Zn status. After the depletion period, three supplementation strategies were utilized. Steers received either a trace mineral injection and supplemental dietary TM at national recommended concentrations (ITM), increased concentrations of dietary TM at 150% of national recommended concentrations from a blend of 25% organic and 75% inorganic TM (BLEND), and increased concentrations of dietary TM at 150% of recommended national concentrations from only inorganic TM (ING; NASEM, 2016). Even in the face of dietary antagonists, all TM supplementation strategies improved the TM status of steers, such that Cu and Se status was increased in ITM on d 14, BLEND was improved on d 28, and ING reached similar concentrations as ITM and BLEND on d 42. Liver Mn status was affected by repletion strategy
and there were no effects of depletion diet or TM supplementation on liver Zn concentrations. The lack of a reliable biomarker for Mn and Zn status may be the reason for the lack of differences in status. To the author’s knowledge, this is the first time a study has reported the effect of injectable TM Multimin90 on TM status of cattle fed high antagonist diets. Additionally, there were no effects of repletion strategy within depletion diet, suggesting all three supplementation strategies can improve TM status even in the presence of a high antagonist diet. Previous research has shown injectable TM to improve TM status for at least 30 d, and the present study shows the effect may extended to at least 42 d. There were few recorded effects of treatment on the performance of beef steers in the two trials, and neither experiment reported change in final BW or ADG. In the trial that utilized a diet high in S and Mo, dietary antagonisms decreased DMI and increased G:F during the TM repletion period. The improved G:F could be due to the increase TM in the repletion period, which may have indirectly caused some compensatory gain. Overall, this research supports that of others indicating diets containing high S and high antagonists decrease the TM status of feedlot cattle. The effects of hydroxy TM supplementation in diets containing high S were inconsistent, especially within Mn status, however these differences may be attributed to the interactions of S and TM within diet type. Regardless of if steers were fed a control or antagonist diet during the depletion period of the second trial, all TM supplementation strategies improved TM status of Cu and Se by 42 d. These results suggest injectable TM and high concentrations of dietary TM may be able to overcome TM deficiencies in feedlot cattle and improve status. Further research is warranted to solidify the implications of how hydroxy TM may improve TM status in a high S diet, and additional research should be done to determine reliable and repeatable biomarkers for Mn and Zn status in cattle.
CHAPTER I
GENERAL INTRODUCTION

There are 7 trace minerals required by cattle for health and growth: Co, Cu, I, Fe, Mn, Se, and Zn (Hambidge, 2003). These are required in small concentrations for processes such as cell signaling and enzyme cofactors, and play a role in growth, development, and immune response for the production of healthy cattle (Underwood and Suttle, 1999; NASEM, 2016). This thesis will focus specifically on how rates of supplementation and sources of Cu, Mn, Se, and Zn can impact performance and TM status of beef cattle.

The National Academies of Engineering, Science and Medicine (2016) has reviewed recent literature and published recommended guidelines for minimum and maximum inclusions of TM in cattle diets. The current national minimum recommended concentrations are suggested to be 10 mg Cu, 20 mg Mn, 0.1 mg Se, and 30 mg Zn/kg DM. However, a variety of factors can alter these recommendations based on symbiotic or antagonistic interactions in the gastrointestinal tract (Suttle, 2010). Furthermore, the addition of high S or Mo concentrations have been shown to greatly antagonize the absorption of TM (Spears, 2003; Pogge et al., 2014). Producers have a variety of options to supplement TM, yet little is known about how antagonists in the diet may influence availability of TM from different sources.

Trace mineral form can alter the absorption and metabolism of TM due to modifications in the chemistry of the supplemented form (Kegley and Spears, 1994; Du et al., 1996). Inorganic TM are highly available in the rumen and are easily reduced, which ultimately results in decreased bioavailability for absorption (Engle and Spears, 2000; Spears et al., 2004). In contrast, research indicates organic TM may be less available in the rumen, and thus have greater
bioavailability in ruminants as they are able to avoid ruminal antagonisms (Spears, 1996; Engle et al., 1997; Andrieu, 2008, Genther and Hansen, 2015). Recently, the introduction of hydroxy and injectable TM sources have been reported to greatly improve TM status in ruminants (Arthington, 2015; Pogge et al., 2014; Genther and Hansen, 2015; Caldera et al., 2016). Improvements in TM bioavailability could result in decreased dietary inclusion rates, improved efficiency, and ultimately a lesser economic input for the same health and growth response in cattle. Presently, there has been little research on the effects of using these TM sources in conjunction with diets containing high S or other antagonists. This research was designed to evaluate the effects of supplemental TM source in feedlot cattle fed diets high in antagonists, including S, and Mo.

**Thesis Organization**

The following chapter will provide a detailed review of the literature regarding the impact of TM source on bioavailability in the presence of high antagonist diets, and what supplementation strategies may be utilized to best overcome these antagonisms in feedlot cattle. The remaining chapters, III and IV, will present research intended for publication in the *Journal of Animal Science*. Chapter III contains research conducted to study the effect of supplemental inorganic or hydroxy TM on the mineral status and performance of beef steers fed diets containing low (0.27%) or high (0.54%) sulfur. Chapter IV contains research on the effectiveness of three TM supplementation strategies on cattle fed an antagonist diet containing 0.3% supplemental S and 2 mg supplemental Mo/kg DM. Finally, chapter V will conclude the findings of this thesis and present overall findings, conclusions, and implications for future research.
CHAPTER II

REVIEW OF THE LITERATURE.

Chemistry of Trace Minerals

There are a variety of forms of trace minerals (TM) that producers can supplement to cattle, and research has shown these sources do not have the same bioavailability. Trace minerals must be solubilized to be absorbed in the small intestine, and while some TM sources can pass through the rumen without going into solution, ruminally soluble TM have the capacity to bind with feed and other minerals in the rumen, thus decreasing their bioavailability further down the digestive tract (Kegley and Spears, 1994). Inorganic TM have been shown to have a high ruminal bioavailability, which makes them susceptible to ruminal antagonism and furthermore decreases their absorption (Ward et al., 1996, Spears et al., 2004). In contrast, TM that are not ruminally soluble are potentially available for absorption in the small intestine, and some research has suggested metal-hydroxy bound TM have the capacity to avoid ruminal interactions (Spears, 2004; Genther and Hansen, 2015). Additionally, injectable TM bypass the gastrointestinal tract entirely, resulting in a source that avoids ruminal competition (Pogge et al., 2012). Ultimately, the bioavailability of TM depends on how the chemical form interacts in the rumen and gastrointestinal tract.

Inorganic

Trace minerals that are not bound to C are inorganic, and common forms include sulfates (SO$_4$), chlorides (Cl), or oxides (O; Taylor and Field, 1998). Inorganic TM have been the primary option for feed grade TM supplementation for decades due to their affordability and
high rumen solubility (Cunningham et al., 1953; Ammerman and Goodrich, 1983). Inorganic TM dissociate in the reticulo-rumen, omasum, and abomasum, and can form compounds with plant polyphenols (McDonald et al., 1996) or with other TM that may have precipitated out of the digesta (Spears, 2003). These complexes are insoluble and cannot be absorbed in the small intestine, which decreases the availability of the inorganic form to the host (Wright et al., 2008). The antagonisms that can occur are related to the chemistry of the TM; while selenate and selenite are reduced by rumen microbes due to their similar structure to S (Galbraith et al., 2016), Cu has a strong attraction to thiomolybdates (Humphries et al., 1983), Zn can be rapidly bound to fiber (Genther and Hansen, 2015), and the absorption of Se, Cu, Mn, and Zn can be decreased in the presence of high dietary S (Pogge et al., 2012). Greater concentrations of TM have been supplemented as a method to overcome these ruminal interactions, however this strategy is wasteful (Ledoux and Shannon, 1983). Therefore, alternative TM sources have been researched extensively to determine which sources have greater bioavailability, in order to increase production by optimizing TM absorption and utilization in the animal.

Organic

Organic TM vary in regards to the ligand or ligands used to form the metal complex, and are typically classified as complexes, chelates, or proteinates (Spears, 1996). Complex is used as a general term to describe the product of a soluble metal salt and at least one amino acid (Table 1, AAFCO, 1998). In other words, while all chelates and proteinates are complexes, a complex can be defined as either a chelate or a proteinate. Specifically, metal proteinates are defined as the product of the chelation of a soluble salt with either an amino acid or partially hydrolyzed
protein (AAFCO, 1998). However, the definition of a chelate is slightly differently between chemists and animal scientists.

In chemistry, complexes are formed when one or more ligands containing electron donor groups surround an electron deficient central atom, such as a metal ion (Murphy, 2009). If the ligand binds the metal ion with only one ion, the structure is referred to as unidentate (Vella, 1993). However, if the ligand attaches to the metal ion with more than one ion, the structure becomes polydentate, and is officially referred to as a chelate (Vella, 1993). The increased number of binding points results in greater stability for the molecule compared to unidentate molecules, and is known as the chelation effect (Vella, 1993). Though chelates can form four, five, six, or seven membered rings, five membered rings have been shown to have the greatest stability (Murphy, 2009). For this reason, the hexadentate ligand ethylenediamine tetraacetic acid (EDTA) is an excellent chelate because it has six binding groups available (Essilfie-Dughan, 2007).

According to the Association of American Feed Control Officials (AAFCO, 1998), an organic mineral is produced by the complexing of a soluble metal salt with an organic molecule, such as an amino acid. The term “complex” is defined as any species formed when a metal ion reacts with a molecule or ligand containing an atom with a lone pair of electrons (Murphy, 2009). Similarly, a chelated mineral is defined as the reaction of a metal ion of a soluble salt with a mole ratio of between one and three moles of amino acid to form a covalent bond, with a preferable ratio of two moles amino acid per metal ion (AAFCO, 1998). While AAFCO places restrictions on the range of the final molecular weight of the chelate (must not exceed 800 Da) and the average weights of the hydrolyzed amino acids (approximately 150 Da), the definition does not specify the binding mechanism, which is a key part of the chemical definition of a
chelate. As a result, it is possible complexes that are considered chelates by animal scientists may not technically meet a chemist’s definition.

Regardless of definition, there are several overlapping concepts between the definitions. When molecules are chelated properly, the chemical structure enforces the physical stability of the molecule, thus resulting in a high mineral bioavailability (Mohanta and Garg, 2014). This stability is based on several ligand characteristics, including the basicity of the ligand, the number of metal-chelate rings per ligand, the size of the chelate ring, steric effects, resonance effects, and the physical ligand atom (Murphy, 2009). Additionally, several weak interactions can produce an overall strong bond; this effect is especially strong when multiple amino acids or proteins are involved due to the many points of interaction between the large molecules (Vella, 1993). Chelated TM have been suggested to be a superior alternative to inorganic TM because of their ability to avoid ruminal antagonisms that may inhibit the absorption of the metal (Essilfie-Dughan, 2007). The transition elements, which include Cu, Fe, Mn, and Zn, have the necessary physio-chemical characteristics to form coordinate covalent bonds with amino acids and peptides, making them a biologically stable form.

**Hydroxy**

Another recent advancement in mineral nutrition is the introduction of hydroxylated Cu, Mn, and Zn sources. Hydroxy TM are covalently bound to an OH group instead of the carbon-containing ligands that make up organic TM (Arthington, 2015). The OH group prevents the mineral from going into solution at the neutral pH range of the rumen, and hydroxy-bound forms have been shown to dissociate in the pH range of 1.6 to 2.5, which closely mimics the environment of the abomasum (Merchen, 1988; Spears, 2003). Similarly to complexed organic
TM, hydroxy minerals are very stable, however, the hydroxy sources are much more concentrated than organic forms; data have shown a mineral supplement containing 4,000 mg Zn/kg would require 2.7% inclusion space for organic Zn compared to only 0.73% formulation space for the hydroxy form due to the greater concentration of supplemental TM from the hydroxy form (Arthington, 2015).

In comparison to inorganic sources, hydroxy sources have been shown to be less soluble at ruminal pH, but be equally soluble at a lower pH (Spears, 2003). A test by Spears et al. (2004) demonstrated this with a simple in vitro experiment by incubating CuSO$_4$ and tribasic CuCl [Cu$_2$(OH)$_3$Cl], a metal bound hydroxy Cu source, in either deionized water or 0.1% HCl for 1 or 3 hrs. Of the samples incubated in water, CuCl was found to be much less available than CuSO$_4$, with only 0.06% going into solution compared to 94.5%, respectively. When the samples were incubated at a pH of 2.2, inorganic CuSO$_4$ displayed slightly increased solubility with 98.3% and 96.8% Cu going into solution at 1 and 3 hours, respectively. Similarly, the concentrations of CuCl in solution were much higher, with 76.7% and 86.8% CuCl filtered out of solution at 1 and 3 hours, respectively. This ability for hydroxy TM to avoid going into solution until the abomasum makes them an excellent source to study as a supplementation strategy for ruminants, as these TM would be more resistant to ruminal antagonisms and subsequently have improved opportunities for absorption.

**Injectable**

Injectable TM have also been shown to be an effective method to supplement TM while avoiding potential ruminal antagonisms. The chelator EDTA has been shown to be an effective carrier of the divalent cations Cu, Fe, and Zn. Multimin90 is the primary injectable TM product
that has been studied as a supplementation strategy for cattle (Pogge et al., 2012; Genther and Hansen, 2014, Machado et al., 2014; Teixeira et al., 2014). This product contains 15 mg Cu, 10 mg Mn, and 60 mg Zn from EDTA sources, and 5 mg Se from selenite (per mL of product). The subcutaneous dose circulates in the body and the TM are incorporated into the cells as needed, with any excess mineral being filtered to the liver for either storage or processing (Suttle, 2010). Multiple species have been shown to store injected TM in the liver; Owen and Hazelrig (1968) injected rats intravenously with Cu cupric acetate and reported that while plasma Cu and ceruloplasmin concentrations increased slightly, liver Cu concentrations increased up to 20 fold. At this point, little research has been done with the injectable TM in high antagonist diets, and it would be beneficial to determine the effectiveness of the injectable product.

**Source Bioavailability of Trace Minerals**

The absorption of different TM sources is dependent on the form of the mineral, but within this form can also vary greatly depending on the ligand, stability of the chelate, and molecular weight (Ashmead et al., 1986). Early in vitro studies have shown the intestinal uptake of amino acid chelates to occur more rapidly than metal salts (Ashmead, 1993). Inorganic salts must be presented to the intestinal mucosa as a cation in order for absorption to occur (Ashmead, 1993). When there are no interactions to antagonize the absorption of the salts, the cations enter the intestine, bind amino acids or carrier proteins, and are transported to the cytoplasm via passive diffusion or active transport in the small intestine (Ashmead, 1993). The optimal location for absorption is the duodenum, due to the low pH which causes the metal ions to go into solution and remain soluble (Ashmead et al., 1986).
Conversely, chelated amino acids are not ionized before absorption because of their increased stability. Instead, they are absorbed following the pathway of a low molecular weight peptide into the mucosal cell without luminal hydrolysis (Ashmead, 1993). This process is primarily dependent on the stability of the chelated amino acid; when only one amino acid is chelated, peptidases have the capacity to break the bonds before the complex can be absorbed in the small intestine (Ashmead, 1993). If the molecule is weakened enough, it may be absorbed in the same methods as the inorganic salts, however it loses a significant portion of bioavailable action (Ashmead, 1993).

Overall, providing a TM in a more bioavailable form would effectively decrease the dietary requirement and lessen the environmental impact that may result from fecal excretion of unabsorbed TM. There are a variety of supplementation strategies on the market today, and while there is not one that is greater than the rest, the options allow producers to determine what supplementation strategies would be the best based on the composition of their diets.

**Summary - Trace Mineral Sources**

After a review of the literature regarding TM supplementation in ruminants, it is clear TM source has a major impact on bioavailability. Of the commonly supplemented sources, inorganic TM have the greatest ruminal availability, causing them to be the most susceptible to interactions in the rumen and ultimately decreasing their absorption by the animal. In contrast, organic TM are less available in the rumen as a result of the ligands that complex with metal ions to form chelated structures. Chelated forms have improved physical stability which assists in them having lesser ruminal bioavailability and therefore greater TM availability to the animal. Similarly to organic TM, hydroxy TM have improved physical stability resulting from covalent
bonds that form with OH groups. Not only have hydroxy TM been shown to have improved bioavailability, they have also been shown to be more concentrated than organic TM, and thus require a lesser inclusion for the same concentrations of provided TM. Additionally, ruminal interactions can be bypassed entirely by supplementing TM through injectable TM, which provides TM directly to the tissues via EDTA bound TM. Though the bioavailability of inorganic and organic TM have been studied extensively in ruminants, less research exists to compare the bioavailability of hydroxy and injectable TM. Future research should compare the bioavailability of these sources in cattle, and consider how the addition of dietary antagonisms may impact their ultimate effectiveness.

### Table 1. Classification of organic metals as defined by AAFCO (1998).

<table>
<thead>
<tr>
<th>Class</th>
<th>Definition / description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal polysaccharide</td>
<td>The product resulting from complexing of a soluble salt with a polysaccharide solution.</td>
</tr>
<tr>
<td>Proteinate</td>
<td>The product resulting from the chelation of a soluble salt with amino acids and / or a partially hydrolyzed protein.</td>
</tr>
<tr>
<td>Metal amino acid complex</td>
<td>The product resulting from complexing a soluble metal salt with at least one amino acid.</td>
</tr>
<tr>
<td>Metal amino acid chelate</td>
<td>The product resulting from the reaction of a metal ion of a soluble metal salt with a mole ratio of one to three (preferably two) moles of amino acids to form coordinate covalent bonds. The average weight of the hydrolyzed amino acids must be approximately 150 Da, and the final molecular weight of the chelate must not exceed 800 Da. Metal amino acid chelates are available for Co, Cu, Fe, Mn, and Zn.</td>
</tr>
</tbody>
</table>
Review of the Trace Minerals Copper, Selenium, Manganese, and Zinc

Copper

Copper functions as an essential component in the activity of several enzymes, cofactors, and reactive proteins, and adequate Cu is required for normal growth and development of animals (Suttle and Angus, 1976; Suttle, 2010). Depending on the concentrations of S and Mo in the diet, Cu requirements can range from 4 to 15 mg Cu/kg DM, however the addition of 10 mg Cu/kg DM has been found to be sufficient for beef cattle assuming there is less than 0.25% S and 2 mg Mo/kg DM (NASEM, 2016). In situations where S and Mo exceed these concentrations, the Cu requirement is greatly increased (Spears, 2003; Hansen et al., 2008; Pogge et al., 2014). Likewise, supplementation of less than 10 mg Cu/kg DM may be sufficient in diets containing low amounts of S and Mo (NASEM, 2016).

The maximum tolerable concentration of Cu has been estimated at 40 mg Cu/kg DM (NRC, 2005), however this is greatly dependent on the concentrations of dietary S and Mo. Copper toxicity can occur through excessive Cu supplementation or by consuming feeds that may have been contaminated with Cu from agriculture or industrial sources (NASEM, 2016). Due to the excellent storage mechanisms of the liver, ruminants have the capacity to absorb large concentrations of Cu before symptoms of toxicity are noted (NASEM, 2016). Hemolytic crisis occurs when the liver surpasses its maximum tolerable concentration of Cu, and can result in hemolysis, methemoglobinemia, hemoglobinuria, jaundice, icterus, widespread necrosis, and ultimately death (NRC, 2005; NASEM, 2016).

Symptoms of Cu deficiency include decreased ADG, DMI, and feed efficiency, and changes in hair pigmentation and texture (Gould and Kendall, 2011). Copper deficiency can be
caused by either a lack of sufficient dietary Cu or via secondary deficiencies due to antagonisms in the rumen (Minson, 2012; NASEM, 2016). The action of thiomolybdate antagonism on Cu absorption has been well researched, and current feedlot practices have been to provide greater dietary concentrations of Cu to overcome these antagonisms. A recent survey found nutritionists recommend between 10 and 40 mg Cu/kg DM, with an average inclusion of 17.0 mg Cu/kg DM (Samuelson et al., 2016). In order to prevent wasteful addition of Cu to diets, it is crucial to balance the nutrients in each diet to attain optimal growth and development in cattle.

**Dietary Sources of Cu**

Feed sources vary widely in Cu concentrations and bioavailability, as plants grown in Cu deficient soils will absorb low amounts of Cu (Alloway and Tills, 1984). The presence of high soil N and P may also decrease Cu uptake by plants, and some research has suggested plant concentrations of Cu decrease as they mature (Minson, 2012). Brassicas, cereal grains, and certain cereal byproducts have been reported to be a better source of Cu than fresh grass (Suttle, 2010; NASEM, 2016). Additionally, the method by which forages are stored may impact Cu concentrations; Suttle (1980) reported Cu in fresh forages to be much less available than Cu from dried forage of a similar composition. While there are less consistent data on Cu concentrations between forage species, some research has suggested Cu to be more available from concentrate based diets than forage based diets (Minson, 2012; NASEM, 2016). Additionally, if producers chose to use byproducts from the ethanol industry, it should be considered these products have the potential to contain excessively high concentrations of S, which may decrease the availability of Cu provided to the animal.
Copper Supplementation

Copper is often added to the diet through a TM supplement which may contain other essential TM including Se, Mn, and Zn. It is well accepted S has a pronounced antagonistic effect on Cu by combining with Cu in the rumen to form Cu sulfide (Suttle, 1974b). Similarly, thiomolybdates formed in the rumen will greatly decrease the absorption of Cu further down the digestive tract (Suttle, 1974b). Therefore, it has been highly desirable to find a product which may be insoluble in the rumen and thus have greater resistance to binding S or thiomolybdates. The solubility of TM greatly affects the total concentration available to rumen microbes, as the minerals must be solubilized to be absorbed. Numerous studies have been undertaken to compare the bioavailability of free (inorganic) or complexed (chelated) Cu. Chelated minerals are thought to have greater bioavailability because they are more stable in the rumen, and thus less likely to be bound to an antagonist before entering the intestines for absorption (Nelson, 1988; Genther and Hansen, 2015).

There are a variety of Cu sources that have been researched as potential Cu supplementation strategies. Many experiments have shown inorganic CuSO₄ to have high ruminal availability (Engle and Spears, 2000; Arthington, 2005; Arthington and Spears, 2007). While this is useful for providing Cu in a form the animal can utilize, this availability allows rumen microbes to easily bind supplemented Cu, thus research has been undertaken to determine the bioavailability of alternative Cu sources that may be able to avoid the antagonisms of the rumen. Chapman and Bell (1963) reported cattle given one oral dose of CuSO₄ had greatly increased liver Cu concentrations compared to those dosed with CuO. Similarly, in vitro work by Kegley and Spears (1994) reported CuO was completely insoluble in water, and only slightly soluble in acid. An in vivo study supported this work; researchers supplemented beef calves with
CuSO$_4$ or CuO and reported the oxide form to be essentially unavailable for absorption, and resulted in calves displaying similar plasma Cu and ceruloplasmin concentrations to calves from a control group not supplemented with Cu (Kegley and Spears, 1994).

While CuO has been shown to have very low bioavailability as a dietary supplement, dosing cattle with oxidized Cu wire, also known as a CuO needle or bolus, has been shown to improve the Cu status of cattle (MacPherson, 1984; Cameron et al., 1989; Yost et al., 2002). Recently, Arthington (2005) dosed steers with boluses containing 12.5 g CuO and reported liver Cu concentrations were nearly two-fold greater after 33 d in comparison to steers that did not receive a CuO bolus. It is likely that the difference in availability of CuO between the supplemented powder form and the needle dosage is related to a much faster passage rate of the powder compared to the rumen-dwelling needle. Consequently, the usage of a CuO needle may provide adequate supplementation, however the strategy may not be ideal for animals consuming diets already high in Cu.

