The influence of dietary zinc concentration during periods of rapid growth induced by ractopamine hydrochloride or dietary energy and dietary fiber content on trace mineral metabolism and performance of beef steers

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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NOMENCLATURE

AC	Adenylate cyclase
ADG	Average daily gain
ADF	Acid detergent fiber
BF	12^{th} rib fat thickness
BW	Body weight
cAMP	Cyclic adenosine monophosphate
CON	Control
CT	Cycle threshold
Cu	Copper
d	Day
DM	Dry matter
DMI	Dry matter intake
Fe	Iron
G:F	Gain to feed
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GPCR	G-protein coupled receptor
HE	Heat energy
hr	Hour
HCl	Hydrochloride
HCW	Hot carcass weight
HSL	Hormone sensitive lipase
IGF-1	Insulin-like growth factor-1

INZN	Inorganic Zinc
KPH	Kidney, pelvic, and heart fat
LE	Low energy
ME	Metabolizable energy
MHC	Myosin heavy chain
Mn	Manganese
MRE	Metal response element
MS	Marbling score
MT	Metallothionein
MTF-1	Metal transcription factor-1
mTOR	Mammalian target of rapamycin
N	Nitrogen
NEFA	Plasma non-esterified fatty acids
NEg	Net energy of gain
NEm	Net energy of maintenance
NDF	Neutral detergent fiber
OM	Organic matter
PA	Perilipin A
PCR	Quantitative real-time polymerase chain reaction
PDE	Phosphodiesterase
PKA	Protein kinase A
PKC	Protein kinase C
QG	Quality grade
RE	Retained energy

REA	Longissimus dorsi muscle area
SLC30	Solute carrier 30
SUN	Serum urea nitrogen
TM	Trace mineral
TMR	Total mixed ration
W	Metabolic body weight
YG	Yield grade
ZIP	Zrt/Irt-like protein
Zn	Zinc
ZNBLD	Zinc blend
Zn-AA	Zn-amino acid complex
ZnT	Zn transporter
ZNTRT	Zinc treatment
β-AA	β-adrenergic agonist
β-AR	β-adrenergic receptor

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ABSTRACT

The trace mineral zinc (**Zn**) is an important constituent of many biological processes associated with growth and maintenance. While Zn is currently recommended to be supplemented to cattle at 30 mg Zn/kg dry matter (**DM**), this recommended concentration stems from studies conducted in cattle genetically and phenotypically very different than the feedlot cattle of today. Prior research has shown a greater growth response when cattle fed ractopamine hydrochloride were also fed Zn at increasing supplemental concentrations as a blend of inorganic $ZnSO_4$ and Zn-amino acid complex. Therefore, the objective of this dissertation was to investigate if improvements in cattle growth due to greater supplemental Zn concentrations are specific to ractopamine hydrochloride induced growth and if Zn source differentially influences the growth response. It was hypothesized that periods of rapid growth increase the body's physiological need for Zn above current recommendations, regardless of how this growth is achieved. The influence of dietary energy on growth is well established and dietary energy concentrations can be altered to manipulate rates of growth in beef steers. Therefore, research reported in this dissertation investigates the interaction of Zn and rapid rates of growth. Rapid growth was induced either by ractopamine hydrochloride supplementation or diets differing in energy, designed to imitate the rates of growth typically noted during ractopamine hydrochloride supplementation, in a series of metabolism and performance studies. Results of this research indicated that supplementation of ractopamine hydrochloride did not increase Zn absorption or retention, and no final performance advantages were noted with supplemental Zn at 120 mg Zn/kg DM as either $ZnSO_4$ or a blend of $ZnSO_4$ and Zn-amino acid complex.

However, Zn supplementation from the Zn source blend was found to increase N retention similar to the increase in N retention seen from ractopamine hydrochloride administration. Ractopamine hydrochloride acts through a G-protein coupled receptor, increasing circulating cAMP concentrations. Zinc has been shown to potentiate greater cAMP concentrations by Zrt/Irt-like protein 14 (**ZIP14**) import of Zn into the cell, decreasing activity of the cAMP degrading enzyme phosphodiesterase. Expression of the Zn importer ZIP14 was unaffected due to Zn supplementation or growth method, but the Zn-sequestering protein metallothionein appeared to be increased due to supplemental Zn concentration and source, and further influenced by ractopamine hydrochloride supplementation. This may be indicative of a function of ractopamine hydrochloride to influence Zn sequestering and homeostasis following administration.

The metabolism portion of the dietary energy growth model became focused on differences in dietary fiber content due to greater silage quality than expected. The supplemental Zn blend led to increased dry matter and organic matter digestibility and tended to increase N digestibility over unsupplemented controls. Additionally, the high fiber diet led to greater absorption and retention of Zn as mg/d in steers receiving supplemental Zn. Growing steers receiving greater supplemental Zn concentrations had decreased rates of absorption and retention of Zn as a portion of intake. This is in contrast to late stage finishing steers where no differences in rate of apparent absorption of Zn were observed between the control and supplemental Zn treatments. These results suggest differences in capacity or ability to absorb Zn due to age or diet composition. However, increasing supplemental Zn resulted in greater retention of Zn as mg/d for both growing and finishing steers.

Performance of beef steers was increased in steers receiving the high energy diet compared to the low energy diet. However, following transition to the high energy diet, cattle receiving the blend of $ZnSO_4$ and Zn-amino acid complex at 120 mg Zn/kg DM had improved performance relative to $ZnSO_4$ and control, suggesting a protective action of the blend of Zn in during this time. Supplementation of differing Zn sources at similar concentrations resulted in greater mRNA expression of the Zn storage protein metallothionein in liver relative to control. Metallothionein expression, while inconsistent, differed between sources in both cattle supplemented ractopamine hydrochloride and diets differing in energy. Additionally, supplementing Zn did not change liver Zn concentrations relative to controls when supplemented at 5 times the concentrations of the controls, confirming liver Zn concentrations are not an adequate biomarker for Zn status excluding deficiency. Tissue and plasma concentrations were shown to differ due to source, but direct comparisons of sources were not completed in the metabolism portions of this dissertation and further research of effect of source on Zn tissue and plasma concentrations is warranted.

Overall, increasing supplemental Zn concentrations as either $ZnSO_4$ or a blend of $ZnSO_4$ and Zn-amino acid complex over unsupplemented controls (basal diets just above NASEM (2016) recommendations) influenced interim performance but did not improve overall performance of steers experiencing increased rates of growth. However, ractopamine hydrochloride influenced expression of Metallothionein, suggesting an influence on Zn sequestering and trafficking post-absorption.

CHAPTER 1. GENERAL INTRODUCTION

Zinc is a trace mineral required by all mammals for growth and is critical in the function of insulin-like growth factor (**IGF-1**), growth hormone (**GH**), and DNA synthesis. The requirement for Zn in cattle was established more than 30 years ago to prevent signs of deficiency and support normal physiological function (NRC, 1984a). However, the type of cattle today are quite different, inherently exhibiting greater and more efficient growth while also receiving growth technologies to support even more efficient growth, and according to Capper (2011) average daily gain (**ADG**) across of the life of feedlot cattle has increased 44% since 1977. Movement toward nutrient regulation to regulate environmental impacts, specifically nutrients and additives fed to livestock, has already begun impacting perception and practices of the agricultural industry. Therefore, the industry as a whole has begun to move towards the balance of efficiency, environmental safety, and productivity, and need to aspire to reach a more strategic supplementation strategy of nutrients within diets.

The application of growth promoting technologies as well as the more efficient type of cattle seen today have not been considered relative to current recommended Zn requirements. Current practices involve the use of growth enhancing technologies such as β-adrenergic agonists (**β-AA**) and anabolic implants that increase cattle efficiency while utilizing less inputs of feed, water and land. In the final month prior to harvest, the β-AA ractopamine hydrochloride shifts animal growth toward lean tissue accretion and away from adipose tissue synthesis by abruptly altering the physiological process of protein synthesis. The use of ractopamine hydrochloride results in greater ADG, final live body weight (**BW**), and hot carcass weight (**HCW**) compared to non-supplemented cattle when fed the last 28-42 d before harvest (Avendaño-Reyes et al., 2006; Gruber et al., 2007; Winterholler et al., 2007;

Scramlin et al., 2010). Since Zn is required for protein synthesis and growth, cattle receiving ractopamine hydrochloride or experiencing periods of rapid growth may require greater amounts of Zn in the diet. According to Genther-Schroeder et al. (2016) steers receiving ractopamine supplementation for the last 28 d on feed saw increased growth when steers received 150 mg Zn/kg DM vs. 90 mg Zn/kg DM, suggesting greater Zn supplementation rates could prove beneficial during periods of rapid growth. Recently, it has been suggested that Zn may interact with the β-adrenergic cascade by inhibiting phosphodiesterase (Hojyo et al., 2011), a negative feedback mechanism for prolonged β-AA supplementation that diminishes growth induced by β-AA (Spurlock et al., 1994), suggesting Zn may support further activation of the β-adrenergic cascade with ractopamine hydrochloride supplementation. In general, the role of Zn in multiple growth processes suggests that greater supplemental Zn may be beneficial during periods of rapid growth.

This research was designed to evaluate the effect of increased supplemental Zn concentrations on performance, gene expression, and the muscle proteome during periods of rapid growth such as that induced by ractopamine hydrochloride or increased plane of nutrition. Additionally, this work aims to identify if Zn apparent absorption increases during periods of rapid growth. Overall, an improved understanding of how greater supplemental Zn supports rapid growth will allow producers to more strategically supplement Zn and maximize gain in an environmentally responsible manner and was evaluated in this research.

Dissertation Organization

The following (Chapter 2) contains a review of the literature related to physiological Zn in ruminant diets, Zn trafficking and Zn involvement in growth processes. Additionally, the literature review will cover relevant information involving growth induced by plane of

nutrition, β-adrenergic agonists, and anabolic implants, and possible interactions with Zn. The following 4 chapters are related to research completed within these subjects. Chapter 3, published in the Journal of Animal Science, investigates the impacts of ractopamine hydrochloride and dietary Zn supplementation on N and Zn retention in finishing beef steers. Chapter 4, to be submitted to the Journal of Animal Science, investigates the influence of ractopamine hydrochloride and dietary Zn supplementation from differing sources on beef steer performance, blood metabolites, and gene expression. The research completed for Chapter 5 determines the influence of increasing digestible dietary fiber and dietary Zn supplementation on N and trace mineral retention and digestibility. Research completed in Chapter 6 determines the impact of increasing dietary energy and supplemental Zn on performance measures, blood metabolites, and gene expression of beef steers. Chapter 7 will summarize the research and overall findings, conclusions, implications, and suggestions for future research to be conducted.

CHAPTER 2. LITERATURE REVIEW

Zinc in Ruminant Diets

Importance of Zn in the Body

The trace mineral Zn is involved in multiple growth pathways and processes including the growth hormone (**GH**) and insulin-like growth factor-1 (**IGF-1**) pathways (Roth and Kirchgessner, 1994) and DNA, RNA, and protein synthesis (MacDonald et al., 1998; O'Dell and Browning, 2011). Over 2000 transcription factors rely on Zn for structure and function, and Zn is required throughout almost every signaling pathway in the body (Cousins et al., 2006). Since being recognized as a necessary mineral for fungal growth more than 100 years ago, Zn has become a highly studied mineral in animal agriculture and nutrition. Early research in Zn function and importance was thwarted by the failed recognition that purified diets designed to restrict Zn were also restrictive for other important vitamins and minerals. The research truly establishing the necessity for Zn in animals was conducted by Todd et al. (1934), where they discovered the essentiality of Zn for normal growth and development in rats, who were provided a semi purified diet deficient in Zn while other known nutrients were supplemented. Since then, deficiency of Zn has been characterized in most animals including swine (Luecke et al., 1956; Lewis et al., 1957), chickens (Young et al., 1958; Sullivan, 1961), and large and small ruminants (Legg and Sears, 1960; Miller et al., 1962a; Ott et al., 1965b; Ott et al., 1965a; Perry et al., 1968a) and clinical signs of Zn deficiency can be recognized as parakeratosis, decreased survivability, and diminished growth. Unfortunately, the first limiting function of Zn in growth is unestablished. Previous research during Zn deficiency has attributed decreased growth to decreased intake; however, force feeding a diet deficient in Zn to rats has resulted in exacerbation of symptoms and even death (Park et al., 1986).

Other researchers have suggested decreased intake may be a protective mechanism during Zn deficiency (MacDonald, 2000), but the answer remains unclear.

Minimum Zn requirements to prevent deficiency in cattle were established more than 30 years ago (NRC, 1984b). Since then, many changes have occurred within the beef industry, including the use of growth promoting technologies, improved management practices, and improved cattle efficiency through genetic and nutritional advances. According to Capper (2011) ADG has increased 44 % since 1977 suggesting increased efficiency. Since Zn is required for growth and efficient protein utilization it stands to reason the requirement for Zn may have increased. In the ovine species, Zn requirements are based on body weight and growth rate (NRC, 2007) rather than a general uniform value across all ages and physiological types. Thus, this may be a beneficial consideration for future application in beef cattle. Regardless, while greater requirements may be expected, Zn absorption rates in cattle have not increased over this same time and even tend to decrease with increased Zn supplementation as a percentage of intake (Mohanna and Nys, 1999; Pogge et al., 2014; Shaeffer et al., 2017; VanValin et al., 2018). Further research is needed to more accurately define beef cattle requirements during periods of increased growth and differing physiological states. Nonetheless, Zn is required for protein synthesis and growth in mammals. In landmark research by Oberleas and Prasad (1969), when rats were fed increasing protein concentrations in the diet, only those with adequate Zn were able to utilize the increasing amount of protein. The addition of adequate Zn in the diet effectively doubled weight gain at 4, 8, 12 and 16% CP, clearly expressing the necessary role of Zn in protein utilization in the body. Collectively, supplying adequate Zn in the diet is unequivocally necessary for utilization of protein and growth of animals. The importance of Zn in protein accretion in cattle will be discussed in greater detail later in this literature review.

Establishing Zn as a Requirement for Cattle

After the establishment of Zn as an essential trace mineral in swine and chickens, it was proposed that Zn could also be an essential nutrient for cattle, but it had also been seen that high concentrations of Zn could negatively impact animal performance. To test this in ruminants, Feaster et al. (1954) maintained Jersey steers for over a year on 1000 and 50 mg Zn/kg DM diets, resulting in slightly lesser gains and higher Zn excretion for the high Zn treatment vs. the control. The results of this study suggest high Zn concentrations may negatively impact performance and that Zn absorption may be regulated by high dietary Zn. Additionally, immediately following an intravenous injection of radiolabeled Zn, 100% of plasma Zn was radiolabeled, while after 4 hours only 3% of plasma Zn was radiolabeled. This result, as well as follow up measurements of Zn transfer from plasma to red blood cells, solidified the importance of Zn presence for the erythrocyte enzyme carbonic anhydrase and the permeability of the erythrocyte cell wall to Zn in cattle (Feaster et al., 1954). However, up to the 1960's little evidence of Zn deficiency had been procured in ruminants. Miller and Miller (1960) set out to and succeeded in establishing visual signs of deficiency, in which Holstein calves fed a semi-purified diet containing 2.7 mg Zn/kg DM developed inflammation of the hocks, alopecia on the rear legs, broken skin around the hooves, and listlessness after 8.5 weeks of low Zn supplementation. Additionally, Zn plasma content as well as carbonic anhydrase activity for these animals was lesser than for calves fed a diet containing 50 mg Zn/kg DM (Miller and Miller, 1960). Monogastrics have displayed signs of Zn deficiency when fed sesame meal (a feedstuff high in phytate) as the protein source when adequate dietary Zn was supplied. However, Miller et al. (1962) observed dairy calves receiving differing protein sources (sesame meal and soybean meal) with adequate Zn did not manifest clinical signs of Zn deficiency. This research was the first indication of the

ruminants ability to lessen or prevent the effects of the mineral antagonist phytate, which has since been shown to bind Zn in monogastrics and limit absorption (O'Dell and Savage, 1960). Collectively, research established in the 1960's studying the impacts of supplemental Zn concentrations on Holstein calves, Jersey calves, and low gaining yearling steers (~1.16 kg ADG) is the basis of the requirement for beef cattle still recognized today (Miller and Miller, 1960; Miller et al., 1962b; Perry et al., 1968b). According to the FAO (2017) HCW has increased 69% from 1961 to 2017 (215 vs. 363 kg, respectively). The increased HCW could be attributable to greater days on feed, genetic selection, growth technologies or a combination. Regardless, the magnitude of this increased growth begs the question of whether today's Zn recommendation still applies to the more efficient and growthier cattle type noted today.

Zn Trafficking

Considered the most widely used transition metal for structural function (Krishna et al., 2003), a catalytic component of numerous enzymes (Vallee and Galdes, 1984), and a cellular signaling mediator (Maret, 2013), Zn is expected to have very refined regulation to control incorporation into cellular processes (Eide, 2004). While Zn can be trafficked through non-specific transporters such as the Fe and Mn transporter divalent metal transporter 1 and various Ca channels, those most associated with Zn trafficking are the SLC30 and SLC39 families of transporters (Glover and Hogstrand, 2002; Wang et al., 2004a; Aydemir et al., 2009; Bosomworth et al., 2012; Martin et al., 2013; Myers et al., 2013; Kambe et al., 2014; Chen et al., 2015; Guthrie et al., 2015; Lin et al., 2017; Thomas et al., 2017; Aydemir and Cousins, 2018; Gordon et al., 2018; Mnatsakanyan et al., 2018; **Figure 1**). These transporter families function to regulate Zn homeostasis in cells. Solute carrier 30 (**SLC30**) transporters, also known as Zn transporter (**ZnT**) proteins, export Zn out of vesicles of storage, cellular

compartments, and the cell itself. The SLC39 family of Zn transporters, or Zrt/Irt-like proteins (**ZIP**), sequester Zn ions from the cell exterior or lumen into the cytoplasm, as well as from the cytoplasm into subcellular compartments. These transporter proteins also function to transport other metal ions such as Fe, Mn, and Cd, creating competitive and even inhibitory routes of transport between Zn and other metals.

Mobilization and cellular homeostasis are important aspects of Zn biology. In total, 14 ZIP and 10 ZnT transporters are contained within mammalian genomes (Cousins et al., 2006). The number of membrane transporters and regulatory mechanisms for Zn are extremely high for a trace mineral, and these regulatory transporters are also located intracellularly. The amount of Zn within the cell is very tightly controlled and the regulation of Zn-dependent or Zn-activated proteins are dependent on concentrations of "free" Zn ions (Maret, 2015). Zinc ions are held within cellular vesicles for storage and release, and in secretory vesicles for exocytosis (Krężel and Maret, 2016; Hershfinkel, 2018). According to Kimura and Kambe (2016) each transporter exhibits tissue-specific, developmental, stimulus-responsive expression patterns, and specific cellular and subcellular localization. Zinc deficiency or excess can cause changes in transporter protein stability and cellular localization in addition to other various stimuli.

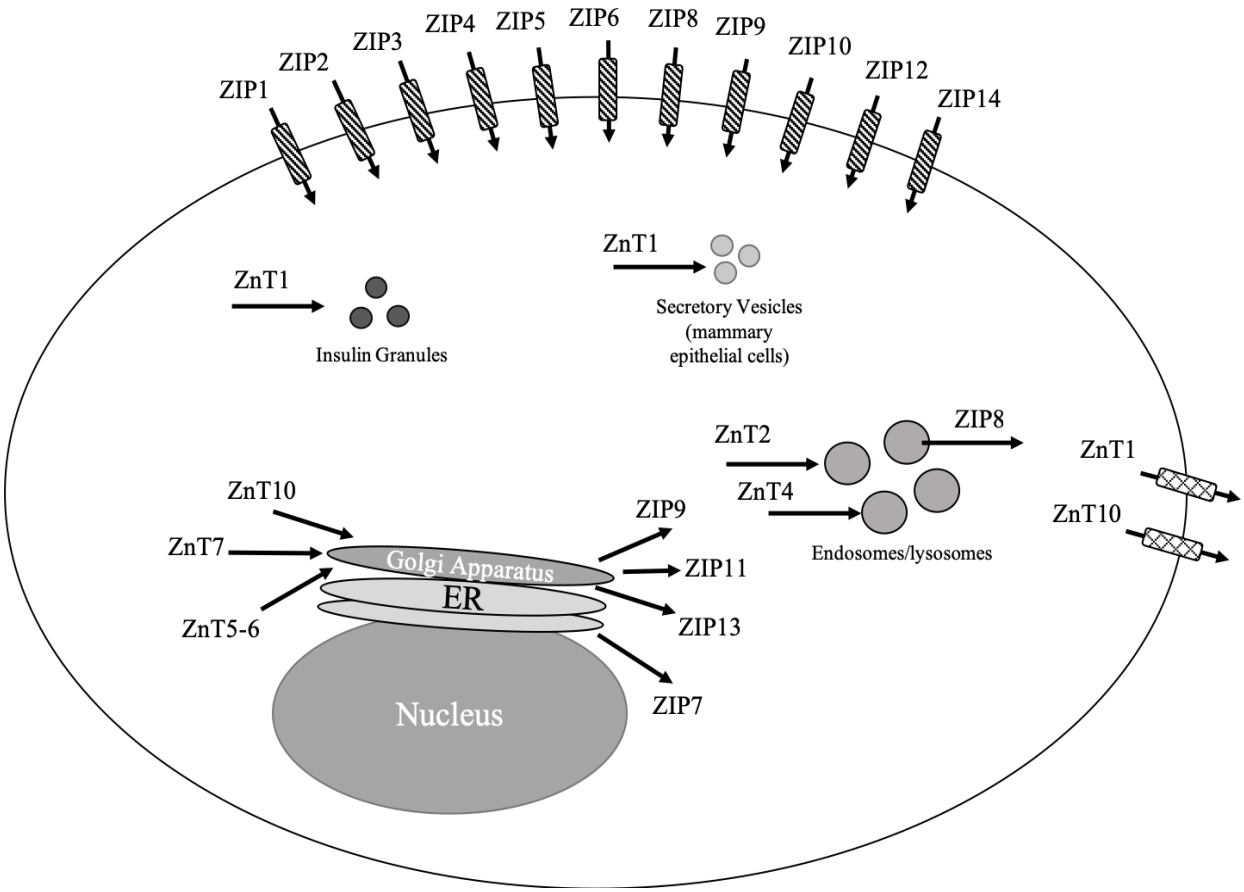

Figure 1. Zinc influx and efflux transporters. Figure adapted from Kimura and Kambe (2016) showing the subcellular localization of ZIP and ZnT. Zrt and Irt-like proteins import Zn in the cytoplasm, while ZnT exports Zn out of the cytoplasm and into the extracellular space. Cytosolic Zn is mobilized in and out of subcellular compartments in the direction of the arrows. ER, endoplasmic reticulum.