Organic TM have been measured extensively in recent years due to the improved stability of the chelated form (Mohanta and Garg, 2014). Wittenberg et al. (1990) supplemented Cu proteinate (Prot) in diets containing 100 mg Mo/kg DM and 3-4 g S to beef steers and reported similar bioavailability to CuSO$_4$ as measured by plasma and liver Cu concentrations. In contrast, Engle and Spears (2000) reported steers fed a high concentrate diet and supplemented with CuProt tended to have higher liver Cu concentrations than steers supplemented similar concentrations of CuSO$_4$. Copper glycinate (Gly) has also been shown to have improved bioavailability in ruminants. Hansen et al. (2008) fed cattle diets containing 2 mg Mo/kg DM and 0.15% S supplemented with either 5 or 10 mg Cu/kg DM sourced from CuSO$_4$ or CuGly, and reported the bioavailability of CuGly tended to be greater than CuSO$_4$ based on greater liver Cu.
concentrations, plasma Cu concentrations, and plasma ceruloplasmin in steers fed CuGly. These results supported early research by Allcroft and Uvarov (1959), which reported a subcutaneous injection of 120 mg CuGly increased liver Cu concentrations rapidly in beef cows. Interestingly, when Hansen et al. (2008) increased the concentration of dietary Mo from 2 mg Mo/kg DM to 6 mg Mo/kg DM, the bioavailability of CuGly was greater than from CuSO₄. In contrast, Muehlenbein et al. (2001) supplemented pregnant cows with either 200 mg CuSO₄ or 100 mg Availa Cu and reported liver Cu to be two-fold greater for cows receiving inorganic TM, however these diets were low in S, and the results may have been largely based on the greater inclusion of CuSO₄. Multiple studies have also compared the bioavailability of Cu lysine (Lys), another organic form of Cu, to CuSO₄. In vivo (Ward et al., 1996; Kegley and Spears, 1994; Rabiansky et al., 1999) and in vitro (Ward and Spears, 1993) studies have suggested CuLys and CuSO₄ may have similar bioavailability in cattle, even in the presence of dietary S and Mo. In contrast, a study by Nockles et al. (1993) reported stressed calves fed CuLys had 53% greater apparent absorption compared to those fed CuSO₄. When Mo and S are present in the diet, it would appear these organic forms of Cu may have increased bioavailability when compared to CuSO₄ in ruminants, and that there may be some stress response affecting the absorption of Cu.

Many studies have been utilized with injectable TM, however few have looked at the potential improvements a TM injection may have on TM status in the face of high antagonist diets. Daughtery et al. (2002) reported cows injected subcutaneously with Multimin had increased liver Cu concentrations. Similarly, Pogge et al. (2012) utilized a TM injection containing 15 mg Cu/mL, 10 mg Mn/mL, 60 mg Zn/mL, and 5 mg Se/mL (Multimin) on beef calves and reported improved liver Cu and plasma Cu concentrations. While both of these
experiments reported improved TM status in cattle, neither utilized a diet containing antagonists, and this area requires more research to clarify the benefit of injectable TM.

Hydroxy TM have been recently introduced as a new supplementation option for improving TM bioavailability to ruminants. The hydroxy source TM have a similar chemical structure to organic TM, but have a slight alteration in the crystalline matrix which prevents the compound from going into solution until they reach a low pH (Arthington, 2015). This technology allows the hydroxy Cu to bypass the rumen, thus avoiding interactions with antagonists, and be available for absorption further in the digestive tract. Genther and Hansen (2015) supplemented cannulated steers with TM from hydroxy source (Intellibond, Micronutrients Inc., Indianapolis, IN), or from inorganic Cu (as CuSO₄) and found HYD Cu to be less available at the pH of the rumen. Hydroxy source Cu and inorganic Cu were equally available in the abomasum, however the authors hypothesized a decreased ruminal availability would translate to an increased availability for absorption in the lower pH of the abomasum (Genther and Hansen, 2015). Additionally, the hydroxy forms have shown to be very stable in premixes, thus they are less likely to degenerate vitamins added to the feed.

Tribasic Cu chloride (Cl) has been shown to be soluble at a neutral or slightly acidic pH, thus making it less ruminally available than CuSO₄ and more available for absorption further down the GIT. In vitro work has shown CuCl to be insoluble in water but have greatly increased solubility following an incubation with 0.1% HCl, suggesting CuCl could be a potential supplementation strategy to bypass the rumen and be absorbed later in the GIT (Spears, 2004). Similarly, Spears (2003) reported CuCl to be less soluble at the pH of the rumen. Interestingly, a follow up in vivo experiment by Spears et al. (2004) reported no differences in the bioavailability of CuSO₄ and CuCl. In contrast, an in vivo study by Ward and Spears (1997) reported CuCl was
more bioavailable than CuSO₄, as shown by increased Cu concentrations in plasma, liver, and ceruloplasmin in steers fed diets high in Mo and S.

It should be noted that the many sources of Cu do not have to be supplemented separately. Bailey et al. (2001) determined a mixture of 50% organic and 50% inorganic TM resulted in slower hepatic loss of Cu in the face of dietary antagonists S, Mo, and Fe, in comparison to heifers supplemented with 100% CuSO₄. Presently, the variety of Cu sources provide opportunities for producers to select the best product based on the health of their animals and on the feedstuffs they utilize. The alternative supplementation strategies discussed may be a strategic mechanism to avoid absorption of thiomolybdates into circulation, while still providing bioavailable Cu for the animal to use.

**Copper Absorption**

Research has indicated that TM may form insoluble complexes in the rumen by interacting with digesta, and sources of Cu differ in their ability to be metabolized based the presence of Cu antagonists (Bremner, 1970; Suttle, 1991; Kegley and Spears, 1994). Copper has been shown to be largely insoluble in the abomasum relative to the rumen in vitro (Ward and Spears, 1993) and in vivo (Bremner, 1970). Early research suggested ruminants to have a net absorption of Cu in the digestive tract with the exception of the reticulorumen and the abomasum (Ivan and Greive, 1976); however, it is now known the primary site of TM absorption is the small intestine, and occurs primarily as simple diffusion or active transport (McDowell, 1992; Andrieu, 2008). The active transport involves the action of metallothioneine and is used primarily when low concentrations of Cu are present, and fluctuations in Cu supply can be
controlled by homeostatic absorption mechanisms, hepatic storage, and biliary secretion (McDowell, 1992).

While Cu is not absorbed in the reticulorumen, the rumen environment is ideal for Cu to interact with S, Fe, and thiomolybdates, thus making Cu less available for absorption in the abomasum and intestines. As a result, sources of Cu that could bypass the reticulorumen could be ideal for Cu supplementation in feedlot diets for optimal bioavailability. Due to the irreversible inhibitions that can occur in the rumen as minerals interact with digesta, new technologies, such as chelation of ionic molecules, have been developed to increase the bioavailability of minerals for absorption.

**Copper Status Biomarkers**

Copper status can be measured as concentrations circulating in plasma or stored in liver, or through one of the numerous Cu enzymes found in these locations. When Cu is provided in excess, a majority of absorbed Cu will end up stored in the liver due to the excellent storage capacity of the tissue (Suttle, 2010). Albumin and transcuprein are responsible for transporting Cu from the intestine to the liver for storage (McDowell, 1992). When concentrations of Cu are adequate or high within the liver, metallothionine is thought to be the primary storage protein, with nearly 90% of absorbed Cu found here in mammalian species (Bremner, 1987; Essilfie-Dughan, 2007). However, as Cu status declines, a greater proportion of Cu will be found in cuproenzymes such as ceruloplasmin as the liver releases its stores to supply Cu to the target organs (McDowell, 1992; Ward and Spears, 1997).

Plasma ceruloplasmin has been shown to be very stable and to be associated with a majority of circulating Cu concentrations (Kincaid, 1999; Prohaska, 2006), and multiple studies
have found ceruloplasmin to be an accurate indicator of Cu status (Kegley and Spears, 1994; Hansen et al., 2008). Superoxide dismutase 1 (SOD) has been suggested to be a reliable marker of Cu status because approximately 60% of Cu in erythrocytes is associated with SOD, however SOD is not a good initial marker of Cu status because it does not show depleted status until after plasma Cu and ceruloplasmin have been depleted (Kincaid, 1999; Ward and Spears, 1997). Chidambaram et al. (1984) found serum Cu and a variety of Cu containing enzymes to be irreversibly inhibited by thiomolybdates. Similarly, Lannon and Mason (1986) reported ceruloplasmin concentrations to be inhibited when thiomolybdates were circulating in plasma. In contrast, an in vitro study by Kelleher and Mason (1986) reported sheep ceruloplasmin supplemented with Mo had reversible inhibition of thiomolybdates on ceruloplasmin. If plasma concentrations or enzymes are to be used as Cu biomarkers, it would be prudent to analyze the trichloroacetic acid (TCA)-insoluble concentrations of Cu, which represent the portion bound to thiomolybdates, as a method to ensure all Cu accounted for is available.

Suttle and Angus (1976) suggested the concentration of Cu in hair may be correlated with Cu plasma concentrations. Later research suggested this may only be the case in cases of intense Cu deficiency, and that the method may not be suitable when Cu absorption is decreased by high dietary Mo (Suttle and McMurray, 1983). However, it should be noted the high risk of sample contamination for measurements from hair. Additionally, due to the tight homeostatic regulations on circulating Cu concentrations, plasma and serum are often not responsive to changes in Cu status until extreme toxicity or deficiently. Presently, it appears liver tissue may be one of the most reliable and repeatable indicators of Cu status.
Copper Excretion

Copper is poorly absorbed in most animals, and has been shown to be absorbed in greater concentrations in young animals compared to older animals (Ammerman et al., 1995; McDowell, 2003). Interactions occurring in the rumen that decrease the absorption of Cu will be excreted as an un-absorbed compound in the feces (McDowell, 2003). Absorbed Cu is primarily excreted in the bile, which ultimately is excreted in the feces (Gooneratne et al., 1989). Urinary Cu excretion has been shown to be relatively constant and unaffected by Cu intake in comparison to biliary excretion, and urine Cu is primarily correlated with Cu supplementation when Cu is added in excess concentrations (Suttle, 1991; Suttle, 2010).

Selenium

Selenium was identified as an essential nutrient in the 1950’s (Schwarz and Foltz, 1957) and functions as a component of approximately 25 selenoproteins (Beckett and Arthur, 2005). Glutathione peroxidase is a major Se dependent enzyme that catalyzes the conversion of hydrogen peroxide to water (Rotruck et al., 1973). In addition to having function in an antioxidant, Se impacts metabolic function through iodothyronine 5’-deiodinase, which converts the less biologically active thyroxine (T4) to the more active triiodothyronine (T3) form (Arthur et al., 1990).

The current recommendation for Se supplementation is 0.1 mg Se/kg DM, with a maximum tolerable concentration of 5 mg Se/kg DM (NRC, 1980; NASEM, 2016). Symptoms of acute Se toxicity include labored breathing, diarrhea, abnormal posture, and respiratory failure ultimately resulting in death (NRC, 1980; NRC, 2005). Subacute toxicity may also occur in animals grazing pasture high in Se, such as the Rocky Mountains, and will be expressed as
stumbling, anorexia, and eventually blindness, excessive swallowing, and labored respiration (NRC, 2005). Interestingly, cattle fed 0.28 mg Se/kg DM or greater from seleno-methionine (SeMet) or sodium selenite displayed some symptoms of Se toxicity, but none of the neuropathological symptoms associated with high Se concentrations, suggesting the neurological issues previously observed to accompany high Se concentrations may be a result of combined high Se and alkaloid poisoning, starvation, or PEM (O’Toole and Raisbeck, 1995).

While Se toxicity has negative effects on performance and neurological activity, Se deficiency has been classified as having a greater impact due to its widespread effect across the Northern Plains (NRC, 2005). Nutritional muscular dystrophy, which is also known as white muscle disease, is the primary symptom of Se deficiency and has been known to result in degeneration of skeletal and cardiac muscle, however reproductive failure and decreased immune function have also been associated with Se deficiencies (NRC, 1983; Underwood and Suttle, 1999; Oliveira et al., 2016). Studies have shown beef cows and calves fed a receiving forage-based diet containing between 0.02 and 0.05 mg Se/kg DM to display clinical and subclinical symptoms of Se deficiency (Morris et al., 1984; Spears et al., 1986). Interestingly, calves fed a semi-purified diet containing between 0.02 and 0.03 mg Se/kg DM for multiple months had low glutathione peroxidase activity but did not display symptoms of deficiency (Siddons and Mills, 1981; Reffett et al., 1988). As a result, it has been proposed that clinical signs of Se deficiency may result from a combined deficiency of Se and vitamin E (Miller et al., 1988). Interestingly, it appears neither a strict dietary Se deficiency nor toxicity results in symptoms typically associated with low or high Se, consequently signifying there are interactions occurring between Se and other dietary additions.
Dietary Sources of Selenium

Many feedstuffs grown in North America are deficient or marginally deficient in Se, especially those grown in the Northern Plains; however, most soil contains between 0.1 and 2 mg Se/kg DM (Podoll et al., 1992; NRC, 2005; NASEM, 2016). Land with soil Se concentrations above this range are known as seleniferous areas, and plants that accumulate Se will thrive in the soil and uptake high concentrations of Se primarily as SeMet and seleno-cystathionine (SeCys; Underwood and Suttle 1999; NRC, 2005). Plants can incorporate SeMet into their structure via protein synthesis due to its similar structure to methionine, thus grazing livestock often consume high concentrations of Se as SeMet (Lawler et al., 2004). Additionally, in areas with pH greater than 7.0, the primary form of Se in plants is selenate, which is highly soluble and easily incorporated into plant matter (NRC, 2005).

Selenium Supplementation

Many forms of Se supplementation have been utilized, including loose TM supplementation, Se injection, Se drench, and slow releasing Se ruminal boluses (McDowell, 2003; NRC, 2005). However, Se supplementation is restrictive, as Se is the only TM regulated as an additive by the United States government. In 1987, the FDA increased the inclusion of Se supplementation from 1 mg Se/kg DM to 3 mg Se/kg DM (Lawler et al., 2004). While originally supplementation of Se could only be provided as an inorganic form, alternative forms have since been approved as long as the concentrations of their inclusion are lesser than 3 mg Se/kg DM (NASEM, 2016).

Elemental Se is largely un-absorbable and has been reported to be excreted primarily in the feces (McDowell, 2003). The inorganic forms sodium selenate and selenite have been shown
to be highly bioavailable and have very similar availability to each other (Podoll et al., 1992; McDowell, 2003). However, similar to S, the inorganic forms of Se can be reduced in the rumen by microbes, thus resulting in decreased Se bioavailability in the intestine (Wright and Bell, 1966). If selenite is reduced to selenide in the rumen, it can serve as a substrate for the synthesis of SeCys, however due to the reducing environment of the rumen it is often excreted as unabsorbed Se before this synthesis can occur (Gould and Kendall, 2011).

Seleno-methionine has been shown to have greater bioavailability for absorption compared to inorganic forms of Se in ruminants (Deagan et al., 1987; O’Toole and Raisbeck, 1995). Research has suggested SeMet has greater bioavailability because it is directly incorporated into proteins, while inorganic Se has to overcome microbe antagonism to be absorbed, and enters the selenoprotein pool by a highly regulated process (Combs and Combs, 1986; Combs, 2015). Not only is the incorporation of SeMet into the nonspecific Se pool unregulated (Combs, 2015), it also has a much greater bioavailability than the inorganic forms; Pehrson et al. (1989) reported supplementation of SeMet or yeast Se product to Se-depleted heifers resulted two times as much glutathione peroxidase activity as heifers supplemented selenite. Selenocysteine has also been utilized as an organic supplementation form, however due to the lack of discrimination between SeMet and methionine, SeCys is absorbed in lesser concentrations (NASEM, 2016). Accordingly, SeMet supplementation is more effective at increasing tissue concentrations of Se than inorganic Se supplementation (Combs, 2015).

Injectable TM have also been utilized as a strategy for rapid Se supplementation. Pogge et al. (2012) injected growing calves subcutaneously with sodium selenite (5 mg Se/mL given at 1 mL/45 kg BW as part of a multi-element product), and found glutathione peroxidase activity, and plasma and liver Se concentrations were all increased compared to calves injected with a
control saline solution. The improvement in liver Se in particular was rapid and lasted for up to 15 d post injection (Pogge et al., 2012). Differing results were noted in sheep injected with 250 µm sodium selenite intravenously; at 4 hr post dosing only 20% of the original dose was recovered suggesting a rapid clearance from the blood stream; however, liver samples collected at 4 hr post dosing did show increased liver Se concentrations (Wright and Bell, 1966). Although these studies used the same form of Se, the dosage was different and the authors measured liver and blood samples at different times post injection, so it is difficult to compare these results directly. However, it is clear Se provided as an injection can be rapidly utilized by the ruminant and improve Se status.

Previous research has also studied the efficacy of slow releasing Se boluses in cattle and sheep as a method to overcome Se deficiencies. Hidiroglou and Proulx (1985) dosed cows with Se boluses and reported higher plasma Se and greater glutathione peroxidase concentrations compared to cows not receiving a bolus. Similarly, Campbell et al. (1990) found cows dosed with a Se bolus into the reticulorumen to have greater blood Se concentrations up to 220 d from the original dosage. Millar et al. (1988) found sheep dosed with Se boluses had greater whole blood Se concentrations compared to control sheep, however they also noted a high bolus loss rate where 40% of dosed sheep had lost the bolus after 6 months. The authors calculated blood selenium concentration to fall with a half-life of 43 ± 10 days for sheep that lost a bolus (Millar et al., 1988). The average loss of Se-pellets has been calculated to be at a rate of 0.14 mg Se/day over a period ranging from 218 to 349 d, and it is generally accepted that two pellets each containing 30 g Se is appropriate for cattle (Hemingway, 2003). While dosing grazing livestock with a Se bolus has been shown to improve status over a period of time, some have speculated the variation in bolus types may require greater oversight or regulation.
Selenium Absorption

Selenium is absorbed primarily in the duodenum, with little absorption occurring in the rumen and abomasum (Spears et al., 1986). There is lesser absorption of Se in ruminants compared to monogastric species, which has been suggested to be due to ruminal interactions which reduce selenite to insoluble forms (Write and Bell, 1966). The metabolism of organic Se revolves around the absorption of SeMet and SeCys as selenoproteins through an active amino acid uptake mechanism (NRC, 2005). Inorganic forms of Se that manage to escape ruminal antagonism are absorbed via passive diffusion and incorporated into selenoenzymes (Juniper et al., 2011). Regardless of original form, organic and inorganic-bound forms of Se can be used to meet the Se requirement in the body once they have been absorbed and incorporated into Se-dependent enzymes and proteins in the body (NRC, 2005; NASEM, 2016).

Selenium Status Biomarkers

It is well known that plasma, serum, and whole blood concentrations of Se are highly correlated with Se status (Stowe and Herdt, 1992; Juniper et al., 2011). Although whole blood accounts for serum and glutathione peroxidase Se, it is a less sensitive measurement of Se status because a majority of the glutathione peroxidase in whole blood is incorporated into red blood cells at erythropoiesis (Stowe and Herdt, 1992). Rotruck et al. (1973) determined glutathione peroxidase was a Se-dependent enzyme and research has shown this is a reliable indicator of Se status. Similarly, McMurray and Branchflower (1976) found whole blood glutathione peroxidase activity and dietary Se concentrations to be positively correlated in sheep and cows. Kincaid (1999) suggested glutathione peroxidase assays to produce inconsistent results between
laboratories due to differences in assay temperature, however with modern technology it is much easier to create repeatable tests.

Tissue also can be a reliable indicator of Se status; Langland et al. (1984) measured Se concentrations in sheep and reported between 4% and 9% of total body Se to be located in the liver. Consequently, many studies have used liver Se as an indicator of Se status (Netto et al., 2014; Pogge et al., 2014), especially because studies have shown the liver had the capacity to store more Se when there is excess Se in the diet (Koenig et al., 1997). Additionally, studies have noted that while muscle has low to moderate concentration of total Se, it does accounts for the largest pool of Se due to its mass (McDowell, 2003). However, studies across many species have consistently shown Se concentrations to be greatest in the kidney, then the liver, and finally the muscle when animals are not deficient in Se (Combs and Combs, 1986; Lawler et al., 2004). It would appear the order of tissue accretion of Se is independent of Se source as well; Pavlata et al. (2001) supplemented calves with either 40 mg Se from two intramuscular injections over two weeks, or fed a dietary supplement containing 0.3 mg yeast bound Se/kg DM, and reported Se concentrations were greatest in the kidney, followed by the liver, and then the muscle. It should be noted the authors did note calves supplemented with organic Se had higher blood and tissue concentrations of Se than those dosed with the injectable supplement, thus it is difficult to compare tissue accretion of Se when the mineral is provided as two different sources and through two different application methods (Pavlata et al., 2001). Overall, there are a variety of methods to measure Se status, and it would appear no one measurement has been determined to be the best indicator, however, circulating glutathione peroxidase and liver Se concentrations have been shown to be reliable measurements of Se status in cattle.
Selenium Excretion

Selenium can be excreted in feces and urine, however the primary route of excretion is in feces due to interactions in the rumen that convert Se to insoluble forms (NASEM, 2016). McDowell (1996) has reported excessive Se supplementation to cattle will also increase fecal excretion of Se. When Se is provided in a bioavailable form, the strict homeostatic mechanisms in the body will increase urinary output of Se to maintain normal circulating concentrations (Ivancic and Weiss, 2001). Wright and Bell (1966) reported sheep receiving an intravenous dose of 2.5 μc labeled $^{75}$Se to be primarily excreted in the urine, while sheep orally dosed with 0.35 mg Se/kg DM excreted Se primarily in the feces. Additionally, pulmonary excretion of Se has been shown to be important for rats fed high concentrations of Se, thus it is possible excessive concentrations of Se may require pulmonary Se excretion in cattle as well (Ganther et al., 1966).

Manganese

Manganese is required for skeletal development of young animals, and plays a major role in reproductive performance of adults (Rojas et al., 1965). Gycosyltransferase requires Mn to function and is necessary for the synthesis of mucopolysaccharides in cartilage for the formation of bone (Hurley et al., 1958). In addition to playing a role in physical development, Mn functions as a component of a number of enzymes, including hydrolases, kinases, transferases, and decarboxylases (NASEM, 2016). Pyruvate carboxylase, a Mn dependent metalloprotein, plays a role in normal lipid and carbohydrate metabolism (Scrutton et al., 1966), and in the mitochondria, a Mn-SOD assists in the protection of cells from free radicals (Weisiger and Fridovich, 1973).
The current recommended inclusion of Mn for growing and finishing cattle is 20 mg Mn/kg DM (NASEM, 2016), however this concentration may not be adequate for optimal growth or reproduction. Research has suggested lower concentrations of Mn may be sufficient for finishing steers, Legleiter et al. (2005) reported steers consuming a corn based finishing diet (analyzed to contain 8.1 mg Mn/kg DM) performed equally as well as cattle receiving 20 mg Mn/kg DM of supplemental Mn. Similarly, heifers have been reported to perform well when supplemented less than the national recommended concentration, however there have been reports of decreased reproductive efficiency as a result of low dietary Mn. Rojas et al. (1965) reported 20 mg of supplemental Mn/kg DM to be adequate for the growth and reproduction of heifers. In contrast, Bentley and Phillips (1951) supplemented 10 mg Mn/kg DM to heifers and reported adequate growth, however the heifers displayed decreased conception rates and delayed estrus cycles in comparison to heifers supplemented with 30 mg Mn/kg DM. More recent data has reported 16 mg Mn/kg DM to be sufficient for heifers to grow and reproduce, however Angus and Simmental calves did not have proper gestational development in comparison to calves from heifers fed 66 mg Mo/kg DM (Hansen et al., 2006).

It appears cattle have the capacity to consume very high concentrations of Mn as well, Cunningham et al. (1966) supplemented calves 0, 820, 2460, and 4820 mg MnSO₄/kg DM for 84 d, and decreased DMI and ADG were noted between 820 and 2460 mg SO₄/kg DM. These results contradicted a previous study where calves were supplemented 0, 1000, 2000, and 3000 mg MnSO₄/kg DM for 100 d and reported no difference in average daily gain, body weight, or feed efficiency (Cunningham et al., 1966). In contrast, Jenkins and Hidiroglou (1991) reported supplementation of Mn at 5000 mg Mn/kg DM to pre-ruminant calves to be lethal. Calves supplemented more than 1000 mg Mn/kg DM displayed decreased ADG and feed efficiency, and
liver samples collected at necropsy were reported to contain 26.7 mg Mn/kg DM, which is close to two times the Mn concentrations found in a normal liver. While the maximum tolerable concentration of Mn is relatively high compared to other minerals, once neurological symptoms of Mn toxicity are established, they become progressive and irreversible, thus it is vital for producers to avoid these toxic concentrations in diets (Zheng et al., 2011).

Because Mn is so tightly regulated, symptoms of toxicity or deficiency do not appear immediately. Heifers with Mn deficiencies will produce calves with a low birth weight, dwarfism, superior brachygnathism, swollen joints, decreased bone strength, and an unsteady gait (Hurley and Keen, 1987; Hansen et al., 2006). Some of these symptoms are likely due to the inability to properly synthesize mucopolysaccharides, resulting in decreased amounts of cartilage (Suttle, 2010). Clinical deficiencies are rarely reported, as most feedstuffs contain adequate concentrations of Mn (Weiss and Socha, 2005).

Dietary Sources of Manganese

Manganese concentrations in forage are greatly dependent on soil pH, plant species, and soil drainage (McDowell, 2003). Increased soil pH results in decreased concentrations of retained Mn in crops and pastures (NRC, 2005), and Mn deficiency is most likely to occur in cattle grazing on alkaline soils (Suttle, 2010). Forages have been reported to contain adequate Mn (NASEM, 2016), and corn silage has been shown to contain low concentrations of Mn (Berger, 1995) while cereal grains vary widely (Underwood and Suttle, 1999).
Manganese Supplementation

There have been a variety of Mn sources studied in recent years to determine the most bioavailable form. Supplementation of Mn from organic sources has become increasingly popular, and a variety of forms have been researched including Mn methionine (MnMet), Mn proteinate, Mn polysaccharide complex, and Mn amino acid chelate (NASEM, 2016). Henry et al. (1992) supplemented wether lambs with 900, 1800, or 2700 mg Mn/kg DM from MnSO₄ or MnMet and based on a multiple linear regression of bone and kidney estimated the bioavailability of MnMet to be approximately 120% compared to MnSO₄. In contrast, dairy cows orally dosed with a bolus containing 0.62 g MnSO₄ had similar apparent absorption and retention to cows orally dosed with a bolus containing 2.5 g MnMet (Weiss and Socha, 2005). Cows fed the same inclusion of an organic or inorganic Mn had similar liver Mn concentrations (9 mg Mn/kg DM vs. 10 mg Mn/kg DM for organic and inorganic, respectively), however, the supplementation concentration was 200 mg Mn/kg DM, and it is unlikely normal absorption mechanisms occurred in the small intestine due to the pharmacological concentration of supplementation (Olson et al., 1999).

Recently, Genther and Hansen (2015) measured the availability of Mn hydroxychloride in rumen fluid and reported it to be less ruminally soluble than MnSO₄. In contrast, steers supplemented with sulfate, or hydroxy TM included at increasing concentrations were reported to have no differences in liver or plasma Mn concentrations (Caldera et al., 2016). Injectable TM are another recent strategy that have been tested as a method to improve Mn status. Pogge et al. (2012) injected feedlot steers (weight) with a product containing 15, 10, 5, and 60 mg/mL of Cu, Mn, Se, and Zn, respectively, at a dose of 1 mL/45 kg BW, and using repeated measures analysis reported liver Mn concentrations tended to increase across the first 15 d post-injection. Genther
and Hansen (2014) also injected yearling steers with a multi-element injection containing 15, 10, 5, and 60 mg/mL of Cu, Mn, Se, and Zn, respectively, at a dose of 1 mL per 68/kg BW and using repeated measures analysis for samples collected over 90 d post injection, reported Mn concentrations tended to increase in Mn-SOD from red blood cell lysate, but did not report a change in liver Mn concentrations. The injectable TM may provide an opportunity to supplement TM in a highly available method, and these data suggest there may be lesser ruminal solubility from the hydroxy Mn source, indicating it bypass the rumen and overcome ruminal antagonisms.

**Manganese Absorption**

Manganese has been shown to be poorly absorbed (less than 1%) in ruminants (Van Bruwaene, 1984). This is in part due to the parallels between Mn and Fe metabolism, in which competition for the non-specific apical transporter DMT1, and also competition for binding to ferritin, may ultimately decrease the absorption of Mn (Arrenondo and Nunez, 2005). When Mn is consumed in excess, it is stored in the bone, liver, and kidney (O’Neal and Zheng, 2015). Pogge et al. (2012) reported liver Mn concentrations were greater in Simmental steers compared with Angus cattle, regardless of injectable TM treatment, however this result could be due to changes in diet utilization or excretion, and is not necessarily representative of absorption. There has also been some data suggesting there may be differences in Mn status between sexes, however these results have not been repeated and more research should be done on this topic before drawing conclusions (Hansen et al., 2006).
Manganese Status Biomarkers

Presently, there is no widely recognized Mn biomarker. In contrast to other TM, plasma and serum Mn concentrations have been shown to be poor biomarker and not reflect TM status, and there is no established concentration for toxic liver Mn concentrations (NASEM, 2016). This makes it difficult to determine when plasma or liver Mn are marginally deficient or deficient in Mn (Hansen et al., 2006), however studies have suggested cattle liver statuses greater than 13 mg Mn/kg DM to be adequate (NRC, 2005). Liver Mn concentrations in steers increased linearly as dietary MnSO₄ supplementation increased from 0 to 240 mg Mn/kg DM (Legleiter et al., 2005). Underwood (1999) reported reserves of Mn to be highest in bone, liver, and kidney, and multiple other studies have assessed bone Mn concentrations to determine bioavailability (Schroeder et al., 1966; Leach and Harris, 1997). Leach and Harris (1997) suggested bone Mn could be utilized as a passive reserve with substantial fluctuations in Mn concentrations occurring as intakes of Mn were raised or lowered. However, this measure is impossible for live animal measurements, thus it would be in the best interest of the industry to determine a reliable Mn biomarker.

Whole blood Mn concentrations have been shown to be an unreliable marker of Mn status (Underwood and Suttle, 1999). Some studies have suggested blood may be a useful measure to compare Mn concentrations within a population, however the concentrations would not be comparable to other studies (Zheng et al., 2011). Early studies reported Mn-SOD gene expression to be related to concentrations of Mn in heart and lung (Masters et al., 1988), and liver Mn concentrations (Underwood and Suttle, 1999). This has recently been supported by Genther and Hansen (2015), who noted a similar trend between liver Mn concentrations and Mn-SOD activity from red blood cell lysate.
Manganese Excretion

Similar to other TM, excess Mn is excreted primarily via bile (Suttle, 2010), and Mn is quickly and efficiently eliminated from circulation by the liver (Gibbons et al., 1976). As a result, blood Mn levels are not representative of Mn status. When Mn is absorbed in excess, tissues such as the brain and the liver will accumulate Mn (Crossgrove and Zheng, 2004). In cattle duodenally infused with 4000 µg Mn/min, biliary excretion of Mn was shown to increase up to 200 fold for a few hours with no effects of apparent toxicity in steers; however, the authors noted this drastic removal of Mn is unlikely to occur for extended periods of time (Hall and Symonds, 1981). Additionally, the presence of high Fe concentrations in the diet will limit Mn absorption and result in greater endogenous fecal losses of Mn (Davis et al., 1992).

Zinc

Zinc is essential for normal growth, immune function, and reproductive function (Chesters, 1997; Engle et al., 1997; NRC, 2005). In mammals, over 300 Zn-dependent metalloenzymes and 2,000 Zn-dependent transcription factors have been identified that play roles in the metabolism of carbohydrates, proteins, lipids, and nucleic acids (Hambidge et al., 1986; Suttle, 2010). These enzymes include but are not limited to Cu-Zn superoxide dismutase (Tainer et al., 1983), alcohol dehydrogenase, carboxypeptidase, and RNA polymerase (Hambidge et al., 1986).

The current National Academies of Engineering, Science and Medicine (2016) recommends a minimum inclusion of 30 mg Zn/kg DM for cattle fed a concentrate based diet, however the requirements for beef cattle fed a forage based diet are less well defined. In cases of Zn deficiency, livestock may have decreased performance resulting in lesser growth, DMI, and
efficiency (NASEM, 2016). Additional symptoms of Zn deficiency include listlessness, excessive salivation, and parakeratotic lesions on the legs, neck, and head (NASEM, 2016). The estimated maximum tolerable concentration is much greater for Zn than for other TM, and is currently listed at 500 mg Zn/kg DM (NRC, 2005; NASEM, 2016). However, research has shown that even greater Zn inclusion concentrations, while causing symptoms of toxicity, may not be lethal; studies that fed beef calves 700 mg Zn/kg DM (Jenkins and Hidiroglou, 1991) and 900 mg Zn/kg (Ott et al., 1966) noted decreased gain, intake, and efficiency, but had no other adverse effects on the calves.

Dietary Sources of Zinc

The concentration of Zn in forage sources varies greatly depending on the plant, maturity, and soil Zn (Minson, 2012) On average, plant sources have been shown to contain between 50 and 70 mg Zn/kg DM, with legumes containing higher Zn concentrations than grasses on average, and cereal grains containing on average between 20 and 30 mg Zn/kg DM (NASEM, 2016). From forage based sources, a relatively large portion of Zn is associated with the plant wall (Whitehead et al., 1985). The standard concentration of Zn in drinking water is on average 5 mg/L and the NRC (1980) recommends cattle not consume greater than 25 mg/L Zn from water, however these concentrations are almost never reached in surface water (NRC, 2005).

Zinc absorption can be affected by the source of Zn, phytate, and the presence of other minerals including Fe, Ca, Cu, and S (NRC, 2005; Pogge et al., 2014). Pogge et al. (2014) reported provided steers a subcutaneous injection of Multimin90 TM product, and reported liver Zn to be decreased in steers high S diets (0.68% S) compared to those fed control diets (0.24% S). It should be noted some of these antagonisms only apply to animals with a functional rumen;
for example, phytate does not affect Zn in ruminants with a functional rumen (NASEM, 2016). Other factors, such as amino acids histidine and cysteine, have been shown to actually enhance Zn absorption in ruminants (NRC, 2005).

**Zinc Supplementation**

The bioavailability of Zn sources determine how much Zn should be supplemented and how much will be retained. Zinc supplements can be provided in a variety of forms including dietary Zn oxide (ZnO), Zn sulfate (ZnSO₄), methionine (ZnMet), proteinate (ZnProt), hydroxy forms, as well as through injectable TM. In general, solubility is key for TM absorption in the rumen, and Zn is absorbed more efficiently from aqueous sources (NRC, 2005). Some research has suggested there is no difference between the absorption of the inorganic and the organic forms of Zn (Rojas et al., 1996; Kessler et al., 2003). Similarly, Kegley and Spears (1994) found ZnSO₄ and ZnO forms to have a similar bioavailability in ruminants. However, Spears (1989) found the absorption of ZnMet to be similar to ZnO, but that they were metabolized in the body differently. Holstein calves supplemented with 20 mg Zn/kg DM from ZnProt had lower liver Zn concentrations than calves receiving supplemental ZnSO₄; however, when calves were supplemented 500 mg Zn/kg for 14 d, ZnProt calves had greater concentrations of duodenal, liver, kidney, and plasma Zn compared to calves receiving ZnSO₄ (Wright and Spears, 2004); these data support the hypothesis that the concentration of supplemental Zn greatly influences how it is absorbed from the separate sources.

For many years, the organic forms of Zn have been cited to have greater bioavailability compared to inorganic forms, with the some of the strongest data having been reported from Spears (1989). However, recent data has suggested the hydroxy form of Zn may be one of the
more bioavailable forms (Spears et al., 2004; Genther and Hansen, 2015). Ruminally soluble Zn can bind with fiber in the rumen and be captured in the non-soluble fraction, and sources with lesser ruminal availability would be beneficial for providing greater concentrations of available Zn.

**Zinc Absorption**

The absorption of Zn occurs primarily in the abomasum and small intestine, and does not occur in the rumen (Miller and Cragle, 1965; Georgievskii et al., 1979). Homeostatic regulation plays an important role in Zn uptake (Miller et al., 1975). Genther and Hansen (2015) provided high and low concentrations of Zn from inorganic and hydroxy sources and found the hydroxy source to be more ruminally soluble than ZnSO$_4$. However, research has shown Zn can bind to particulate matter in the rumen, thus limiting interactions with the rumen microbes and falsely inflating the appearance of ZnSO$_4$ (Eryavuz and Dehority, 2009).

Zinc can be absorbed through a nonmediated or a mediated process. The nonmediated process is non-saturated, and is unaffected by Zn uptake in the digestive tract (Solomons and Cousins, 1984). This process does not require energy and has been suggested to reflect paracellular Zn uptake of Zn diffusion into cells (Cousins, 1996). Zinc can also be absorbed through a mediated process which is saturable and can be stimulated in the presence of low Zn (Solomons and Cousins, 1984). When Zn uptake is rapidly decreased, it is believed to be a reflection of hepatic Zn uptake which could result from hormonal activations (NRC, 2005).
Zinc Status Biomarkers

While plasma and liver samples have been used to diagnose severe Zn deficiencies, because of the range of metalloenzymes Zn functions in, there are few reliable biomarkers to determine Zn status (Hambidge, 2003; NASEM, 2016). Plasma and serum samples have been commonly used to test circulating Zn concentrations, however it is difficult to determine the form or purpose for Zn measured. The concentrations of Zn in plasma are highly responsive to external stimuli, such as fluctuations in Zn intake, fasting, and acute stress (King and Keen, 1999). Research has suggested there are two primary proteins involved with Zn transport and binding: albumin, and metallothioneine. Total plasma Zn is typically bound to albumin or alpha-2-macroglobulin (20-30%) (NRC, 2005). Metallothioneine is involved in the regulation of Zn metabolism, and there is an excess of albumin compared with Zn, ensuring adequate transport always exists (NRC, 2005).

Steers fed a basal diet containing 26 mg Zn/kg DM did not have different plasma Zn concentrations compared to steers receiving 20 mg supplemental Zn/kg DM for 196 d (Spears and Kegley, 2002). Additionally, Spears and Kegley (2000) supplemented steers 25 mg supplemental Zn from ZnO or ZnProt, and reported very similar plasma Zn concentrations compared to control fed steers. These results make it apparent that even in the face of differing Zn status, plasma concentrations may appear similar between treatments. Liver samples are not the most reliable indicator due to their poor tracking of Zn concentrations (Herdt and Hoff, 2011); while researchers have recorded increases in liver Zn after TM injections (Pogge et al., 2014), this is not always the case. Genther and Hansen (2014) injected steers with Multimin90 and reported no effect on liver Zn concentrations, and commented on the difficulty of measuring Zn due to the number of metalloenzymes it has function in.
Zinc Excretion

Zinc is excreted primarily in feces from unabsorbed dietary and endogenous Zn (Miller, 1970). Calves and goats fed low Zn had decreased total and endogenous excretion (Hambidge et al., 1986). Urinary Zn losses comprise less than 20% of total Zn loss under normal conditions, and do not change in response to differences in Zn intake unless the diet is essentially lacking all Zn (Underwood and Suttle, 1999; King and Keen, 1999). Even with urine loss accounted for, the total Zn excreted in the urine is less than 1 mg Zn/kg per day (NRC, 2005).

Trace Mineral Conclusions

Trace minerals are incorporated into a variety of enzymes and proteins, and have an essential role in growth, development, and reproduction. For the most part, soil concentrations, and thus feedstuff concentrations, of TM vary widely, thus supplementation is necessary to ensure adequate status for optimal livestock production. Trace mineral absorption occurs primarily in the small intestine, and it is desirable to supplement TM from a source that may avoid ruminal antagonisms and be available for absorption in the small intestine. Supplements commonly include Cu, Se, Mn, and Zn together, and the effectiveness of inorganic, organic, hydroxy, and injectable forms have been reviewed in the previous pages.

Though there are a number of reliable biomarkers for Cu and Se, there are fewer biomarkers to accurately measure Mn and Zn status. Liver samples appear to be one of the most consistent indices for all four TM of interest, and many research trials have collected liver samples to analyze TM concentrations. Plasma samples are also used to determine TM status of Cu and Se, however, in contrast to the other TM, plasma Mn and Zn have been shown to be very poor indicators of status. Some studies have suggested Mn-SOD may be a reliable biomarker for
Mn status, however, more research is necessary to determine the extent of this interaction. It would be advantageous to compare how TM source may impact individual TM absorption, especially in the presence of a high antagonist diet, and more work should be done to elucidate the most reliable sample(s) to collect for TM analysis.

**Trace Mineral Interactions**

Trace mineral interactions in the rumen can present substantial challenges to TM absorption and mineral status in cattle. These interactions primarily occur in the rumen prior to intestinal absorption, and competition for absorption between minerals may also ultimately decrease the concentrations of absorbed mineral. Specifically, there are a variety of known interactions that can occur between Fe, Cu, Mn, and Zn. Iron has been shown to antagonize the absorption of Cu and Mn in ruminants, which is likely due to competition for a common intestinal absorption mechanism, divalent metal transporter 1 (DMT1; Arredondo et al., 2003). Although Cu has an intestinal transporter, Ctr1, dedicated to Cu absorption, research has shown DMT1 has the capacity to transport Cu (Lee et al., 2002) and Mn (Gunshin et al., 1997). Research has also revealed Fe to be antagonistic towards Cu absorption and storage in the body (Standish et al., 1969). More recently, Hansen et al. (2010) supplemented young dairy calves 750 mg Fe/kg DM for 56 d and found the expression of DMT1 was decreased compared to calves not supplemented with Fe, and reported decreased intestinal concentrations of Mn. It appears the absorption of Mn and Cu may be inversely related to the concentrations of Fe in the diet (Arredondo et al., 2003), and other TM can impact their absorption as well. Hansen et al. (2009) reported the supplementation of excessive Mn (500 mg Mn/kg DM) decreased plasma and liver Cu concentrations in beef cattle. High concentrations of Zn have also been shown to decrease Cu
concentrations; Ott et al. (1966) reported feeder calves (24 steers and 36 heifers) supplemented with ZnO at concentrations ranging from 0 to 2100 mg Zn/kg DM had a linear decrease in serum Cu concentrations. Bremner and Beattie (1995) have suggested this antagonism to be a result of competition for metallothionein, a storage protein that can bind to either Cu or Zn.

In addition to TM having antagonistic effects on the absorption of other TM, S has the capacity to decrease the TM bioavailability as well. It is well known sulfide can decrease Cu absorption and liver Cu concentrations (Suttle, 1974a; Spears et al., 2011). More recently, Pogge et al. (2014) pair fed steers a low S (0.24%) or high S (0.68%) diet and supplemented ING TM at NRC (2001) recommended concentrations and reported high S to decrease the percent retention and apparent absorption of Cu, Mn, and Zn, compared to the low S diet. Similarly, Se absorption has been shown to be decreased by high dietary S in multiple species including rats (Muth, 1970), lambs (Van Ryssen, 1988, Netto et al., 2014) and cattle (Amat et al., 2014). It is clear there are many ruminal interactions occurring that may alter the bioavailability of TM, therefore it would be highly beneficial to determine a supplementation strategy that may overcome these antagonisms, thus decreasing the concentrations required to supplement and increasing the efficiency of absorption.

**Review of Trace Mineral Antagonisms**

**Sulfur**

Sulfur is essential for ruminants to produce S-containing amino acids, B-vitamins, and a variety of enzymes (Spears et al., 1976; NASEM, 2016). Methionine is required for initiating amino acid synthesis for nearly all eukaryotic proteins, while cysteine and cystine are crucial for protein metabolism (Goodrich and Garrett, 1986, Brosnan and Brosnan, 2006). The B-vitamins
thiamin and biotin are required for decarboxylation reactions, and are functional in transketolase and carbon fixation in rumen microbes, respectively (Goodrich and Garrett, 1986). Meanwhile, many enzymes require S for disulfide bond formation and enzyme activity (Goodrich and Garrett, 1986). Due to the many diverse compounds and enzymes containing S, adequate dietary S is important to maintain normal production and performance in cattle.

**Dietary Sources of Sulfur**

The current National Academies of Engineering, Science and Medicine (2016) recommends a minimum inclusion of 0.15% S to the diet. However, due to the increased S concentrations of current feedstuffs, high dietary concentrations have become the greater cause for concern. Though previous work suggested the maximum tolerable inclusion of S in beef cattle to be 0.4% (NRC, 1980), recent studies have shown the percentage of roughage in the diet can affect this upper limit. Current beef national recommendations include a maximum inclusion of 0.30% S for a diet containing less than 15% forage, and 0.50% S for a diet containing at least 40% forage (NASEM, 2016). Though S is not stored well in the body, the concentration of S in each ingredient is additive in the diet; therefore, a combination of ingredients well below the maximum tolerable inclusion could result in a diet with considerable S.

High concentrations of S can be introduced to the diet through a variety of feedstuffs including molasses, high sulfate water, dried whey, S salts, and more recently, by-products of the ethanol industry (NASEM, 2016). These byproducts include corn gluten feed, steep liquor, distillers grains with solubles (DGS), and condensed corn distillers solubles. Corn byproducts from the distilling process have concentrated energy and protein nearly threefold of the original value, making them a valuable feed source for livestock (Klopfenstein et al., 2008). However,
the use of sulfuric acid to stabilize the pH for ethanol processing increases the S concentration in feeds from between 0.6% to in excess of 1.0% (Klopfenstein et al., 2008; Kerr et al., 2008). Spiehs et al. (2002) analyzed dried DGS from 10 separate ethanol plants over a 3-year period and found the S concentration ranged from 0.33% to 0.74% S across plants. Similarly, Buckner et al. (2011) measured DGS from 6 ethanol plants and found S concentrations across plants ranged from 0.71% to 0.84% S, and that variation between loads at the same plant ranged from 3 to 13%. The wide variation of S concentrations within and between plants intensifies the need for accurate feed analysis before diet formulation.

Elemental S is one of the least toxic forms of dietary S, as it has very low bioavailability and is less soluble in rumen fluid compared to other sources; however, in large concentrations SRB can reduce elemental S to H₂S (NRC, 2005). When Rumsey et al. (1978) fed 0.42% elemental S to beef steers there were no reported effects; however, an inclusion of 0.98% elemental S resulted in dramatically decreased intakes. In contrast, sulfate from water can represent a significant amount of S consumed (Gould, 1998) and is highly available to rumen microbes because the S is already in solution. Similarly to dietary S, the maximum tolerable concentration of sulfate from water is dependent on the diet formulation. Cattle consuming a high concentrate diet should not drink water containing greater than 600 mg sulfate/L, however cattle fed a diet with at least 40% forage may be able to safely drink water containing 2,500 mg sulfate/L (NRC, 2005). Gould et al. (2002) collected water samples from beef operations across the United States and found a range of approximately 100 to 480 mg sulfate/L, with the greatest concentrations appearing in the Northern central states. To estimate S intake from water, one must record average water intake and convert sulfate to sulfur (Gould, 1998). Gould (1998) calculated an intake of 50 L of water containing 600 mg sulfate/L would equal 200 mg S/kg, or
0.02% S. Depending on the percent roughage of the diet, water S can make up a large proportion of total S consumed, and could introduce excessive S to the diet.