Metallothionein: Zn Sequestering Protein

Metallothionein (**MT**) is the primary intracellular Zn sequestering protein in the body (Kimura and Kambe, 2016). This protein was first identified containing heavy metals (Cd and Zn), functioning to sequester and bind these metals to decrease their concentration at critical sites in the cell (Kagi and Vallee, 1960). Metallothionein functions by chelating Zn from the environment and increases intracellular Zn. In one mouse study, when Zn concentrations were too low to stabilize MT, MT was proteolyzed and Zn was released into the cell, keeping intracellular Zn concentrations constant (Palmiter, 1995). Two isoforms of

MT, MT1/2, were shown to scavenge extracellular Zn without competing with essential Zn requiring proteins and to degrade when Zn concentrations were unable to stabilize MT (Palmiter, 1995). Additionally, MT also function as a "Zn buffer" through low-affinity binding, providing a Zn supply for target proteins and enzymes when Zn is limiting (Suhy et al., 1999).

Zinc strongly induces MT expression in mammals, but MT also appears to have a very high affinity for Cu (Oestreicher and Cousins, 1985), potentially explaining the known interaction where excess dietary Zn decreases Cu absorption in mammals. The regulation of MT by Zn is accomplished by MTF-1, a zinc-finger transcription factor that regulates metal-response element (**MRE**) induced gene expression (Kimura et al., 2009). A MRE is a *cis*-acting DNA sequence located on MT gene promoters, which can be bound by MTF-1 to induce MT gene transcription (Li et al., 2008). This is the only known metal-sensing transcription factor, and Zn is the only heavy metal that can directly activate MTF-1 binding activity of DNA (Kimura et al., 2009). Homeostatic regulation of Zn is extremely important as Zn serves multiple functions in proteins as co-factors, structural components and as a signaling molecule, and concentrations in the pico- and femtomolar range result in high affinity for binding sites in structural and catalytic roles (Kochańczyk et al., 2015; Maret, 2017). Cadmium, Cu, and oxidative stress have been shown to activate MTF-1 also, but mostly through redistribution of cellular Zn (Kimura et al., 2009). Activation of MTF-1 also up-regulates ZnT1, a member of the SLC30 family responsible for Zn efflux from the cell, suggesting MTF-1 directly influences the regulation of genes responsible for Zn homeostasis in the cell since ZnT1 appears to be localized to the plasma membrane (Langmade et al., 2000).

Zinc Influx and Homeostasis

The transporter ZIP4 is located on the apical membrane of enterocytes and is responsible for the majority of Zn absorption in mammals. Acrodermatitis enteropathica has been identified to be caused by a mutation in ZIP4, where individuals with this condition cannot fully synthesize ZIP4 and therefore cannot adequately absorb dietary Zn from the intestinal lumen (Dufner-Beattie et al., 2003). A similar syndrome has been noted in cattle of Friesian decent, resulting in loss of hair on the legs and certain parts of the body as well as parakeratosis and reduced growth (Brummerstedt, 1977). Treatment of this condition is via greater supplementation of dietary Zn, suggesting the disease is caused by reduced Zn uptake from the lumen due to the mutation (Cousins, 1985; Kasana et al., 2015). Low dietary Zn appears to upregulate ZIP4 expression in enterocytes, with more Zip4 localized to the apical membrane, lower expression of MT, and internalization of ZIP5 (Dufner-Beattie et al., 2005). ZIP5 is present on the basolateral membrane to import Zn from plasma, and internalization of this transport protein would allow Zn to be transported throughout the body by efflux from ZnT1. Adequate dietary Zn supply increases expression of MT within the enterocyte, translocation of ZIP5 to the basolateral membrane, and down regulation and internalization of ZIP4 from the apical membrane (Dufner-Beattie et al., 2005). All of these changes in dietary Zn transporter abundance and localization are thought to restore Zn homeostasis within the body during Zn deprivation or excess, since regulation of bodily Zn content is primarily controlled through absorption and reabsorption. The SLC39A4 gene, or ZIP4, is also expressed in the colon and kidney, suggesting a role for ZIP4 in homeostasis of Zn beyond the small intestine. Reduction of endogenous Zn loss is seen in humans during Zn deficiency, suggesting importance of Zn homeostasis regulation during Zn deficiency by

ZIP4 (O'Dell and Sunde, 1997). Additionally, ZIP5 has been shown to be internalized during Zn deficiency in kidney cells, possibly decreasing excreted Zn (Wang et al., 2004b).

Zinc Exporters

As stated previously, Zn exporters, or ZnT, regulate cytosolic Zn by efflux from the cytosol into the extracellular space or intracellular vesicles. The first Zn mammalian transporter gene to be identified was ZnT1, and absence of this gene was proposed to result in susceptibility of BHK Zn sensitive cells to Zn toxicity (Palmiter and Findley, 1995a). Additionally, ZnT1 appears to be the primary regulator of cellular Zn efflux (Palmiter and Findley, 1995b) and is regulated by Zn concentrations within the cytoplasm, having been shown to be regulated by MTF-1 (Langmade et al., 2000) but not MT (Davis et al., 1998). The ZnT proteins 5 and 7 are hypothesized to be involved with incorporation of Zn into enzymes, namely alkaline phosphatase, by the efflux of Zn^{2+} into the lumen of the Golgi apparatus (Suzuki et al., 2005b; Suzuki et al., 2005a). Disruption of ZnT5 resulted in a 45% reduction in alkaline phosphatase activity, ZnT7 a 20% reduction, and the disruption of both resulted in activity of alkaline phosphatase decreased to less than 5% of normal activity (Suzuki et al., 2005a). Alkaline phosphatase dephosphorylates compounds and is instrumental in metabolism in liver and bone (Millán, 2006). During mitosis, ZnT9 is translocated to the nucleus suggesting this transporter and therefore efflux of Zn from the cytoplasm into the nucleus plays a role in transcriptional regulation (Sim and Chow, 1999).

Zinc and Diet Digestibility

Diet composition can play a large role in Zn utilization, where factors such as phytate in monogastric animal diets and high Ca inclusion exacerbate the effects of Zn deficiency (Heth et al., 1966). While phytate is not considered a problem in most ruminant diets because microbes in the rumen possess phytase activity (Suttle, 2010), interactions with other

minerals and fiber can be detrimental to absorption and utilization of Zn (Arelovich et al., 2000). The rumen is one of the most complex environments on the planet, with billions of microorganisms competing for nourishment and synergistic relationships that benefit both parties. The added complexity of dietary ingredient also influences microorganism relationships (Coop and Kyriazakis, 1999). Additionally, study and characterization of rumen microorganisms is a complex procedure, adding another elusive layer to the mystery of rumen interactions. The challenge of dietary impacts on supplemental Zn is further complicated by Zn source and potential interactions within the rumen, not only with known antagonists but other components such as fiber.

Studies have been conducted to determine impacts of supplemental concentration and source on fiber digestibility and absorption of Zn as well as interactions with other minerals (Arelovich et al., 2000; Arelovich et al., 2014; Faulkner et al., 2017; Faulkner and Weiss, 2017; VanValin et al., 2018). Sources evaluated have included inorganic (sulfates, chlorides, oxides, hydroxy) and organic (chelates, amino acid complexed). VanValin et al. (2018) evaluated the effect of Zn sulfate, Zn methionine, and Zn hydroxychloride (supplemented at 40 mg Zn/d) on Zn retention and diet digestibility in lambs, and found Zn methionine decreased DM and NDF digestibility compared to Zn hydroxychloride. They suggested the two sources may have interacted differently within the rumen to change fiber digestibility. Furthermore, hydroxy minerals have been shown to support greater NDF digestibility when compared to sulfate (Faulkner et al., 2017), but this study also included hydroxy sources of Cu and Mn, therefore the results could be impacted by inter-mineral relationships.

Dietary fiber and Zn interactions have been evaluated in ruminant diets; however, these studies lack uniformity of dietary fiber source, Zn source, length of study, and biological type of animal, making conclusions as to how Zn impacts dietary fiber digestibility obscure. In a

study conducted in lambs fed 20 mg Zn/kg DM as Zn methionine or $ZnSO_4$ with wheat straw as the roughage source, cellulose and acid detergent fiber digestibility were shown to be decreased due to $ZnSO_4$ treatment, suggesting more soluble Zn in the rumen may have detrimental effects on fiber digestibility (Garg et al., 2008). In this same study, Zn retention was greater for the Zn methionine treatment compared to the $ZnSO_4$ treatment, indicating dietary fiber may have a similar detrimental effect on Zn retention when the source is more soluble in the rumen (Garg et al., 2008). Eryavuz and Dehority (2009) reported that when Zn concentrations of 50 μg/mL in rumen fluid were added to purified cellulose medium in vitro, cellulose digestion was decreased following 24 hr of incubation, and it was suggested that Zn has an inhibitory effect on enzymes secreted by cellulolytic bacteria to degrade cellulose. However, it was determined that the necessary concentration in the diet to achieve ruminal Zn concentrations at 50 μg/mL were approximately 2000 mg Zn/kg DM (Eryavuz and Dehority, 2009), four times the maximum tolerable level defined by NASEM (2016). Collectively, further research is necessary to more fully comprehend the dietary interactions in the rumen between Zn concentration and source, and dietary fiber or concentrate content and the resulting effects on dietary digestibility.

Dietary Energy

Dietary Energy History

Newton's first law of thermodynamics, the law of conservation, states that in a closed system energy cannot be created or destroyed but can be transformed. Based on this principle, Antoine Lavoisier created the world's first calorimeter and measured the heat produced by a guinea pig and the capacity of heat release to melt ice (Kleiber, 1961). According to Ferrell and Oltjen (2008) the second law of thermodynamics, is that all forms of energy can be quantitatively converted to heat, and the law of Hess, where products lost in

a chemical reaction are independent of the reaction, are the platform for all energetic measurements. Lavoisier also discovered combustion and oxidation, the basis for energy production, and determined the generic equation for aerobic respiration as $6O_2 + C_6H_{12}O_6 = 6CO_2 + 6H_2O$ + heat. Aerobic respiration is achieved through combustion, and heat production composes a large portion of energy production in the animal from feedstuffs (Lofgreen and Garrett, 1968). In ruminant animals, rate of fermentation, type of feedstuff, and rate of passage all contribute to this expenditure of energy (Geay, 1984). Metabolizable energy (**ME**) is defined as the sum of heat energy (**HE**) and retained energy (**RE**) and equates to the amount of energy not expended in urine, feces, or gasses lost. The measurement of HE and RE therefore can be used to assess ME for feedstuffs or a combination of feedstuffs in an animal. Energetic values of protein, carbohydrates and fats differ due to different metabolism within the body.

Dietary Energy Systems in Cattle

Body size of the organism is highly related to metabolic rate and more specifically surface area in relation to volume determines metabolic rate. Max Rubner devised a law in 1883 that states fasting metabolism decreases with increasing size of the animal. Due to difficulty of measuring animal surface area, an equation relative to body weight was established. Evaluation of heat production at maintenance across species led to the equation established by Kleiber (1972) and Brody (1945), where the allometric equation of fasting heat production equals *a* (the coefficient *a* being 70 when applied to young animals across species) multiplied by metabolic body weight (**W**) raised to 0.75. The equation $W^{0.75}$ was adopted in place of the initial equation and since has not been changed because no major errors occur with use (Guilbert and Loosli, 1951). However, some current systems sometimes implement changes in the coefficient to account for fasting heat production and maintenance

requirements (Geay, 1984; Van Amburgh et al., 2015). Nutrient Requirements of Beef Cattle (1984) established an in depth schematic relating to utilization in the ruminant animal describing the intake, digestibly, metabolizable, and recoverable energy. However, regardless of energy losses, evaluation factors such as intake, products of fermentation and their utilization, heat loss during fermentation, and feedstuff digestibility will improve accuracy of results for energy requirements and systems (Van Amburgh et al., 2015). Additionally, feedstuff substitution for established valued feedstuffs can improve accuracy, since a known value can be compared to the value being ascertained. It should be mentioned that all current evaluations of energy requirements and net energy are rooted in concepts provided by direct calorimetry work founded by Lavoisier. Regardless, rather than being concerned with the minutia of building or breaking down of living material, nutritionists are generally more concerned with the energy demands of the animal for normal function and maintenance, as well as gain, and discovering the best combination of feedstuffs to most effectively meet these demands.

The development of the California Net Energy System led to more accurate feeding standards for beef producers. Lofgreen and Garrett (1968) completed a comparative serial slaughter in over 200 steers and heifers fed with varying intake and roughage contents. The combination of the five trials completed for this study eventually led to the assignment of two values to a feedstuff, Net energy of Maintenance (**NEm**) and Net Energy of Gain (**NEg**; Lofgreen and Garrett, 1968). The development of this system was based on the principle presented by Kleiber (1961) that energy required for maintenance is greater than the energy required for gain. Additionally, it was shown that when animals were fed above maintenance requirements, the energy attributable to gain was linearly increased in relation to ad libitum feeding, suggesting the NEg from a feedstuff can be considered constant (Lofgreen and

Garrett, 1968). However, the NEm was shown to be more constant than NEg because it depends on the relatively constant heat production at basal metabolism (Kleiber, 1961). Therefore, net energy was assigned two values since NEg can vary depending on intake over maintenance, rather than assigning one value for feedstuffs. In this same study, the average NEm (Mcal) was expressed as 0.077 per $W^{0.75}$kg ($0.077W^{0.75}$) and this NEm value was found to be the same between heifers and steers, while the increase in requirement of NEg was shown to be greater in heifers (Lofgreen and Garrett, 1968).

Net Energy and Gain

The impact of energy intake on body composition has been extensively researched, and more specifically, the plane of nutrition or dietary energy content and resulting effects on body composition. A classical definition of growth in cattle according to Owens et al. (1995) is energy intake is presumed to control both rate and quantity of tissue accretion, and Oltjen and Garrett (1988) state that gain of beef cattle is directly related to the energy available for gain (Mcal/kg) and energy content of that gain. However, growth can be manipulated in many ways, namely by maturity, body size and prior plane of nutrition (Fox and Black, 1984). According to Byers (1980), both large and small mature sized cattle exhibited greater ADG when fed whole shelled corn vs. corn silage, indicating that a plane of nutrition/dietary energy effect existed regardless of mature body size. Furthermore, when growing heifers were fed isonitrogenous pellets containing 75% concentrate vs. 75% alfalfa, they produced less heat energy and retained more tissue energy, suggesting concentrate pellets were more efficiently utilized to provide dietary energy (Reynolds et al., 1991). Additionally, Byers (1980), Slabbert et al. (1992), and Old and Garrett (1987) all saw that restricting intake, and therefore dietary energy, reduced protein accretion but did not alter final body composition. Since body composition can be considered constant (Old and Garrett, 1987b), it can also be

interpreted that decreasing energy intake during development will increase mature body size and therefore weight at which cattle will achieve an empty body weight of 28% fat. This concept can be assessed in compensatory feedlot studies, where decreased energy density, greater roughage, and more days on feed in growing diets result in larger mature body weights, increase in protein accretion, and greater carcass transfer in cattle after placement on higher concentrate diets in the feedlot (Gill et al., 1993). In this study, steers weaned at 3.5 months and immediately placed into the feedlot had higher a percentage of fat and lower percentage of protein than steers entering the feedlot at 11.6, 15.4, and 17.4 months after being allowed to graze following weaning at 7.9 months old, suggesting steers allowed to graze during the growing period mature at heavier weights (Gill et al., 1993). Energy requirements depend on the proportion of fat in the gain, and therefore weights where cattle have similar body composition must be used to calculate energy requirements (Garrett et al., 1959). Collectively, steers similar in previous plane of nutrition, expected mature body size, and composition will gain at a similar rate when fed diets containing similar energy content (Owens et al.). Furthermore, manipulation of growth can occur with manipulation of energy content and current models allow beef producers to strategically target rates of growth with differing energy in the diet when used with groups of cattle of similar mature weight.

Growth Technology Impacts on Net Energy Requirements

The relationship of dietary energy and body composition is strongly correlated. According to Simpendorfer (1973) changes in body composition are strong indicators of nutritional adequacy of the diet. A growth curve of an animal is simply shown by plotting weight against age of the animal and is generally a sigmoidal shape, possessing a strong slope, inflection point, and plateau. The inflection point encompasses the end of greatest weight gain and beginning of slowing growth (Simpendorfer, 1973). This point occurs during

12 to 18 months in Hereford and Friesian breeds, generally during puberty (Berg and Butterfield, 1976). While body composition is heavily affected by energy, castration and hormonal implants will also have a marked effect on the growth curve. According to Forrest (1968) the combination of progesterone (200 mg) and estradiol-17-β-benzoate (20 mg) in implants could exert a synergistic effect and replace lost testosterone due to castration. In a study with genetically identical (cloned) beef steers, NEg requirements were reduced 19% with administration of either estrogenic (20 mg estradiol benzoate and 200 mg progesterone) or combination implants (120 trenbolone acetate and 24 mg estradiol 17-β; Hutcheson et al., 1997) improving efficiency, as well as shifting the growth curve upward when fed to 28% empty body fat. Additionally, a study conducting meta-analysis on 13 implant trials reported increased ME values of 4.2 and 3.1% for steers and heifers, respectively, regardless of implant regimen or type (Guiroy et al., 2002). Collectively, these studies show that implanting steers will shift their growth curve towards that achieved by bulls and shift the heifer growth curve upwards also, changing the necessary NEg requirements and making the animal more efficient. It is also pertinent to note that while implanting will shift the growth curve higher, it will also shift it right, increasing days on feed needed to achieve a uniform empty body fat of 28% (Hutcheson et al., 1997b). While β-AA have been shown to increase ADG and gain efficiency in feedlot heifers and steers in the last 28-42 d before harvest (Avendaño-Reyes et al., 2006; Walker et al., 2006; Gruber et al., 2007; Winterholler et al., 2007; Scramlin et al., 2010; Quinn et al., 2016), the increase in efficiency is minimal relative to the whole feeding period and likely will not change NEg during the life of the animal.

Regardless of growth technologies, cattle type has changed significantly from the early studies of NE requirements. According to Capper (2011) ADG has increased 44% since 1977 while using 81.4% of feedstuffs, meaning quality of the feed provided has increased, cattle

efficiency has increased, or more likely a combination of the two. Additionally, cattle today are generally more Angus-influenced rather than Hereford or Brahman type, and have heavier carcass weights (Capper, 2011). Regardless, improvements in energy efficiency are due to increased yield per animal, and will dilute the energy cost of maintenance (Brody, 1939). Increases in efficiency of cattle currently call into question the so-called "price" of efficiency, namely the impact or change on metabolic processes to support energy utilization in the animal and the dietary requirements necessary to support this increase in growth.

Beta-Adrenergic Agonists

Beta-Adrenergic Agonist use in Cattle

Previous studies have shown that a decrease in muscle growth and increase in fat accretion in feedlot cattle occurs with increasing empty body weight (Fox and Black, 1984). This shift towards fat accretion during the finishing period, generally as subcutaneous fat, decreases efficiency and productivity of the animal and represents a loss to the producer. Beta-adrenergic receptor agonists (**ß-AA;** i.e. ractopamine hydrochloride, zilpaterol hydrochloride) are phenethanolamines (a trace amine with a similar structure to the catecholamine neurotransmitters norepinephrine and epinephrine) acting on the ß-adrenergic receptor (**ß-AR**). Beta-adrenergic receptor agonists are termed as repartitioning agents because they share a similar structure with two catecholamines present in the body, epinephrine and norepinephrine, that act on the ß-AR.

The ß-AR through which ß-AA elucidate their response is a G-protein coupled receptor (**GPCR**), containing seven hydrophobic transmembrane domains (Liggett, 2002). Activation of β-AR results in decreased protein degradation and increased protein synthesis and lipolysis. The ß-AR contains three extracellular segments at the N-terminus and three intracellular segments associated with the C-terminus (McGraw and Liggett, 2005). Two

subtypes of ß-AR are in skeletal muscle, $ß_1$ and $ß_2$, while the $ß_2$ is the dominant isoform expressed in skeletal tissue (Kim et al., 1991). Currently, two phenethanolamines are approved in the United States for supplementation in feedlot animals, zilpaterol hydrochloride ($ß_2$-adrenergic agonist) or ractopamine hydrochloride ($ß_1$-adrenergic agonist).

The effect of ß-AA in cattle have been well documented (Avendaño-Reyes et al., 2006; Gruber et al., 2007; Winterholler et al., 2007; Scramlin et al., 2010; Quinn et al., 2016). In a small pen study assessing supplementation of zilpaterol hydrochloride (60 mg·steer^{-1}·d^{-1}) and ractopamine hydrochloride (300 mg·steer^{-1}·d^{-1}) in feedlot steers for the last 33 d on feed, while not differing between β-AA improved ADG by 26 and 24%, respectively, compared to control, while G:F improved from ß-AA supplementation versus control (0.253 and 0.248, respectively, vs. 0.185; Avendaño-Reyes et al., 2006). Another study comparing zilpaterol (75 mg·steer^{-1}·d^{-1}) and ractopamine hydrochloride (200 mg·steer^{-1}·d^{-1}) supplementation in feedlot steers for the last 33 d on feed saw similar improvements in performance measures compared to control, while zilpaterol decreased ADG, average daily feed intake, and final BW, but increased HCW compared to ractopamine (Scramlin et al., 2010). In a large pen study, ractopamine hydrochloride as either Optaflexx (Elanco Animal Health, Greenfield, IN) or Actogain (Zoetis, Kalamazoo, MI) added an additional 1.5% BW, 15% ADG, 17% G:F, and 2% HCW (Quinn et al., 2016). Gruber et al. (2007) investigated the impact of 200 mg·steer^{-1}·d^{-1} ractopamine hydrochloride supplementation on feedlot steers differing in biological type (British, Continental crossbred, and brahman) and saw similar results for performance across biological type, improving ADG (15%), G:F (17%), HCW (1.6%), and REA (2.8%). Additionally, Bohrer et al. (2014) saw a 16% improvement in ADG and G:F and 1.87% improvement in HCW of feedlot steers fed 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride for the final 35 d on feed. Collectively, it is clear that ß-AA supplementation

improves performance in feedlot steers. Although speculation still remains on the exact mechanisms by which ß-AA impact growth, multiple studies have sought to uncover the general route of action. In a broad sense, it is understood that ß-AA increase protein accretion and decrease protein degradation in skeletal muscle tissue.

Mode of Action

Mechanistically, the GPCR is stimulated by extracellular catecholamines or phenethanolamines, leading to binding of the heterotrimeric signal transduction elements (the α, ß, and γ subunits of G-proteins). The α subunit will dissociate and activate adenylate cyclase (**AC**) to release the secondary messenger cyclic adenosine monophosphate (**cAMP**) by hydrolyzing ATP. Cyclic-AMP will then bind to Protein Kinase A (**PKA**), leading to phosphorylation of serine residues on hormone sensitive lipase (Miyoshi et al., 2006) downstream activation of PKB/Akt and mTOR pathways (Sneddon et al., 2001a; Berdeaux et al., 2007; Kline et al., 2007), and depression of the Ca^{2+} proteolysis and ATP/Ubiquitin pathways (Higgins et al., 1988; Parr et al., 1992; Costelli et al., 1995; Harcourt et al., 2007a; Kline et al., 2007; Lynch and Ryall, 2008a; Ryall and Lynch, 2008a).