**Sulfur Metabolism in Ruminants**

While non-ruminants must be supplied with S in the organic form, ruminants can use both inorganic and organic forms of S because of the ruminal microbe activity (Goodrich and Garrett, 1986). Rumen bacteria, such as sulfate reducing bacteria (SRB), can rapidly reduce inorganic sulfate to sulfide, which can be absorbed or incorporated into microbial protein (Goodrich and Garrett, 1986; Gould, 1998). This reduction can occur though either assimilatory or dissimilatory pathways (Emery et al., 1957; Lewis, 1953). In the assimilatory reaction, bacteria use anaerobic respiration to reduce sulfate to hydrogen sulfide (H$_2$S), which is then absorbed and incorporated into S-containing amino acids or B-vitamins (Bradley et al., 2011). Sulfate reducing bacteria preferentially use the dissimilatory sulfate reduction pathway, which results in the formation of sulfide as the end-product (Coleman, 1960; Bradley et al., 2011).

In general, sulfate and sulfide form a recycling system in the rumen (Gould, 1998). When sulfide is absorbed, it is oxidized to sulfate in the liver and distributed in the extracellular fluid where it can be recycled through saliva to the rumen (Bray and Till, 1975). However, greater activity of SRB will result in the production of excess H$_2$S gas, which is eructated from the rumen (Coleman, 1960; Goodrich and Garrett, 1986, Doughtery and Cook, 1962b). Although SRB comprise less than 1% of ruminal bacterial populations (Callaway et al., 2010), SRB greatly contribute to H$_2$S concentrations eructated from the rumen because of their rapid conversion of sulfate (Drewnoski et al., 2014). Early studies by Doughtery and Cook (1962a) suggested that 70-80% of gas eructated was re-inhaled by the animal. Though H$_2$S is a normal product of rumen...
microbial metabolism, in large concentrations it can pose a threat if toxic amounts of S are inhaled and absorbed (Lewis, 1953).

There are a number of factors that can affect SRB activity in the rumen, including increased dietary S, length of S adaptation, and decreased pH. Cummings et al. (1995) found ruminal microbes had greater capacity to reduce sulfate after adaptation to a high S diet. Similarly, Lewis (1953) found a repeated dose of sulfate increased the rate of sulfate reduction, thus increasing sulfide production in sheep. This response has been suggested to be an adaptation of SRB to increased dietary S, resulting in greater reduction activity and more SRB in the rumen. Gould et al. (1997) found concentrations of H₂S peaked about 1 to 3 weeks after calves were placed on a diet containing 0.37% S, suggesting there is an adaptation period for rumen microbes to metabolize S. These data were supported by Drewnoski and Hansen (2013) who found a 14 d adaptation period was optimal for SRB activity, and suggested this may be due to an increase in efficiency as SRB utilize different electron donors for the dissimilatory reaction.

Greater inclusions of corn and ethanol byproducts to diets have increased the concentrations of S introduced while simultaneously decreasing rumen pH. More sulfide is converted to H₂S by SRB at more acidic pH (Beauchamp et al., 1984). Additionally, as pH decreases, H₂S becomes less soluble, and greater concentrations may be released into the gas cap of the rumen (Gould, 2000). Data from Morine et al. (2014) indicate the concentration of H₂S produced from the rumen can be reliably predicted when the pH is below 5.6 ± 0.08, but is not a good estimate of H₂S production when the rumen pH is greater than 5.68.
Sulfur Toxicity- H$_2$S production

Increased ruminal concentrations of H$_2$S have also been shown to be positively associated with neurological issues (Gould, 1998). Hydrogen sulfide gas and its ionic forms have been shown to be highly toxic when concentrated, and polioencephalomalacia (PEM), a neurological disease where lesions appear in the grey matter of the brain, has been suggested to be a form of subacute H$_2$S toxicity (Gould, 1998). When inhaled, H$_2$S bypasses detoxification in the liver and can act immediately on the brain (Gould, 1997).

One of the earliest recorded cases of H$_2$S poisoning was noted by Coghlin (1944), after approximately 13.6 kg of S were fed to 40 calves as a preventative measure against lice and ringworm. A diagnosis of S toxicity was made after the cattle exhibited sudden symptoms of muscle twitching, staggering, blindness, and scours, in addition to the “offensive odor” of S surrounding the cattle, and was supported by necropsy data (Coghlin, 1944). A later project by Doughtery et al. (1965) infused the rumen of sheep with H$_2$S gas and found those with open tracheas collapsed after several eructations, while those with an artificially blocked trachea showed no symptoms of toxicity. Concentrations of H$_2$S have been shown to be greater in the gas cap compared to sulfide concentrations in the rumen fluid (Gould et al., 1997; Loneragan et al., 1998). Later data by Loneragan et al. (2005) showed cases of S-PEM in feedlot cattle to appear while ruminal concentrations of H$_2$S were high. Sulfur toxicity and H$_2$S production in the rumen are closely linked in ruminants, likely through a respiratory mechanism (Coghlin, 1944; Doughtery et al., 1965; McAllister et al., 1992; Gould, 1998).
Ruminal Availability of Sulfur

While multiple sources of S can be metabolized in the rumen, total S intake does not necessarily equal the concentration of S available for metabolism by rumen microbes. Some research has suggested that passage rate may affect the concentration of S absorbed. Delfiol et al. (2013) reported sheep fed 0.9% and 1.2% S to have less rumen motility than sheep consuming only 0.2% S. Similarly, Uwituze et al. (2011) suggested high concentrations of H$_2$S to decrease the activity of smooth muscle in steers. If high S concentrations decrease passage rate of feed from the rumen, the possibility of toxicity from lesser concentrations of S could be increased in the presence of many SRB.

The concept of ruminally available S (RAS) accounts for the availability of S for ruminal reduction to sulfide, and disregards ruminal recycling of S (Sarturi et al., 2013). For instance, inorganic sulfates have been shown to have very high ruminal availability. Other sources of S including sulfolipids, glutathione, β-thioglucose, succinyl-CoA, and CoA, have been suggested to be 100% available for ruminal reduction (Sarturi et al., 2013). However, organic sources, such as S-amino acids, have lesser availability in the rumen because the amino acids can escape degradation or be incorporated into rumen microbes (Sarturi et al., 2013), and thus should not be calculated as having the same bioavailability for ruminal metabolism by microbes. Sarturi et al. (2013) found the concept of RAS to explain 65% of variation in H$_2$S production, compared to only 29% of the variation being explained by the total dietary S. Ruminally available S can be calculated using in vitro dry matter digestibility analytical procedures, and is a good indicator of available S for absorption.
Effects of Sulfur on Performance

It has long been known high S diets can decrease gain and carcass characteristics of feedlot steers. Thompson et al. (1972) found steers consuming elemental S had decreased ADG and HCW compared to steers consuming a diet with no added S. Studies have shown negative effects on performance when S is provided in-excess of 0.2% from ammonium sulfate (Zinn et al., 1997; Spears et al., 2011), sulfuric acid (Uwituze et al., 2011), and sodium sulfate (Richter et al., 2012; Pogge and Hansen, 2013). However, not all sources of S have the same effect on live and carcass performance. A review by Drewnoski et al. (2014) has estimated the addition of 0.1% inorganic S beyond 0.2% results in an approximate decrease of 0.43 kg/d DMI, 0.08 kg/d ADG, and 6.6 kg HCW. These estimations can be calculated based on the assumption that the inorganic S is mostly available for absorption. Therefore, it is difficult to make similar exact calculations for other dietary sources of S which may include a mixture of organic and inorganic S forms.

Sulfur Excretion

After S has been metabolized, there are several routes for excretion from the body. As previously mentioned, H2S is eructated and can be re-inhaled where it gains entry into general circulation through the lungs (Gould, 1998). Sulfide can be rapidly absorbed across the rumen wall into the blood stream, transported in the portal blood to the liver where it is metabolized to sulfate and excreted via urine (Bray, 1969; Kandylis, 1984). Meanwhile, sulfate is not easily absorbed into circulation, and as microbial protein synthesis increases in the rumen undegraded organic S is excreted in greater concentrations in the feces (Bray, 1969; Kandylis and Bray,
Johnson et al. (1970) observed this in lambs fed either inorganic or organic S where urinary excretion of S was greater for lambs fed NaSO₄ compared to those fed L-methionine.

**Summary - Sulfur**

Sulfide produced in the rumen will be eructated as H₂S when produced in excess, which may result in symptoms of toxicity classified as PEM. Additionally, it is clear rumen microbes have an adaptation period, as H₂S concentrations peak early in the feeding period, thus increases in dietary S must be monitored to prevent toxicity. The period of optimal H₂S production appears to be between 2 and 3 weeks on high grain, high S diets, thus future research should be undertaken to determine methods to modify the rumen and SRB in this time frame. Additionally, the S concentrations in feedstuffs have been a concern in the livestock industry due to the wide variability of S concentrations between batches and between ethanol plants. It would be useful for producers for a standardization process to be developed for these feedstuffs to create batches of byproducts with repeatable S concentrations.

It has been shown that S can decrease performance characteristics and TM concentrations of feedlot cattle. Considering the vital role TM play in growth, development, and immunity, it would be helpful to determine the best supplementation strategy for feedlot cattle consuming high S diets, to ensure optimal performance. Trace minerals can be supplemented in multiple forms which can be absorbed through different routes, and differences in metabolism of these sources may be exploited to overcome limitations of dietary S on ruminant TM status. Previous studies have used multiple forms of TM including inorganic, organic, and injectable. However, the effects of all products have not been tested on cattle fed high antagonist diets. This is an area that should be researched as the knowledge could have extensive effects on the industry.
Molybdenum

Molybdenum functions in the enzymes xanthine oxidase, sulfite oxidase, and aldehyde oxidase (Mills and Davis, 1987). There currently is no established Mo requirement for beef cattle, as there have been no reported cases of Mo deficiencies (NASEM, 2016). An early study by Ellis et al. (1958) reported lambs fed 0.35 mg Mo/kg from semi-purified sources had improved weight gain compared to lambs not supplemented with Mo, and suggested the growth could be due to improved cellulose digestion in the rumen. However, these results were not repeated in a later study (Ellis and Pfander, 1960), and there have been multiple reported cases of Mo having a negative effect on performance and absorption of Cu. The recommended maximum tolerable inclusion is estimated to be between 5 and 10 mg Mo/kg DM, and Mo has been shown to have negative impacts on growth when fed in concentrations greater than 20 mg Mo/kg DM (Underwood, 1962; Ward, 1978; NRC, 2005).

Symptoms of toxicity are similar for Mo toxicity and Cu deficiency, and may include anorexia, weight loss, diarrhea, anemia, and joint and bone deformities (NRC, 2005). Suttle (1974a) recorded cattle had diarrhea, growth retardation, anemia, and achromotrichia while consuming a diet adequate in Cu but high in Mo. Ruminants have been shown to be especially susceptible to Mo toxicity due to the formation of thiomolybdates which can cause a secondary deficiency in Cu (Underwood, 1962; Ward, 1978; Spears, 2003), thus it is vastly important to have accurate measurements of Mo, S, and Cu in feedstuffs before formulating a diet.

Dietary Sources of Molybdenum

Forages can vary greatly in Mo concentrations depending on the soil pH and soil type (NASEM, 2016). For instance, sandy soils have been shown to contain low concentrations of
Mo, while marine-origin soils have been shown to contain high concentrations of Mo ranging from 0.1 to 20 mg Mo/kg DM (Underwood and Suttle, 1999); however, the concentrations of Mo available to the plant are rarely greater than 1 mg Mo/kg DM (Aubert and Pinta, 1980). As soil pH increases, the total Mo uptake by plants will increase, resulting in excessively high concentrations of Mo from alkaline soils (McDowell, 2003; NRC, 2005). As a result, Mo toxicity is uncommon for animals grazing pasture, especially if the pasture is acidic or well drained, and feedstuffs on average contain 0.2 and 1.5 mg Mo/kg DM (McDowell, 2003). Water can also be a source of Mo; Kincaid (1986) suggested the minimal toxic concentrations of Mo in drinking water to be between 10 to 50 mg/L, with a critical Cu:Mo ratio of less than 0.5. However, the ratio suggested by Kincaid (1980) is based on beef cattle consuming a diet containing 0.29% S and 13 mg Cu/kg, and the ratio may be lesser for diets containing greater concentrations of S.

**Thiomolybdate formation**

Molybdenum and S metabolism are closely intertwined in ruminants, and the relationship has been researched extensively in recent years. Sulfur has a strong attraction to Mo, and can dramatically decrease the ruminal absorption and increase the retention of Mo post-absorption (Grace and Suttle, 1979). Thiomolybdates form when sulfide is substituted for O in the molybdate anion (Suttle, 1991). In ruminants, this occurs when SRB in the rumen produce sulfide which binds to dietary Mo. As a result, there was some interest in finding a method to supplement Mo as a method to slow the production of H₂S. Brayden and Bray (1972) proposed that Mo could inhibit SRB without compromising rumen fermentation. This theory was supported with data showing molybdate can inhibit SRB in rumen fluid (Taylor and Oremland, 1979), and was further reinforced by in vitro work where H₂S gas production was inhibited by
adding 100 mg/kg Na$_2$MoO$_4$ to sulfide (Kessler et al., 2012). However, Kessler et al. (2012) found 5 mg Mo/kg DM was not sufficient to entirely halt H$_2$S production in steers, and concluded that while chemically Mo can bind SRB and decrease H$_2$S production, the high concentrations of Mo that would be necessary to decrease SRB would be impractical as a preventative for PEM.

Originally, Dick (1956) reported only the inorganic form of S interacted with Mo to form thiomolybdates, which then bound to Cu in the rumen. Suttle (1974a) later disproved this by reporting organic and inorganic forms of S to be equally effective at binding with Mo, and at decreasing the availability of Cu. Because rumen microbes can reduce both organic and inorganic forms of S to sulfide and S has a high affinity for Mo, there are ample opportunities for Mo to bind and form thiomolybdates in the rumen (Dick, 1956). This interaction may decrease the absorption and post-absorptive metabolism of Mo (Mills and Davis, 1987).

Molybdenum is poorly absorbed in the rumen and abomasum, however, thiomolybdates can be absorbed in the small intestine (Kelleher et al., 1983). When Mo was dosed as an abomasal infusion instead of a ruminal infusion, Suttle (1980) reported fewer thiomolybdates were formed and therefore suggested the rumen to be the primary site of Mo and S complex formation. As previously discussed, concentrations of S in beef cattle diets have increased due to the often high amount of S in ethanol co-products; thus proper diet formulation and analysis of feedstuffs for nutrient concentrations have become increasingly important to balance diets that value these interactions.
Thiomolybdate Chemistry

The formation of the thiomolybdate complex is pH dependent, with most thiomolybdates forming at a neutral pH close to 7.0, and thiomolybdate breakdown occurring at a pH below 3.0 (Suttle, 1974b). As sulfate ions are reduced in the rumen, thiomolybdate formation progresses via sulfide binding with Mo in the fluid concentration. This results in one of four thiomolybdate forms which develop at different rates; mono-thiomolybdates are produced transiently, di-thiomolybdates will form in 5 minutes, tri-thiomolybdates form in 30 minutes, and tetra-thiomolybdates will only form after several hours of exposure at a pH of 7.5 (Mason et al., 1982a). These forms are produced at different rates and absorbed in different concentrations; sheep fed diets containing 6 mg Mo/kg DM and 0.43% S and ruminally dosed with labeled $^{99}$Mo or $^{99}$tetra-thiomolybdate were found to have 41% and 34% of ruminal thiomolybdates in the tri- and tetra- forms, respectively (Price et al., 1987). However, plasma samples collected from these animals showed that di- and tri- $^{99}$thiomolybdates were circulating, suggesting the absorbed concentrations of thiomolybdates are not always reflective of ruminal concentrations (Price et al., 1987). Similarly, Mason et al. (1982b) collected plasma samples from sheep fed 0.3% S and dosed with 30 mg $^{99}$Mo/kg DM, and found only di- and tri- thiomolybdate to be circulating in plasma. These data support a hypothesis by Suttle (1974b) which suggested the tetra-thiomolybdate form to have a greater affinity for binding solid digesta, thus making the tetra-form less available for absorption.

Interestingly, tetra-thiomolybdates have been detected in plasma samples, but only after sheep were dosed with 50 mL of $^{99}$tetra-thiomolybdate directly to the rumen (Price et al., 1987). In combination, these data suggest while the tetra- form has the capacity to circulate if it is absorbed, it is more likely to be bound to digesta before the small intestine than the di- and tri-
forms, and thus the di- and tri- forms have the capacity to be absorbed in the small intestine. Additionally, while thiomolybdates may exist in the rumen or plasma as a variety of forms, research has suggested there is little difference in behavior of these forms (Kelleher et al., 1983), and regardless of form, the total available amount of thiomolybdates should be the primary focus.

Thiomolybdate antagonism on copper

The addition of low Mo concentrations has been shown to antagonize Cu absorption. Suttle (1983) found a dietary ratio of 1 mg Mo/kg DM to 1 mg Cu/kg DM decreased Cu by a factor of three. Similarly, Marston (1999) recommended that 8 mg Cu/kg DM should be added to the diet for each increase of 1 mg Mo/kg DM, however the negative effect of Mo on Cu concentrations has been shown to plateau after the diet contains greater than 5 mg Mo/kg DM (Underwood and Suttle, 1999). However, the effect is most greatly noted when S and Mo are added together; Suttle (1974a) reported the addition of 4 mg Mo/kg DM had little effect on sheep plasma Cu concentrations and the addition of 0.3% dietary S slightly decreased plasma Cu, but when S and Mo were supplemented together there was a reported 50% decrease in plasma Cu concentrations. This dramatic decrease in Cu availability has been seen in many studies (Dick et al., 1975, Suttle, 1991).

While clinical Cu deficiency has been characterized as a primary deficiency in Cu, it can be considered a secondary deficiency when caused by Mo, S, or Fe (Suttle, 1991). A secondary Cu deficiency was induced in beef heifers fed 7 to 16 mg Mo/kg DM and 3 to 6 mg Cu/kg DM (Mo:Cu ratio of 2.5:1) in a diet containing 0.3% S (Arthington et al., 1996). Heifer calves consuming a diet with 0.28% S were supplemented 0 or 800 mg Fe/kg DM, and 0 or 5 mg
Mo/kg, and were reported to display clinical symptoms of Cu deficiency when fed Mo but there were no effects of Fe (Humphries et al., 1983). It is clear Mo and S have antagonistic effects on Cu absorption, and that this action is much stronger than the antagonism of Fe alone. Additionally, recent work by Sinclair et al. (2017) reported silage source may alter Cu status when supplemented in diets containing high S and Mo. Dairy cows fed diets containing grass silage and supplemented with 20 mg Cu/kg DM, 7.5 mg Mo/kg DM, and 0.35% S were reported to have more greatly decreased liver Cu concentrations than cows receiving corn silage (Sinclair et al., 2017). Further work is needed to determine the influence of dietary components on the interaction among Cu and antagonists.

Research by Phillippo et al. (1987) suggested thiomolybdates could only have antagonistic effects in the rumen; however, when Mo concentrations are in excess of Cu in the rumen, thiomolybdates may be absorbed into the blood where they scavenge Cu from albumin and other Cu containing enzymes (Mason, 1982a; NASEM, 2016). Once thiomolybdates have formed and bound irreversibly to Cu in the rumen, Cu will be excreted without being absorbed, and if the thiomolybdates are circulating, they will be incorporated into enzyme functions (Suttle, 1974a). The thiomolybdate fraction that binds to Cu in plasma is referred to as the TCA-insoluble Cu. Research by Suttle and Small (1993) suggested the TCA-insoluble portion of plasma Cu could serve as a pool of slowly releasing Cu, however this work has not been repeated or supported to the author’s knowledge, and it is a widely-held belief that the TCA-insoluble Cu may only serve as an indirect measurement of circulating thiomolybdates.

The effect of Mo on Cu status is decreased considerably if the rumen is bypassed and Mo is provided through a subcutaneous injection (Suttle and Field, 1974) or an intravenous infusion (Suttle, 1974), however multiple studies have shown the presence of thiomolybdates in the blood
stream to decrease available Cu. Gooneratne et al. (1984) showed a subcutaneous injection of ammonium tetrathiomolybdate to sheep suffering from Cu toxicity led to recovery as circulating Cu concentrations decreased extremely rapidly. Similarly, Kelleher et al. (1983) reported that a dosage of 20 mg Mo/kg from either $^{99}$Mo, $^{99}$tri-, or $^{99}$tetra-thiomolybdate resulted in increased concentrations of TCA-insoluble $^{99}$Mo in plasma. When Lannon and Mason (1986) infused ruminants intravenously with a pharmacological dose (100 mg Mo/kg DM) from either tri- or tetra-thiomolybdates, they reported while there was a decrease in ceruloplasmin activity of plasma, there was an increase in Cu bound to plasma albumin. Considering thiomolybdates can bind Cu in albumin, it is possible the increased Cu concentrations recorded were simply an increase in TCA-insoluble Cu concentrations. Additionally, Suttle (1999) reported that using ceruloplasmin as an indicator of Cu status to have little value due to the 20% loss of activity during the blood clotting process, and it is possible alternative indicators including plasma and liver may be a better indicator of Cu status in cattle.

In-vitro work by Chidambaram et al. (1984) reported thiomolybdates irreversibly inhibited ceruloplasmin activity, and also greatly decrease the activity of the Cu containing enzymes cytochrome oxidase, superoxide dismutase, ascorbate oxidase, and tyrosinase. In contrast, Lannon and Mason (1986) reported the effect of thiomolybdate concentrations to be reversible in vitro. It is clear thiomolybdates have the capacity to antagonize Cu absorption through not only the gastrointestinal tract, but also that they can be absorbed and decrease available circulating Cu concentrations in the blood.
Summary - Thiomolybdates

The formation of ruminal thiomolybdates has the potential to detrimentally affect Cu status of ruminants consuming high concentrations of S and Mo. Research has shown Mo to have a very strong affinity for S in the rumen, however, the interaction is not only ruminal. High concentrations of Mo in the rumen may decrease Cu absorption, however the effect will plateau after Mo is added to the diet in concentrations greater than 5 mg Mo/kg DM (Underwood and Suttle, 1999). When S is present and Mo concentrations are in excess of ruminally available Cu, thiomolybdates can be absorbed into the blood where they have the capacity to bind with Cu from albumin and other Cu containing enzymes (Mason, 1982c; NASEM, 2016). This plasma TCA-insoluble fraction has been utilized as an indicator of thiomolybdate absorption by scientists, and there has been an attempt to determine the reliability of Cu biomarkers in the blood in the presence of circulating TCA-insoluble Cu. It appears ceruloplasmin activity is inhibited somewhat by thiomolybdates, and that concentrations of Cu in plasma are more responsive to circulating thiomolybdates. Therefore, it would be advantageous to locate a ruminally insoluble Cu source that would be able to by-pass the irreversible antagonisms occurring in the rumen, and have greater availability for absorption in the small intestine.

Literature Cited


CHAPTER III

Effect of trace mineral source on mineral status and performance of beef steers fed low or high sulfur diets.