Rothwell et al. (1987) noted that in rats supplemented with clenbuterol (a $ß_2$-adrenergic agonist) blood flow increased in white and brown adipose tissue and adipose tissue concentration was reduced by 80% in skeletal muscle, suggesting treatment reflects increases in lipolytic activity or increase in body temperature due to brown adipose tissue activity. To more thoroughly illustrate this downstream action, β-AR activation of PKA results in phosphorylation of perilipin A (**PA**), a lipid droplet associated protein, and hormone sensitive lipase (**HSL**), the main lipase associated with adipocytes (Miyoshi et al., 2006). When PKA phosphorylates HSL, the catalytic activity of this enzyme increases and promotes translocation of HSL from the cytosol to the lipid droplet (Brasaemle et al., 2000; Egan et al.,

2006). It has been suggested that perilipin is a key regulator of basal and PKA regulated lipolysis, acting as a barrier between HSL and the lipid droplet during basal lipolysis to limit HSL action and is necessary for HSL translocation into the lipid droplet (Sztalryd et al., 2003); however, it has been shown that phosphorylation by PKA is not necessary for translocation of HSL to the lipid droplet but necessary for the lipolytic action of the lipid droplet associated HSL in adipocytes (Miyoshi et al., 2006). Hormone sensitive lipase is involved in lipolysis of triglycerides within adipose tissue, releasing non-esterified fatty acids (**NEFA**) and glycerol into the bloodstream. These free fatty acids are then available in the bloodstream for uptake by the target tissue, in this case skeletal muscle, to be broken down via β-oxidation within the mitochondria via the TCA cycle to produce ATP. The glycerol released from lipolysis is transported to the liver, converted to glucose, and transported to muscle tissue.

The administration of β-AA is well known to increase protein synthesis in muscle. This hypertrophy results from activation of the β-AR/AC/cAMP and PKA pathway, which initiates transcription of proteins and thus protein synthesis (Berdeaux et al., 2007). Protein kinase A is able to passively diffuse into the nucleus following activation by cAMP, which will regulate transcription of multiple genes by phosphorylating the cAMP response element binding protein (Berdeaux et al., 2007)**.** Additionally, according to Kline et al. (2007) β-AR promote protein synthesis by acting on the PKB/Akt signaling pathway, which leads to activation of several pathways involved in protein synthesis, gene transcription, cell proliferation and survival. Downstream of this pathway, β-AA induced hypertrophy is associated with phosphorylation of both $p70^{s6k}$ and the e1F4E binding protein 1 (Sneddon et al., 2001b). Additionally, Kline et al. (2007) noted activation of mammalian target of rapamycin (**mTOR**) through the PKB/Akt pathway with administration of clenbuterol in rats

for 14 d. This mTOR activation was also shown to be differentially regulated within fast and slow twitch muscle fibers, and when rapamycin was introduced, inhibited clenbuterol activation of mTOR in fast twitch muscle fibers (Kline et al., 2007).

Muscle fiber types are classified according to their contractile and metabolic qualities: type I slow twitch-oxidative, type II fast twitch-glycolytic (including three subtypes as IIa, IIb, and IIx), with fast or slow twitch being so named according to the myosin heavy chain (**MHC**) ATP hydrolysis capacity and succinate-dehydrogenase based histochemistry (Bär and Pette, 1988; Bortolotto et al., 2017). This suggests hypertrophy induced by β-AA is differentially regulated and induced between fast and slow twitch muscle fibers. Not only are fast twitch type muscle fibers more susceptible to β-AA administration, the transition of slow to fast type muscle fiber generally occurs with treatment of a β-AA (Zeman et al., 1988; Ryall et al., 2007). Due to the relative plasticity of skeletal muscle, fiber type can change with a variety of activities, including physical activity, electrical stimulation, regeneration after injury and age (Gutmann and Hanzlíková, 1966; Geiger et al., 2001; Houmard et al., 2017)

According to Lynch and Ryall (2008) the major mechanisms by which protein degradation is decreased during ß-AA use are the Ca^{2+} dependent proteolytic and ATP/ubiquitin pathways. The Ca^{2+} dependent proteolysis pathway involves a family of cysteine proteases called calpains (μ-calpain, m-calpain, and muscle specific calpain-3) which are regulated by kinases and the calpain-specific inhibitor calpastatin (Sorimachi et al., 1989; Navegantes et al., 2002; Costelli et al., 2005). In skeletal muscle some but not all calpains are generally associated with the Z-disc of sarcomeres and are activated by an intracellular messenger, Ca^{2+}, to begin the degradation of contractile proteins (Bullard et al., 1990). As a regulatory system for protein degradation, the Ca^{2+} dependent proteolysis

pathway has been a well-developed area of study for β-AA mechanism of action. A two-fold increase in calpastatin was seen in the latissimus dorsi muscle following administration of clenbuterol in lambs and cimaterol in Friesian steers for 6 and 16 weeks, respectively (Higgins et al., 1988; Parr et al., 1992). This suggests calpain activity following β-AA supplementation is regulated by increased calpastatin activity, decreasing contractile protein degradation. Additionally, according to Hawkins et al. (1995) PKA dependent phosphorylation of calpastatin and some sarcoplasmic reticulum regulatory proteins are necessary for β-AA effects on Ca^{2+} dependent proteolysis. Furthermore, the calpastatin gene contains a cAMP response element motif in the promoter region, suggesting PKA activation by the β-AR cascade phosphorylates the CREB, promoting calpastatin transcription (Sensky et al., 2006).

The ATP dependent pathway of ubiquitination has also been implicated in decreased protein turnover due to β-AA. Proteins destined for ubiquitination are tagged by the 26S proteasome, ubiquitin is bound by the ubiquitin binding enzyme, and transferred to a ubiquitin conjugating enzyme, which then binds ubiquitin binding enzyme and ubiquitin ligase proteins (Ryall and Lynch, 2008b). The ubiquitin ligase proteins are then responsible for transferring multiple ubiquitin proteins to the protein targeted for degradation. One of the first studies to examine the effects of a β-AA on the ubiquitination pathway was with rats receiving clenbuterol subcutaneously for 8 days, resulting in a 2-3-fold increase of ubiquitin mRNA (Costelli et al., 1995). Further studies have confirmed this (Harcourt et al., 2007b; Kline et al., 2007) and have also seen a greater response to ubiquitin fold increases in fast twitch vs. slow twitch muscle fibers (Yimlamai et al., 2005). Additionally, some studies have shown that the 26S proteasome involved in ubiquitination is down-regulated by β-AA administration (Busquets et al., 2004; Yimlamai et al., 2005). Collectively, β-AA

administration slows protein degradation to the point where protein synthesis exceeds the degradation process, resulting in net protein accretion.

Zinc and Growth Pathways

Interactions between Zn and the β-Adrenergic Agonist Cascade

Constant exposure to a ß-AA is necessary to cause an increase in cAMP release, since this cascade is transient. However, increased and constant exposure to a ß-AA will eventually lead to desensitization of the receptor, lessening the impact of a supplemental ß-AA on muscle growth (McElligott et al., 1989). In rats injected with clenbuterol for 18 d, a 50% reduction in receptor density was seen with no change in the ratio of $ß_1$-AR to $ß_2$-AR, suggesting desensitization is not receptor type specific (Rothwell et al., 1987). Abney et al. (2007) confirmed this in feedlot steers, where a quadratic effect was noted with increasing duration of ractopamine hydrochloride, where ADG increased 14.8% in cattle up to 35 d on ractopamine hydrochloride relative to those receiving no ractopamine hydrochloride, but did not increase further through 42 d. In the same study, increasing supplemental concentration of ractopamine hydrochloride from 100 $mg \cdot steer^{-1}d^{-1}$ to 200 $mg \cdot steer^{-1}d^{-1}$ diminished the maximal values observed for ADG and G:F from 42 to 35 d (Abney et al., 2007) suggesting greater concentrations of supplemental ractopamine hydrochloride will decrease effectiveness of the ß-AA more quickly through desensitization. Currently, ractopamine hydrochloride is approved to be fed the last 28-42 d on feed with no withdrawal period before slaughter. As mentioned previously, the ß-AR cascade that causes protein accretion and lipolysis involves the hydrolysis of ATP to cAMP (**Figure 2**). During desensitization, the enzyme phosphodiesterase (**PDE**) prohibits prolonged stimulation of the ß-adrenergic GPCR by degrading cAMP (Morris and Malbon, 1999). Previous research in vitro and in rodents has shown an increase in circulating cAMP via inhibition of PDE by Zn (von Bülow et al.,

2005; Haase and Rink, 2007; Hojyo et al., 2011) suggesting an influence on the ß-AA cascade. Moreover, SLC39A14 Zn transporter (**ZIP14**) knock out mice exhibited decreased growth and impaired gluconeogenesis, caused by increased action of PDE and decreased cAMP concentrations (Hojyo et al., 2011), suggesting ZIP14 transport of Zn may be involved in attenuating the PDE action on cAMP. Recent research has shown a trend for a linear increase in circulating cAMP with increasing supplemental concentrations of Zn as Zn-Amino Acid complex (Genther-Schroeder et al., 2016), possibly because of the aforementioned Zn inhibition of PDE. Overall, supplementation of greater concentrations of Zn during β-AA supplementation could in theory extend the period that β-AA are effective at increasing protein synthesis and decreasing degradation. Further studies should be conducted to determine the extent that increasing supplemental Zn concentrations may impact β-AA efficacy.

Additionally, other recent research has shown an interaction of Zn with AC, the enzyme activated within the ß-AR cascade by the G-protein α subunit. Klein et al. (2002) reported that $ZnCl_2$ in cell culture inhibited AC activity of the 1, 5 and 6 isoforms; however, AC2, an isoform specific to type II fast twitch glycolytic muscle fibers (Hanoune and Defer, 2001; Berdeaux and Stewart, 2012), exhibits a distinct dose response curve where action increases with increasing concentrations up to 10 μM. Adenylate cyclase activation leads to increased lipolysis and protein synthesis through downstream cAMP release, and cAMP signaling participates in muscle precursor cell differentiation, migration, and fusion to repair muscle and regenerate adult skeletal muscle. Additionally, Berdeaux and Stewart (2012) show that increasing activation of AC leads to a shift from oxidative to more glycolytic activity in muscle fibers as well as increasing myofiber size. Hergenreder et al. (2016) demonstrated in semimembranosus muscle that steers fed 750 mg/d of Zn as Zn methionine possessed

increased MHC II protein abundance over control. The MHC II is most prevalent in fast twitch glycolytic muscle fibers and Hergenreder et al. (2016) also observed an increased fiber cross-sectional area of MHC-IIA and MHC-IIX. The study by Hergenreder et al. (2016) is one of the only studies to look at muscle fiber type in cattle following Zn supplementation and suggests a positive effect of Zn methionine on transition of type I slow twitch to type II fast twitch fibers. This study also concluded that Zn methionine supplementation led to a greater percentage of oxidative type I muscle fibers over control; however, there were no treatments without the inclusion of zilpaterol hydrochloride, and it was hypothesized that overstimulation of $ß_2$-adrenergic receptors with this combination led to the transition to more type I oxidative fibers (Hergenreder et al., 2016). Regardless, further studies need to be conducted to determine the mechanism by which supplemental Zn influences skeletal muscle fiber type and if the activation of AC by increased supplemental Zn occurs in feedlot cattle.

Furthermore, Hanoune and Defer (2001) suggest that AC2 is increased following differentiation, suggesting a critical time to supply adequate Zn for cell growth. Protein Kinase C (**PKC**), an activator of AC2, contains Zn-finger structures important for activity and Zn supplementation induces membrane translocation of PKC (Hanoune and Defer, 2001), and therefore possible activation of AC2, which potentially explains the increases seen in cAMP concentrations with Zn supplementation (Genther-Schroeder et al., 2016) or Zn application in vitro (Klein et al., 2002). If Zn supplementation inhibits PDE, and therefore increases cAMP action downstream of AC, more type II muscle fibers may be transitioned from type I slow twitch oxidative muscle fibers. Increasing type II muscle fibers theoretically would increase the quantity of the type II muscle fiber specific AC2 isoform, creating an opportunity for Zn supplementation to further improve muscle growth through activation of this cascade. As aforementioned, β-AR promote protein synthesis by acting on the PKB/Akt

signaling pathway (Kline et al., 2007). A role for the Zn importer ZIP7 in myoblast differentiation through activation of the P13K/Akt pathway has been established, whereby impairment of ZIP7 reduced P13K/Akt activation, decreased the number of multinucleated myofibers, and decreased myotube development (Mnatsakanyan et al., 2018) suggesting import of Zn from the endoplasmic reticulum may be necessary for muscle hypertrophy. Overall, activation of the β-AR cascade may improve due to inhibitory action of Zn on PDE and a concurrent activation of AC2. However, as Zn may elicit further action of the β-AA cascade and therefore increase the amount of cAMP, the inhibitory feedback mechanisms could cause desensitization sooner due to increased action of the β-AR cascade. Regardless, research in vitro and vivo warrants further investigation into the possible interactions of Zn and the β-AA cascade in feedlot animals.

Zinc and Anabolic Implants

One growth promoting technology used prolifically (Samuelson et al., 2016) in the beef industry is estrogenic, androgenic, and combination implants. In a large pen study, a combination implant containing 140 mg trenbolone acetate and 28 mg estradiol utilized in feedlot steers for 140 to 168 d increased ADG and G:F 18 and 10%, respectively, over non-implanted steers, effectively improving growth performance (Bartle et al., 1992). These anabolic implants induce growth by activating the GH/IGF-1 pathway (Johnson et al., 1996; Johnson et al., 1998). Zinc is a critical part of the GH and IGF-1 pathway by acting on protein tyrosine phosphatases. Li and Maret (2009) found that during cell growth and replication, two phases of growth exhibited accompanying peaks in Zn concentrations; the fluctuations were found to modulate phosphorylation signaling, where low intracellular Zn concentrations inhibited protein tyrosine phosphatases for DNA synthesis, effectively halting cell proliferation. Hyperplasia, hypertrophy, and wound healing all rely heavily on cell

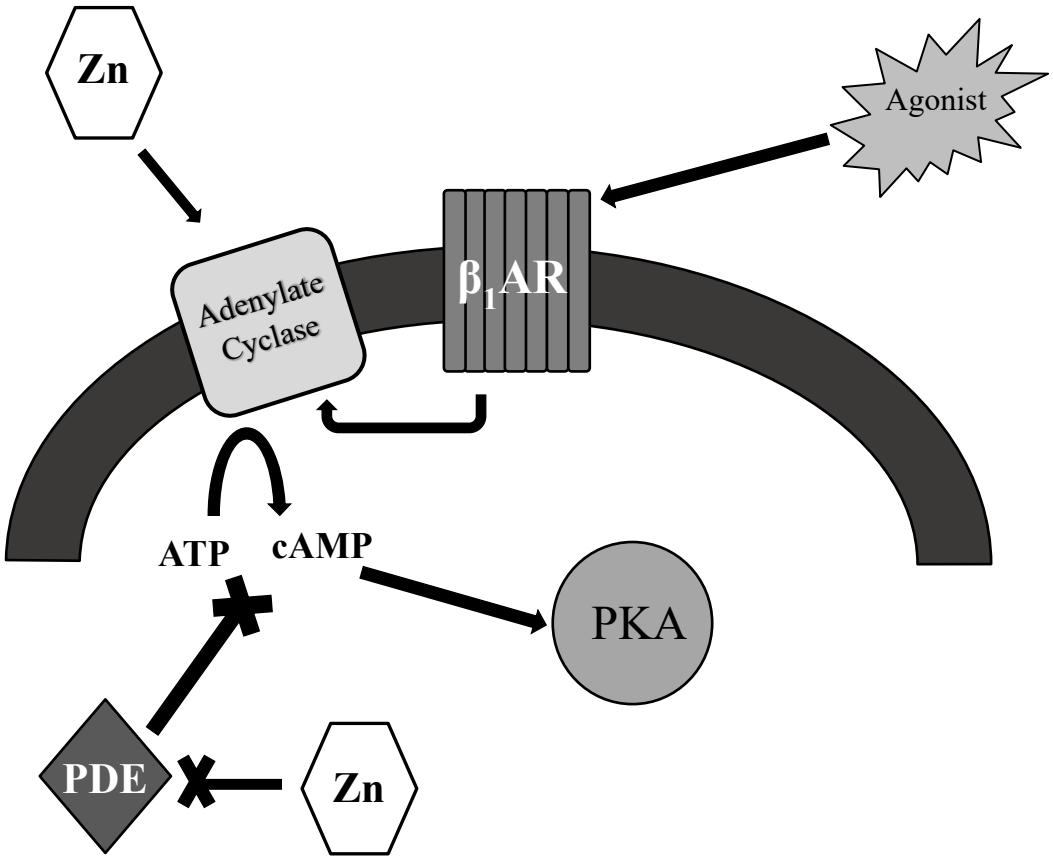

Figure 2. The β-adrenergic agonist cascade. Figure adapted from Hojyo et al. (2011) showing the ß-AA cascade as well as the possible interactions with Zn. Lines ending with an arrow indicate a positive effect while lines ending with a cross suggest an inhibitory effect.

proliferation and growth (Chiakulas and Pauly, 1965; Goldberg et al., 1975), making Zn instrumental in growth. This control of DNA synthesis demonstrates that cellular Zn homeostasis is extremely important for proper cellular function.

During hypertrophy of the muscle cell, muscle growth can be limited by the amount of DNA present in the cell to support protein. Satellite cells are responsible for donation of nuclei to support muscle growth. However, satellite cell number declines over the lifetime of the animal, resulting in less contribution of DNA for hypertrophy. Pax7, a paired-box transcription factor, plays a key role in myogenesis during post-natal growth and muscle

generation in adult muscle (Berdeaux and Stewart, 2012; Maltzahn et al., 2013) and loss of expression compromises satellite cell proliferation. Pax7 is also important in cell survival in general, as deletion of Pax7 results in apoptosis (Buckingham and Relaix, 2015). Beginning in the late fetal stage, satellite cells expressing Pax7 begin to accumulate under the basal laminae in muscle fibers which contribute to hypertrophy and muscle cell regeneration postnatally (Buckingham and Relaix, 2015). Hergenreder et al. (2016) demonstrated muscle satellite cell culture from steers treated with 720 mg·steer^{-1}·d^{-1} as Zn methionine possessed increased expression of Pax7 in satellite cells, suggesting the animals treated with Zn methionine had the ability to increase total nuclei through proliferation and differentiation of the Pax7 satellite cells. Zac1, a zinc-finger protein involved in regulation of apoptosis and cell cycle arrest, is expressed in satellite cells and activated by Pax7 binding to a novel binding motif on Zac1, demonstrating Zac1 is a direct target gene during myogenesis (Yang et al., 2018). The ghrelin receptor family A receptor ZnR/GPR39 (previously believed to exist as two separate receptors) is activated by Zn and has been shown to be involved in adipocyte and myoblast proliferation and differentiation (Yang et al., 2018). GPR39 has been demonstrated to be exclusively regulated by Pax7 through activation of Zac1. Additionally, the Zac1/ZnR/GPR39 system downstream promotes the formation of type II fast twitch muscle fibers. This signaling cascade, particularly the ZnR/GPR39 receptor, has been shown to be activated by extracellular changes in Zn, and may also be affected by endogenous sources of Zn, such as that stored in the vesicles (Hershfinkel, 2018).

Zinc clearly has a role in proliferation and maintenance of muscle tissue, and as mentioned previously, has multiple support roles for growth of feedlot cattle. In a study completed by Niedermayer et al. (2018), implanted cattle (Component TE-IS [16 mg estradiol and 80 mg trenbolone acetate] on d 0 and component TE-200 [20 mg estradiol and

200 mg trenbolone acetate] on d 56) receiving current industry mineral supplementation rates (Samuelson et al., 2016) demonstrated increased HCW over NASEM (2016) recommendations or unsupplemented controls; however, multiple TM were changed in this study so a synergistic effect may have been present. Implants act through activation of the GH-IGF-1 axis, and according to Hojyo et al. (2011), impaired GH synthesis occurs in ZIP14 knockout mice, possibly due to decreased stimulation of the GH releasing hormone receptor, a GPCR located on pituitary somatotroph cells. Decreased pituitary concentrations of cAMP and Zn were seen in ZIP14 knock out mice, and when a growth hormone releasing hormone (**GHRH**) bolus was introduced, the ZIP14 knockout mice had a smaller subsequent increase in circulating GH (Hojyo et al., 2011). In conclusion, Zn may be considered an integral piece necessary for skeletal muscle fiber hypertrophy through involvement in the GH/IGF-1 axis, DNA synthesis, and Pax7 proliferation in feedlot animals. Since these processes have been shown to be essential for growth induced by anabolic implants, greater Zn supplementation strategies may be necessary to support the rapid growth seen during this time.

While Zn has been established to be involved in multiple growth processes in rodents and cell culture, information on the influence of supplemental Zn concentrations on these growth processes in ruminants is lacking. Although improvements in performance have been seen following use of ractopamine HCl and Zn supplementation together, the mechanisms behind improvements in growth are poorly understood. Additionally, while growth processes initiated by β-agonists have been shown to involve Zn, previous literature in ruminants has not investigated effects on these processes due to greater supplemental Zn concentrations. Therefore, this research aims to improve the understanding of the influence of greater supplemental Zn concentrations on growth processes during periods of rapid growth induced either by ractopamine HCl or differing dietary energy.

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CHAPTER 3.

THE INFLUENCE OF SUPPLEMENTAL ZINC AND RACTOPAMINE HYDROCHLORIDE ON TRACE MINERAL AND NITROGEN RETENTION OF BEEF STEERS

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Abstract: The study objective was to determine if N retention was improved with supplemental Zn above NRC concentrations with or without ractopamine hydrochloride inclusion. Angus crossbred steers (n = 32, 485 ± 26 kg BW) with Genemax gain scores of 4 or 5 were utilized in a 2×2 factorial arrangement (8 steers/treatment). Steers were blocked by BW to a finishing diet with one of two mineral supplementation strategies (**ZNTRT**), no supplemental Zn (analyzed 32 mg Zn/kg DM; **CON**) or supranutritional Zn (CON + 60 ppm $ZnSO_4$ + 60 ppm Zn-amino acid complex; analyzed 145 mg Zn/kg DM; **SUPZN**), fed 56 days in pens equipped with Growsafe bunks and assigned to beta agonist (**BA**) supplementation strategies of 0 (**NON**) or 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (**RAC**) fed the last 30 d before harvest. Initial 56 d ADG was not affected by ZNTRT (P = 0.66) but DMI was greater in CON vs. SUPZN (P < 0.01). On d 56 (d 1 of BA supplementation), steers (4 groups; 8 steers/group; 2 steers/treatment) were moved to metabolism crates and adapted for 10 d, followed by 5 d of total fecal and urine collection. Total retention of Zn, Mn, Fe, Cu, and N were calculated. Data were analyzed as a 2 x 2 factorial arrangement, with group as a fixed effect and the three-way interaction of ZNTRT × BA × group as random. No interactions between ZNTRT and BA were noted for any data (P

≥ 0.19). Collection DMI did not differ among treatments ($P \geq 0.23$); however, Zn intake was lesser in CON vs. SUPZN ($P < 0.01$). Fecal and urinary Zn excretion and Zn and Mn retention were lesser in CON vs. SUPZN ($P \leq 0.03$); however, Zn retention was not different between NON and RAC ($P = 0.43$). Retention of Cu and Fe were unaffected by strategies ($P \geq 0.49$). Urine output and urine N excretion were greater in NON vs. RAC ($P \leq 0.05$). Nitrogen retention (as percent of N intake) was lesser ($P = 0.05$) in CON (40.0%) vs. SUPZN (44.3%) and lesser ($P = 0.02$) in NON (39.5%) vs. RAC (44.8%). Zinc and N retention were found to be positively correlated ($r = 0.46$, $P < 0.01$). Average daily gain and G:F across the 86 d trial were lesser in NON vs. RAC ($P < 0.03$). Overall, SUPZN appears to improve N retention, suggesting that increasing dietary Zn may be important for cattle growth beyond that induced by ractopamine hydrochloride.

Keywords: beef cattle, nitrogen, ractopamine hydrochloride, zinc

Introduction

The trace mineral Zn is critical in numerous biological growth processes. Current recommendations of 30 mg Zn/kg DM were established more than forty years ago to prevent deficiency in healthy animals and support growth (National Academies of Sciences, Engineering, and Medicine, 2016); however, beef cattle ADG from birth to slaughter has increased by 44% since 1977 (Capper, 2011). Genetics, improved management practices, and growth technologies have all contributed to this increase in cattle growth. Ractopamine hydrochloride (HCl), a ß-adrenergic agonist, increases animal growth rates when fed 28 to 42 d prior to harvest (Ricks et al., 1984; Abney et al., 2007; Gruber et al., 2007), and is one reason for continuous improvement in cattle growth performance in the United States. In swine, increasing concentrations of lysine in the diet improves growth (Ross et al., 2011) and

improvements in protein utilization regardless of CP content (Xiao et al., 1999) have been seen when ractopamine HCl is fed.

Nitrogen retention has been shown to increase in Holstein steers receiving supplemental ractopamine HCl (Walker et al., 2007), and Zn has long been established as critical in protein utilization in the body (Oberleas and Prasad, 1969; Somers and Underwood, 1969; Greeley et al., 1980). Genther-Schroeder et al. (2016a,b) reported that in steers receiving ractopamine HCl, supplementation of Zn at 150 mg Zn/kg DM increased growth performance compared to steers receiving 90 mg Zn/kg DM. In these studies, additional growth responses to Zn were only noted in cattle receiving ractopamine HCl, suggesting an interaction between ractopamine HCl action and Zn may exist. However, it is unclear if this is an interaction in the ß-adrenergic signaling cascade, or if Zn requirements are increased due to greater growth rate induced by ractopamine HCl.

The objective of this study was to ascertain the impacts of ractopamine HCl and dietary Zn supplementation on N and Zn retention in beef steers. The hypothesis was that supranutritional supplementation of Zn would increase both Zn and N retention in steers consuming ractopamine HCl.

Materials and Methods

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (8-15-8073-B).

Experimental Design

Cattle consisting of 75% or greater Angus genetic background from two separate sources were acquired and gentled for 1 month prior to application of initial treatment.

Gentling steers consisted of frequent handling, haltering with rope halters, and decreasing flight zone of the animal. The study was conducted as a 2 × 2 factorial, with mineral (**ZNTRT**) supplementation strategies of no supplemental Zn (analyzed 32 mg Zn/kg DM; **CON**) or supranutritional Zn (CON + 60 ppm $ZnSO_4$ + 60 ppm Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM; **SUPZN**) beginning on d 0, and beta agonist (**BA**) strategies of 0 (**NON**) or 300 $mg \cdot^{-1} steer \cdot^{-1} d$ ractopamine HCl (**RAC;** Actogain45, Zoetis, Parsippany, NJ) beginning on d 56. Steers (n = 32; 485 ± 26 kg) with Genemax gain scores of 4 or 5 (Zoetis), indicating the top 40% in growth potential of Angus cattle, were utilized in this study. Steers were separated into four groups (n = 8; 2 per treatment combination) and stagger-started on diets to accommodate space limitations in the metabolism facility. On d 0 for each group, steers were blocked by BW and Genemax scores to receive ZNTRT diets for 56 d in pens equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Diet composition and analysis is shown in **Table 1**. On d 0 of the study, steers were implanted with Component TE-IS with Tylan (80 mg trenbolone acetate, 16 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health, Greenfield, IN). Steers were weighed prior to feeding on d -1 and 0 and 55 and 56 to determine initial weights and final weights of the feedlot period, respectively. After weighing on d 56, steers were transported 6.3 km to the metabolism facility in Kildee Hall (Iowa State University, Ames, IA). Steers continued to receive their respective ZNTRT diets in the metabolism facility from d 56-71 (d 0-15 of metabolism period). Within ZNTRT strategy steers were randomly assigned to BA strategies fed from d 56 to 84.

Metabolism Period

From d 56 to 71 (d 1 to 10 adaptation, d 11 to 15 collection) steers (570 ± 30.9 kg BW) were housed in individual (213.4 cm [length] × 182.9 cm [height] × 91.4 cm [width]) stainless steel crates with rubber fatigue mats. Each morning, steers were offered the appropriate BA supplement (either 0.226 kg dried distillers grains without ractopamine HCl or 0.226 kg premix of dried distillers grains with ractopamine HCl included at 300 $mg \cdot steer^{-1} \cdot d^{-1}$, respectively) along with 1.362 kg of the appropriate ZNTRT total mixed ration (**TMR**). The initial offering of TMR and BA supplement were consumed by steers before remaining daily TMR was offered. Feed delivery was 110% of the previous days intake. All offered feed and refused feed for each steer was recorded daily, and daily intake was determined by subtracting refused feed from offered feed. During the acclimation period, cattle adjusted to crates and allowed space to lie down. On the morning of d 10 (d 66 of study) of metabolism period, cattle were removed from crates and crates were thoroughly cleaned. Preparation of metabolism crates prior to return of the steers, as well as daily fecal and urine collection procedures were as described by Pogge et al. (2014).

During the collection period (d 11 to 15; d 67 to 71 of study), feed orts were removed and weighed, and aliquots (≥ 600 g, as-fed) were collected. Control and SUPZN TMR as well as NON and RAC premixes were sampled daily. All feed and orts samples were dried in a convection oven at 70ºC for 48 h. Fecal and urine aliquots were collected as described by Pogge et al. (2014) with the modification of a 3% aliquot of daily urine output. Determination of fecal DM was achieved according to procedures described by Pogge et al. (2014). Dried fecal, TMR, and orts samples were subsequently ground through a 2mm screen

(Wiley Mill; Thomas Scientific, Swedesboro, NJ; Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and stored in airtight bags until analysis.

On d 15 (d 71 of study) of the metabolism period, steers were removed from metabolism crates and transported 6.3 km to the Iowa State Beef Nutrition Farm, where they continued to receive their respective diets from d 71 to 86 of study. Prior to fecal collection and subsampling on d 71, crates were hand-scraped with acid washed plastic paint scrapers and deionized water to collect all remaining feces excreted during the collection period.

Pre-feeding weights were collected on d 85 and 86 to determine final BW. A 4% pencil shrink was applied to all live weights recorded during the trial. Steers were harvested at a commercial abattoir (Iowa Premium Beef, Tama, IA) and individual animal ID was maintained throughout slaughter. Liver samples were collected at harvest for analysis of mineral content.

Analytical Procedures

Total mixed ration samples of each diet were collected weekly during the feedlot period, and after steers returned to the feedlot. Samples were dried for 48 h at 70°C and the resulting DM value was multiplied by as-fed feed intake to determine individual steer DMI during the feedlot period. Dry matter and organic matter of feed, orts, and fecal matter were determined according to AOAC (1990) procedures. Nitrogen content of feed, orts, fecal matter, and urine was determined using the combustion method (TruMac N, LECO Corporation, Saint Joseph, MI; Lundy et al., 2015). Nutrient digestibility (DM, OM) was calculated as described by Pogge et al. (2014). Nitrogen digestibility was calculated as described by Lundy et al. (2015).

Dried, ground, and composited feed, orts, and fecal samples were acid digested prior to mineral analysis according to the methods described by Richter et al. (2012) and Pogge et al. (2014). Liver samples were digested according to Pogge and Hansen (2013). Urine samples were diluted 1:2 with 2% trace metal grade nitric acid for the analysis of Cu, Fe, Mn, and Zn. No additional dilutions were necessary for mineral analysis of feed, orts, or fecal matter for Cu, Fe, Mn, and Zn. Mineral analysis was conducted using inductively coupled plasma optical emission spectrometry (ICP; Optima 7000 DV, Perkin Elmer, Waltham, MA). A bovine liver standard from the National Institute of Standards and Technology (Gaithersburg, MA) was utilized to verify instrument accuracy and yttrium (Inorganic Ventures, Christianburg, VA) was used as an internal standard to account for any variation in sample introduction within a run.

Analyzed trace mineral concentrations (mg/kg or mg/L) were multiplied by total amounts of feed, orts, fecal matter and urine, to determine total mineral content of each. Mineral intake was calculated by subtracting mineral refused from mineral consumed during the collection period. Daily mineral intake, fecal mineral output, and urine mineral output were determined by dividing total mineral content of each by number of days of collection. Apparent absorption was determined by subtracting fecal mineral content from mineral intake, dividing by mineral intake and multiplying by 100. Mineral retention was determined by subtracting excreted mineral (urine and fecal mineral) from mineral intake. Mineral retention as a percentage of intake was determined by subtracting retained mineral from consumed mineral, divided by mineral intake, and multiplying by 100.

Statistical Analysis

All data were analyzed as a randomized complete design. Growth and intake data for the initial 56 d period prior to entering metabolism crates (steer as experimental unit; n = 16 per ZNTRT) were analyzed using the Mixed procedures of SAS (SAS Institute Inc., Cary, NC). The model included the fixed effect of ZNTRT and group. Dry matter intake data were analyzed as repeated measures with week as the repeated effect. Data collected from d 56 onward were analyzed as a 2 × 2 factorial arrangement utilizing the Mixed procedure of SAS. Pearson correlation analyses (PROC CORR) was used to identify and quantify the relationship between Zn retention and N retention. The model for the analysis of the metabolism period, final BW, and liver mineral included the fixed effects of ZNTRT, BA, group, and the interaction of ZNTRT × BA, with the three-way interaction of ZNTRT × BA × group as random. Steer was the experimental unit (n = 8 per treatment combination) for all analyses. Data for a steer from SUPZN-NON were removed from collection analysis due to negative retention values for Cu, Fe, Mn, and Zn throughout the collection period. Significance was declared at $P \leq 0.05$ and tendencies identified at $P = 0.06$ to 0.10. Values reported are least square means and SEM.

Results

Pre-BA Growth Period. After 56 d of Zn supplementation there was no treatment × time interaction ($P = 0.99$) for DMI. Dry matter intake was increased ($P \leq 0.01$; **Table 2**) in CON vs. SUPZN. No differences in BW, ADG or G:F were detected for the initial 56 d period ($P \geq 0.22$).

Collection Period. There were no interactions detected between ZNTRT × BA during the collection period ($P \geq 0.19$) for any variable. No effects of ZNTRT or BA were detected for

DMI, DM digestibility, OM digestibility, N intake, fecal output and fecal N, or mg N retained/d ($P \geq 0.20$; **Table 3**). Urine output was increased ($P = 0.05$) and urine N output was increased ($P = 0.01$) for NON vs. RAC. Nitrogen retention as a percentage of N intake was lesser in NON (39.7%) vs. RAC (44.8%; $P = 0.02$; **Table 3**). Additionally, less N was retained as a percentage of N intake for CON (40.0%) vs. SUPZN (44.4%; $P = 0.05$).

Influence of ZNTRT and BA strategies on mineral intake, excretion, apparent absorption and retention values on a mg/d and percent of intake basis are reported in **Tables 4** and **5**, respectively. Dietary concentrations of Zn did not influence intake, excretion, absorption, or retention of Cu or Fe when measured as mg/d ($P \geq 0.24$) or as a percent of intake ($P \geq 0.25$). Intake, fecal excretion, urinary excretion, and retention of Zn (mg/d) were lesser in CON vs. SUPZN ($P \leq 0.01$). Urinary Zn excretion (%) was greater in CON vs. SUPZN ($P \leq 0.01$). A positive correlation between Zn retention and N retention was detected ($r = 0.46$, $P < 0.01$). There was a tendency for increased fecal excretion of Mn ($P = 0.06$) and lesser apparent absorption ($P = 0.06$) and retention ($P = 0.05$) of Mn as a percentage of intake in CON vs. SUPZN. Intake of Mn (mg/d) tended to be lesser ($P = 0.06$) in CON vs. SUPZN and Mn retention (mg/d) was decreased ($P = 0.03$) in CON vs. SUPZN. The BA strategy had no effect on trace mineral fecal excretion, urine excretion, apparent absorption or retention when reported as mg/d ($P \geq 0.29$) or as a percentage of intake ($P \geq 0.25$).

Overall performance and liver mineral concentrations. No ZNTRT × BA interactions were detected for overall performance data ($P \geq 0.17$). Across the feeding period G:F and ADG were unaffected by ZNTRT strategy ($P \geq 0.30$; **Table 6**). Both G:F and ADG were lesser in NON vs. RAC ($P = 0.03$). Liver mineral concentrations (mg/kg DM) were

unaffected by both ZNTRT ($P \geq 0.15$) and BA ($P \geq 0.30$) strategies (Cu 296 ± 23, Fe 187 ± 17, Mn 8.0 ± 0.3, Zn 148 ± 13).

Discussion

Previous research by Genther-Schroeder et al. (2016 a,b) reveals increasing dietary Zn concentrations (as Zn-AA complex) of ractopamine HCl-fed cattle may result in improved growth. Beta-adrenergic receptors, when activated, stimulate the membrane-bound enzyme adenylate cyclase, resulting in the production of cyclic adenosine monophosphate (**cAMP**; Lefkowitz et al., 1983). Cyclic AMP is a potent downstream intracellular messenger involved in activation of cAMP-dependent protein kinase A (Yang and McElligott, 1989), eventually leading to activation of hormone sensitive lipase. As a result, β-agonists act as repartitioning compounds, shifting anabolism in the late stage finishing animal from adipose to protein accretion, resulting in increased ADG, G:F, and HCW in pigs and cattle (Mersmann, 1998; Beermann, 2002; Johnson et al., 2014).

In swine, β-agonists increase efficiency of dietary protein utilization (Xiao et al., 1999). This has been further refined in pigs, where increasing dietary lysine in conjunction with increasing supplemental ractopamine improves ADG and G:F (Webster et al., 2007; Ross et al., 2011). It is possible that feeding β-agonists to livestock alters nutrient requirements, unfortunately, this is poorly understood in ruminants.

In the present study, N retention as a percent of intake was independently improved by both ractopamine HCl supplementation and Zn supplementation. Ractopamine HCl supports cattle growth in part through increased N retention (Walker et al., 2007), likely due to a combination of decreased protein degradation (Hill, 1974; Li and Jefferson, 1977; Tischler, 1981) and increasing protein accretion (Maltin et al., 1990). Wheeler and

Koohmaraie (1992) theorized that increased muscle hypertrophy due to β-agonist supplementation results from increased calpastatin activity, therefore decreasing proteolytic capacity. However this phenethanolamine (L644,969) tested by these authors targets the β2-adrenergic receptor, while ractopamine HCl primarily targets the β1-adrenergic receptor (Anderson et al., 2005). In the present study urine output was lesser in steers receiving ractopamine HCl, contributing to the lesser overall N excretion by ractopamine HCl-fed steers. It is unclear if others have noted similar effects of ractopamine HCl supplementation, as no studies completed with cattle supplemented ractopamine HCl report total urine output (Abney et al., 2007; Walker et al., 2007; Koontz et al., 2010). However, research in swine suggests a decrease in urine output can occur with ractopamine HCl supplementation (Ross et al., 2011).

Results of the present study support a role for ractopamine HCl in N retention, but also suggest a critical role for Zn in N metabolism. Given the increased protein accretion experienced by β-adrenergic agonist-fed cattle, the positive correlation between N and Zn retention observed in this study suggests adequate Zn nutrition may be important in this growth response. Indeed, work in other species has displayed an interdependency of Zn and protein on growth, establishing that even with sufficient dietary protein, Zn is necessary for adequate protein utilization (Oberleas and Prasad, 1969; Greeley et al., 1980). Although the CON diet used in the present study met current supplementation recommendations for Zn (National Academies of Sciences, Engineering, and Medicine, 2016), increased N retention due to SUPZN suggests that the Zn requirement of feedlot cattle needs further refinement.

These results clearly define a role for Zn in bodily N retention, which is required for accretion and deposition of lean body mass. Increasing rates of growth due to growth promoting technologies, genetic selection, and improved animal husbandry of modern cattle

presents the opportunity for establishing supplemental Zn concentrations to optimize gain. However, increasing supplementation of Zn should be approached with caution, as some countries have begun to place limits on supplementation rates based on concerns of excess manure concentrations (Jondreville et al., 2003). Determining the precise mechanisms by which Zn influences cattle growth is necessary to move the industry toward more strategic supplementation of Zn, potentially per unit of gain.

Increasing concentrations of Zn-AA increases plasma cAMP in cattle (Genther-Schroeder et al., 2016a) and increasing Zn-AA in combination with ractopamine HCl linearly increases BW, ADG, and G:F, suggesting an enhancement of the biological action of ractopamine HCl. Zinc has been shown to inhibit cyclic nucleotide phosphodiesterase (Spurlock et al., 1994)**,** which is responsible for degrading cAMP and decreasing the response to β-agonists (von Bülow et al., 2005; Haase and Rink, 2007). Zinc deficiency results in an increase in phosphodiesterase expression and a decrease in cAMP concentrations in mice (Hojyo et al., 2011). It has been suggested that Zn may interact with the $ß_2$-adrenergic receptor, potentiating cell signaling and increasing internalization of the receptor; however, Hergenreder et al. (2016) reported that Zn fed as Zn methionine has no effect on internalization of the $ß_1$-adrenergic receptor, which ractopamine HCl preferentially binds to. As there was no interaction between BA and ZNTRT in the present study for measures of N retention it appears there may be opportunity to utilize Zn to improve N retention in cattle even when ractopamine HCl is not fed.

Ractopamine HCl did not change the coefficient of absorption of Zn in the current study and does not appear to upregulate Zn absorption or retention. Coefficients of absorption for Zn in this study (34.5%) are greater than those reported in previous studies in beef steers (22.2% for receiving calves, Nockels et al., 1993; 10.0% ($ZnSO_4$) and 19.6%

(ZnOHCl) for growing steers, Shaeffer et al., 2017; 16.0% for growing steers, Pogge et al., 2014a; and 9.9% for growing steers, Pogge et al., 2014b). The reasons for the high coefficient of absorption of Zn in the present study are unclear. To the authors knowledge, Zn absorption resulting from differing concentrations of Zn supplementation in late stage finishing steers (570 ± 30.9 kg) has not been studied previously, and may be reflective of stage of production or BW (167 ± 5 kg, Nockels et al., 1993; 371 kg, Shaeffer et al., 2017; 368 ± 12 kg [fistulated] and 388 ± 10 kg [unmodified], Pogge et al., 2014a; 370 ± 9.5 kg, Pogge et al., 2014b). Growth in this period of production has been shown to shift away from protein accretion towards adipose tissue deposition. Previous studies have shown increased carcass attributes associated with adipose tissue with Zn supplementation (quality grade, marbling score, yield grade, and backfat; Greene et al., 1988; Spears and Kegley, 2002), and could possibly be influencing the effect on Zn absorption seen in the current study.

The lack of difference reported for the coefficient of absorption of Zn in SUPZN vs. CON are in contrast to those published previously in rats and chickens (Weigand and Kirchgessner, 1979; Mohanna and Nys, 1999), where increasing supplemental Zn concentrations decreased the coefficient of absorption. In general, Zn absorption is down-regulated when dietary Zn is fed well above animal requirements, which may suggest that late stage finishing steers require greater dietary Zn concentrations than currently recommended. Additionally, steers in the SUPZN treatment retained a greater amount of Zn (mg/d) than CON steers, similar to previous reports in rats (Weigand and Kirchgessner, 1979), supporting that increasing supplemental concentrations of Zn increases the amount of Zn retained. Given the positive correlation observed in this study and others between Zn and N retention (Oberleas and Prasad, 1969; Greeley et al., 1980), it appears that increasing dietary Zn may increase N capture in animals, and may be of particular value in rapidly

growing individuals. Further work is needed to refine beef cattle requirements for Zn based on stage of production, BW, and rate of growth.

Supplementing ractopamine HCl at 300 mg·steer^{-1}·d^{-1} did not change absorption or retention of Cu, Fe, or Mn. Additionally, liver mineral concentrations of Cu, Fe, Mn, and Zn were unaffected by RAC or SUPZN. A well-established antagonism between dietary Zn and Cu exists, where Zn tends to increase tissue Metallothionein expression, which then binds to Cu, decreasing Cu absorption (Oestreicher and Cousins, 1985). Regardless, no effects on Cu absorption due to SUPZN were noted in the present study. Metallothionein expression tends to increase when transitioning to a greater concentration of supplemental dietary Zn (Cousins, 1985), but absorption and fecal excretion of endogenous Zn have been shown to adjust within 6 d of increasing supplemental concentrations in rats (Weigand and Kirchgessner, 1978). Therefore, adjustment to increased dietary concentrations of Zn may have preceded the metabolism period of the current study. Furthermore, ruminants store Cu very effectively in the liver (Suttle, 2010), and the cattle in this study had highly adequate Cu status according to harvest liver Cu concentrations, therefore SUPZN may have had little relevant influence on Cu status. It is necessary to be cognizant of the relationship between Cu and Zn as cattle requirements for both essential elements continue to be refined.

Interestingly, Mn absorption and retention were increased by SUPZN treatment. To the authors knowledge, this has not been reported by others and may be due to late finishing stage of these animals, as few metabolism studies have examined beef steers of this weight. Manganese supports enzymes in N recycling (Hellerman and Perkins, 1935) and antioxidant capacity (McCord and Fridovich, 1969; Borgstahl et al., 1992). Absorption rates of Mn in the current study are greater than seen in previous work in humans (Greger et al., 1978) and

cattle (Pogge et al., 2014b) and further work is needed to determine if the findings of the present study are repeatable.