S. J. Hartman*, O. N. Genther-Schroeder*, and S. L. Hansen*

*Department of Animal Science, Iowa State University, Ames, IA 50011

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Corresponding author: slhansen@iastate.edu
ABSTRACT: A 190 d trial was designed to measure the effect of trace mineral (TM) sources fed within low or high sulfur (S) diets on the mineral status and performance of cattle. Angus-crossbred steers (n = 48; 6/pen) were blocked by BW (316 ± 16.6 kg), assigned to a 2 × 2 factorial with low (0.27%; LS) or high S (0.54%; HS; added as CaSO₄) diets, and supplemented TM as 10 mg Cu, 30 mg Zn, and 20 mg Mn/kg DM from hydroxy (HYD; Intellibond; Micronutrients USA, LLC, Indianapolis, IN) or inorganic (sulfates; ING) sources (n = 12 steers/treatment). Steers were fed corn-silage growing period (GP; 84 d) and corn-based finishing period (FP; 77 d) diets via GrowSafe bunks. Plasma and liver were collected at trial initiation, and end of GP and FP for mineral concentrations. End of GP and FP red blood cell lysate superoxide dismutase (SOD) and Mn-SOD activity and liver glutathione concentrations were measured. Data were analyzed as a 2 × 2 factorial using Proc Mixed of SAS with initial plasma and liver status as covariates in analysis. High S decreased (P < 0.01) liver Cu and tended (P ≤ 0.1) to decrease plasma Cu concentrations. Liver Cu concentrations were lesser in HYD than ING (P < 0.01). High S decreased (P = 0.04) GP plasma Zn concentrations and tended to decrease (P = 0.1) GP liver Zn. There were GP (P = 0.05) and FP (P = 0.02) S × TM effects for liver Mn concentrations where GP LS-HYD was greater than all other treatments, while FP LS-HYD was lesser than HS-HYD and LS-ING, and FP HS-ING was less than LS-ING. Glutathione, SOD, and Mn-SOD were not different (P ≥ 0.13) in the GP, but S × TM tended to affect FP Mn-SOD (P = 0.10), where LS-HYD tended to be lesser than LS-ING. Oxidized glutathione in FP tended to be lesser (P = 0.06) for HYD than ING. In the GP, there were S × TM effects on performance where LS-HYD had greater ADG and G:F (P ≤ 0.05) than HS-HYD, while LS and HS-ING were intermediate. For FP performance S × TM effects were noted where LS-HYD and HS-ING tended (P = 0.10) to gain more than HS-HYD, and HS-HYD had lesser...
G:F ($P = 0.04$) than HS-ING. There were no effects of $S \times TM$ on final BW, DMI, or ADG ($P \geq 0.11$); however, HS-HYD had lesser G:F than others overall ($P = 0.05$). High S decreased back fat ($BF$) and YG ($P = 0.03$), and REA was smaller for HYD than ING ($P = 0.02$). In this study HS decreased markers of Cu and Zn status, and differential effects of HYD vs. ING minerals were noted across dietary phases, though all steers maintained adequate TM status.

**KEYWORDS:** cattle, hydroxy, inorganic, sulfur, trace mineral

**INTRODUCTION**

In recent years, increased inclusions of ethanol co-products such as distillers grains with solubles have resulted in greater S concentrations in feedlot diets. While beef cattle have a requirement for dietary S for enzyme activity, amino acid production, and B vitamin formation by ruminal bacteria, excessive S antagonizes trace mineral absorption in ruminants (Drewnoski et al., 2014; Pogge et al., 2014). Trace minerals such as Cu, Mn, and Zn are critical in growth processes such as collagen and protein formation (Mills et al., 1976) and have several functions in essential antioxidant enzymes in the body, including Cu-Zn and Mn superoxide dismutase (SOD; Andrieu, 2008). It is well known that high S decreases Cu absorption in ruminants (Spears, 2003; Suttle, 2010), and there is recent evidence that Mn and Zn may also be antagonized by the presence of sulfide in the rumen (Pogge et al., 2014). Hydroxychloride trace minerals are less soluble in the rumen than inorganic sources, and have been suggested to face lesser ruminal antagonism and be more bioavailable for absorption in the intestine (Spears, 2003; Spears et al., 2004). This concept has been supported by Genther and Hansen (2015), who reported basic Cu chloride to be less soluble in the rumen, but have similar solubility compared to inorganic trace minerals under simulated abomasal conditions. Similarly, Shaeffer (2006)
reported Zn hydroxychloride to be less soluble in the rumen of feedlot cattle in comparison with ZnSO₄. Presently, there is little information about how high S diets may impact absorption of hydroxy trace minerals in ruminants. The objective of the present study was to determine the effects of supplementation of hydroxy or inorganic sourced Cu, Mn, and Zn within low or high S diets on mineral status, antioxidant concentrations, and growth and carcass performance of beef steers.

**MATERIALS AND METHODS**

All procedures involving the use of animals were approved by the Iowa State University Institutional Animal Care and Use Committee (log # 2-15-7939-B).

*Experimental Design and Sampling Procedures.* This experiment was conducted at the Iowa State University Beef Nutrition Research Center (Ames, IA). Angus crossbred steers (n = 48, 316 ± 16.6 kg) were housed in partially covered pens (6 steers per pen), fed via GrowSafe-equipped bunks, and had ad libitum access to water. At the initiation of the study steers were vaccinated against viral (Bovi Shield Gold 5; Zoetis Inc., Florham Park, NJ) and clostridial (Vision 7; Merck Animal Health, Summit, NJ) pathogens and were dewormed with eprinomectin (Eprinex, Merial Ltd., Iselin, NJ). Steers received an electronic identification tag (Allflex US Inc., Dallas–Fort Worth Airport, TX) to track intakes in the GrowSafe bunk system (GrowSafe Systems Ltd., Airdrie, AB, Canada). Steers were fed a receiving diet consisting of 50% corn silage, 25% corn, 20% DDGS, and 5% supplement (salt, Rumensin, vitamin premix, mineral premix, DDGS as carrier) containing no supplemental Cu, Mn, or Zn for 4 weeks, then blocked by BW and initial liver Cu concentrations to a 2 × 2 factorial design. The factors included two
concentrations of dietary S; low (LS, averaging 0.27% S across all phases of the trial) or high (HS, averaging 0.54% S across all phases of the trial) and two sources of supplemental trace mineral (TM) providing 10 mg Cu, 30 mg Zn, and 20 mg Mn/kg DM from either hydroxy (HYD; IntelliBond; Micronutrients USA LLC, Indianapolis, IN) or inorganic (ING; sulfates) sources. This resulted in 12 steers per treatment combination: 1) LS with ING (LS-ING), 2) LS with HYD (LS-HYD), 3) HS with ING (HS-ING), and 4) HS with HYD (HS-HYD). Steers were fed a corn silage-based diet for an 84 d growing period (GP), and were transitioned with 3 step up diets over 28 d to a corn-based finishing diet for a 77 d finishing period (FP). All diets were formulated to meet or exceed NRC (1996) recommendations. Diet composition and analyzed concentrations of S, Cu, Mn, Mo, and Zn for the GP and FP are shown in Tables 1 and 2, respectively. Growing period dietary S concentrations were 0.23% for LS and 0.51% for HS, and S concentrations in the FP were 0.30% for LS and 0.56% for HS. Steers were implanted on d 84 with a combination implant containing 80 mg trenbolone acetate and 16 mg estradiol (Component TE-IS; Elanco Animal Health, Greenfield, IN).

Steers were weighed on consecutive days on a single animal scale that had been calibrated at the start of the GP (d -1, d 0), end of the GP (d 83, d 84), start of the FP (d 111, d 112), and end of the FP (d 188, d 189). Additional interim weights were collected during the GP (d 28, d 56) and FP (d 140, d 169). Liver biopsy samples were collected using the methods of Engle and Spears (2000) for trace mineral analysis on d -15 (initial), d 80 (end of GP), and d 185 (end of FP). Liver samples collected on d 80 and d 185 were separated into two containers with one placed on ice for transport to the laboratory, and the other flash frozen in liquid nitrogen. Jugular blood samples were collected on d 0, d 84, and 190 into 7 mL trace mineral potassium-
EDTA vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood samples were transported on ice to the laboratory for preparation prior to analysis.

**Tissue and Diet Analysis.** Diet total mixed ration (TMR) samples were collected on a weekly basis and dried in a forced air oven at 70°C for 48 hr for DM calculations. Dry matter intakes were calculated using as-fed intake data from the GrowSafe system and were corrected for DM content using results from the weekly TMR sample analysis. Diet samples were ground and composited by month within treatment for trace mineral analysis. Feed samples were analyzed for CP, NDF, and ether extract (EE) concentrations (Dairyland Laboratories, Arcadia, WI). Feed efficiency (G:F) was calculated using the total gain and total DMI for each period, and ADG was calculated using the total weight gained for each period divided by the length of each period.

Blood samples were centrifuged at 1000 × g for 10 min at 4°C, and plasma was removed and stored at -20°C until analysis. Plasma samples were prepared for trace mineral analysis according to the methods of Pogge and Hansen (2013). Red blood cell lysate (RBCL) was processed using the methods of Russell et al. (2016) for analysis of total SOD and Mn-SOD, and CuZn-SOD was calculated by subtracting Mn-SOD from total SOD. The CV for inter-assay analyses of SOD and Mn-SOD were 10.41 and 15.68, respectively, with an intra-assay CV of 3.28. Hemoglobin (Hb) analysis was conducted on the RBCL as previously described by Hansen et al. (2009). One unit of SOD activity is defined as the amount of enzyme required to exhibit 50% dismutation of the superoxide radical, and activity is expressed per g of Hb.

Liver samples were transported on ice to the laboratory where they were dried to completion in a forced air oven at 70°C for approximately one wk. Flash frozen liver samples were stored at -80°C until further sample preparation. Diet TMR composites and dried liver
samples were digested using nitric acid prior to mineral analysis using the methods of Pogge et al. (2014). Plasma samples were analyzed for Cu, Fe, and Zn, and liver samples were analyzed for Cu, Mn, and Zn using an ICP-OES (Optima 7000, Perkin Elmer, Waltham, MA). All runs included serum (UTAK Laboratories, Inc., Valencia, CA) or liver (National Institute of Standards and Technology, Gaithersburg, MD) standards as appropriate for verification of instrument accuracy.

For the analysis of glutathione concentrations, flash frozen liver samples were ground into a fine powder with a mortar and pestle cooled by liquid nitrogen. Ground liver samples were weighed into a 50 mL tube and homogenized with 5 mL of 2-(N-morpholino) ethanesulfonic acid monohydrate buffer. Samples were centrifuged at 10,000 × g for 15 min at 4°C and the supernatant was aliquoted into a conical tube and vortexed with an equal volume of metaphosphoric acid. The deproteinated solution was left at room temperature for 5 min, then centrifuged at 3000 × g for 3 min. The supernatant was removed and stored at -80°C. Total glutathione and oxidized glutathione (GSSG) concentrations were determined using the commercial glutathione assay kit (Cayman Chemical Company, Ann Arbor, MI). Reduced glutathione (GSH) concentrations were calculated by subtracting oxidized glutathione concentration from total glutathione concentration, and the ratio of oxidized:reduced was calculated. The CV for inter-assay analyses of total glutathione and GSSG were 8.76 and 14.71, respectively, with an intra-assay CV of less than 10.0.

**Carcass Data Collection.** Steers were harvested at a commercial abattoir (Iowa Premium Beef, Tama, IA) on d 190, and electronic and visual identification tags were matched with each individual carcass before HCW collection. Carcasses were chilled for 48 hr, and on d 192 carcass
data were collected including 12th rib back fat thickness (BF), KPH, ribeye area (REA), marbling score (as called by the USDA grader), and plant quality grade. Yield grade was calculated using the following equation:

\[ 2.5 + (2.5 \times BF) + (0.2 \times KPH) + (0.0038 \times HCW) - (0.32 \times REA) \]

**Statistical Analyses.** Data were analyzed as a 2 × 2 factorial using the MIXED procedures of SAS 9.4 (SAS Institute Inc., Cary, NC) with steer as the experimental unit (n = 12 per treatment). The model included the fixed effects of S diet, source of trace mineral supplementation, and the interaction, and liver and plasma mineral data were analyzed using pre-trial values as a covariate in the analysis. Individual treatment means were separated using the pdiff command in SAS. Outliers were determined using Cook’s D statistic with a cut-off of 0.5. One initial liver Zn outlier was established as having an abnormally high value, and this steer was removed from all subsequent Zn analysis because of the need for initial liver Zn as a covariate (LS-ING). One additional sample was removed from liver Zn analysis at the end of the GP because of abnormally high Zn concentration (LS-ING), and 4 liver samples were unable to be collected on d 185 (three HS-ING, one LS-HYD). Significance was declared at \( P \leq 0.05 \) and tendencies were declared as \( 0.06 \leq P \leq 0.10 \).

**RESULTS**

**Trace Mineral Concentrations.** Plasma and liver trace mineral concentration data are shown in Tables 3 and 4, respectively. There were no S × TM interactions or effects of TM source on plasma mineral concentrations during the GP or FP (\( P \geq 0.21 \)). High S decreased plasma Zn (\( P = 0.04 \)), and tended to decrease plasma Fe (\( P = 0.06 \)) and Cu (\( P = 0.08 \)) concentrations in the GP.
In the FP, HS tended to decrease \((P \leq 0.1)\) plasma Cu and Fe concentrations, but did not affect plasma Zn concentrations \((P = 0.81)\).

There were no interactions between S × TM for liver concentrations of Cu or Zn during the GP or FP \((P \geq 0.25)\). High S decreased liver Cu concentrations in the GP and FP \((P < 0.01)\), and tended to decrease liver Zn concentrations in the GP \((P = 0.1)\), but did not affect FP liver Zn concentrations \((P = 0.98)\). There were no effects of TM source on GP liver Cu concentrations \((P \geq 0.12)\), but FP liver Cu concentrations were lesser in HYD compared to ING \((P < 0.0001)\). Liver Zn concentrations were unaffected by TM source in the GP and FP \((P \geq 0.34)\). There was a S × TM effect on GP liver Mn concentrations \((P = 0.05)\) where LS-HYD was greater than all other treatments, which were intermediate. In the FP, there was a S × TM effect on liver Mn concentrations \((P = 0.02)\) where LS-HYD and HS-ING tended to be lesser than LS-ING and HS-HYD.

**Antioxidant Analysis.** There were no effects of S, TM, or the S × TM interaction on any measurements of SOD activity \((P \geq 0.13\), Table 5\) or glutathione concentration \((P \geq 0.32\), Table 6\) at the end of the GP. At the end of the FP, there were no differences in total SOD or CuZn-SOD activity due to the main effects of S or TM, or their interaction \((P \geq 0.13)\); however, there was a tendency for a S × TM effect \((P = 0.10)\) for FP Mn-SOD activity where LS-HYD tended to be lesser than LS-ING, while steers receiving HS were intermediate. Total and reduced glutathione concentrations displayed a S × TM effect at the end of the FP \((P \leq 0.02)\) where LS-HYD concentrations were less than all other treatments. There was no S × TM effect in the FP for GSSG \((P = 0.96)\); however, there was a tendency for a TM effect \((P = 0.06)\), with less GSSG in steers supplemented with HYD compared with ING. Additionally, in the FP there was a
tendency for a $S \times TM$ effect in GSSG:GSH ($P = 0.09$) where HS-HYD tended to have a lesser ratio than HS-ING, while steers receiving LS were intermediate.

**Live Performance and Carcass Characteristics.** There were no effects of $S$, $TM$, or the $S \times TM$ interaction on DMI during the GP, FP, or overall trial ($P \geq 0.11$, Table 7). At the end of the GP, there were $S \times TM$ interactions for G:F ($P = 0.01$), ADG ($P = 0.05$), and BW ($P = 0.09$), where LS-HYD gained more and were more efficient, and tended to weigh more than HS-HYD at the end of the FP, while animals receiving ING were intermediate. Following the transition period there were no effects of $S$, $TM$, or the $S \times TM$ interaction on BW at the start of the FP ($P \geq 0.12$) or the end of the FP ($P \geq 0.12$). There was a tendency for a $S \times TM$ effect ($P = 0.10$) on FP ADG where LS-HYD and HS-ING tended to gain more than HS-HYD, while LS-ING were intermediate. In the FP ($P = 0.04$) and overall ($P = 0.05$), there were $S \times TM$ effects where HS-HYD steers were less efficient than HS-ING, while those consuming low $S$ were intermediate. Additionally, ADG assessed across the entire trial was not different ($P \geq 0.11$) due to $S$, $TM$, or the $S \times TM$ interaction.

There were no effects of $S$, $TM$, or the $S \times TM$ interaction on HCW, marbling score, KPH, or dressing percent ($P \geq 0.12$, Table 8). High $S$ decreased BF and yield grade ($P = 0.03$), and HYD had smaller REA than ING ($P = 0.02$).

**DISCUSSION**

The current recommended $S$ concentration in cattle diets is 0.15% $S$, with maximum inclusion limits of 0.30% $S$ for diets containing less than 15% forage, or 0.50% $S$ for diets containing at least 40% forage (NASEM, 2016). However, ethanol coproducts such as distillers
grains have been shown to contain variable S concentrations ranging from 0.33% to greater than 1.0% (Spiehs et al., 2002; Buckner et al., 2011), and water sulfate concentrations vary widely due to geographical region (Gould, 1998). Increased dietary S concentrations have been shown to decrease the absorption of Cu (Suttle, 1991; Spears, 2003), as well as Mn and Zn in ruminants (Pogge et al., 2014), which may result in decreased growth and immune function, as trace minerals play essential roles in these processes (Spears, 2003; NASEM, 2016). Hydroxy trace mineral sources are less soluble in the rumen than inorganic sources, and it has been suggested they may be supplemented as an alternative source to avoid ruminal antagonisms caused by high S (Spears et al., 2004; Genther and Hansen, 2015). As a result, research was undertaken to determine the effect of HYD trace mineral on performance and trace mineral status of beef cattle fed high S diets.

In the present study, DMI did not differ due to dietary treatment, therefore changes in liver concentrations are likely a result of differences in absorption or excretion of the trace minerals. High S decreased liver Cu concentrations and tended to decrease plasma Cu concentrations during the GP and FP, which is consistent with previous research (Spears, 2003; Spears et al., 2011; Richter et al., 2012; Pogge et al., 2014). Unexpectedly, liver Cu concentrations in the present study were greater for ING compared with HYD. In contrast, Spears et al. (2004) reported steers supplemented with hydroxy Cu in a diet supplemented with 0.15% S and 5 mg Mo/kg had increased liver Cu, plasma Cu, and ceruloplasmin activity in comparison to steers supplemented with CuSO₄, and suggested the relative bioavailability of hydroxy Cu to be greater than CuSO₄. While the results from the present study do not match those previously reported in regards to bioavailability of Cu sources, it is clear there are some
interactions occurring in the rumen between the trace minerals and the diet type that may impact the absorption of trace minerals.

While the presence of sulfide in the rumen can decrease Cu absorption, thiomolybdates formed by the ruminal interaction of S and Mo can also greatly decrease Cu absorption by forming irreversibly insoluble complexes in the rumen (Suttle, 1991). In the presence of ruminally available Cu, thiomolybdates will preferentially bind Cu and be excreted as an insoluble compound (Suttle, 1991); however, in the absence of soluble ruminal Cu, thiomolybdates can be absorbed and inhibit Cu-dependent enzymes in the body (Kelleher et al., 1983; Chidambaram et al., 1984). The capacity for thiomolybdates to form and decrease ruminal Cu concentrations would have been much greater in the work by Spears et al. (2004), as dietary Mo concentrations were much greater than in the present study where no additional Mo was supplemented and dietary concentrations averaged only 0.77 mg Mo/kg DM. The ruminally insoluble nature of hydroxy Cu would have avoided interactions with thiomolybdates formed in the rumen, and could have resulted in absorbed thiomolybdates that ultimately decreased Cu hepatic stores, as was observed in the present study. Additionally, recent work by Sinclair et al. (2017) supplemented dairy cows with 20 mg Cu/kg DM, 7.5 mg Mo/kg DM, and 0.35% S and reported cows consuming grass silage had more greatly decreased liver Cu concentrations than those consuming corn silage. There has been little research on how silage source may impact Cu status when thiomolybdates are present in the rumen, and though Sinclair et al. (2017) supplemented greater concentrations of S and Mo than the present study, it should be noted Cu status may be affected by multiple factors. While thiomolybdate presence was not assessed in the present study, more work is needed to refine the potential of ruminally soluble Cu to serve a sacrificial role in the rumen and prevent the absorption of thiomolybdates.
High S had deleterious effects on Zn and Fe status of steers in the GP. The antagonistic effect of S has been noted in previous studies on Fe (Bremner et al., 1987; Phillippo et al., 1987) and Zn (Pogge et al., 2014). Though there was a tendency for HS to decrease plasma Fe concentrations in the FP, there were no effects of HS on liver or plasma Zn concentrations during this period. Similarly, steers consuming forage containing between 0.37% to 0.40% S were not reported to have changes in serum Zn concentrations (Spears et al., 1985). There were no effects of TM source on liver or plasma Zn concentrations during the present trial. These data concur with of Caldera et al. (2017), which stated steers supplemented with iso-concentrations of Zn from hydroxy or inorganic trace minerals had no difference in plasma or liver Zn concentrations.

Few studies have examined how Mn supplementation from a hydroxychloride source impacts the metabolism of Mn in the ruminant. In the present study, liver Mn concentrations at the end of the GP were greater for LS-HYD steers than for all other treatments. Due to the ruminally insoluble nature of Mn hydroxychloride (Genther and Hansen, 2015), it is possible there were increased concentrations of Mn available for absorption in the small intestine. In contrast, during the corn-based FP, LS-HYD liver Mn concentrations were lesser than HS-HYD and LS-ING. When Caldera et al. (2017) fed steam-flaked corn based diets (< 0.2% S, no supplemental Mo) to feedlot cattle supplemented with either hydroxy or inorganic sources of Cu, Mn, and Zn, there were no differences in the trace mineral status of the steers when hydroxy trace minerals were supplemented at roughly one third to one half the rate of supplementation of inorganic sources. These data suggest the bioavailability of hydroxy trace minerals may be greater than that of inorganic trace minerals, or that finishing steers with adequate to high trace minerals status had no need for additional trace minerals supplementation.
Some researchers have suggested Mn-SOD to be a reliable biomarker of Mn status (Masters et al., 1988; Genther and Hansen, 2014). In the present study, FP Mn-SOD activity was similar to FP liver Mn concentrations in steers fed low S, where LS-HYD was lesser than LS-ING. Liver Mn concentrations and RBCL Mn-SOD activity were not statistically correlated in either the GP ($r = -0.08, P = 0.60$) or FP ($r = 0.20, P = 0.19$). However, data from the FP do trend more closely together, perhaps due to the longer duration on diets and the likely turnover of red blood cells during this time. Further work is needed to assess potential Mn biomarkers, especially when assessed within the same tissue. Additionally, liver Mn concentrations at the end of the FP were greater in LS-ING than HS-ING. Pogge et al. (2014) reported cattle tended to have decreased Mn absorption when 0.68% S was included in the diet, and suggested the decrease in Mn status could be a result of a complex formed between sulfide and Mn in the rumen. At this point, there is still much to learn about the potential usefulness of Mn-SOD activity as a Mn biomarker, as well as how source may affect Mn absorption, and more research should be done to clarify these interactions.

Glutathione has been shown to be highly concentrated in the liver (Lu, 2009), and it has been suggested a GSSG:GSH ratio of greater than 0.1 may be indicative of oxidative stress (Ithayaraja, 2011). Based on the reference range of Ithayaraja (2011), it would appear all steers were experiencing some amount of oxidative stress by the end of the finishing period. Total glutathione and GSH concentrations measured at the end of the FP were lesser in cattle fed LS-HYD diets compared to the other three treatments. Similarly, lesser Mn-SOD activity in cattle fed LS-HYD during the FP which may indicate steers from this treatment had less oxidative stress and thus a lesser demand for antioxidants. Alternatively, a greater ratio of GSSG:GSH in steers fed LS-HYD could also indicate greater oxidative stress in these cattle, however at this
time it in unclear what is driving this interaction in the FP. Additional research has shown GSSG concentrations increase with age in humans (Liu et al., 2004), mice (Lu, 2009) and cattle (Pogge and Hansen, 2013; Russell et al., 2016). The results in the present study support this numerical increase of GSSG from the growing through the finishing period, and steers fed HYD tended to have lesser concentrations of GSSG in the FP compared to those fed ING.