There is opportunity in the industry to refine feeding strategies for livestock when utilizing growth-promoting technologies. It does not appear that ractopamine HCl alters apparent absorption of trace minerals, suggesting that if increased retention of trace minerals such as Zn are needed for an optimal growth response to ractopamine HCl, greater dietary concentrations of trace minerals than those utilized in this study may be needed to provide these nutrients. Future work is needed to elucidate the mechanism behind increased N retention in beef steers due to SUPZN supplementation, but clearly Zn has a positive role on N retention. Further research is needed to move the industry toward more strategic supplementation of trace minerals to support optimum livestock production while concurrently lessening environmental impact.

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Table 1. Diet ingredient composition and nutrient content

Ingredient	**CON Diet (% DM)**
Cracked corn	62
Modified distillers grains with solubles	25
Hay	8
Micronutrients and carrier[1]	5
Analyzed components	
Crude protein[2], %	14.6
NDF[2], %	19.2
Ether extract[2], %	5.19
Cu, mg/kg DM	12
Fe, mg/kg DM	164
Mn, mg/kg DM	33
Zn[3], mg/kg DM	32

[1]Basal includes dried distillers grains with solubles as carrier, micronutrients provided as % DM; Limestone (1.5%), Rumensin (0.0135%), and Salt (0.31%). Trace minerals and Vitamins provided per kg of DM: 0.15 mg Co (cobalt carbonate), 10 mg Cu (copper sulfate), 20 mg Mn (manganese sulfate), 0.1 mg Se (sodium selenite), 0.5 mg I (calcium iodate), and Vitamin A 2,200 IU (ROVIMIX A 1000 [1000 kIU/g], DSM, Parsippany, NJ).

[2]Chemical analysis completed by Dairyland Laboratories (Arcadia, WI).

[3]Control (CON) diet received no supplemental Zn (32 mg Zn/kg DM); Supranutritional Zn (SUPZN) diet received formulated Zn inclusion of 120 mg Zn/kg DM (CON + 60 ppm $ZnSO_4$ and 60 ppm Availa-Zn [Zinpro Corporation, Eden Prairie, MN] which contains (DM basis) 12% Zn and AA complex). CON diet analyzed 32 mg Zn/kg DM; SUPZN diet analyzed 145 mg Zn/kg DM.

Table 2. Dietary Zn influence on 56 d performance preceding metabolism period.

	ZNTRT[1]			
Item	CON[2]	SUPZN[3]	*P*-value	SEM
Steers (n)	16	16		
Dry matter intake[4], kg/d	12.8	12.1	0.01	0.36
BW[5], kg				
d 0	484	487	0.68	4.0
d 56	570	570	0.99	5.6
ADG[5], kg				
d 0 to 56	1.53	1.49	0.66	0.064
Gain to feed[5]	0.114	0.120	0.37	0.0124

[1]ZNTRT (mineral supplementation strategy)
[2]CON (no supplemental Zn; analyzed 32 mg Zn/kg DM)
[3]SUPZN (CON + 60 ppm $ZnSO_4$ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM)
[4]Daily dry matter intake, repeated measures (no treatment × time interaction).
[5]All body weight values include 4% pencil shrink in calculations.

Table 3. Influence of dietary Zn and ractopamine supplementation on dry matter intake, diet digestibility, and daily urine and fecal output during 5 d collection period

Dietary Treatment	ZNTRT[1]			BA[1]			
	CON[2]	SUPZN[2]	*P*-value[4]	NON[3]	RAC[3]	*P*-value[4]	SEM
Steers (n)	16	15		15	16		
Dry matter intake[5], kg/d	9.43	10.12	0.40	9.89	9.67	0.78	0.558
DM digestibility[6], %	80.71	80.76	0.97	81.09	80.38	0.61	0.946
OM digestibility[7], %	81.95	82.19	0.84	82.49	81.65	0.51	0.869
N intake, g/d	210.3	224.0	0.45	218.3	216.0	0.90	12.31
Daily output							
Fecal, kg DM/d	1.85	1.96	0.66	1.90	1.91	0.98	0.173
Urine, L/d	8.85	8.56	0.82	10.13	7.23	0.05	0.876
Fecal, N g/d	46.8	49.8	0.58	48.2	48.4	0.97	3.79
Urine, N g/d	78.5	75.8	0.58	84.5	69.8	0.01	3.35
N retention, g/d	85.1	98.8	0.20	86.1	97.8	0.27	7.03
N retention[8], %	40.0	44.3	0.05	39.5	44.8	0.02	1.38

[1]ZNTRT (mineral supplementation strategy); BA (ß-adrenergic agonist supplementation strategy)

[2]CON (no supplemental Zn; analyzed 32 mg Zn/kg DM); SUPZN (CON + 60 ppm $ZnSO_4$ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM)

[3]NON (no supplemental ractopamine HCl), RAC (300 $mg \cdot^{-1} steer \cdot^{-1} d$ ractopamine HCl; Actogain 45, Zoetis, Parsippany, NJ)

[4]ZNTRT × BA interaction was not significant ($P \geq 0.19$)

[5]Dry matter intake over 5 d period during collection.

[6]Dry matter digestibility.

[7]Organic matter digestibility.

[8]Reported as percentage of intake.

Table 4. Influence of dietary Zn and Ractopamine inclusion on daily micro mineral intake, fecal and urine excretion, and mineral retention of steers during 5 d collection period.

Dietary Treatment	ZNTRT[1]			BA[1]			
	CON[2]	SUPZN[2]	*P*-value[4]	NON[3]	RAC[3]	*P*-value[4]	SEM
Steers (n)	16	15		15	16		
Mineral intake							
Cu, mg/d	124	134	0.45	129	128	0.92	9.0
Fe, mg/d	1617	1678	0.71	1656	1640	0.92	111.4
Mn, mg/d	299	361	0.06	330	330	0.98	20.5
Zn, mg/d	322	1534	<0.01	916	940	0.75	50.5
Fecal excretion							
Cu, mg/d	94	103	0.24	97	100	0.72	4.7
Fe, mg/d	999	1004	0.96	1005	998	0.94	66.1
Mn, mg/d	229	247	0.34	236	239	0.87	12.8
Zn, mg/d	206	1004	<0.01	578	632	0.38	41.1
Urinary excretion							
Cu, mg/d	0.15	0.13	0.24	0.15	0.14	0.66	0.013
Fe, mg/d	1.92	1.98	0.77	2.00	1.90	0.65	0.160
Mn, mg/d	0.50	0.46	0.81	0.55	0.41	0.42	0.115
Zn, mg/d	1.09	1.99	<0.01	1.64	1.44	0.29	0.121
Mineral retention							
Cu, mg/d	29	31	0.87	32	28	0.71	6.7
Fe, mg/d	616	673	0.49	649	640	0.91	56.2
Mn, mg/d	70	114	0.03	93	90	0.86	12.1
Zn, mg/d	114	529	<0.01	337	306	0.43	26.8

[1]ZNTRT (Mineral supplementation strategy); BA (ß-adrenergic agonist supplementation strategy)

[2]CON (no supplemental Zn; analyzed 32 mg Zn/kg DM); SUPZN (CON + 60 ppm $ZnSO_4$ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM)

[3]NON (no supplemental ractopamine HCl), RAC (300 $mg \cdot^{-1}steer \cdot^{-1}d$ ractopamine HCl; Actogain 45, Zoetis, Parsippany, NJ)

[4]ZNTRT × BA interaction was not significant ($P \geq 0.19$)

Table 5. Influence of dietary Zn and ractopamine inclusion on daily micro mineral fecal and urine excretion, and mineral retention of steers as a percent of intake during 5 d collection period.

	ZNTRT[1]			BA[1]			
Item	CON[2]	SUPZN[2]	*P*-value[4]	NON[3]	RAC[3]	*P*-value[4]	SEM
Steers (n)	16	15		15	16		
Fecal excretion							
Cu, %	78.2	78.9	0.89	76.7	80.5	0.49	3.67
Fe, %	62.0	60.8	0.61	60.6	62.2	0.53	1.71
Mn, %	76.7	69.4	0.06	71.9	74.2	0.50	2.32
Zn, %	64.9	65.7	0.83	63.1	67.4	0.25	2.45
Urinary excretion							
Cu, %	0.14	0.11	0.25	0.13	0.12	0.81	0.017
Fe, %	0.12	0.12	0.89	0.12	0.12	0.81	0.018
Mn, %	0.16	0.12	0.37	0.15	0.13	0.50	0.027
Zn, %	0.35	0.13	<0.01	0.26	0.22	0.41	0.035
Apparent absorption							
Cu, %	21.8	21.1	0.89	23.3	19.5	0.49	3.67
Fe, %	38.0	39.3	0.51	39.4	37.8	0.53	1.71
Mn, %	23.3	30.6	0.06	28.1	25.8	0.50	2.32
Zn, %	35.1	34.3	0.83	36.9	32.6	0.25	2.45
Mineral retention							
Cu, %	21.6	21.0	0.90	23.2	19.4	0.49	3.68
Fe, %	37.9	39.1	0.61	39.3	37.7	0.53	1.72
Mn, %	23.2	30.5	0.05	28.0	25.7	0.50	2.32
Zn, %	34.8	34.2	0.88	36.6	32.3	0.25	2.46

[1]ZNTRT (mineral supplementation strategy); BA (ß-adrenergic agonist supplementation strategy)
[2]CON (no supplemental Zn; analyzed 32 mg Zn/kg DM); SUPZN (CON + 60 ppm $ZnSO_4$ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM)
[3]NON (no supplemental ractopamine HCl), RAC (300 $mg \cdot^{-1} steer \cdot^{-1} d$ ractopamine HCl; Actogain 45, Zoetis, Parsippany, NJ)
[4]ZNTRT × BA interaction was not significant ($P \geq 0.19$)

Table 6. Influence of dietary Zn and Ractopamine inclusion on performance measurements for d 0-86

	ZNTRT[1]			BA[1]			
Item	CON[2]	SUPZN[2]	*P*-value[4]	NON[3]	RAC[3]	*P*-value[4]	SEM
Steers (n)	16	16		16	16		
Final BW[5], kg	600	604	0.54	598	606	0.23	4.38
Gain to feed[5]	0.121	0.126	0.30	0.114	0.133	<0.01	0.0039
ADG[5], kg	1.34	1.36	0.77	1.27	1.43	0.03	0.042

[1]ZNTRT (Mineral supplementation strategy); BA (ß-adrenergic agonist supplementation strategy)
[2]CON (no supplemental Zn; analyzed 32 mg Zn/kg DM); SUPZN (CON + 60 ppm $ZnSO_4$ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM)
[3]NON (no supplemental ractopamine HCl), RAC (300 $mg \cdot^{-1} steer \cdot^{-1} d$ ractopamine HCl; Actogain 45, Zoetis, Parsippany, NJ)
[4]ZNTRT × BA interaction was not significant ($P \geq 0.19$)
[5]All body weight values include 4% pencil shrink in calculations.

CHAPTER 4.

THE INFLUENCE OF RACTOPAMINE HYDROCHLORIDE SUPPLEMENTATION AND SUPPLEMENTAL ZINC CONCENTRATION AND SOURCE ON PERFORMANCE, TRACE MINERAL STATUS, AND GENE EXPRESSION IN BEEF STEERS

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Abstract: The objective of this study was to determine the impacts of increasing supplemental Zn as either $ZnSO_4$ or $ZnSO_4$ blended with Zn-AA complex on associated growth processes in steers supplemented with ractopamine HCl. High-percentage Angus steers (n = 72, 428 ± 2.8 kg) with Genemax gain scores of 3, 4, or 5 were blocked by BW and stratified by Genemax score to 12 pens of 6 steers each. Pens were randomly assigned by weight block to 1 of 3 Zn strategies (**ZNTRT**) for 56 d: 1) no supplemental Zn (analyzed 37 mg Zn/kg DM; **CON**) 2) 120 mg supplemental Zn/kg DM as $ZnSO_4$ (CON + 120 mg Zn/kg DM; **INZN**) or 3) 120 mg supplemental Zn/kg DM as a blend of $ZnSO_4$ and Zn-amino acid complex [**Zn-AA** ; CON + 60 mg Zn/kg DM $ZnSO_4$ + 60 mg Zn/kg DM as Zn-AA (Availa-Zn; Zinpro, Eden Prairie, MN); **ZNBLD**]. After 56 d of ZNTRT serum urea N was greater for supplemental Zn vs. CON ($P = 0.03$). Liver Zn tended to be lesser in INZN vs. ZNBLD on d 28 and 54 ($P \leq 0.08$) while no differences were noted between supplemental Zn and CON ($P \geq 0.32$). Liver MT1A expression was lesser in CON vs. supplemental Zn ($P = 0.01$) and lesser in INZN vs. ZNBLD ($P = 0.02$). A ZNTRT × time effect was detected for plasma Zn concentrations ($P = 0.003$) where on d 28 and 56 both INZN and ZNBLD were greater than CON but did not differ from each other. No ZNTRT effects were noted on performance

measures during the initial 56 d ($P \geq 0.13$). On d 57 pens were assigned to 1 of 2 β_1-adrenergic agonist (**BA**) inclusion rates for the remaining 32 d on trial utilizing a 3 × 2 factorial arrangement (n = 12 steers/treatment combination): 1) no ractopamine hydrochloride (**NON**) or 2) 300 mg ractopamine hydrochloride·steer^{-1}·d^{-1} (**RAC**; Optaflexx, Elanco Animal Health, Greenfield, IN). No ZNTRT × BA effects were noted for any performance measures ($P \geq 0.30$) and no ZNTRT effects were noted for ADG or G:F ($P \geq 0.57$) while both measures were greater for RAC vs. NON ($P \leq 0.04$). Serum urea N concentrations tended to be lesser ($P = 0.09$) and liver Mn was lesser on d 71 ($P = 0.05$) in RAC vs. NON. Dressing percentage was greater ($P = 0.03$) and REA tended to be greater ($P = 0.08$) in RAC vs. NON. No ZNTRT effects were noted for any carcass characteristics ($P \geq 0.33$) or plasma IGF-1 concentrations assessed as repeated measures across the BA period ($P = 0.40$). Expression of ZIP14 in muscle and liver remained unaffected due to BA, ZNTRT, or the interaction ($P \geq 0.16$). However, a ZNTRT × BA tendency for liver MT1A was detected ($P = 0.08$), where MT1A mRNA expression was greater in INZN-RAC and both ZNBLD-NON and ZNBLD-RAC vs. CON-NON, CON-RAC and INZN-NON. No improvements in performance were noted due to the combination of ractopamine HCl and greater supplemental Zn, regardless of source, but differences in MT1A mRNA expression due to supplemental Zn and ractopamine HCl suggest a role of the Zn sequestering protein in Zn homeostasis during ractopamine HCl supplementation.

KEY WORDS: beef cattle, beta agonist, Metallothionein, ractopamine hydrochloride, zinc

Introduction

The trace mineral Zn is a critical structural, catalytic, and signaling component in multiple growth processes including DNA and protein synthesis (Maret, 2013). Current Zn recommendations were established more than 34 years ago to support growth and prevent deficiency (NRC, 1984). However, since 1977 ADG has increased 44% across the life of feedlot animals, which can be partially attributed to improved management, growth promoting technologies, and genetics. The growth technology ractopamine hydrochloride (**HCl**) is a β-adrenergic agonist (**β-AA**) approved to be fed the last 28-42 d prior to slaughter in feedlot cattle and has been shown to increase performance measures such as ADG and G:F (Lean et al., 2014). The β-AA ractopamine hydrochloride is classified as a repartitioning agent in late stage finishing cattle, shifting growth away from adipose tissue to protein accretion, similar to the endogenous catecholamines in the body epinephrine and norepinephrine (Mersmann, 1998). Prior research has shown performance advantages of supplementing ractopamine HCl in tandem with increasing supplemental Zn as Zn-amino acid complex (**Zn-AA**) in swine (Patience and Chipman, 2011) and as a blend of Zn-AA and $ZnSO_4$ in feedlot steers (Genther-Schroeder et al., 2016a,b). While Genther-Schroeder et al. (2016a,b) noted greater performance in steers receiving supplemented ractopamine HCl in combination with a blend of $ZnSO_4$ and Zn-AA complex, they did not evaluate inorganic $ZnSO_4$ as the supplemental Zn source. Zinc has been established as a critical component for protein utilization in the body (Oberleas and Prasad, 1969; Greeley et al., 1980), and Carmichael et al. (2018) noted that Zn supplemented as a blend of 60 mg Zn/kg DM as $ZnSO_4$ and 60 mg Zn/kg DM as Zn-AA increased N retention regardless of ractopamine HCl supplementation. However, cattle are generally supplemented Zn as $ZnSO_4$ or Zn oxide in

feedlot operations. Beta-adrenergic agonists act on the G-protein couple receptor, increasing concentrations of the secondary messenger cAMP (Ryall and Lynch, 2008). When the Zn importer ZIP14 is knocked out in mice, increased activity of phosphodiesterase results in more rapid degradation of cAMP, lessening activity of the β-AA cascade (Hojyo et al., 2011). The objective of this study was to determine the effects of greater supplemental Zn concentrations, from either $ZnSO_4$ alone or as a blend with Zn-AA complex, on performance of beef steers supplemented ractopamine HCl and resulting Zn trafficking mechanisms by which Zn may influence the growth processes involved. The hypothesis was that supplemental Zn would improve performance in beef steers receiving ractopamine HCl and increase expression of the Zn transport protein ZIP14.

Materials and Methods

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (8-15-8073-B).

Experimental Design

High-percentage Angus cattle from a single source with Genemax (Zoetis, Parsippany, NJ) gain scores of 3, 4, or 5 were utilized in this study, indicating the top 60% of growth potential for genetically tested Angus cattle. Seventy-two steers (428 ± 2.8 kg BW) were blocked by body weight (**BW**; 2 blocks) and stratified by Genemax gain score to 12 pens of 6 steers each. Pens were randomly assigned within block to one of three Zn supplementation strategies (**ZNTRT**; n = 24 steers per ZNTRT) for 56 d: 1) no supplemental Zn (analyzed 37 mg Zn/kg DM; **CON**) 2) 150 mg supplemental Zn/kg DM as inorganic $ZnSO_4$ (CON + 120 mg Zn/kg DM; **INZN**) or 3) 150 mg supplemental Zn/kg DM as a blend of $ZnSO_4$ and Zn-amino acid complex [CON + 60 mg Zn/kg DM $ZnSO_4$ + 60 mg Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN); **ZNBLD**]. Before the

initiation of the study, steers were given a booster vaccination for Bovine Viral Diarrhea Virus type I and II (Bovi-Shield Gold, One Shot, Zoetis) and received a dose of broad range dewormer pour-on solution (Dectomax, Zoetis). Diet composition is shown in **Table 1**. On d 57, half of the pens in each ZNTRT were assigned to one of two β_1-adrenergic agonist inclusion rates (**BA**): 1) no ractopamine hydrochloride (**NON**) or 2) 300 mg steer^{-1} d^{-1}ractopamine hydrochloride (**RAC**; Optaflexx, Elanco Animal Health, Greenfield, IN) and fed for an additional 32 d (n = 12 steers/treatment combination). Steers were fitted with unique electronic identification tags so daily individual as-fed intake could be determined from pens equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Steers were fed daily at 0800 h and allowed ad libitum access to feed and water and pre-feeding BW were collected on d -1, 0, 26, 56, 57, 70, 88 and 89. A 4% pencil shrink was applied to all body weight (**BW**), average daily gain (**ADG**) and feed efficiency (**G:F**) measures reported. On d 0, all steers were implanted with a combination Component TE-IS implant with Tylan (80 mg trenbolone acetate, 16 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health) and, on d 39, a combination Component TE-S implant with Tylan on d 39(140 mg trenbolone acetate, 24 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health).

Liver and muscle biopsies were performed on d 28, 54, and 71 on half of the steers (stratified by BW and chosen randomly) within each ZNTRT. Liver biopsies were collected with methods established by Engle and Spears (Engle and Spears, 2000). Muscle biopsies were collected by clipping and then scrubbing the area between the 11th and 12th ribs with betadine and 70% ethanol and subsequently injecting the area with 10 mL of 2% lidocaine into the Longissimus dorsi. A modified Jamshidi bone marrow biopsy/aspiration needle (8 g × 10 cm needle) was utilized to collect muscle tissue (~0.5 g wet basis). After rinsing with

0.1 M phosphate buffered saline (pH 7.0) to remove remaining blood from collected tissue, tissues were subsequently flash frozen and stored at -80°C until analysis. Blood samples were collected on d 0, 26, 56, 70, and 88 for various blood metabolites and plasma trace mineral analysis. Following the end of the experiment (d 89) all steers were shipped 100 km to a commercial abattoir (Iowa Premium, Tama, IA) and held in lairage for ~13 h before harvest. Following a 48 h chilling period, a team blinded to treatment was dispatched to collect hot carcass weight (**HCW**), longissimus dorsi muscle area (**REA**), 12th rib fat thickness (**BF**), kidney, pelvic and heart fat percentage (**KPH**), and marbling score (**MS**). These measures were then utilized to determine yield (**YG**) and quality grade (**QG**).

Feed and Tissue Analysis

Weekly total mixed ration (**TMR**) samples were collected during the feedlot period (d 0 to 89), dried in a forced-air oven for 48 h at 70°C, and the resulting dry matter (**DM**) value was multiplied by individual as-fed intakes for each steer to determine dry matter intake (**DMI**). Dry matter of TMR was determined according to AOAC (1990) procedures, and feed efficiency was calculated from the total gain and total DMI determined for each weighing interval. Dried TMR was ground through a 2mm screen (Retsch Zm100 Grinder; Glen Mills Inc., Clifton, NJ) and stored in sealed plastic bags until compositing and nutrient analysis.

Analysis of TMR, plasma, and liver mineral was conducted using inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 7000 DV, Perkin Elmer, Waltham, MA). Dried, ground, and composited TMR samples were acid digested prior to mineral analysis according to the methods described by Pogge et al. (2014). Liver and plasma samples were digested and analyzed for Zn and Cu according to Pogge and Hansen (2013) and instrument accuracy was determined with a bovine liver standard from the National Institute of Standards and Technology (Gaithersburg, MA) and a trace mineral quality

control for serum (UTAK Laboratories, Inc., Valencia, CA). Yttrium (PerkinElmer, Waltham, MA) was utilized as an internal standard to account for within run variation. Blood was collected by jugular venipuncture into serum, sodium heparin, K_2 EDTA Plus, and trace element K_2 EDTA blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Serum tubes were allowed to clot at room temperature for 90 min and centrifuged at 1500 x g for 20 minutes. A colorimetric assay kit (HR series NEFA-HR(2) assay kit; Wako Pure Chemical Industries, Ltd., Osaka, Japan; inter-assay CV of 7.8%) was used to measure plasma non-esterified fatty acid (**NEFA**) concentrations. Serum urea N (**SUN**) concentrations were determined from serum samples by colorimetric assay (B551; TECO Diagnostics, Anaheim, CA; inter-assay CV of 9.5%). Plasma insulin-like growth factor-1 (**IGF-1**) concentrations were analyzed via a commercial ELISA kit (SG100; R&D Systems, Minneapolis, MN; inter-assay CV of 4.3%) utilizing heparinized plasma; the assay has been shown to have 100% cross-reactivity with bovine IGF-1 (Moriel and Arthington, 2013).

Liver and muscle tissue collected from steers were kept frozen with liquid nitrogen while ground with a mortar and pestle. The Trizol method (Life Technologies, Carlsbad, CA) and the RNeasy Mini kit (Qiagen GmbH, Qiagen Strasse 1, Hilden, Germany) were used to isolate RNA per manufacturer's instructions from the powdered samples. Integrity and quality as well as quantity of RNA was determined using a Qubit 4 Fluorometer (Invitrogen, ThermoFisher Scientific, Waltham, MA). One half (0.5) microgram was reverse transcribed using the Superscript III kit (ThermoFisher Scientific) for each sample per manufacturer's instructions. Melting temperature during the 40 amplification cycles was 94°C and resulting cycle threshold (**Ct**) values were normalized to the abundance of RPS9. Values for gene expression are presented as the ΔCt technique, calculated by subtracting Ct values of RPS9

from the Ct value of the gene of interest. Primers sequences and annealing temperature for each gene are shown in **Table 2**.

Statistical Analysis

Performance and intake data for the initial 57 d period prior to ractopamine HCl supplementation (steer as experimental unit; n = 24 per ZNTRT) were analyzed as a randomized complete block design using the Mixed procedures of SAS (SAS Institute Inc., Cary, NC) with the fixed effects of ZNTRT and block. Data collected following d 57 were analyzed as a 3 × 2 factorial arrangement utilizing the Mixed procedures of SAS with the fixed effects of ZNTRT, BA, block, and the interaction of ZNTRT and BA. Finishing period plasma trace mineral (**TM**) and metabolite data were analyzed as repeated measures with time as the repeated effect. The compound symmetry variance structure for repeated measures was utilized for plasma TM and metabolite data to achieve the lowest Akaike information criterion value. Data were tested for normal distribution by the Shapiro-Wilk test of normality; NEFA were log transformed for analysis and treatment means and SEM shown are reverse transformed. Steer was the experimental unit for all analyses (n = 12/treatment combination). Determination of outliers was accomplished using Cook's D statistic and removed if Cook's D ≥ 0.5. One steer from each of the treatment combinations INZN-NON and CON-RAC were removed from all analysis following d 57 due to illness unrelated to treatment. Significance was declared at $P \leq 0.05$ and tendencies identified at $P = 0.06$ to 0.10. The PDIFF statement in SAS was utilized to determine pairwise differences and tabular values reported reflect the least square means.

Results

Pre-Ractopamine Period

Effects of dietary Zn supplementation on performance, blood metabolite measures, liver mineral concentrations, and gene expression data are shown in **Table 3**. No ZNTRT × time effects were detected for plasma NEFA or SUN ($P \geq 0.87$). Plasma NEFA concentrations (153, 131, and 167 mEq/L for d 0, 26, and 56, respectively) changed over time ($P = 0.0001$), being least on d 26. Serum urea N concentrations were lesser on d 0 than other days (effect of time; $P = 0.0006$; 9.3, 10.2, and 10.3 for d 0, 26, and 56, respectively). Additionally, SUN were affected by ZNTRT ($P = 0.03$) and were greater for supplemental Zn than CON. Liver Zn tended to be lesser in INZN compared to ZNBLD on d 28 and 54 ($P \leq 0.08$) but no differences were noted between supplemental Zn and CON ($P \geq 0.32$). Liver MT1A mRNA expression was lesser in CON vs. supplemental Zn ($P = 0.01$) and lesser in INZN vs. ZNBLD ($P = 0.02$). No effects of ZNTRT were noted during the 57 d period before ractopamine HCl supplementation on plasma NEFA and Cu, DMI, BW, ADG, or G:F, or d 28 and 56 liver Cu, Mn, or d 54 liver ZIP14 mRNA expression ($P \geq 0.17$). A ZNTRT × time effect was detected for plasma Zn concentrations ($P = 0.003$; **Figure 1A**), where on d 28 and 56 both INZN and ZNBLD were greater than CON but did not differ from each other. No ZNTRT × time effects were noted for plasma Cu during the initial 56 d feeding period ($P = 0.39$) but generally increased over the first 56 d of the trial ($P = 0.0001$; **Figure 1B**).

Ractopamine HCl Supplementation Period

The effects of dietary Zn and ractopamine HCl supplementation on performance and blood metabolite measures during the BA period are shown in **Table 4**. No ZNTRT, BA, or ZNTRT × BA effects were noted for DMI or BW ($P \geq 0.30$). No ZNTRT × BA or ZNTRT

effects were noted for ADG and G:F during the BA period ($P \geq 0.57$); however, there was a BA effect where ADG and G:F were lesser for NON than RAC ($P \leq 0.04$). No ZNTRT, BA, ZNTRT x BA, or interactions with time were noted for plasma NEFA concentrations ($P \geq 0.33$); however, concentrations (166, 145, and 153 mEq/L for d 56, 70, and 88, respectively) differed over time ($P = 0.002$), being greatest on d 56. No ZNTRT × BA, ZNTRT or interactions with time were noted for SUN ($P \geq 0.34$), but SUN concentrations tended to be greater in NON compared to RAC ($P = 0.09$) and increased over time ($P = 0.01$) being lesser on d 56 and 70 than d 88 (10.3, 10.6, and 11.2 for d 56, 70, and 88, respectively). The effects of dietary Zn and ractopamine HCl supplementation on carcass characteristics are shown in **Table 5**. No ZNTRT × BA effects were noted for any carcass characteristic ($P \geq 0.31$). However, NON decreased DP ($P = 0.03$) and tended to decrease REA ($P = 0.08$) relative to RAC, while BA had no effect on HCW, BF, KPH, MS, or YG ($P \geq 0.12$). No ZNTRT effects were detected for any carcass characteristic ($P \geq 0.33$).

The effects of dietary Zn and ractopamine HCl supplementation on liver TM concentration and gene expression for liver and muscle on d 71 are shown in **Table 6**. No ZNTRT × BA, ZNTRT, or BA effects were detected for d 71 liver Zn concentrations, liver ZIP14 mRNA expression, or muscle MT1A and ZIP14 mRNA expression ($P \geq 0.16$). A ZNTRT × BA effect was identified for liver Cu, where NON was greater than RAC within CON ($P = 0.03$) while BA did not affect liver Cu concentrations within INZN and ZNBLD ($P \geq 0.99$). A ZNTRT × BA tendency for liver MT1A was detected ($P = 0.08$), where MT1A mRNA expression was lesser in CON-NON, CON-RAC and INZN-NON vs. INZN-RAC and both ZNBLD-NON and ZNBLD-RAC. No ZNTRT × BA or ZNTRT effects were noted for liver Mn ($P \geq 0.41$) but concentrations were greater in NON compared to RAC on d 71 (*P*

= 0.05). Liver MT1A and liver Zn were negatively correlated on d 54 (r = -0.54; P = 0.0006) and d 71 (r = -0.72; P = 0.0001), which can be interpreted to mean that as liver Zn increases MT1A increases as well, since a more negative value for ΔCT means greater gene expression.

No ZNTRT × BA × time effects were noted for plasma Zn or IGF-1 concentrations ($P \geq 0.39$). A ZNTRT × BA × time effect was noted for plasma Cu (P = 0.05; **Figure 2**) where steers receiving RAC with the exception of ZNBLD increased plasma Cu during the BA period, while CON-NON and INZN-NON decreased from d 56 to d 71 and increased from d 71 to 88, with ZNBLD-NON and ZNBLD-RAC remaining relatively steady across the 32 d RAC period. No ZNTRT × BA effects were noted for plasma Zn, SUN, or plasma IGF-1 concentrations ($P \geq 0.14$). A ZNTRT × BA effect was detected for liver Cu (P = 0.03), where the addition of RAC to CON resulted in concentrations more similar to those of ZNBLD, which regardless of BA were lesser than CON-NON, with INZN-NON and INZN-RAC being intermediate. A tendency for a BA × time effect was detected for plasma IGF-1 concentrations (P = 0.06), where on d 56 concentrations for steers assigned to NON were greater than for those assigned to RAC (P = 0.03) but BA did not affect IGF-1 concentrations on d 70 and 88 ($P \geq 0.78$; **Figure 3**). No ZNTRT effects were noted for plasma IGF-1 concentrations ($P \geq 0.40$). Plasma Zn was greater in ZNBLD and INZN than CON ($P \leq$ 0.005) but did not differ between supplemental Zn treatments (**Figure 4A**) and was unaffected by BA ($P \geq 0.38$). Plasma Zn was greater on d 88 than d 56 or 70 (P = 0.04; **Figure 4B**).

Discussion

Beef cattle performance and efficiency has improved over the past 50 years due to improved genetics, management practices, and the use of growth promoting technologies. One of these technologies is the β_1-AA ractopamine HCl, which is approved to be fed the last 28-42 prior to slaughter, and leads to advantages in ADG and feed efficiency (Lean et al., 2014). The trace mineral Zn is critical for growth in mammals, supporting growth pathways such as DNA and protein synthesis (Maret, 2013), and prior research has shown improvements in swine and cattle performance when Zn-AA complex is fed together with the β-AA ractopamine HCl (Patience and Chipman, 2011; Genther-Schroeder et al., 2016a,b). However, cattle are most frequently supplemented with inorganic Zn, such as $ZnSO_4$ or Zn oxide. Therefore, the objective of this study was to determine the impacts of greater supplemental Zn concentrations, from either inorganic $ZnSO_4$ alone or blended with Zn-AA complex, on performance of beef steers supplemented ractopamine HCl and the mechanisms by which Zn may influence growth processes.

While Zn is heavily involved in growth processes, across the current study no effects of ZNTRT on performance were noted. Previous research indicated no improvements in ADG or final BW in heifers receiving either 30, 60, or 90 mg supplemental Zn/kg DM for 144 d as $ZnSO_4$ vs. unsupplemented heifers for (Van Bibber-Krueger et al., 2019). Contrary to the present study, Spears and Kegley (2002) indicated 25 mg supplemental Zn/kg DM from Zn oxide or Zn proteinate in addition to the control (basal Zn concentration 33 mg Zn/kg DM) in steers improved ADG during the growing phase vs. control alone, while Zn proteinate increased HCW compared to Zn oxide and the control, even with concentrations much lower than the present study. Additionally, no synergisms between supplemental Zn and ractopamine HCl were noted on performance in the current study, which is in contrast to

prior research by Genther-Schroeder et al. (2016b) where steers receiving similar Zn and ractopamine HCl concentrations displayed increased carcass adjusted ADG, BW, and G:F vs. those receiving only 60 mg Zn/kg DM as $ZnSO_4$. Increasing supplemental Zn during β-AA supplementation has yielded mixed results related to growth improvements of cattle. Synergistic improvements were reported when supplementing Zn beyond suggested requirements to pigs (NRC, 2012), increasing growth rates and feed efficiency beyond that induced only by ractopamine HCl (Patience and Chipman, 2011). However, increasing Zn supplementation beyond NASEM (2016) concentrations yielded no improvements in performance in heifers (Van Bibber-Krueger et al., 2017) or steers (Bohrer et al., 2014) fed ractopamine HCl. Furthermore, Hergenreder et al. (2016) reported no performance advantage when supplying 720 mg $Zn \cdot steer^{-1} \cdot d^{-1}$ as Zn methionine to steers receiving the β-AA zilpaterol HCl.

Plasma Zn concentrations were considered adequate (Kincaid, 2000) in all steers but were greater in steers receiving supplemental Zn in the current study, which is in accordance with previous literature where greater supplemental Zn increased plasma Zn concentrations in heifers fed 100 mg supplemental Zn/kg DM as $ZnSO_4$ (Van Bibber-Krueger et al., 2017; Van Bibber-Krueger et al., 2019) over controls receiving 30 mg Zn/kg DM and steers fed 20 mg supplemental Zn/kg DM as Zn-glycine complex or Zn methionine vs. controls receiving 19 mg Zn/kg DM (Spears et al., 2004). However, plasma Zn concentrations can be influenced by physiological status of the animal, and with the exception of severe deficiency plasma Zn is an unreliable indicator of Zn status (Hambidge, 2003).

Prior research has shown supplementation of Zn up to 120 (Genther-Schroeder et al., 2016b) or 100 mg Zn/kg DM (Niedermayer et al., 2018) has minimal effect on liver Zn concentrations of beef steers. Detrimental effects of Zn deficiency on growth appear to occur

before measurable differences in tissue Zn concentrations occur in young animal models (Chesters, 1978), and liver is likely an unreliable status index unless an animal is deficient (Suttle, 2010). Accumulation of free Zn in the cytosol, or Zn not bound to proteins, induces MT mRNA expression by binding metal transcription factor-1 (Fukada and Kambe, 2014; Kimura and Kambe, 2016). Liver MT mRNA expression has been shown to increase due to supplemental Zn in a variety of species (Rojas et al., 1996; Cao et al., 2002; Wright and Spears, 2010) and was investigated as a more sensitive indicator of Zn status. Liver Zn only tended to be decreased in INZN vs. ZNBLD while liver MTA1 expression was lesser in steers receiving $ZnSO_4$ vs. steers receiving $ZnSO_4$ and Zn-AA blend, suggesting liver Zn is a less sensitive indicator of differences between sources than MT1A expression. If greater concentrations of Zn induce greater MT1A expression it would seem more Zn is appearing in the liver of steers fed the ZNBLD diet; however, regardless of the apparent increase in available Zn no benefits on performance were noted.

After 14 d of BA supplementation (d 71 of trial) it appeared ractopamine HCL was affecting MT1A expression in the liver, though this effect was different due to both concentration and source of Zn. In general BA supplementation appeared to increase expression of MT1A, with greatest evidence of this within steers receiving inorganic supplemental Zn. Norepinephrine administration in the rat increases MT expression in brown adipose tissue (Beattie et al., 2000), suggesting there may be a direct relationship between catecholamines and Zn metabolism. Alternately, it was also suggested MT expression is responsive to inflammation (Beattie et al., 2000) and Genther-Schroeder et al. (2016a) noted increases in circulating inflammatory markers and increased plasma Cu in steers receiving 300 mg ractopamine HCl $\cdot steer^{-1} \cdot d^{-1}$. The acute phase protein ceruloplasmin is Cu-dependent (Hellman and Gitlin, 2002), and increased plasma Cu noted in the current study, which

appears driven by ractopamine HCl supplementation, may have been due to inflammation; however, no markers of inflammation were measured in the present study.

Serum urea N concentrations were increased in supplemental Zn treatments in the first 56 d, which may suggest Zn influences N metabolism. Carmichael et al. (2019) fed similar concentrations of Zn and noted an improvement in total tract N digestibility over unsupplemented controls, regardless of fiber content of the diet. If the increase in N digestibility occurred in the rumen there may have been more ammonia produced in the rumen, ultimately resulting in more SUN following detoxification of ammonia via the hepatic urea cycle. During the BA period SUN tended to decrease in steers receiving ractopamine HCl. This has been similarly reported by others and attributed to decreased muscle catabolism in response to feeding the β-AA (Bryant et al., 2010; Walker et al., 2010; Van Bibber-Krueger et al., 2017). While both Zn and ractopamine HCl have been shown to impact N metabolism, no interactions between Zn and ractopamine HCl were detected for SUN in the current study.

The trace mineral Mn is involved in N recycling (Hellerman and Perkins, 1935) and is an integral constituent in the antioxidant protein Mn-superoxide dismutase (McCord and Fridovich, 1969; Borgstahl et al., 1992). In the current study β-AA administration decreased liver Mn, which may be related to the decrease in SUN observed due to β-AA. Manganese-dependent arginase is a component of the urea cycle (Hellerman and Perkins, 1935) and it is possible that with more N being utilized for protein deposition and subsequently less muscle catabolism less urea cycle activity was needed in β-AA-fed steers . However, N recycling in the ruminant is hardly a static process and involves factors beyond the scope of this study.

A typical response to ractopamine HCl supplementation is increased ADG and feed efficiency (~16 and 17%, respectively) in beef cattle (Avendaño-Reyes et al., 2006; Gruber

et al., 2007; Lean et al., 2014; Quinn et al., 2016). Similarly, in the current study ADG and G:F were improved in RAC over NON, as were dressing percentage and REA. Beta-adrenergic receptor agonists are classified as phenethanolamines, sharing similar activities in the body to the catecholamines epinephrine and norepinephrine (Mersmann, 1998). The β-AA act through activation of a G-protein coupled receptor, increasing intracellular concentrations of the secondary messenger cAMP which during desensitization is degraded by the enzyme phosphodiesterase (Tetsi et al., 2017). When ZIP14, a Zn importer, is knocked out in mice there is increased phosphodiesterase (**PDE**) degradation of cAMP and mice exhibit decreased growth because of limited Zn availability (Hojyo et al., 2011). In the current study ZIP14 expression in liver and muscle was examined with the hypothesis that ZIP14 mRNA expression would increase due to β-AA supplementation to support greater muscle demand for Zn, because previous literature has shown cAMP concentrations increase in bovine satellite cells treated with 10 μM ractopamine HCl and 1 μM Zn (Johnson et al., 2015) and mice deficient in Zn possess greater PDE activity (Hojyo et al., 2011). No differences in ZIP14 expression in muscle or liver were noted due to supplemental Zn which is in agreement with previous work (Lichten et al., 2009; Aydemir and Cousins, 2018). Additionally, supplemental ractopamine HCl had no effect on liver or muscle ZIP14 expression in the current study, suggesting that if greater amounts of Zn are required in the skeletal muscle during ractopamine HCl supplementation ZIP14 is not responsible for this action. More recently, Paskavitz et al. (2018) saw differential expression of several importers and transporters during the course of myogenesis, suggesting the importance of influx and efflux of Zn for myoblast differentiation and further research should be conducted to establish impacts of supplemental Zn concentrations on muscle Zip and ZnT expression in feedlot cattle.

Insulin-like growth factor 1 is an anabolic hormone in the body involved in growth and maintenance of skeletal muscle (Maggio et al., 2013). Plasma IGF-1 concentrations were greater on d 56 for NON than RAC; however, since plasma samples were collected pre-feeding at the beginning of the BA period these are inherent differences between sampling groups prior to starting treatment. By day 71 plasma IGF-1 concentrations did not differ, which is in accordance with prior literature where ractopamine HCl supplementation did not alter circulating IGF-1 concentrations (Winterholler et al., 2008; Bryant et al., 2010). However, others have noted effects of β-AA supplementation, Walker et al. (2010) saw increases in plasma IGF-1 in steers fed 200 mg ractopamine HCl $\cdot steer^{-1} \cdot d^{-1}$ and Beermann et al. (1987) saw decreases in circulating IGF-1 by 47 and 22% when cimaterol was fed to lambs for 6 and 12 weeks, respectively. Collectively, conflicting results regarding circulating IGF-1 concentrations require further research in regard to supplemental β-AA concentrations, type of β-AA, and length of supplementation. Zinc has been suggested to alter the IGF-1 signaling pathway through inhibition of protein tyrosine phosphatases, resulting in increased activation of receptor tyrosine kinases, thereby triggering growth factor signaling (Haase and Maret, 2003). Numerous studies in animal models have shown greater IGF-1 circulation in Zn supplemented animals vs. Zn deficient animals (Estívariz and Ziegler, 1997). Collectively, IGF-1 concentrations appear to increase due to supplemental Zn only when compared to states of Zn deficiency, suggesting cattle receiving CON were supplied adequate Zn concentrations to support similar concentrations of circulating IGF-1 as those receiving either supplemental Zn treatment.

Opportunity remains in the beef industry to improve supplemental trace mineral strategies during the implementation of growth technologies. Collectively, increasing supplementation of Zn, regardless of source, to cattle fed the β-AA ractopamine HCl has led

to mixed performance results across a variety of studies (Genther-Schroeder et al., 2016a,b) with no improvement in performance noted in the present study. Differences in MT mRNA expression due to supplemental Zn and ractopamine HCl indicates this may be a possible point of interaction between the two and given the critical role of MT in cellular Zn homeostasis further investigation is warranted. Regardless, providing dietary concentrations of Zn as 150 mg Zn/kg DM to finishing cattle receiving ractopamine HCl resulted in no performance advantages, suggesting that currently recommended concentrations of Zn are sufficient to support growth induced by ractopamine HCl.

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Table 1. Diet ingredient composition and nutrient content.

Ingredient (%DM)	CON[1]	INZN[1]	ZNBLD[1]
Cracked corn	62	62	62
Modified distillers grains with solubles	25	25	25
Chopped grass hay	8	8	8
Micronutrients and carrier[2]	5	5	5
Calculated components			
NEm	2.11	2.11	2.11
NEg	1.43	1.43	1.43
Analyzed components[3]			
Crude protein[4], %	15.0	15.0	15.0
NDF[4], %	20.5	20.5	20.5
Ether extract[4], %	4.7	4.7	4.7
Cu, mg/kg	13	12	12
Fe, mg/kg	85	70	83
Mn, mg/kg	33	32	32
Zn[2], mg/kg	37	133	133

[1]ZNTRT: CON = no supplemental Zn (diet analyzed 36 mg Zn/kg DM); INZN = CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; ZNBLD = CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ + 60 mg supplemental Zn/kg DM as ZN-AA complex (Availa-Zn; Zinpro, Eden Prairie, MN); Separate basals with dried distillers grain as carrier were made for each treatment containing supplemental Zn.

[2] Basal includes dried distillers grains with solubles as carrier, micronutrients provided as % DM; limestone (1.5%), Rumensin (0.0135%), and salt (0.31%). Trace minerals and vitamins provided per kg of DM: 0.15 mg Co (cobalt carbonate), 20 mg Cu (copper sulfate), 20 mg Mn (manganese sulfate), 0.1 mg Se (sodium selenite), 0.5 mg I (calcium iodate), and Vitamin A 2,200 IU (ROVIMIX A 1000 [1000 kIU/g], DSM, Parsippany, NJ.

[3] Analyzed components on DM basis.

[4] Chemical analysis completed by Dairyland Laboratories (Arcadia, WI).

Table 2. Nucleotide sequences and conditions of primers used for qPCR amplifications.

Primer[1]	Forward (5' to 3')	Reverse (3' to 5')	Annealing Temperature (C)
Rps9	CCTCGACCAAGAGCTGAAG	CCTCCAGACCTCACGTTTGTTC	52.0
ZIP14	AGGCTCCTGCTCTACTTC	AGCGTCTCAGAGGTATAATG	56.0
MT1a	ATGGACCCGAACTGCTCCTGC	GCGCAGCAGCTGCACTTGTCCG	59.0

[1]Rps9 (40S ribosomal protein S9; Janovick-Guretzky et al., 2007; Hergenreder et al., 2016; Kim et al., 2018); ZIP14 (Zrt- and Irt-like protein 14; Hansen et al., 2010); MT1a (Metallothionein 1a; Fry et al., 2013).

Table 3. Dietary Zn influence on initial performance, liver trace mineral concentrations and gene expression before ractopamine hydrochloride supplementation.