It was not the intent of this study to induce S toxicity; subsequently, 12% bromegrass hay was included in the finishing diet to increase the ruminal pH and lessen the activity of sulfate reducing bacteria. This hay inclusion likely contributed to the limited performance differences observed in this study due to dietary S. Morine et al. (2014) demonstrated steers fed roughage concentrations similar to the present study were able to consume diets of 0.5% S without impacting DMI or growth; however, multiple studies have shown high S to decrease DMI and carcass performance in feedlot cattle (Zinn et al., 1997; Spears et al., 2011; Richter et al., 2012). During the GP in the present study, an interaction between dietary S and TM source was observed as LS-HYD steers outperformed HS-HYD. Additionally, HS-HYD steers were overall less efficient than HS-ING steers, which was likely driven by numerical lesser gains and greater DMI by HS-HYD steers compared with HS-ING steers throughout the entire trial. Arthington (2015) has suggested calves may have an aversion for the taste of inorganic minerals included in high concentrations of a creep feed supplement. It should be noted the concentrations of TM in the feed in Arthington (2015) were much greater than those included in the present diets, however this could possibly explain the numerical differences in DMI between HS steers in the present study. High S decreased BF and yield grade; however, unlike others (Zinn et al., 1997; Richter et al., 2012), no negative impacts of dietary S on HCW or quality grade were observed. Although HS decreased select trace mineral concentrations in plasma and liver, all steers
continued to have adequate trace mineral status (Kincaid, 2000; Hansen et al., 2006) throughout
the trial, which, in combination with the increased dietary roughage concentration, may have
decreased the expression of the negative performance characteristics often associated with high S.

Overall, HS decreased Cu status of steers and tended to decrease Zn status in the GP.
Liver Cu concentrations were greater for ING than HYD at the end of the FP; however, all
animals maintained adequate status throughout the trial. Based on the $S \times TM$ interaction for
liver Mn concentrations observed across the GP and FP, it is possible that dietary factors such as
fiber or protein, and concentrations of Fe, Mo, and S may have influenced the availability of
different Mn sources. Similar trends between liver Mn concentrations and RBCL Mn-SOD
activity further support the need for investigation of this enzyme as a Mn biomarker in
ruminants. As producers continue to supplement combinations of inorganic and chelated trace
minerals, additional work is needed to determine what the optimal inclusion concentrations of
these sources are, especially when supplemented in the presence of high antagonist diets.
LITERATURE CITED


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**Analyzed composition**

| CP, % of DM⁷                      | 14.7          | 14.9                | 14.7                | 14.5                |
| NDF, % of DM⁷                     | 25.3          | 25.8                | 25.8                | 25.9                |
| EE, % of DM⁷                      | 6.4           | 6.9                 | 7.2                 | 7.0                 |
| S, % of DM                        | 0.22          | 0.23                | 0.51                | 0.51                |
| Cu, mg/kg DM                      | 14.3          | 13.7                | 12.7                | 12.7                |
| Mn, mg/kg DM                      | 36.3          | 33.3                | 34.4                | 32.0                |
| Mo, mg/kg DM                      | 0.55          | 0.53                | 0.55                | 0.50                |
| Zn, mg/kg DM                      | 54.7          | 56.7                | 61.5                | 58.4                |

¹Inorganic trace mineral provided per kg diet DM: 10 mg of Cu (copper sulfate), 30 mg of Zn (zinc sulfate), 20 mg of Mn (manganese sulfate), 0.5 mg of I (calcium iodate), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).

²Hydroxy trace mineral provided from Intellibond products per kg diet DM: 10 mg of Cu (basic copper chloride), 30 mg of Zn (zinc hydroxychloride), 20 mg of Mn (manganese hydroxychloride), 0.5 mg of I (calcium iodate), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).

³Modified distillers grains with solubles.

⁴Dried distillers grains with solubles.

⁵Provided 200 mg monensin·steer⁻¹·d⁻¹ (Elanco Animal Health, Greenfield, IN).

⁶Contained 4,400,000 IU/kg Vitamin A premix.

⁷Composite TMR samples analyzed at Dairyland Laboratories (Arcadia, WI).
### Table 2. Ingredient composition of finishing steer diets

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<th>Ingredient, % DM basis</th>
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<th>Low Sulfur</th>
<th>High Sulfur</th>
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<td>Inorganic$^1$</td>
<td>Hydroxy$^2$</td>
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**Analyzed composition**

| CP, % of DM$^7$ | 14.2 | 14.1 | 14.4 | 13.6 |
| NDF, % of DM$^7$ | 22.9 | 21.3 | 20.3 | 20.8 |
| EE, % of DM$^7$ | 5.3  | 5.7  | 6.3  | 5.6  |
| S, % of DM      | 0.26 | 0.34 | 0.59 | 0.53 |
| Cu, mg/kg       | 13.0 | 16.5 | 12.5 | 11.5 |
| Mn, mg/kg       | 36.7 | 32.6 | 34.6 | 32.7 |
| Mo, mg/kg       | 0.75 | 0.60 | 0.66 | 0.71 |
| Zn, mg/kg       | 57.9 | 54.2 | 63.8 | 60.3 |

1. Inorganic trace mineral provided per kg diet DM: 10 mg of Cu (copper sulfate), 30 mg of Zn (zinc sulfate), 20 mg of Mn (manganese sulfate), 0.5 mg of I (calcium iodate), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).
2. Hydroxy trace mineral provided from Intellibond products per kg diet DM: 10 mg of Cu (basic copper chloride), 30 mg of Zn (zinc hydroxychloride), 20 mg of Mn (manganese hydroxychloride), 0.5 mg of I (calcium iodate), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).
3. Modified distillers grains with solubles.
4. Dried distillers grains with solubles.
5. Provided 200 mg monensin·steer$^{-1}·d^{-1}$ (Elanco Animal Health, Greenfield, IN).
6. Contained 4,400,000 IU/kg Vitamin A premix.
7. Composite TMR samples analyzed at Dairyland Laboratories (Arcadia, WI).
Table 3. Effect of hydroxy or inorganic trace minerals supplementation within low or high S diets on plasma mineral concentrations of feedlot steers.

<table>
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<tr>
<th>Plasma mineral, mg/L</th>
<th>Sulfur$^1$</th>
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$^1$Low sulfur diets contained 0.23% S and high sulfur diets contained 0.51% S during growing period (d 0-84); low sulfur diets contained 0.30% S and high sulfur diets contained 0.56% S during finishing period (d 112-189).

$^2$Cu, Zn, and Mn supplemented as inorganic or hydroxy sources.

$^3$Comparison of low sulfur vs. high sulfur.

$^4$Comparison of trace mineral supplemented as inorganic vs hydroxy source.

$^5$Interaction between dietary sulfur concentration and trace mineral source.

$^6$Data were analyzed with d 0 values as covariates.
Table 4. Effect of hydroxy or inorganic trace minerals within low or high S diets on liver mineral concentrations of feedlot steers.

<table>
<thead>
<tr>
<th>Liver mineral, mg/kg DM</th>
<th>Sulfur&lt;sup&gt;1&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>Low sulfur</td>
<td>High sulfur</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Trace minerals&lt;sup&gt;2&lt;/sup&gt;</td>
<td>SEM</td>
<td>Sulfur&lt;sup&gt;3&lt;/sup&gt;</td>
<td>TM&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Sulfur*TM&lt;sup&gt;5&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Inorganic</td>
<td>Hydroxy</td>
<td>Inorganic</td>
<td>Hydroxy</td>
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</tr>
<tr>
<td>d -15</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>245</td>
<td>253</td>
<td>249</td>
<td>261</td>
<td>20.2</td>
<td>0.77</td>
<td>0.61</td>
<td>0.92</td>
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</tr>
<tr>
<td>Mn</td>
<td>8.9</td>
<td>8.7</td>
<td>9.3</td>
<td>8.8</td>
<td>0.37</td>
<td>0.48</td>
<td>0.39</td>
<td>0.76</td>
<td></td>
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<tr>
<td>Zn</td>
<td>137.8</td>
<td>125.3</td>
<td>131.1</td>
<td>130.0</td>
<td>4.55</td>
<td>0.82</td>
<td>0.13</td>
<td>0.20</td>
<td></td>
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<tr>
<td>d 80&lt;sup&gt;6&lt;/sup&gt;</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>320</td>
<td>295</td>
<td>247</td>
<td>228</td>
<td>14.0</td>
<td>&lt;0.0001</td>
<td>0.12</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>8.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.29</td>
<td>0.14</td>
<td>&lt;0.01</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Zn&lt;sup&gt;7&lt;/sup&gt;</td>
<td>123.4</td>
<td>119.8</td>
<td>114.3</td>
<td>110.2</td>
<td>6.04</td>
<td>0.10</td>
<td>0.50</td>
<td>0.97</td>
<td></td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 185&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>315</td>
<td>248</td>
<td>267</td>
<td>202</td>
<td>15.4</td>
<td>&lt;0.01</td>
<td>&lt;0.0001</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>10.1&lt;sup&gt;x&lt;/sup&gt;</td>
<td>9.2&lt;sup&gt;y&lt;/sup&gt;</td>
<td>9.2&lt;sup&gt;y&lt;/sup&gt;</td>
<td>10.0&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.40</td>
<td>0.84</td>
<td>0.92</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Zn&lt;sup&gt;7&lt;/sup&gt;</td>
<td>117.6</td>
<td>116.3</td>
<td>111.0</td>
<td>122.6</td>
<td>5.76</td>
<td>0.98</td>
<td>0.34</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Within a row, means without a common superscript differ: (P < 0.05)

<sup>x,y</sup> Within a row, means without a common superscript tend to differ: (P < 0.1)

<sup>1</sup>Low sulfur diets contained 0.23% S and high sulfur diets contained 0.51% S during growing period (d 0-84); low sulfur diets contained 0.30% S and high sulfur diets contained 0.56% S during finishing period (d 112-189).

<sup>2</sup>Cu, Zn, and Mn supplemented as inorganic or hydroxy sources.

<sup>3</sup>Comparison of low sulfur vs. high sulfur.

<sup>4</sup>Comparison of trace mineral supplemented as inorganic vs hydroxy source.

<sup>5</sup>Interaction between dietary sulfur concentration and trace mineral source.

<sup>6</sup>Data were analyzed with d -15 values as covariates.

<sup>7</sup>Highest SEM of any treatment is reported due to unequal treatment numbers.
Table 5. Effect of hydroxy or inorganic trace minerals within low or high S diets on superoxide dismutase (SOD) activity in red blood cell lysate of feedlot steers.

<table>
<thead>
<tr>
<th>SOD, U × 10³/g Hb</th>
<th>Sulfur¹</th>
<th>Trace minerals²</th>
<th>SEM</th>
<th>Sulfur³</th>
<th>TM⁴</th>
<th>Sulfur*TM⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low sulfur</td>
<td>High sulfur</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inorganic</td>
<td>Hydroxy</td>
<td>Inorganic</td>
<td>Hydroxy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.9</td>
<td>4.6</td>
<td>4.6</td>
<td>4.4</td>
<td>3.08</td>
<td>0.32</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>2.1</td>
<td>2.0</td>
<td>2.1</td>
<td>1.9</td>
<td>1.76</td>
<td>0.80</td>
</tr>
<tr>
<td>CuZn-SOD</td>
<td>2.8</td>
<td>2.6</td>
<td>2.5</td>
<td>2.5</td>
<td>4.41</td>
<td>0.36</td>
</tr>
<tr>
<td>d 185</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.5</td>
<td>5.0</td>
<td>5.4</td>
<td>5.1</td>
<td>6.08</td>
<td>0.97</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>2.8x</td>
<td>2.2y</td>
<td>2.4xy</td>
<td>2.6xy</td>
<td>4.18</td>
<td>0.91</td>
</tr>
<tr>
<td>CuZn-SOD</td>
<td>2.8</td>
<td>2.7</td>
<td>2.8</td>
<td>2.5</td>
<td>9.87</td>
<td>0.59</td>
</tr>
</tbody>
</table>

x,y: Within a row, means without a common superscript tend to differ: (P < 0.1)

¹Low sulfur diets contained 0.23% S and high sulfur diets contained 0.51% S during growing period (d 0-84); low sulfur diets contained 0.30% S and high sulfur diets contained 0.56% S during finishing period (d 112-189).

²Cu, Zn, and Mn supplemented as inorganic or hydroxy sources.

³Comparison of low sulfur vs. high sulfur.

⁴Comparison of trace mineral supplemented as inorganic vs hydroxy source.

⁵Interaction between dietary sulfur concentration and trace mineral source.
Table 6. Effect of hydroxy or inorganic trace minerals within low or high S diets on liver total, reduced and oxidized concentrations of glutathione, and the oxidized to reduced ratios of feedlot steers.

<table>
<thead>
<tr>
<th>Liver</th>
<th>Sulfur$^1$</th>
<th>Trace minerals$^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low sulfur</td>
<td>High sulfur</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inorganic</td>
<td>Hydroxy</td>
<td>SEM</td>
</tr>
<tr>
<td>d 84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total$^6$</td>
<td>1.96</td>
<td>1.84</td>
<td>2.04</td>
</tr>
<tr>
<td>GSH$^7$</td>
<td>1.65</td>
<td>1.57</td>
<td>1.77</td>
</tr>
<tr>
<td>GSSG$^8$</td>
<td>0.15</td>
<td>0.15</td>
<td>0.17</td>
</tr>
<tr>
<td>Ratio$^9$</td>
<td>0.086</td>
<td>0.087</td>
<td>0.093</td>
</tr>
<tr>
<td>d 188</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total$^6$</td>
<td>2.72$^a$</td>
<td>2.18$^b$</td>
<td>2.54$^a$</td>
</tr>
<tr>
<td>GSH$^7$</td>
<td>2.47$^a$</td>
<td>1.96$^b$</td>
<td>2.27$^a$</td>
</tr>
<tr>
<td>GSSG$^8$</td>
<td>0.27</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>Ratio$^9$</td>
<td>0.100</td>
<td>0.116</td>
<td>0.121</td>
</tr>
</tbody>
</table>

$^{a,b}$Within a row, means without a common superscript differ: ($P < 0.05$)

$^1$Low sulfur diets contained 0.23% S and high sulfur diets contained 0.51% S during growing period (d 0-84); low sulfur diets contained 0.30% S and high sulfur diets contained 0.56% S during finishing period (d 112-189).

$^2$Cu, Zn, and Mn supplemented as inorganic or hydroxy sources.

$^3$Comparison of low sulfur vs. high sulfur.

$^4$Comparison of trace mineral supplemented as inorganic vs hydroxy source.

$^5$Interaction between dietary sulfur concentration and trace mineral source.

$^6$Total liver glutathione (uM/g wet tissue).

$^7$Reduced glutathione (uM/g wet tissue).

$^8$Oxidized glutathione (uM/g wet tissue).

$^9$Ratio of oxidized to reduced glutathione.
Table 7. Effect of hydroxy or inorganic trace minerals supplementation within low or high S diets on growth and feed efficiency of feedlot steers during the growing period, finishing period, and overall trial.

<table>
<thead>
<tr>
<th>Live performance</th>
<th>Sulfur(^1)</th>
<th>Low sulfur</th>
<th>High sulfur</th>
<th>Trace minerals(^2)</th>
<th>Inorganic</th>
<th>Hydroxy</th>
<th>Inorganic</th>
<th>Hydroxy</th>
<th>SEM</th>
<th>Sulfur(^3)</th>
<th>TM(^4)</th>
<th>Sulfur*TM(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growing (d 0-84)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Start of growing BW, kg</td>
<td>317</td>
<td>315</td>
<td>316</td>
<td>316</td>
<td>2.8</td>
<td>0.95</td>
<td>0.80</td>
<td>0.85</td>
<td></td>
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</tr>
<tr>
<td>End of growing BW, kg</td>
<td>423(^xy)</td>
<td>437(^x)</td>
<td>426(^xy)</td>
<td>418(^y)</td>
<td>6.0</td>
<td>0.19</td>
<td>0.58</td>
<td>0.09</td>
<td></td>
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</tr>
<tr>
<td>DMI, kg/d</td>
<td>9.25</td>
<td>9.46</td>
<td>8.78</td>
<td>9.07</td>
<td>0.283</td>
<td>0.13</td>
<td>0.38</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.27(^ab)</td>
<td>1.45(^a)</td>
<td>1.31(^ab)</td>
<td>1.22(^b)</td>
<td>0.066</td>
<td>0.16</td>
<td>0.47</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G:F</td>
<td>0.137(^ab)</td>
<td>0.152(^a)</td>
<td>0.149(^ab)</td>
<td>0.135(^b)</td>
<td>0.0053</td>
<td>0.63</td>
<td>0.93</td>
<td>0.01</td>
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<tr>
<td><strong>Finishing (d 112-189)</strong></td>
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</tr>
<tr>
<td>Start of finishing BW, kg</td>
<td>487</td>
<td>492</td>
<td>481</td>
<td>474</td>
<td>7.7</td>
<td>0.12</td>
<td>0.86</td>
<td>0.47</td>
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<tr>
<td>End of finishing BW, kg</td>
<td>615</td>
<td>622</td>
<td>611</td>
<td>589</td>
<td>11.8</td>
<td>0.12</td>
<td>0.53</td>
<td>0.24</td>
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<tr>
<td>DMI, kg/d</td>
<td>13.44</td>
<td>13.50</td>
<td>12.88</td>
<td>12.78</td>
<td>0.398</td>
<td>0.12</td>
<td>0.97</td>
<td>0.84</td>
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</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.66(^xy)</td>
<td>1.69(^x)</td>
<td>1.69(^x)</td>
<td>1.50(^y)</td>
<td>0.066</td>
<td>0.21</td>
<td>0.24</td>
<td>0.10</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>G:F</td>
<td>0.124(^ab)</td>
<td>0.126(^ab)</td>
<td>0.131(^a)</td>
<td>0.117(^b)</td>
<td>0.0038</td>
<td>0.87</td>
<td>0.14</td>
<td>0.04</td>
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<tr>
<td><strong>Overall (d 0-189)</strong></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>DMI, kg/d</td>
<td>11.11</td>
<td>11.26</td>
<td>10.61</td>
<td>10.74</td>
<td>0.313</td>
<td>0.11</td>
<td>0.66</td>
<td>0.99</td>
<td></td>
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</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.58</td>
<td>1.62</td>
<td>1.56</td>
<td>1.45</td>
<td>0.059</td>
<td>0.11</td>
<td>0.55</td>
<td>0.20</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>G:F</td>
<td>0.142(^ab)</td>
<td>0.144(^ab)</td>
<td>0.147(^a)</td>
<td>0.135(^b)</td>
<td>0.0035</td>
<td>0.49</td>
<td>0.14</td>
<td>0.05</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a,b}\) Within a row, means without a common superscript differ: (P < 0.05)

\(^{x,y}\) Within a row, means without a common superscript tend to differ: (P < 0.1)

\(^1\)Low sulfur diets contained 0.23% S and high sulfur diets contained 0.51% S during growing period (d 0-84); low sulfur diets contained 0.30% S and high sulfur diets contained 0.56% S during finishing period (d 112-189).

\(^2\)Cu, Zn, and Mn supplemented as inorganic or hydroxy sources.

\(^3\)Comparison of low sulfur vs. high sulfur.

\(^4\)Comparison of trace mineral supplemented as inorganic vs hydroxy source.

\(^5\)Interaction between dietary sulfur concentration and trace mineral source.
Table 8. Effect of hydroxy or inorganic trace minerals within low or high S on carcass variables HCW, MS, QG, dressing %, BF, YG, and REA.

<table>
<thead>
<tr>
<th>Carcass Characteristics</th>
<th>Sulfur</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low sulfur</td>
<td>High sulfur</td>
<td>Trace minerals</td>
<td>Inorganic</td>
<td>Hydroxy</td>
<td>Inorganic</td>
<td>Hydroxy</td>
</tr>
<tr>
<td>HCW, kg</td>
<td>394</td>
<td>393</td>
<td>388</td>
<td>376</td>
<td>7.7</td>
<td>0.51</td>
<td>0.42</td>
</tr>
<tr>
<td>Dressing %</td>
<td>65</td>
<td>64</td>
<td>64</td>
<td>62</td>
<td>0.0</td>
<td>0.53</td>
<td>0.44</td>
</tr>
<tr>
<td>BF, cm</td>
<td>2.1</td>
<td>2.1</td>
<td>1.7</td>
<td>1.9</td>
<td>0.14</td>
<td>0.03</td>
<td>0.37</td>
</tr>
<tr>
<td>REA, cm²</td>
<td>83.9</td>
<td>77.7</td>
<td>82.5</td>
<td>79.3</td>
<td>1.95</td>
<td>0.98</td>
<td>0.02</td>
</tr>
<tr>
<td>KPH</td>
<td>2.33</td>
<td>2.29</td>
<td>2.21</td>
<td>2.25</td>
<td>0.086</td>
<td>0.34</td>
<td>1.00</td>
</tr>
<tr>
<td>Marbling score⁶</td>
<td>548</td>
<td>522</td>
<td>568</td>
<td>499</td>
<td>29.4</td>
<td>0.97</td>
<td>0.12</td>
</tr>
<tr>
<td>Yield grade</td>
<td>4.1</td>
<td>4.5</td>
<td>3.7</td>
<td>4.0</td>
<td>0.19</td>
<td>0.03</td>
<td>0.11</td>
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¹Low sulfur diets contained 0.23% S and high sulfur diets contained 0.51% S during growing period (d 0-84); low sulfur diets contained 0.30% S and high sulfur diets contained 0.56% S during finishing period (d 112-189).

²Cu, Zn, and Mn supplemented as inorganic or hydroxy source.

³Comparison of low sulfur vs. high sulfur.

⁴Comparison of trace mineral supplemented as inorganic vs hydroxy source.

⁵Interaction between dietary sulfur concentration and trace mineral source.

⁶400 = small, 500 = modest
CHAPTER IV

Comparison of trace mineral repletion strategies in beef cattle to overcome a high antagonist diet.

S. J. Hartman*, O. N. Genther-Schroeder*, and S. L. Hansen*

*Department of Animal Science, Iowa State University, Ames, IA 50011

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Corresponding author: slhansen@iastate.edu
ABSTRACT: The objective was to compare trace mineral (TM) repletion strategies in feedlot steers after depletion by S and Mo. Seventy-two Red Angus steers were blocked by BW (253 ± 14 kg) and assigned equally (6 steers per pen, fed via GrowSafe bunks) to corn silage-based depletion diets (DEP) supplemented with NRC (2016) recommended concentrations of Cu, Mn, Se, and Zn (CON) or supplemented with 0.3% S (CaSO₄), 2 mg of Mo/kg DM, and no added Cu, Mn, Zn or Se (ANT). On d 89 steers were assigned to a TM repletion strategy (REP): 1) Multimin90 injection (contains Cu, Mn, Se, Zn) and 100% of NASEM (2016) recommended dietary TM supplementation from inorganic sources (ITM), 2) saline injection, and 150% of NASEM from inorganic sources (ING), or 3) saline injection, and 150% of NASEM provided as 25% organic and 75% inorganic sources (BLEND). Subcutaneous injections were given at a rate of 1 mL/68 kg BW. Inorganic sources were Cu, Mn, and Zn sulfate, and sodium selenite, while organic sources were Availa Cu, Mn and Zn, and SelPlex Se. Liver and blood were collected on d -10, 14, 28, and 42 for TM and enzyme analysis. Data were analyzed as a 2 × 2 factorial (n = 12 per treatment) using Proc Glimmix of SAS with plasma and liver TM analyzed as repeated measures. Liver Cu, Se, and Mn were decreased (P < 0.0001) by ANT during DEP. Interactions of DEP × REP × day were not present in liver TM (P ≥ 0.18). There were REP × day effects on liver Cu (P < 0.0001) and Se (P < 0.0001) where status was improved by ITM by d 14, increased in BLEND by d 28, and ING was the same by d 42. A DEP × day effect was noted for liver Cu (P < 0.0001) and Mn (P = 0.07), where ANT Cu increased linearly from d 0 to d 42 and CON Cu was slightly improved on d 14 and d 28, and ANT Mn was lower than CON Mn on all d except d 42 and status fluctuated. Liver Se (P < 0.0001) concentrations were lesser in ANT vs. CON throughout repletion. Liver Zn was greater (P < 0.0001) on d 0 than d 14, 28, and 42, and concentrations were greater on d 42 than
Average daily gain and final BW were not affected by treatment, and ANT had decreased DMI ($P = 0.04$) and improved G:F ($P = 0.006$) during repletion. All repletion strategies were effective at improving TM status of steers, and ITM had the most rapid recovery of Cu and Se status, followed by BLEND, and ING.