Item	CON[1]	INZN[1]	ZNBLD[1]	SEM	C vs. Z[2]	ZB vs. ZS[2]
Steers (*n*)	23	23	24			
Dry matter intake, kg/d	11.3	11.7	11.7	0.198	0.13	0.97
BW[3], kg						
d 0	428	428	428	3.8	0.85	0.74
d 57	546	550	545	3.7	0.75	0.33
ADG[3], kg	2.07	2.14	2.07	0.045	0.61	0.26
Gain to feed[3]	0.184	0.183	0.177	0.0035	0.34	0.25
Steers (*n*)	12	12	12			
Liver						
d 28, mg/kg DM						
Cu	254	231	222	13.6	0.11	0.64
Mn	9.2	9.2	9.3	0.33	0.88	0.92
Zn	106	102	108	2.4	0.84	0.08
d 54, mg/kg DM						
Cu	211	219	215	7.2	0.53	0.64
Mn	8.6	9.1	9.0	0.36	0.38	0.95
Zn	108	108	116	3.1	0.32	0.08
d 54, ΔCT						
MT1A	-0.713	-1.181	-2.260	0.3184	0.01	0.02
ZIP14	1.127	1.053	1.006	0.2046	0.91	0.43
Plasma NEFA[5], mEq/L	145	153	151	5.9	0.37	0.86
SUN[6], mg/dL	9.3	10.0	10.6	0.36	0.03	0.25

[1] ZNTRT: CON = no supplemental Zn (diet analyzed 36 mg Zn/kg DM); INZN = CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; ZNBLD = CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ + 60 mg supplemental Zn/kg DM as ZN-AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).
[2]Contrasts: C vs. Z = CON (C) vs. ZNBLD and INZN (Z); ZB vs. ZS = ZNBLD (ZB) vs. INZN (ZS).
[3]All weight values include 4% pencil shrink in calculations.
[4]Relative abundance of the Metallothionein 1A (MT1A) and Zrt- and Irt-like protein 14 (ZIP14) genes were normalized with the 40S Ribosomal Protein 9 (RPS9) endogenous control by using the change in cycle threshold (ΔCT). More negative ΔCT values equal greater expression.
[5]NEFA = non-esterified fatty acids; Analyzed as repeated measures utilizing compound symmetry covariate structure. No ZNTRT × time effect ($P = 0.87$). A time effect was noted ($P = 0.0001$) where plasma NEFA concentrations (153, 131, and 167 mEq/L for d 0, 26, and 56, respectively) were least on d 26 while d 0 and 56 did not differ.
[6]SUN = serum urea N; Analyzed as repeated measures utilizing compound symmetry covariate structure. No ZNTRT × time effect ($P = 0.86$). A time effect was noted for SUN concentrations ($P = 0.0001$; 9.3, 10.2, 10.3 mg/dL for d 0, 26, and 56, respectively) and were least on d 0 while d 26 and 56 did not differ from each other.

Table 4. Dietary Zn and ractopamine hydrochloride inclusion influence on performance and blood metabolite measures for ractopamine hydrochloride supplementation period.

	CON[1]		INZN[1]		ZNBLD[1]			*P*-value		
Item	NON[2]	RAC[2]	NON[2]	RAC[2]	NON[2]	RAC[2]	SEM	ZNTRT	BA	ZNTRT × BA
Steers (*n*)	12	11	11	12	12	12				
Dry matter intake, kg/d	11.8	11.6	11.9	12.3	11.9	11.9	0.308	0.49	0.80	0.64
BW[3], kg										
d 57	548	545	555	545	542	548	5.2	0.59	0.59	0.30
Final (d 89)	608	610	616	612	600	612	6.50	0.45	0.51	0.47
ADG[3], kg	1.86	2.06	1.90	2.08	1.80	1.97	0.096	0.57	0.02	0.99
Gain to feed[3]	0.158	0.174	0.160	0.168	0.151	0.166	0.0075	0.58	0.04	0.86
Steers (*n*)	6	6	6	6	6	5				
Plasma NEFA[4], mEq/L	164	143	157	155	155	156	9.5	0.94	0.33	0.44
SUN[5], mg/dL	10.8	9.9	10.9	10.1	11.7	10.7	0.63	0.34	0.09	0.99
Plasma IGF-1[6], ng/L	254	309	294	274	255	268	17.7	0.40	0.28	0.14

[1]ZNTRT: CON = no supplemental Zn (diet analyzed 36 mg Zn/kg DM); INZN = CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; ZNBLD = CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ + 60 mg supplemental Zn/kg DM as ZN-AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).

[2] BA: NON (no supplemental ractopamine HCl); RAC (300 mg ractopamine HCl·steer^{-1}·d^{-1}; Optaflexx, Elanco, Greenfield, IN; Fed for 32 d prior to slaughter.

[3]All weight values include 4% pencil shrink in calculations.

[4]NEFA = non-esterified fatty acids; Analyzed as repeated measures utilizing compound symmetry covariate structure. No time interactions for plasma NEFA ($P \geq 0.35$). Plasma NEFA concentrations were greater on d 56 than 70 and 88 (time effect $P = 0.003$; 167, 145, and 153 mEq/L for d 56, 70, and 88, respectively).

[5]SUN = serum urea N; Analyzed as repeated measures utilizing compound symmetry covariate structure. No time interactions for SUN ($P \geq 0.34$). Serum urea N concentrations were greater on d 88 than 56 and 70 (time effect $P = 0.01$; 10.3, 10.6, 11.2 mg/dL for d 56, 70, and 88, respectively).

[6]IGF-1 = Insulin-like growth factor-1; Analyzed as repeated measures utilizing compound symmetry covariate structure; A tendency for a BA × time effect was noted for plasma IGF-1 concentrations ($P = 0.06$; Figure 4).

Table 5. Dietary Zn and ractopamine hydrochloride influence on carcass performance measures.

	CON[1]		INZN[1]		ZNBLD[1]			*P*-value		
Item	NON[2]	RAC[2]	NON[2]	RAC[2]	NON[2]	RAC[2]	SEM	ZNTRT	BA	ZNTRT × BA
Steers (*n*)	12	11	11	12	12	12				
Carcass characteristics										
HCW, kg	388	390	391	392	380	394	4.6	0.68	0.12	0.31
DP[3], %	63.8	63.9	63.4	64.1	63.4	64.5	0.33	0.81	0.03	0.33
REA, cm^2	89.1	90.4	88.3	92.2	87.5	92.6	2.38	0.98	0.08	0.70
Back fat, cm	1.55	1.61	1.82	1.71	1.63	1.67	0.123	0.33	0.99	0.78
KPH, %	2.4	2.3	2.3	2.3	2.4	2.4	0.08	0.41	0.86	0.64
Marbling score[4]	525	465	521	519	508	519	28.9	0.68	0.48	0.44
Yield grade	3.3	3.3	3.6	3.4	3.4	3.3	0.19	0.67	0.45	0.79

[1]ZNTRT: CON = no supplemental Zn (diet analyzed 36 mg Zn/kg DM); INZN = CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; ZNBLD = CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ + 60 mg supplemental Zn/kg DM as ZN-AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).
[2]BA: NON (no supplemental ractopamine HCl); RAC (300 mg ractopamine HCl·$steer^{-1}$·d^{-1}; Optaflexx, Elanco, Greenfield, IN); Fed for 32 d prior to slaughter.
[3]DP = dressing percentage.
[4]400 = small; 500 = modest.

Table 6. Dietary Zn and ractopamine hydrochloride influence on liver trace mineral concentrations and liver and muscle relative mRNA expression for MT1A and ZIP14 during the BA period.

	CON[1]		INZN[1]		ZNBLD[1]			*P*-value		
Item[3]	NON[2]	RAC[2]	NON[2]	RAC[2]	NON[2]	RAC[2]	SEM	ZNTRT	BA	ZNTRT × BA
Steers (*n*)	6	6	6	6	6	5				
Liver										
d 54, mg/kg DM										
Cu[3]	261	193	222	209	205	202	17.7	0.42	0.07	0.16
Mn	8.8	8.5	9.4	8.8	8.3	9.7	0.49	0.65	0.70	0.10
Zn	111	105	108	107	112	120	4.5	0.13	0.88	0.34
d 71, mg/kg DM										
Cu[3]	277^{a}	201^{b}	217^{ab}	226^{ab}	198^{b}	191^{b}	13.2	0.04	0.08	0.03
Mn	8.4	7.8	9.4	7.8	8.1	7.7	0.53	0.41	0.05	0.46
Zn	112	106	107	121	116	123	5.5	0.17	0.26	0.16
d 54[4], ΔCT										
MT1A	-1.398	-0.963	-1.460	-0.902	-2.563	-1.958	0.4239	0.01	0.02	0.57
ZIP14	0.823	1.4319	1.037	1.069	0.9646	1.047	0.293	0.92	0.32	0.56
d 71[4], ΔCT										
MT1A[3]	-0.429^{x}	0.008^{x}	-0.133^{x}	-2.177^{y}	-1.738^{y}	-2.046^{y}	0.5454	0.02	0.16	0.08
ZIP14	0.817	1.566	0.898	0.850	0.838	0.664	0.2746	0.27	0.44	0.21
Muscle										
d 71[4], ΔCT										
MT1A	3.069	3.275	3.262	3.279	3.327	3.110	0.3031	0.95	0.99	0.81
ZIP14	6.102	6.313	6.588	6.355	6.587	6.966	0.2756	0.16	0.60	0.51

[1]ZNTRT: Control (CON) received no supplemental Zn (diets analyzed 33 mg Zn/kg DM); Inorganic Zn (INZN) diet was CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; Zinc blend (ZNBLD) diet was CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ and 60 mg supplemental Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN)].

[2]BA: NON (no supplemental ractopamine HCl); RAC (300 mg ractopamine HCl·steer^{-1}·d^{-1}; Optaflexx, Elanco, Greenfield, IN); Fed for 32 d prior to slaughter.

[3]Within a row, means with differing superscripts are different (a,b; $P \leq 0.05$) or tend to be different (x,y; $P \leq 0.10$).

[4]Relative abundance of the Metallothionein 1A (MT1A) and Zrt- and Irt-like protein 14 (ZIP14) genes were normalized with the 40S Ribosomal Protein 9 (RPS9) endogenous control by using the change in cycle threshold (ΔCT). More negative ΔCT values equal greater expression.

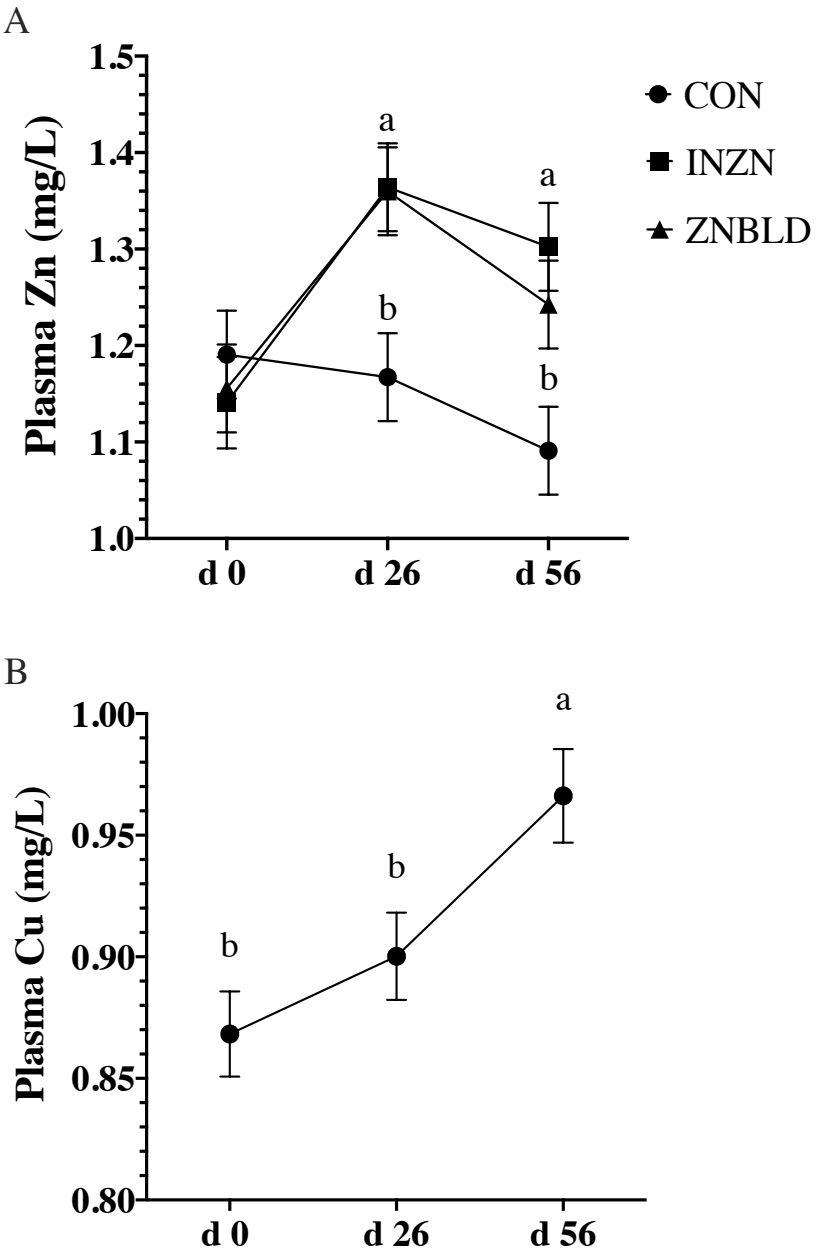

Figure 1. Effect of Zn treatment as no supplemental Zn (37 mg dietary Zn/kg DM; CON), 150 mg Zn/kg DM (CON + 120 mg supplemental Zn/kg DM from $ZnSO_4$; INZN), or 150 mg Zn/kg DM (CON + 60 mg supplemental Zn/kg DM from $ZnSO_4$ + 60 mg supplemental Zn/kg DM from Availa-Zn, Zinpro corporation, Eden Prairie, MN; ZNBLD). **A**) A ZNTRT × time effect was detected for plasma Zn concentrations ($P = 0.003$). **B**) Plasma Cu increased over time ($P = 0.0001$). Superscripts that differ (a,b) indicate means differ ($P \leq 0.05$).

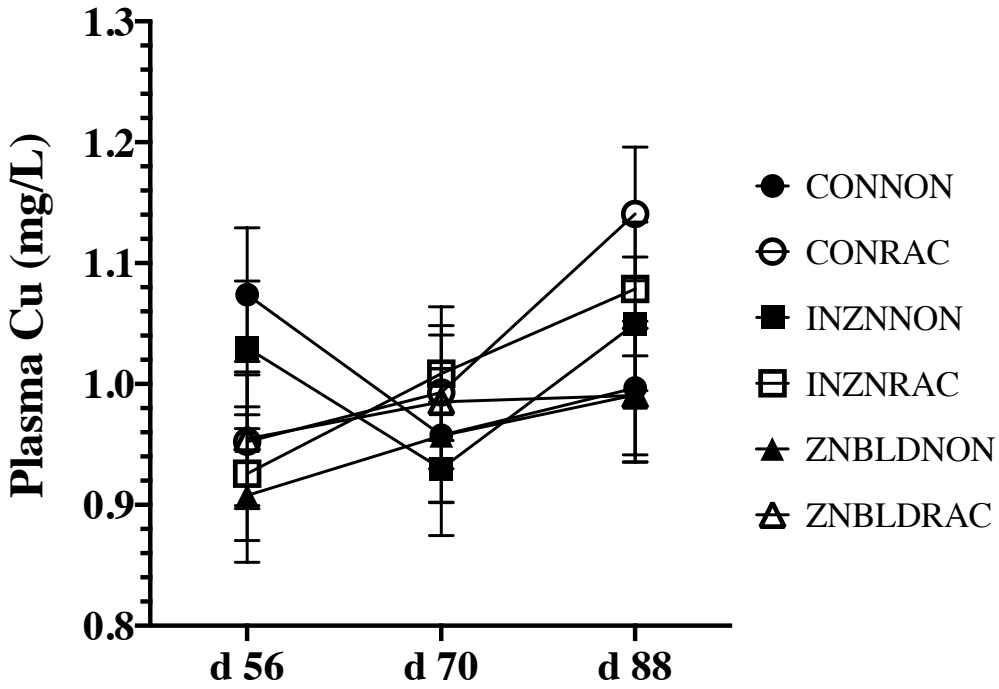

Figure 2. Effect of Zn treatment as no supplemental Zn (37 mg dietary Zn/kg DM; CON), 150 mg Zn/kg DM (CON + 120 mg supplemental Zn/kg DM from $ZnSO_4$; INZN), or 150 mg Zn/kg DM (CON + 60 mg supplemental Zn/kg DM from $ZnSO_4$ + 60 mg supplemental Zn/kg DM from Availa-Zn, Zinpro corporation, Eden Prairie, MN; ZNBLD) and either no supplemental ractopamine HCl (NON) or 300 mg ractopamine HCl·steer^{-1}·d^{-1} (RAC; Optaflexx, Elanco, Greenfield, IN). A ZNTRT × BA × time effect was detected ($P = 0.05$).

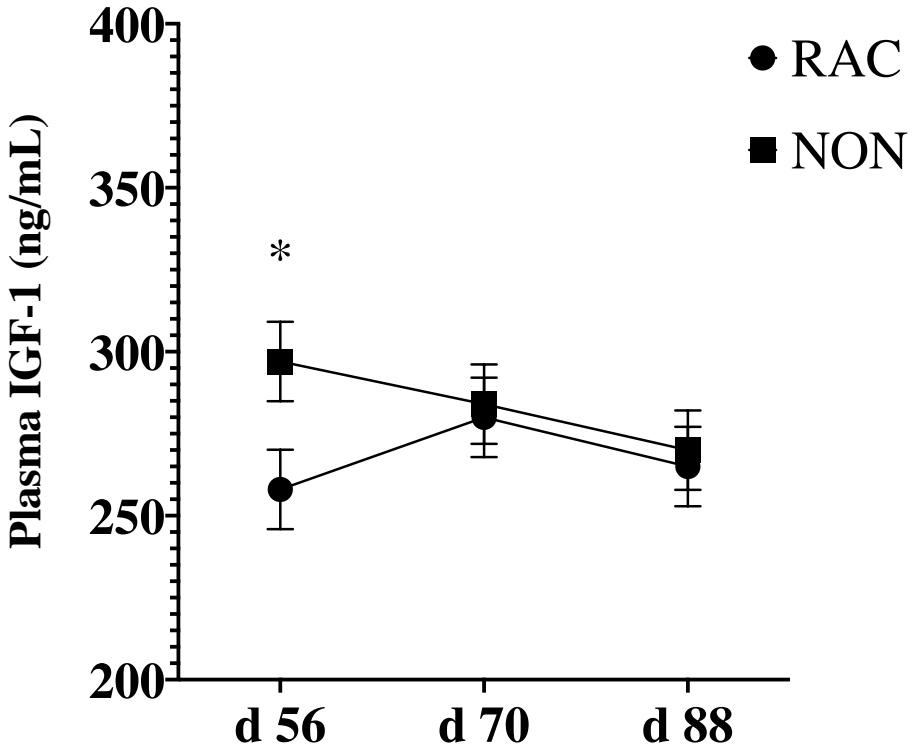

Figure 3. Effect of either no supplemental ractopamine HCl (NON) or 300 mg ractopamine HCl ·steer^{-1}·d^{-1} (RAC; Optaflexx, Elanco, Greenfield, IN). * = denotes a significant difference between treatments on that day ($P \leq 0.05$).

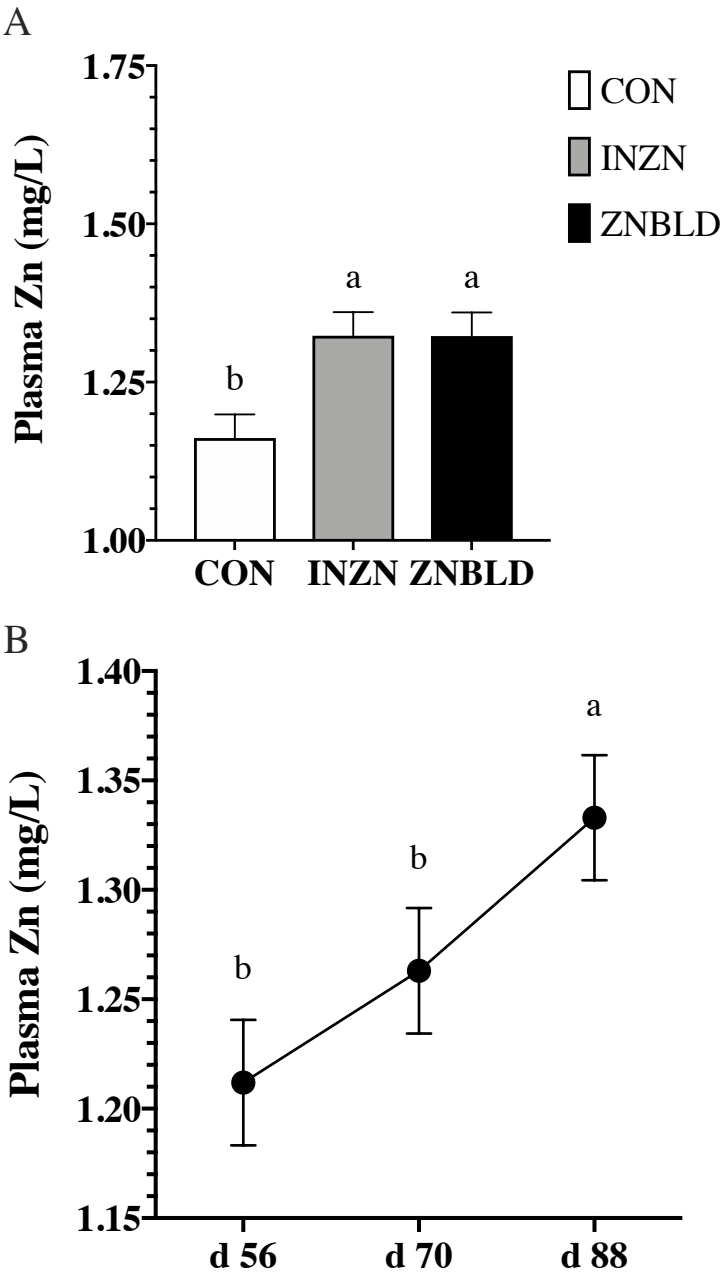

Figure 4. Effect of Zn treatment as no supplemental Zn (37 mg dietary Zn/kg DM; CON), 150 mg Zn/kg DM (CON + 120 mg supplemental Zn/kg DM from $ZnSO_4$; INZN), or 150 mg Zn/kg DM (CON + 60 mg supplemental Zn/kg DM from $ZnSO_4$ + 60 mg supplemental Zn/kg DM from Availa-Zn, Zinpro corporation, Eden Prairie, MN; ZNBLD) and either no supplemental ractopamine HCl (NON) or 300 mg ractopamine HCl $\cdot steer^{-1} \cdot d^{-1}$ (RAC; Optaflexx, Elanco, Greenfield, IN). **A**) Plasma Zn affected by ZNTRT ($P \leq 0.005$). **B**) A time effect was detected for plasma Zn ($P \leq 0.04$); no ZNTRT × BA × time, ZNTRT × time, BA × time, or BA effects were noted for plasma Zn. Superscripts that differ (a,b) indicate means are different ($P \leq 0.05$).