**KEYWORDS:** cattle, sulfur, thiomolybdates, trace minerals

**INTRODUCTION**

Trace minerals (TM) are essential in cattle for adequate growth, health, and reproduction (Underwood and Suttle, 1991). In recent years, greater inclusions of ethanol by products into cattle diets have resulted in higher concentrations of dietary S (Drewnoski et al., 2014). Excessive concentrations of S have been shown to decrease TM availability through antagonistic reactions in the rumen (Spears et al., 2011; Pogge et al., 2014). This effect is especially powerful when Mo is included in the diet, as thiomolybdates form, and cause an irreversible inhibition on Cu function (Suttle, 1974). These interactions create a unique challenge of supplementing TM to cattle in a method that is effective at bypassing rumen antagonisms but still available for absorption in the small intestine. While inorganic forms of TM are typically highly ruminally available, the chemistry of many organic TM makes them ruminally insoluble and thus potentially more available for absorption in the small intestine (Spears, 2003). A recent survey (Samuelson et al., 2016) found a majority of consulting feedlot nutritionists supplement a combination of organic and inorganic TM at concentrations that are numerically greater than those recommended by the NRC (1996). Greater inclusions of TM can increase TM status over time, however, alternative methods have been suggested to cause more rapid improvement. Injectable TM completely bypass the rumen and instead provide TM
directly to the tissues through a subcutaneous injection, and have been shown to quickly increase TM status (Pogge et al., 2012; Genther and Hansen, 2014). There has been little research conducted regarding how a high antagonist diet may influence the ability of TM sources to improve TM status of cattle. Therefore, the objective of this study was to compare the effectiveness of various TM repletion strategies on mineral status and performance of steers fed diets containing the antagonists S and Mo.

MATERIALS AND METHODS

All procedures involving the use of animals were approved by the Iowa State University Institutional Animal Care and Use Committee (log # 10-15-8110-B).

Experimental Design and Sampling Procedures. This experiment was conducted at the Iowa State University Beef Nutrition Research Center (Ames, IA). Seventy-two Red Angus steers were blocked by BW (254 ± 14 kg) and assigned equally to one of two corn-silage based depletion diets (DEP; Table 1), either supplemented with Cu, Mn, Se, and Zn at NRC (1996) recommendations (control, CON), or not supplemented with these TM and supplemented with 0.3% S (CaSO₄) and 2 mg of Mo/kg DM to deplete TM status (antagonist, ANT). Both treatments received Co and I supplemented at NRC (1996) recommendations. Steers were fed these diets for the entirety of the study. Steers were housed in pens (6 steers per pen) equipped with GrowSafe bunks to determine individual feed intake, and had ad libitum access to water. At the initiation of the study, steers received an electronic identification tag (Allflex US Inc., Dallas–Fort Worth Airport, TX) to assist in recording of individual intakes in the GrowSafe bunk system (GrowSafe Systems Ltd., Airdrie, AB, Canada). Steers were vaccinated against
viral (Bovi Shield Gold 5; Zoetis Inc., Florham Park, NJ) and clostridial (Vision 7; Merck Animal Health, Summit, NJ) infections and dewormed with eprinomectin (Eprinex, Merial Ltd., Iselin, NJ). Steers were implanted on d 0 with a combination implant containing 200 mg progesterone USP, 20 mg estradiol benzoate, and 29 mg tylosin tartrate (Component E-S; Elanco Animal Health, Greenfield, IN), and re-implanted with a combination implant containing 80 mg trenbolone acetate, 16 mg estradiol USP, and 29 mg tylosin tartrate (Component TE-IS; Elanco Animal Health, Greenfield, IN) at the start of repletion.

On d 88 and 89 respectively, the heavy and light blocks were assigned randomly within block to one of three trace mineral repletion (REP; Table 2) strategies: 1) an injectable TM (Multimin90) containing Cu, Mn, Se, and Zn, and dietary TM supplemented at 100% of nationally recommended concentrations (NASEM, 2016) from strictly inorganic sources (ITM), 2) a sterilized saline injection and TM supplemented at 150% of NASEM (2016) recommendations from strictly inorganic sources (ING), 3) or a sterilized saline injection and TM supplemented at 150% of NASEM (2016) recommendations from a blend of 75% inorganic and 25% organic sources (BLEND). This 2 x 3 factorial resulted in 6 treatments for the 62 d repletion period (n = 12 steers per treatment). Multimin90 contains 15 mg Cu, 10 mg Mn, 5 mg Se, and 60 mg Zn per mL, and was dosed at a rate of 1 mL per 68 kg BW. Steers for ING and BLEND treatments were dosed with a sterilized saline solution at the same rate as ITM steers, and all injections were subcutaneous.

Steers were weighed on consecutive days on a single animal scale that had been calibrated at the start (d -4, d -3) and end of the depletion period for the heavy (d 88, d 89) and light (d 89, 90) groups, respectively. Liver biopsies were conducted on all steers and were collected over two days, thus, steers were stagger started for the repletion period, and the last
day of depletion was determined to be d 0 for the repletion period (Figure 1). Steers were weighed on consecutive dates at the start of repletion (d -1, d 0) and end of repletion (d 61, d 62), and received treatment injections and received repletion diets starting on d 0. Liver biopsy samples were collected for TM analysis at the start (d -2 or d -1) and end (d 79 or d 80) of the depletion period, and during the repletion period on d 14, d 28, and d 42, using the methods of Engle and Spears (2000). Jugular blood samples were collected on these days two hours post feeding (d -10, 14, 28, 42) into 7 mL trace mineral potassium-EDTA vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for analysis of plasma TM concentration, and red blood cell lysate (RBCL) Mn superoxide dismutase and glutathione peroxidase activity. All samples were transported to the laboratory on ice.

**Tissue Analysis.** Liver samples were dried in a forced air oven at 70°C for approximately one wk until they were dried completely. Liver tissue was then digested with nitric acid using the methods of Pogge et al. (2014) in preparation for TM determination. Blood samples were centrifuged at 1000 x g for 10 minutes at 4°C, then plasma was removed and stored at -20°C until preparation for TM analysis. Plasma samples were diluted 1:20 with 1% nitric acid and vortexed before analysis. Liver and plasma samples were analyzed for Cu, Mn, Fe, Se, Mo, and Zn at the Iowa State University Veterinary Medicine Diagnostic Laboratory using an ICP-MS (Analytik Jena Inc. Woburn, MA, USA). Several elements served as internal standards (Bi, Sc, In, Li, Y, and Tb), and all runs included serum (UTAK Laboratories, Inc., Valencia, CA) or liver (National Institute of Standards and Technology, Gaithersburg, MD) standards as appropriate for verification of instrument accuracy. Red blood cell lysate was prepared from packed red blood cells using the methods of Pogge et al. (2012) and stored at -80°C until
analysis. Red blood cell lysate Mn superoxide dismutase (Mn-SOD) and RBCL glutathione peroxidase (GSH-px) were analyzed using commercial assay kits (Cayman Chemical, Ann Arbor, Mo; catalog 706002 and 703102, respectively). One unit of Mn-SOD is defined as the amount of enzyme required to exhibit 50% dismutation of the superoxide radical. One unit of GSH-px activity is defined as the amount of enzyme that is necessary for the oxidation of 1.0 nmol of reduced nicotinamide adenine dinucleotide phosphate to oxidized nicotinamide adenine dinucleotide phosphate per minute at 25°C. Hemoglobin (Hb) analysis of RBCL was conducted using the methods of Hansen et al. (2010), and Mn-SOD and GSH-px activities are expressed per unit Hb. The CV for intra-assay and inter-assay were 3.05 and 4.08 for GSH-px.

Samples of the total mixed ration (TMR) were collected on a weekly basis and dried in an air forced oven at 70°C for 48 hr, and DM were calculated. Feed samples were composited within treatment by month and acid digested for analysis of TM using the methods of Pogge et al (2014) for S, Cu, Mn, Mo, and Zn concentrations. Dry matter intakes were calculated using GrowSafe intake data corrected for DM using the weekly TMR sample analyses. Average daily gain was calculated using the total weight gained in each period divided by the length of each period. Feed efficiency was calculated using the total gain and the total DMI for each period of interest.

**Statistical Analysis.** Data for the depletion period were analyzed using the GLIMMIX procedures of SAS 9.4 (SAS Institute, Cary, NC) with steer as experimental unit (n = 36 per treatment) and the fixed effect of the depletion period diet (DEP). After repletion period treatments (REP) were applied, data were analyzed as a 2 × 3 factorial using the GLIMMIX procedures of SAS 9.4 (SAS Institute, Cary, NC) with steer as the experimental unit (n = 12
per treatment). The repletion period model included the fixed effects of depletion diet and repletion strategy and the interaction. Means were separated using the slice diff command in SAS. Plasma and liver TM concentration, as well as RBCL enzyme activity data, were analyzed as repeated measures, with day of sampling as the repeated effect. Covariance structures were selected based on the least Akaike information criterion; autoregressive 1 was used for liver data and variance component was used for blood data. Transformations were necessary for liver Zn and Cu data from the repletion period, and were calculated using the logarithmic function and exponential function, respectively. Start of repletion BW was used as a covariate for DMI, G:F, and final repletion period BW. Data presented are LSMEANS and SEM, and back-transformed data are presented for liver Zn and Cu concentrations.

**Removed Samples.** Red blood cell lysate samples were not collected on d 42 for 13 steers in the ANT group (5 ANT-ING, 4 ANT-ITM, 4 ANT-BLEND) due to errors in initial sample processing, and no liver sample was collected for 1 steer (ANT-ING) on d 42 due to technical errors while sampling. Several samples were removed prior to statistical analysis because of identification as a statistical or physiological outlier. These included one sample for each of the following sample collections unless otherwise stated: ANT-ITM liver Zn on d 42 (two), plasma Mo on d 0; ANT-BLEND liver Mn on d 28 (three), liver Zn on d 42 (four), and plasma Mo on d 0, plasma Fe on d 14; ANT-ING liver Cu on d 0, liver Se and Zn on d 14, liver Zn on d 42 (two), plasma Mo on d 0, and plasma Cu on d 14; CON-BLEND liver Zn on d 14, liver Mn and Zn on d 28, plasma Fe on d 0, plasma Cu on d 14, and plasma Mo on d 28; CON-ITM plasma Fe on d 14 and d 28; and CON-ING liver Se on d 14, liver Mn on d 28, and plasma Cu on d 28.
RESULTS

Depletion Period Liver and Plasma TM. Based on biopsies collected immediately prior to start of the depletion period steer liver mineral concentrations (± SEM) were: 252 ± 15.0 Cu, 7.4 ± 0.20 Mn, 2.10 ± 0.069 Se, and 101 ± 2.9 mg Zn/kg DM.

Liver and plasma TM concentrations at the end of the depletion period are displayed in Table 3. At the end of the depletion period, liver concentrations of Cu and Se were decreased in ANT compared to CON (\( P < 0.0001 \)). Regardless of initial liver Cu concentrations liver Cu decreased approximately 90% (± 4.9%) due to ANT, resulting in 23 ± 5.3 mg Cu/kg for ANT while CON maintained their status at 251 mg Cu/kg DM. Similarly, liver Se concentrations (mg/kg DM) were decreased by 40% such that ANT had 1.22 while CON maintained 2.00. Meanwhile, liver Mn concentrations were lesser in ANT than CON at the end of depletion (\( P = 0.0003 \)) and liver Zn concentrations were unaffected (\( P = 0.42 \)). Liver TM concentrations at the end of depletion served as d 0 values for repeated measures analysis of liver TM concentrations during the repletion period. At the end of the depletion period plasma concentrations of Cu, Fe, Se, and Zn were decreased in ANT compared to CON (\( P \leq 0.03 \)). Meanwhile, plasma concentrations of Mo were greater in ANT than CON (\( P < 0.0001 \)) at the end of the depletion period.

Repletion Period Liver TM. Across the repletion period, there were no DEP × REP × day (\( P \geq 0.19 \)) or DEP × REP effects (\( P \geq 0.15 \)) on liver concentrations of Cu, Mn, Se, or Zn. Interactions of DEP × day and REP × day in liver TM concentrations through the repletion period are displayed in Figure 1 and Figure 2, respectively. Repletion period liver Cu was affected by the DEP × day interaction (\( P < 0.0001 \); Figure 1A), where concentrations in ANT
were greater at each subsequent sampling day, while within CON, concentrations were greater on d 14 and 28 relative to d 0 and 42. There was also a REP × day effect on liver Cu concentrations ($P < 0.0001$; Figure 2A), where concentrations among REP strategies were similar on d 0 but were greater in ITM than BLEND and ING on d 14, d 28 liver Cu concentrations were not different between ITM and BLEND but were greater in ITM and BLEND than ING, and d 42 values were similar among treatments.

There was no DEP × day interaction for repletion period liver Se concentrations ($P = 0.52$). However, there was a REP × day effect on liver Se concentrations ($P < 0.0001$; Figure 2B), where concentrations within REP strategy were similar on d 0 but were greater in ITM than BLEND and ING on d 14, liver Cu concentrations on d 28 were not different between ITM and BLEND and were greater than ING, and d 42 values were similar among treatments. There was a main effect of DEP on liver Se concentrations (mg/kg DM) where ANT (1.47 ± 0.054) was lesser than CON (2.12 ± 0.053; $P < 0.0001$).

There was a tendency for a DEP × day interaction for repletion period liver Mn concentrations ($P = 0.07$; Figure 1B) where ANT was less than CON on d 0 and d 14, tended to be lesser than CON on d 28, and was not different from CON on d 42. There was a REP × day interaction for liver Mn ($P = 0.0001$; Figure 2C) where steers randomly assigned to receive ITM had lesser liver Mn concentrations than BLEND and ING on d 0, and ITM and ING were greater than BLEND on d 14; however, there were no differences in liver Mn concentrations due to treatment on d 28 and d 42.

There were no effects of DEP × day or REP × day on repletion period liver Zn concentrations ($P \geq 0.48$). Additionally, there were no effects of DEP diet ($P = 0.14$) or REP strategy ($P = 0.64$) on liver Zn concentrations in the repletion period. There was a day effect ($P$
< 0.0001) where liver Zn concentrations were greater on d 0 than d 14, 28, and 42, and concentrations were greater on d 42 than d 28. Average liver Zn concentrations (mg/kg DM) during the repletion period were as follows: d 0 (121 ± 2.4), d 14 (104 ± 1.8), d 28 (102 ± 1.6), and d 42 (107 ± 2.0).

**Repletion Period Plasma TM.** There was a DEP × REP × day interaction ($P = 0.009$, **Figure 3A**) for plasma Mo concentrations. On d 0, ANT had greater plasma Mo concentrations than all CON treatments where ANT-BLEND was the greatest, followed by ANT-ITM, and lastly by ANT-ING. All treatments had similar plasma Mo concentrations on d 14, on d 28 ANT-ING was greater than all other treatments, and on d 42 there was a cross-over where ANT treatments were lesser than CON. There was a tendency for a DEP × REP × day interaction for plasma Cu concentrations ($P = 0.09$; **Figure 3B**). On d 0 ANT-BLEND and ANT-ING did not differ and were lesser than ANT-ITM and all CON treatments, on d 14 all ANT treatments and CON-ING were lesser than CON-ITM and CON-BLEND, and there were no differences in plasma Cu due to treatment on d 28 and d 42, with the highest concentrations occurring on d 42 for all treatments. There was a tendency for a DEP × REP × day interaction on plasma Zn concentrations ($P = 0.07$; **Figure 3C**). On d 0, all ANT treatments and CON-BLEND were lesser than CON-ING and CON-ITM; CON-ING continued to have greater plasma Zn concentrations on d 14 than all others, there were no differences between plasma Zn concentrations on d 28, and on d 42 all ANT treatments, CON-BLEND, and CON-ING were lesser than CON-ING.

There were no effects of DEP × REP × day, DEP × REP, DEP × day, or REP × day ($P \geq 0.21$) on plasma Se concentrations. There was a main effect of depletion diet ($P < 0.0001$)
where ANT (137 ± 1.4 ug Se/L) had lesser plasma Se concentrations than CON (145 ± 1.4 ug Se/L), and a day effect ($P < 0.0001$) where plasma Se concentrations (ug/L; ± 1.5) were less on d 0 (132) and d 14 (133) than on d 28 (151) and d 42 (152). There was also a tendency ($P = 0.07$) for REP diet to affect Se plasma concentrations (ug/L; ± 1.7) such that ITM (144) were greater than BLEND (139) while ING was intermediate (142). There was no DEP × REP × day interaction for plasma Fe concentrations ($P = 0.90$); however, there was a tendency for a DEP × day interaction ($P = 0.10$; data not shown) where plasma Fe tended to be greater in ANT than CON on d 0, d 14, and d 28, and concentrations were not different on d 42.

**Repletion Period Antioxidant Activity.** There were no effects of DEP × REP × day, DEP × day, or REP × day effects on RBCL GSH-px activity ($P ≥ 0.78$); however, there was a DEP × REP interaction ($P = 0.04$, Figure 4A) where ANT-ITM, ANT-BLEND, CON-ITM, and CON-ING were lesser than CON-BLEND, and ANT-ITM was lesser than ANT-ING. Additionally, d 14 GSH-px activity tended to be lesser ($P = 0.07$, Figure 4B) on d 14 than d 0, d 28, and d 42.

**Steer Growth Performance.** At the end of the depletion period, there were no differences in BW due to DEP diet (472 ± 27.1 kg, $P = 0.73$). Body weights collected at the end of depletion were used as covariates for the analysis of repletion period DMI, G:F, and final BW data. There was no DEP × REP interaction for DMI, ADG, G:F, or final BW data ($P ≥ 0.60$) and the main effects of DEP diet and REP strategy are shown in Table 4. There was no effect of DEP diet on ADG or final BW ($P = 0.11$); however, steers fed ANT consumed less DM ($P = 0.04$) than CON and have improved G:F in the repletion period ($P = 0.006$). There was no effect of
REP on final BW, ADG, or G:F; however, there was a tendency ($P = 0.09$) for DMI to be affected, where ITM consumed more than ING, while BLEND was intermediate.

**DISCUSSION**

High dietary S may result from the inclusion of a variety of feedstuffs including ethanol coproducts and byproducts such as molasses, and also though high sulfate water, which has been further concentrated in recent years as water has become more scarce. This, in combination with the presence of forages containing high concentrations of Mo, especially in the western United States, have created likelihoods for unavoidable trace mineral antagonisms in cattle. In the present study, consuming a diet containing high amounts of S and moderate amounts of Mo for 90 d decreased steer liver Cu concentrations by 90%. A decrease in Cu status due to inclusions of dietary S and Mo is consistent with previous literature (Underwood, 1962; Suttle, 1974; Ward, 1978); however, few studies have reported the change in liver Cu concentrations over time when antagonists are fed. Regardless of initial Cu liver concentrations of ANT steers (ranging 95-540 mg Cu/kg DM), the percentage decrease was extremely consistent by the end of 90 d (ranging 84%-96%). The average liver Cu concentration of the ANT group following depletion (23 ± 5.3 mg Cu/kg) classified these steers as deficient according to ranges suggested by Mills (1987) and Kincaid (2000). Often producers do not know the TM status of their cattle, and may not realize their cattle have been exposed to high S or Mo until symptoms of TM deficiency appear. Considering the essential role TM play in growth and immune function (Underwood and Suttle, 1999), it would be advantageous to determine a highly available TM source to rapidly improve TM status. As a result, this study
was designed to examine the effectiveness of three common TM supplementation strategies on mineral status and performance of beef steers with depleted TM status.

Injectable trace minerals can improve TM status by avoiding ruminal interactions and competition for absorption in the intestine, and providing TM directly to tissues (Pogge et al., 2012). Recent work by others has shown a dose of Multimin to increase liver Cu concentrations by approximately 50 mg Cu/kg DM, with advantages over saline-injected cattle maintained for at least 30 d post-injection (Genther and Hansen, 2014). In the present study, there were no interactions between DEP diet and REP strategy, suggesting that the pattern of TM repletion within a REP strategy was similar across diets with or without antagonists. This is clearly noted in the effect of ITM on steers fed ANT and CON diets in the repletion period, where although ANT had lesser Cu status (mg/kg DM) at the start of repletion (23 ± 5.3) than CON (251 ± 8.7), both treatments increased liver Cu by approximately 44 mg Cu/kg DM on d 14 (post ITM), suggesting the metabolism of ITM is not affected by the presence of dietary antagonists. Liver Cu was improved by d 14 in ITM, and it took 28 and 42 d for TM status to improve in steers supplemented with 150% of national recommended concentrations from BLEND and ING, respectively. These data suggest any of these strategies would be sufficient depending on the urgency of TM repletion. Additionally, while the first biopsy sample collected in this study was on d 14, Pogge et al. (2012) collected liver biopsies 1 d post Multimin injection from steers consuming at least 0.28% S and reported steers to have improved liver Cu concentrations on this day. These data suggest the increase in TM status is likely as rapid as 1 d post ITM injection, and more work is needed to construct a metabolism curve for ITM early after injection when antagonists are present in the diet.
Research has shown plasma Cu concentrations do not decline until liver Cu stores are depleted below approximately 40 mg Cu/kg DM (Claypool et al., 1975), and plasma Cu has been shown to be an unreliable indicator of Cu status. Although ANT decreased plasma Cu and Se concentrations following depletion, the magnitude of difference in liver Cu between ANT and CON at the end of depletion was not translated to plasma Cu concentrations. In the repletion period, plasma Cu concentrations tended to improve over time with some variation due to treatments, however, there were few differences in physiological Cu concentrations due to treatment, further solidifying that plasma is a poor Cu status biomarker. In contrast, plasma Se has previously been shown to be a reliable indicator of Se status (Deagan et al., 1987; Kincaid, 2000). Previous research has shown injectable trace minerals to increase liver Se compared to steers receiving a saline injection, and for the effect to last for at least 30 d when all steers were supplemented with TM at 100% of NRC (1996) recommended concentrations (Genther and Hansen, 2014). In the present study, plasma concentrations of Se were lesser in ANT than CON during repletion, and increased over time such that d 28 and d 42 concentrations were greater than d 0 and d 14, regardless of DEP or REP treatment; this is consistent with previous literature (Spears et al., 1986). Additionally, plasma Se concentrations followed the trends of liver Se and tended to be greater in ITM than BLEND over the repletion period.

As suggested by Kincaid (2000), ANT steers were marginally Se deficient following depletion. Increased concentrations of dietary S have been shown to decrease Se status of ruminants; the addition of 0.2 or 0.4% dietary S with between 0.1 and 1.34 mg Se/kg (as sodium selenate) has been reported to decrease Se status in dairy cows (Ivancic and Weiss, 2001) and wethers (van Ryssen et al., 1998) in a linear fashion. Studies have also compared the
impact of dietary Mo alone on Se absorption; White et al. (1989) supplemented 10 mg Mo/kg DM to sheep and reported there were no changes in blood or liver concentrations of Se. It appears dietary S may decrease Se absorption, as the combination of increased dietary S and no supplemental Se decreased Se status markedly in 90 d, and the effect of dietary antagonists persisted across the repletion period, regardless of method of Se repletion.

Previous research has shown supplementation of some dietary organic TM to have superior absorption compared to inorganic TM when dietary S and Mo are included in cattle diets (Spears, 1989; Suttle, 1991). The improvement of liver Cu and Se concentrations from BLEND by d 28 and ING by d 42 in the present trial support these data, and suggest the more rapid improvement of liver Cu and Se concentrations in BLEND to be due to the improved bioavailability of the organic TM compared to ING. Hansen et al. (2008) supplemented steers either 5 or 10 mg Cu/kg DM from CuSO$_4$ or CuGly in diets with 2 mg supplemental Mo/kg DM and supplemental 0.15% S for 120 d, and reported the relative bioavailability of the organic source to be 131% compared to the inorganic source. Gao et al. (2014) reported Cu amino acid complex (Availa Cu) had greater absorption across Caco-2 cells when compared to CaSO$_4$, supporting the concept that organic sources often have greater bioavailability in the small intestine. Similarly, while inorganic Se can be easily reduced and made unavailable for absorption by rumen microbes (Wright and Bell, 1966), organic Se has greater bioavailability as it is incorporated into the body through active amino acid uptake (NRC, 2005). It appears the supplementation of Cu and Se at 150% of nationally recommended concentrations (NASEM, 2016) from entirely inorganic TM is sufficient for TM repletion, however the response is slower when compared to ITM or BLEND strategies.
Selenium also has an important function in protecting cellular membranes and tissues from free radical damage through the enzyme GSH-px, and as a result, GSH-px has become a valuable estimator of Se status (Kincaid, 1995), although the measurement of GSH-px has been suggested to be unreliable to compare between experiments (Kincaid, 1999). In the repletion period, ANT steers receiving ITM had lesser GSH-px activity than other treatments. This is in contrast to Pogge et al. (2012) who reported steers that received an injectable trace mineral had greater RBCL GSH-px activity than those steers receiving a saline injection. Both studies analyzed data as repeated measures; however, Pogge et al. (2012) reported the greatest GSH-px activity to occur on d 15, while in the present study GSH-px activity was least on d 14 compared to d 0, d 28, or d 42, potentially due to increased temperature on this day, suggesting there may have been environmental impacts on the REP strategy response. It should be noted the lifespan of bovine erythrocytes has been calculated to be approximately 115 d, thus some of the RBCL measured at the start of the trial may have a carryover effect from depletion (Mizuno, 1959).

When concentrations of S and Mo are in excess in the rumen without sufficient ruminally soluble Cu to bind, thiomolybdates can be absorbed and have inhibitory effects on circulating Cu (Kelleher et al., 1983). In the present study, plasma samples collected on d -10 showed ANT to have much greater plasma Mo concentrations compared to CON; however, after REP strategies began the concentrations of plasma Mo decreased in ANT such that all steers had equal plasma Mo concentrations on d 14. Though this trial did not explicitly measure circulating thiomolybdates, it is possible the increased concentrations of dietary Cu were able to bind thiomolybdates in the rumen, and therefore decrease those absorbed into
circulation. It is unclear why plasma Mo concentrations of CON on d 42 surpassed those of ANT, though dietary Mo supplementation to ANT continued throughout repletion.

In the present study, ANT steers had lesser DMI during repletion, which agrees with previous literature showing high S diets decrease DMI in feedlot cattle (Drewnoski et al., 2014). Interestingly, ANT steers also had more favorable feed efficiency during the repletion period, which may be due to the improvement in TM status of these cattle during this period. Bottje et al. (2002) has reported highly feed efficient birds to have lesser reactive oxygen species and $\text{H}_2\text{O}_2$ production, and all four TM assessed in the present study are critically important in antioxidant activity in the body (Spears and Weiss, 2008; NASEM, 2016). Mitochondria require GSH-px to convert $\text{H}_2\text{O}_2$ to water (Meister, 1984), and depend on the activity of Mn-SOD in particular to combat mitochondrial oxidative stress.

Less research has focused on how dietary antagonists such as S or Mo may affect Mn and Zn status of cattle. In the present study, dietary antagonists decreased liver Mn and plasma Zn assessed at the end of depletion; however, there were no effects of ANT on liver Zn concentrations at the end of depletion, which is in agreement with previous research (Daughtery et al., 2002). As defined by Kincaid (2000), steers at the end of depletion had adequate Zn status, as liver Zn concentrations were easily within the range of 25 to 200 mg Zn/kg DM. In repletion, due to random variation, steers assigned to receive ITM had lesser Mn concentrations on d 0. There was a decrease in liver Mn concentrations of BLEND and ING on d 14, which could have been related to the excessively warm summer temperatures shortly prior to that biopsy collection day. Interestingly, ITM Mn concentrations maintained their status during this time, suggesting ITM had additional available Mn to utilize in body functions.
In conclusion, greater concentrations of S and Mo greatly decreased Cu status in beef cattle, and had negative effects on Se, Mn, and Zn status. Through the repletion period, regardless of dietary antagonists, ITM had the most rapidly improved Cu and Se status, steers receiving BLEND reached similar status by d 28 and ING reached similar status by d 42. Additionally, the improved feed efficiency noted during the repletion period in ANT steers is likely due to the greater inclusion of TM through injection or the diet during repletion, with the improved gain suggesting some kind of compensational gain as TM status of the steers improved. There was no DEP × REP interaction during the trial. Overall, these results indicate TM repletion is similar within REP strategy, regardless of presence of the antagonists S and Mo. It should be noted excessive supplementation of TM may result in greater concentrations of excretion of TM in feces, which may introduce greater concentrations of TM into the environment and ultimately result in an economic loss for producers. The use of an injectable trace mineral most rapidly improved TM status of steers from a deficient to mildly deficient or adequate state when diets were supplemented with 100% national recommended concentrations, and the inclusion of 150% of national recommendations from a blend of organic or inorganic dietary TM did reach similar status by 28 d and d 42, respectively. Additionally, the optimization of supplemental TM concentrations may assist in creating the greatest efficiency of production with the least environmental impact.
LITERATURE CITED


Table 1. Ingredient composition of depletion diets fed throughout the trial (DM basis, %).

<table>
<thead>
<tr>
<th>Ingredient, % DM basis</th>
<th>Depletion Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corn silage</td>
<td>40</td>
</tr>
<tr>
<td>Dry rolled corn</td>
<td>25</td>
</tr>
<tr>
<td>DDGS&lt;sup&gt;3&lt;/sup&gt;</td>
<td>20</td>
</tr>
<tr>
<td>MDGS&lt;sup&gt;4&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>S and Mo premix&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Micro ingredients&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>Calculated composition&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CP, %</td>
<td>15.06</td>
</tr>
<tr>
<td>NDF, %</td>
<td>26.03</td>
</tr>
<tr>
<td>EE, %</td>
<td>5.16</td>
</tr>
<tr>
<td>Analyzed composition&lt;sup&gt;8&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cu, mg/kg DM</td>
<td>6.98</td>
</tr>
<tr>
<td>Fe, mg/kg DM</td>
<td>46.86</td>
</tr>
<tr>
<td>Mn, mg/kg DM</td>
<td>17.53</td>
</tr>
<tr>
<td>Zn, mg/kg DM</td>
<td>29.55</td>
</tr>
</tbody>
</table>

<sup>1</sup>Control diet provided supplemental trace minerals per kg diet DM: 10 mg of Cu (CuSO<sub>4</sub>), 30 mg of Zn (ZnSO<sub>4</sub>), 20 mg of Mn (MnSO<sub>4</sub>), 0.5 mg of I (calcium iodate), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).

<sup>2</sup>Antagonist diet provided supplemental trace minerals per kg diet DM: 0.5 mg of I (calcium iodate) and 0.1 mg of Co (cobalt carbonate).

<sup>3</sup>Modified distillers grains with solubles.

<sup>4</sup>Dried distillers grains with solubles.

<sup>5</sup>S and Mo premix use DDGS as a carrier and provide 0.3% S (as CaSO<sub>4</sub>) and 2 mg of Mo/kg DM.

<sup>6</sup>Microingredients include trace minerals, Vitamin A, limestone, salt, and Rumensin, and use DDGS as a carrier.

<sup>7</sup>Calculated based on individual ingredient analysis from Dairyland Laboratories (Arcadia, WI).

<sup>8</sup>Analysis of TMR from depletion period.
Table 2. Composition of repletion period supplemental dietary trace minerals applied within two depletion period diets (CON\(^1\) vs. ANT\(^2\)) fed to feedlot steers.

<table>
<thead>
<tr>
<th>Mineral, mg/kg DM</th>
<th>ITM(^3)</th>
<th>BLEND(^4)</th>
<th>ING(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(^6)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>I(^7)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Cu (CuSO(_4))</td>
<td>10</td>
<td>11.25</td>
<td>15</td>
</tr>
<tr>
<td>Mn (MnSO(_4))</td>
<td>20</td>
<td>22.5</td>
<td>30</td>
</tr>
<tr>
<td>Se (Na(_2)SeO(_3))</td>
<td>0.1</td>
<td>0.1125</td>
<td>0.15</td>
</tr>
<tr>
<td>Zn (ZnSO(_4))</td>
<td>30</td>
<td>33.75</td>
<td>45</td>
</tr>
<tr>
<td>Cu (Availa – Cu)</td>
<td>-</td>
<td>3.75</td>
<td>-</td>
</tr>
<tr>
<td>Mn (Availa – Mn)</td>
<td>-</td>
<td>7.5</td>
<td>-</td>
</tr>
<tr>
<td>Se (Selplex – Se)</td>
<td>-</td>
<td>0.0375</td>
<td>-</td>
</tr>
<tr>
<td>Zn (Availa – Zn)</td>
<td>-</td>
<td>11.25</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\)Control diet provided supplemental trace minerals per kg diet DM: 10 mg of Cu (CuSO\(_4\)), 30 mg of Zn (ZnSO\(_4\)), 20 mg of Mn (MnSO\(_4\)), 0.5 mg of I (calcium iodate), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).

\(^2\)Antagonist diet provided supplemental trace minerals per kg diet DM: 0.5 mg of I (calcium iodate), 0.1 mg of Co (cobalt carbonate), 2 mg Mo/kg DM, and 0.3% S as CaSO\(_4\).

\(^3\)Injectable trace mineral treatment supplemented inorganic forms of trace minerals at 100% of concentrations recommended by NASEM (2016).

\(^4\)Trace minerals supplemented at 150% of concentrations recommended by NASEM (2016) from 25% organic and 75% inorganic sources.

\(^5\)Trace minerals supplemented at 150% of concentrations recommended by NASEM (2016) from entirely inorganic sources.

\(^6\)Cobalt supplemented as Co carbonate.

\(^7\)Calcium supplemented as Ca iodate.
Table 3. Effect of depletion diet on liver mineral concentrations, plasma mineral concentrations, and red blood cell lysate glutathione peroxidase activity of feedlot steers at the end of the depletion period.

<table>
<thead>
<tr>
<th>Depletion Diet</th>
<th>CON¹</th>
<th>ANT²</th>
<th>SEM</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver mineral, mg/kg DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>251</td>
<td>23</td>
<td>-³</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mn</td>
<td>9.8</td>
<td>8.5</td>
<td>0.24</td>
<td>0.01</td>
</tr>
<tr>
<td>Se</td>
<td>2.00</td>
<td>1.22</td>
<td>0.052</td>
<td>0.0001</td>
</tr>
<tr>
<td>Zn</td>
<td>123</td>
<td>118</td>
<td>2.6</td>
<td>0.42</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu, mg/L</td>
<td>0.81</td>
<td>0.71</td>
<td>0.092</td>
<td>0.01</td>
</tr>
<tr>
<td>Fe, mg/L</td>
<td>2.09</td>
<td>1.86</td>
<td>0.066</td>
<td>0.02</td>
</tr>
<tr>
<td>Mo, µg/L</td>
<td>12.8</td>
<td>21.1</td>
<td>0.47</td>
<td>0.0001</td>
</tr>
<tr>
<td>Se, µg/L</td>
<td>135</td>
<td>129</td>
<td>1.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Zn, mg/L</td>
<td>1.26</td>
<td>1.17</td>
<td>0.019</td>
<td>0.03</td>
</tr>
<tr>
<td>Red blood cell lysate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH-px, U × 10³/g Hb⁴</td>
<td>145.3</td>
<td>143.5</td>
<td>2.52</td>
<td>0.62</td>
</tr>
</tbody>
</table>

¹Control diet provided supplemental trace minerals per kg diet DM: 10 mg of Cu (copper sulfate), 30 mg of Zn (zinc sulfate), 20 mg of Mn (manganese sulfate), 0.5 mg of I (calcium iodate), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).

²Antagonist diet provided supplemental trace minerals per kg diet DM: 0.5 mg of I (calcium iodate), 0.1 mg of Co (cobalt carbonate), 2 mg Mo/kg DM, and 0.3% S (as CaSO₄).

³CON liver Cu SEM is 8.1 mg/kg DM and ANT liver Cu SEM is 2.5 mg/kg DM.

⁴Glutathione peroxidase activity unit is defined as the amount of enzyme necessary for the oxidation of 1.0 nmol of reduced NADPH to NADP+ per minute at 25°C.
Table 4. Effect of depletion diets (CON vs. ANT) and trace mineral repletion strategies (ITM vs. BLEND vs. ING) on performance parameters of feedlot steers.

<table>
<thead>
<tr>
<th>Performance</th>
<th>Depletion Diet</th>
<th>Repletion Strategy</th>
<th>P-value</th>
<th>P-value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON¹</td>
<td>ANT²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of REP BW, kg</td>
<td>531.3</td>
<td>536.1</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg</td>
<td>1.26</td>
<td>1.34</td>
<td>0.11</td>
<td>1.37</td>
<td>1.27</td>
</tr>
<tr>
<td>DMI¹, kg</td>
<td>10.4</td>
<td>10.0</td>
<td>0.04</td>
<td>10.5^x</td>
<td>10.2^y</td>
</tr>
<tr>
<td>G:F¹</td>
<td>0.121</td>
<td>0.134</td>
<td>0.006</td>
<td>0.131</td>
<td>0.125</td>
</tr>
</tbody>
</table>

¹Control diet provided supplemental trace minerals per kg diet DM: 10 mg of Cu (CuSO₄), 30 mg of Zn (ZnSO₄), 20 mg of Mn (MnSO₄), 0.5 mg of I (calcium iodate), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).

²Antagonist diet provided supplemental trace minerals per kg diet DM: 0.5 mg of I (calcium iodate) and 0.1 mg of Co (cobalt carbonate).

³Injectable trace mineral treatment supplemented inorganic forms of trace minerals at 100% of concentrations recommended by NASEM (2016).

⁴Trace minerals supplemented at 150% of concentrations recommended by the by NASEM (2016) from 25% organic and 75% inorganic sources.

⁵Trace minerals supplemented at 150% of concentrations recommended by the by NASEM (2016) from entirely inorganic sources.
Figure 1. Effect of depletion diets × day of repletion period on liver Cu (Panel A, $P < 0.0001$) and liver Mn (Panel B, $P = 0.07$). CON supplemented 10 mg Cu, 30 mg Zn, 20 mg Mn, 0.1 mg Se, 0.5 mg I, and 0.1 mg Co/kg DM; ANT supplemented 0.3% S as CaSO$_4$ and 2 mg Mo, 0.5 mg I, and 0.1 mg Co/kg DM. Within a panel, superscripts that differ are different ($P \leq 0.09$).
Figure 2. Effect of trace mineral repletion strategy × day of repletion period on liver Cu (Panel A, $P < 0.0001$), liver Se (Panel B, $P < 0.0001$), and liver Mn (Panel C, $P = 0.07$) concentrations. ITM is Multimin90 injection containing Cu, Mn, Se, and Zn and 100% NASEM supplemental dietary TM from inorganic sources; BLEND is 150% of NASEM dietary TM supplementation from 25% organic and 75% inorganic sources; ING is 150% of NASEM dietary TM supplementation from entirely inorganic sources.

Within a panel, within a day, superscripts that differ are different ($P \leq 0.06$).
Figure 3. Effect of depletion diets × repletion strategy × day on plasma Mo (Panel A, $P = 0.009$), plasma Cu (Panel B, $P = 0.09$), and plasma Zn (Panel C, $P = 0.07$). CON supplemented 10 mg Cu, 30 mg Zn, 20 mg Mn, 0.1 mg Se, 0.5 mg I, and 0.1 mg Co/kg DM; ANT supplemented 0.3% S as CaSO$_4$ and 2 mg Mo, 0.5 mg I, and 0.1 mg Co/kg DM; ITM is Multimin90 injection containing Cu, Mn, Se, and Zn and 100% NASEM supplemental dietary TM from inorganic sources; BLEND is 150% of NASEM dietary TM supplementation from 25% organic and 75% inorganic sources; ING is 150% of NASEM dietary TM supplementation from entirely inorganic sources.
Figure 4. Effect of depletion diets × repletion strategy (Panel A, $P = 0.04$); and day of repletion (Panel B, $P = 0.07$) on red blood cell lysate glutathione peroxidase activity. Within a panel, superscripts that differ between treatment are different (a, b; $P \leq 0.05$) or tend (x, y; $P \leq 0.1$) to differ. CON supplemented 10 mg Cu, 30 mg Zn, 20 mg Mn, 0.1 mg Se, 0.5 mg I, and 0.1 mg Co/kg DM; ANT supplemented 0.3% S as CaSO$_4$ and 2 mg Mo, 0.5 mg I, and 0.1 mg Co/kg DM; ITM is Multimin90 injection containing Cu, Mn, Se, and Zn and 100% NASEM supplemental dietary TM from inorganic sources; BLEND is 150% of NASEM dietary TM supplementation from 25% organic and 75% inorganic sources; ING is 150% of NASEM dietary TM supplementation from entirely inorganic sources.
Trace minerals play an essential role in a variety of biological processes including but not limited to growth, development, and immune responses. Inorganic TM have been traditionally supplemented to cattle due to their affordability and high rumen solubility, however this improved ruminal availability means they are easily reduced and ultimately less bioavailable to the animal (Ammerman and Goodrich, 1983; Engle and Spears, 2000). Research has shown organic (Mohanta and Garg, 2014) and hydroxy (Genther and Hansen, 2015) TM are less susceptible to ruminal interactions and have greater bioavailability in comparison to inorganic TM. Recent studies have shown TM injections can also improve TM status of cattle (Pogge et al., 2012; Genther and Hansen, 2014). This thesis focused on the effects of TM supplementation strategies on TM status and performance of feedlot cattle fed diets containing high S or high S and Mo.

High S has been shown to decrease TM status (Spears, 2003; Pogge et al., 2014) and have negative effects on the live performance (Thompson et al., 1972; Drewnoski et al., 2014) of feedlot steers. If Mo is added in a diet with S present, the absorption of Cu is greatly decreased as the three bind in the rumen to form an insoluble compound (Humphries et al., 1983). In the first study, high S was a strong Cu antagonist across the feeding period and tended to decrease Zn status in the growing period, however all animals had adequate TM status (Kincaid, 2000) because of supplementation of TM at 100% of NRC recommendations. The second study included 2 mg supplemental Mo/kg DM in addition to 0.3% supplemental S, and markers of Cu, Mn, Se, and Zn status were decreased. The effect of thiomolybdates was especially pronounced on liver Cu concentrations in the second trial, where initial liver Cu
concentrations were decreased by approximately 90% after 90 d on the depletion period diet. The limited effects of antagonists on Zn status in these trials are consistent with some literature (Daughtery et al., 2002), however, research by Pogge et al. (2014) reported steers consuming 0.68% S had decreased liver Zn concentrations on d 27. These contradictions may stem from the lack of an accurate biomarker to measure Zn status, and it would be helpful to determine a consistent and reliable biomarker to compare between studies.

Though there were limited effects of hydroxy TM supplementation on cattle in the first trial, research has shown hydroxy Cu, Mn, and Zn to be less ruminally soluble than inorganic forms of these TM (Genther and Hansen, 2015). In this work, the inconsistent results between the liver Mn concentrations from the growing period to the finishing period may be attributed to interactions of TM and S within diet type. This combined with limited interactions of hydroxy TM and high S on Zn status suggest TM supplementation of NRC (1996) concentrations are adequate to maintain TM status when diets contain up to 0.54% S, as long as cattle have adequate TM status to begin with. Future work should focus on how a blend of inorganic and hydroxy source TM may improve TM absorption, and test how different supplementation concentrations may impact bioavailability in the presence of a high S or high antagonist diet.

Injectable TM are effective due primarily because they avoid ruminal antagonisms and provide TM directly to tissues. Previous research has shown organic sources of Cu (Kegley and Spears, 1994) and Se (Combs, 2015) also have improved bioavailability in cattle in comparison to inorganic TM, and these sources can be included in the diet. Thus, a second trial was undertaken to compare the effectiveness of three TM supplementation strategies on cattle fed high antagonist diets. In this research, TM supplementation strategies were applied to steers following a 90 d depletion period, within a control and antagonist diet. Interestingly, there were no interactions between depletion diet and TM repletion strategy, suggesting that regardless of
the presence of dietary antagonists the pattern of TM repletion was similar within TM repletion strategy. While an injectable TM, along with supplementation at 100% of national TM recommendations improved Cu and Se status most rapidly, steers receiving a blend of 25% organic and 75% inorganic TM supplemented at 150% of nationally recommended concentrations (2016) resulted in improved Cu and Se status by d 28, and inorganic supplementation (also at 150% of national recommendations) alone reached a similar TM status as the other supplementation strategies by d 42.

To the author’s knowledge, this is the first time research has shown how an injectable TM can improve TM status in the face of a high antagonist diet. Though the responses between TM strategies were similar in magnitude, speed of repletion was different between supplementation strategies in the repletion phase. Trace mineral injections have been shown to improve Cu and Se status as soon as 1 d post injection (Pogge et al., 2012), and the length of time a response is recordable from an injectable TM has been shown to last at least 30 d post injection (Genther and Hansen, 2014). In the present study, TM injection improved liver Cu for at least 42 d, increased Se on d 14, and maintained Mn status on d 14 over steers supplemented only dietary TM, but no effects of the injectable TM were noted on liver Zn concentrations. Previous research has shown Mn (Henry et al., 1992) and Zn (Spears, 1989) do have improved bioavailability when supplemented from an organic form, however, biomarkers for Mn and Zn remain elusive, and more research should be done to determine the effectiveness of these supplemental sources in overcoming a high antagonist diet.

There were limited effects of treatment on cattle performance in the trials conducted in this research. Neither study reported differences in final BW or ADG, however DMI was decreased in steers when dietary S and Mo were included. The decreased DMI is consistent with high S research (Drewnoski et al., 2014). Feed efficiency was improved in both studies; in the
first trial, steers consuming high S and inorganic TM had greater feed efficiency than those consuming high S and hydroxy TM. Interestingly, in the second study, steers consuming the high antagonist diet had improved G:F in the repletion period. Considering the deficient TM status of the steers at the start of repletion, it is possible the increased concentrations of TM in the repletion period provided additional nutrients for the animal to utilize as a method to overcome the TM deficient state via some compensatory gain. However, this mechanism is unclear, and future research should be done to determine the impact of TM supplementation on feed efficiency of cattle.

Overall, this thesis research indicates high S decreases the TM status of feedlot cattle, and regardless of depletion diet, all TM supplementation strategies were effective at improving Cu and Se status of feedlot steers. In the first trial, there were interactions with S concentration and hydroxy Cu, Mn, and Zn that differed across growing and finishing diets, suggesting diet may play a factor in bioavailability. In the second trial, a TM injection repleted Cu and Se status by d 14, followed by the supplementation of a blend of organic and inorganic TM (at 150% of NRC) which improved Cu and Se concentrations by d 28, or inorganic TM alone at 150% of NRC by day 42. High S decreased status of Cu, Mn, Se, and some markers of Zn in these studies, and it would be advantageous to determine a more reliable biomarker for Mn and Zn status. Producers should consider the implications of high S or high S and Mo on their cattle, and recognize the ideal TM supplementation strategy depends widely on geographical region and known mineral deficiencies or toxicities in the area. Once there is a better understanding of how TM source may improve TM status of beef cattle, optimal supplementation strategies may be adapted to ensure greatest efficiency in feedlots.