CHAPTER 5.

THE INFLUENCE OF SUPPLEMENTAL ZINC AND DIETARY FIBER CONCENTRATION ON MINERAL RETENTION OF BEEF STEERS

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Abstract: The objective was to determine if Zn retention improved with supplemental Zn above recommended concentrations with increasing dietary fiber concentration. Angus steers (n = 32; 309 ± 4.2 kg BW) with Genemax gain scores of 3, 4, or 5 were utilized in a 2×2 factorial arrangement (8 steers/treatment). Steers were stagger started (4 blocks of 8 steers) and stratified by BW within growing diets to one of two Zn strategies (**ZNTRT**), no supplemental Zn (analyzed 36 mg Zn/kg DM; **CON**) or supranutritional Zn (CON + 60 mg Zn/kg DM as $ZnSO_4$ + 60 mg Zn/kg DM as Zn-amino acid complex; **SUPZN**). Dietary fiber strategies (**FIBER**) were formulated to target two fiber supplementation rates representing high fiber (**HF**; ~35% NDF) or low fiber (**LF;** ~25% NDF). Within block, steers received HF for 60 d, then pens were randomly assigned to LF or HF for finishing. Steers fed LF were transitioned for 15 d; on d 75 steers were moved to metabolism crates and adapted for 10 d, followed by 5 d of total fecal and urine collection. Retention of Zn, Mn, Fe, Cu, and N were calculated. The model for analysis of metabolism data included the fixed effects of ZNTRT, FIBER, block, and the interaction of ZNTRT × FIBER, with the three-way interaction of ZNTRT × FIBER × block as random. Steer was the experimental unit (n = 8/treatment

combination). Zinc did not affect initial 60 d performance ($P \geq 0.62$). Dry matter and OM digestibility were lesser ($P = 0.02$) and N digestibility tended to be lesser ($P = 0.07$) in CON vs. SUPZN. Intake and digestibility of NDF and ADF were greater ($P \leq 0.01$) in HF vs. LF. Digestibility and retention of N as a percentage of intake were greater ($P \leq 0.04$) while N retention as g/d tended to be greater in HF vs. LF ($P = 0.06$). Apparent absorption of Zn tended to be greater ($P = 0.06$) in CON vs. SUPZN. A ZNTRT×FIBER effect was identified for Zn retention (mg/d; $P = 0.01$) where within SUPZN Zn retention was greater in HF vs. LF ($P < 0.01$). Apparent absorption and retention of Zn were greater (% of intake; $P \leq 0.02$) in HF vs. LF. Apparent absorption of Cu, Fe, and Mn were unaffected by ZNTRT or FIBER ($P \geq 0.24$). Increasing dietary Zn increased Zn retained regardless of changes in coefficient of absorption. Additionally, dietary fiber content may impact trace mineral and N metabolism by steers, potentially due to increased release of these nutrients from feed as fiber digestibility increases. It appears dietary Zn concentrations and diet composition influence trace mineral absorption in beef steers.

KEY WORDS: beef cattle, fiber, nitrogen, trace mineral, zinc

Introduction

Zinc, a trace mineral found throughout almost all metabolic systems in mammals (Cousins et al., 2006) is a critical component in many growth pathways in the body including DNA and protein synthesis. Current recommendations for Zn (30 mg Zn/kg DM) were established more than fifty years ago to prevent deficiency in healthy animals and support growth (NRC, 2000). Previous research has shown the combination of Zn-amino acid complex and $ZnSO_4$ increased cattle performance when receiving a relatively low fiber

finishing diet (~20% NDF) and ractopamine hydrochloride (Genther-Schroeder et al., 2016a). Additionally, Carmichael et al. (2018) found that increasing supplemental Zn concentrations positively impacted N retention in finishing beef steers also receiving a low fiber diet (~19% NDF). Recently, some have determined that feeding supplemental trace minerals may influence fiber digestibility (Faulkner et al., 2017; Faulkner and Weiss, 2017; VanValin et al., 2018). Regardless, few studies have been conducted evaluating the relationship of fiber and Zn supplementation exceeding current recommendations (NASEM, 2016). Arelovich et al. (2000) evaluated excessive concentrations of dietary Zn to control ammonia toxicity which decreased nutrient digestibility (Arelovich et al., 2000); however, this concentration of dietary Zn (450 mg Zn/kg DM) approached the maximum tolerable level recommended for beef cattle (NASEM, 2016). To the authors knowledge, no studies have been conducted in ruminants evaluating the relationship between fiber content and supplemental Zn concentrations exceeding current recommendations for beef cattle, yet below pharmacological rates. The objective was to evaluate fiber digestibility and Zn retention in beef steers fed low or high fiber finishing diets when supplemental Zn concentrations are similar to rates supplemented in the feedlot industry (Samuelson et al., 2016). The hypothesis was that increasing dietary fiber and supplemental Zn concentrations would decrease Zn absorption and fiber digestibility.

Materials and Methods

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (8-15-8073-B).

Experimental Design

The study was conducted as a 2 × 2 factorial, with Zn (**ZNTRT**) supplementation strategies of no supplemental Zn (**CON**) or supranutritional Zn [CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN); **SUPZN**] beginning on d 0, and differing dietary fiber strategies (**FIBER**) were formulated to target two fiber supplementation rates representing high fiber (**HF**; ~35% NDF) or low fiber (**LF;** ~25% NDF). The HF treatment was obtained by replacing 20% cracked corn in the LF diet with 14% corn silage and 6% grass hay (DM basis). One month prior to initiation of ZNTRT strategy, high-percentage Angus cattle were acquired from two producer sources and gentled with repeated human exposure. Steers (n = 32; 309 ± 4.2 kg) with Genemax gain scores of 3, 4 or 5 (Zoetis, Parsippany, NJ), which indicates a predicted genetic value belonging in the top 60% for growth potential of tested Angus cattle (Certified Angus Beef LLC, 2012), were utilized in this study. Steers were separated into four blocks (n = 8 steers per block; 2 per treatment combination) and stagger-started on diets because of space limitations in the metabolism facility (treatment initiation interval between blocks averaged 30 d; blocks sorted by BW). On d 0 for each block, steers were stratified by BW and Genemax score to receive ZNTRT diets for 60 d in pens equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). During the initial 60 d feeding period 3 pens per ZNTRT were utilized so pen density did not exceed 6 steers and to limit feed competition. On d 60, within ZNTRT, steers were stratified by weight and Genemax gain score and half selected to begin transition to the LF diet for 15 d. Dry matter intakes during d 60-75 for all blocks were not included for any analyses because steers transitioning to LF

were fed in concrete bunks where individual intake was unavailable and steers in each treatment and block were fed in a single pen. During this transition period, steers transitioning to LF were fed 80% (DM) of previous intake on HF for three days in concrete bunks, with daily increases for the remaining 11 d of transition at 0.227 kg DM/steer. Following return from the metabolism facility (d 90-95), 2 pens per treatment combination were utilized where pen stocking rate did not exceed 6 steers. Diet composition and analysis is shown in **Table 1**. On d 28 of the study, steers were implanted with Component TE-IS with Tylan (80 mg trenbolone acetate, 16 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health, Greenfield, IN). Steers were weighed prior to feeding on d -1 and 0, and 59 and 60 to determine initial and final BW of the initial feedlot period, respectively. Pre-feeding weights were recorded on d 74 and 75 for an initial metabolism weight. After weighing at the farm on d 75, steers were transported 6.3 km to the metabolism facility in Kildee Hall (Iowa State University, Ames, IA). Steers continued to receive their respective ZNTRT and FIBER treatments in the metabolism facility from d 75-90 (d 1-15 of metabolism period). A 4% pencil shrink was applied to all BW measurements, including calculations for ADG and G:F.

Metabolism Period

From d 75 to 90 (d 1 to 10 adaptation, d 11 to 15 collection) steers (437 ± 9.2 kg BW) were housed in individual stainless-steel crates [213.4 cm (length) × 182.9 cm (height) × 91.4 cm (width)], that were fitted with rubber fatigue mats. Each morning steers were offered the appropriate total mixed ration (**TMR**) at 0700 h. As-fed feed delivery was 105% of the previous days as-fed intake. All offered TMR and refused feed for each steer was recorded

daily and daily as-fed TMR intake amount was determined by subtracting refused feed from offered TMR. During the acclimation period cattle adjusted to crates and allowed space to lie down. On the morning of d 10 (d 85 of study) of the metabolism period cattle were removed from crates and crates were thoroughly cleaned. Preparation of metabolism crates prior to return of the steers, as well as daily fecal and urine collection procedures were as described by Carmichael et al. (2018). Feed delivery rate during collection was 105% of the previous days as-fed intake. Water intake was recorded individually throughout metabolism period (DLJ single jet water meter; Daniel L. Jerman Co., Hackensack, NJ).

During the collection period (d 11 to 15; d 85 to 90 of study), refused feed was removed and weighed, and aliquots were collected (~300 g or greater). Total mixed ration samples from CON-HF, SUPZN-HF, CON-LF, and SUPZN-LF were sampled daily. All TMR and refused feed samples were dried in a convection oven at 70ºC for 48 h. Fecal and urine aliquots were collected and determination of fecal DM was achieved according to procedures described by Pogge et al. (2014). Dried fecal, TMR, and refused feed samples were ground through a 2mm screen (Wiley Mill; Thomas Scientific, Swedesboro, NJ; Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and stored in sealed plastic bags until compositing and nutrient analysis.

On d 15 of the metabolism period (d 90 of study), steers were removed from metabolism crates and transported 6.3 km back to the Iowa State Beef Nutrition Farm. Prior to fecal collection and subsampling on d 90 crates were hand-scraped with acid washed plastic paint scrapers and deionized water to collect all remaining feces excreted during the collection period.

Upon return to the farm steers were given 5 days to rest and were maintained on their respective diets until liver biopsies were collected on d 95 with methods established by Engle and Spears (2000).

Analytical Procedures

Total mixed ration samples of each diet were collected weekly during the feedlot period (d 0 to d 75). Weekly TMR samples were dried for 48 h at 70°C and the resulting DM value was multiplied by as-fed feed intake for each steer to determine DMI during the feedlot period. Dry matter and organic matter of TMR, refused feed, and fecal matter during the collection period were determined according to AOAC (1990) procedures. Nitrogen content of TMR, refused feed, fecal matter, and urine was determined using the combustion method (TruMac N, LECO Corporation, Saint Joseph, MI; Lundy et al., 2015). Nitrogen digestibility was calculated as described by Lundy et al. (2015). Digestibility of DM and OM was calculated by dividing fecal DM by DM intake, subtracting from 1 and multiplying by 100. Thirty-hour in vitro digestibility was conducted according to Goering and Van Soest (1970; Dairyland Laboratories, Arcadia, WI).

Inductively coupled plasma optical emission spectrometry (ICP; Optima 7000 DV, Perkin Elmer, Waltham, MA) was used to conduct mineral analysis of TMR, refused feed, urine, and fecal matter. Dried, ground, and composited TMR, refused feed, and fecal samples were acid digested prior to mineral analysis according to the methods described by Richter et al. (2012) and Pogge et al. (2014). Liver samples were digested according to Pogge and Hansen (2013). Urine samples were prepared for the ICP with methods described by Carmichael et al. (2018). No additional dilutions were necessary for mineral analysis of

TMR, refused feeds, or fecal matter for Cu, Fe, Mn, and Zn. A bovine liver standard from the National Institute of Standards and Technology (Gaithersburg, MA) was utilized to verify instrument accuracy and yttrium (PerkinElmer, Waltham, MA) as an internal standard to account for any variation in sample introduction within individual runs.

Calculations to determine TMR, refused feed, urine, and fecal mineral content and intake were described by Carmichael et al. (2018). Daily mineral intake, fecal mineral output, and urine mineral output were determined by dividing total mineral content of each by 5 (number of collection days). Apparent absorption, retention, and retention as a percentage of intake was calculated by methods described by Carmichael et al. (2018). Neutral detergent fiber and ADF analysis was conducted on TMR, feces, and all feed refusals in duplicate with methods established by Van Soest et al. (1991) using an ANKOM 200 fiber analyzer (Ankom Technology, Macedon, NY). Alpha-amylase was used during the NDF analysis. Consistency was verified using a standard brome grass hay sample (inter-assay CV of 2.4% and 3.0% for NDF and ADF analysis, respectively).

Statistical Analysis

All data were analyzed as a randomized complete block design. Performance and intake data for the initial 60 d period prior to transition (steer as experimental unit; n = 16 per ZNTRT) were analyzed using the Mixed procedures of SAS (SAS Institute Inc., Cary, NC). The model included the fixed effects of ZNTRT and block and initial BW values (d 0 of study) were used as a covariate. Dry matter intake data were analyzed as repeated measures with week as the repeated effect and compound symmetry variance structure was selected to achieve the lowest Akaike information criterion value. Data collected following d 75 were

analyzed as a 2 × 2 factorial arrangement utilizing the Mixed procedure of SAS. Pearson correlation analyses (PROC CORR) was used to identify and establish the relationship between Zn retention and N retention. The model for the analysis of the metabolism period and liver mineral included the fixed effects of ZNTRT, FIBER, block, and the interaction of ZNTRT × FIBER, with the three-way interaction of ZNTRT × FIBER × block as random. Data for urine excretion (mg/d and % of intake) were normally distributed after log transformation and treatment means and SEM were reverse transformed for reported results. Steer was the experimental unit (n = 8/treatment combination) for all analyses. Determination of outliers was accomplished using Cook's D statistic and removed if Cook's D ≥ 0.5. Due to negative retention values for Cu, Fe, Mn, and Zn during the collection period, data from one steer from CON-LF were removed from analysis. Significance was declared at $P \leq 0.05$ and tendencies identified at $P = 0.06$ to 0.10. Values reported are least square means and SEM. Tabular values reported reflect the least square means and the PDIFF statement in SAS was utilized to determine pairwise differences.

Results

Pre-metabolism Performance Period

During the first 60 d of Zn supplementation, when all steers received the HF diet, there was no week × ZNTRT effect on steer DMI ($P = 0.55$; **Table 2**). Zinc supplementation did not influence DMI, ADG, G:F, or final BW during the 60 d period preceding the metabolism period ($P \geq 0.62$; Table 2).

Metabolism Period

Effects of ZNTRT and FIBER on nutrient intake, output and digestibility assessments are shown in **Tables 3 and 4**. No ZNTRT × FIBER effects were identified for water intake, urine excretion, or intake, output, retention and digestibility of DM, OM, NDF, ADF and N ($P \geq 0.16$). No effects of ZNTRT were noted on nutrient intake, urine output, and fecal output parameters, NDF and ADF digestibility, or N retention ($P \geq 0.27$). Water intake was greater ($P = 0.03$) in CON vs. SUPZN and lesser ($P = 0.01$) in HF vs. LF. A positive correlation was detected between daily DMI (kg/d) and water intake (L/d; $r = 0.48$, $P = 0.007$) as well as urine output and water intake (L/d; $r = 0.42$, $P = 0.02$). Dry matter and OM digestibility were lesser ($P = 0.02$) while N digestibility tended to be lesser ($P = 0.07$) in CON vs. SUPZN. No differences were detected due to FIBER for DMI, OM intake, N intake, or fecal output of DM, OM and NDF ($P \geq 0.13$). Intake and digestibility of NDF and ADF were greater ($P = 0.01$) in HF vs. LF. Urine daily output, urinary N and fecal N tended to be lesser in HF ($P \leq 0.08$) vs. LF. Nitrogen digestibility and N retention as a percent of intake were greater ($P \leq 0.04$) and N retention expressed as g/d tended to be greater in HF vs. LF ($P = 0.06$).

Influence of ZNTRT and FIBER on trace mineral intake, excretion, apparent absorption, and retention as milligrams per day and percentage of intake are reported in **Tables 5 and 6**, respectively. No ZNTRT × FIBER effects were noted for Cu, Fe, or Mn intake, excretion, apparent absorption and retention, or for Zn intake, fecal excretion (% of intake), urinary excretion, apparent absorption and retention (% of intake; $P \geq 0.13$). Expressed as mg/d or percentage of nutrient intake, Cu, Fe and Mn intake, fecal excretion,

apparent absorption, and retention were unaffected by ZNTRT strategy ($P \geq 0.32$). Urinary excretion of Fe and Mn were unaffected by ZNTRT (mg/d and % of intake; $P \geq 0.21$). Copper urinary excretion tended to increase when expressed as percentage of intake ($P = 0.08$) in CON vs. SUPZN. Intake of Zn was lesser in CON vs. SUPZN (mg/d; $P = 0.01$). When expressed as percentage of Zn intake, fecal excretion of Zn tended to be lesser ($P = 0.06$) while urinary excretion of Zn was greater ($P = 0.01$) and apparent absorption of Zn tended to be greater ($P = 0.06$) in CON vs. SUPZN. A ZNTRT × FIBER interaction was detected for fecal Zn excretion (mg/d; $P = 0.04$) where within SUPZN excretion was lesser in HF vs. LF (979 vs. 1161; $P < 0.01$), while CON-HF (233) was similar to CON-LF (242; $P = 0.88$). Additionally, a ZNTRT × FIBER effect was identified for Zn retention (mg/d; $P = 0.01$) where within SUPZN Zn retention was greater in HF vs. LF (250 vs. 113; $P < 0.01$), while within CON HF was not different from LF (64 vs. 51; $P = 0.65$).

Regardless of manner of expression (mg/d or % of intake) Cu and Mn intake, excretion, absorption and retention were unaffected by FIBER ($P \geq 0.18$). Intake, urinary excretion, apparent absorption, and retention of Fe were not affected by FIBER (mg/d and % of intake; $P \geq 0.20$). Fecal excretion of Fe presented as mg/d tended to be greater ($P = 0.07$) in HF vs. LF. Zinc fecal excretion was lesser (% of intake; $P = 0.03$), while apparent absorption (% of intake) and retention (mg/d or % of intake) were greater ($P \leq 0.02$) in HF vs. LF.

No ZNTRT × FIBER effects were detected for d 95 liver mineral concentration data ($P \geq 0.33$; **Table 7**). Liver Cu concentrations were greater ($P = 0.01$) in CON vs. SUPZN and tended to be greater ($P = 0.08$) in HF vs. LF. Liver Fe, Mn and Zn concentrations were unaffected by ZNTRT ($P \geq 0.61$) or FIBER ($P \geq 0.61$).

Discussion

A combination of Zn-amino acid complex and Zn sulfate supplementation fed at rates near the industry reported average for Zn supplementation of 100 mg Zn/kg DM (Samuelson et al., 2016) positively affects cattle growth. Genther-Schroeder et al. (2016a) found that increasing supplemental Zn concentrations increased final BW and ADG during ractopamine hydrochloride supplementation in finishing steers. Utilizing the same Zn supplementation method, Carmichael et al. (2018) noted increased N retention regardless of ractopamine hydrochloride inclusion. These previous studies utilized relatively low fiber finishing diets (~20% NDF) and recently supplemental mineral inclusion has been shown to impact fiber digestibility (Faulkner et al., 2017; Faulkner and Weiss, 2017; VanValin et al., 2018). Faulkner et al. (2017) noted differential impacts of fiber from by-products vs. forages such as corn silage on trace mineral absorption by dairy cows, and a large amount of by-product (modified distillers grains) were fed in previous studies examining the combination of Zn-amino acid complex and Zn sulfate (Genther-Schroeder et al., 2016a; Carmichael et al., 2018). Therefore, the objective of this study was to evaluate the impact of low or high fiber finishing diets on nutrient digestibility and Zn retention when Zn supplementation is similar to industry rates (Samuelson et al., 2016).

Fiber digestion was greater in HF vs. LF in the present study. Negative associative effects occur when grains decrease voluntary intake or digestion of forages in the rumen (Dixon and Stockdale, 1999). Thirty-hour in vitro NDF digestibility of the silage fed during the metabolism period (62.6%) suggests it was of high feeding value; however, ruminal fermentation of grains can be detrimental to fiber digestibility. Rapid fermentation of carbohydrates in the rumen results in production of volatile fatty acids and can rapidly decrease rumen pH when production exceeds absorption. Low ruminal pH can diminish

ruminal fiber digestibility as fibrolytic microorganisms demand a narrow range in pH (6.6-7.0) to sustain function (Terry et al., 1969; Stewart, 1977; Mould and Ørskov, 1983). The greater NDF digestibility in HF vs. LF could be partially explained by negative associative effects occurring in LF steers because of the greater inclusion of dry rolled corn in the LF diet. However, Beckman and Weiss (2005), comparing diets with similar corn silage concentrations as the present study with varying NDF:starch ratios, observed a change in ruminal VFA profile while ruminal pH was not affected. Possible negative associative effects affiliated with feedstuffs decreasing pH may have lessened NDF digestibility in LF, but ruminal pH and ruminal fiber digestibility were not measured in the present study. Both LF and HF diets contained 32% (DM basis) of a combination of modified and dried distillers grains, and Loy et al. (2007) noted that supplementing distillers grains to heifers allowed ad libitum access to chopped grass hay decreased ruminal pH and NDF disappearance. However, the inclusion of distillers grains for both diets in this study could have impacted rumen environment similarly. It is expected that steers fed the HF diet had increased rumination and should therefore have had greater buffering capacity due to saliva influx into the rumen as well as more stable rumen mat consistency, allowing for greater fiber digestion.

As aforementioned, high concentrate diets can decrease ruminal pH, often resulting in increased solubility of minerals within the rumen (Waghorn et al., 1990). Solubilized minerals are potentially susceptible to binding to phytate or undegraded fiber which negatively impacts mineral absorption (Torre et al., 1991). Fortunately, ruminant microbes possess phytase activity, diminishing the inhibitory action of phytate on mineral absorption (Suttle, 2010). However, phytate may be an important consideration in the study since decreases in passage rate from greater amounts of forage in the diet can increase phytate degradation, while diets higher in concentrates may allow the opposite (Balch, 1950).

The effect within SUPZN for increased fecal excretion could be indicative of a supplemental vs. inherent dietary Zn concentration interaction with undegraded fiber. Minerals such as Zn and Cu are associated with the cell wall of plants, which consists of the NDF fraction of feedstuffs, and due to the increased NDF digestibility of HF may also explain the differences seen in Zn retention between HF and LF. Supplemental Zn in the present study was provided as $ZnSO_4$ and as Zn-AA complex, both of which have been shown to be soluble in the rumen (Spears and Kegley, 2002; Spears et al., 2004). It is possible that supplemental Zn could be more susceptible to binding by undegraded fiber, since supplemental Zn solubilization may coincide with fiber digestion, allowing solubilized supplemental Zn to bind fiber not yet digested. Therefore, the increase in the amount of undegraded fiber noted in LF, coupled with potentially increased solubilized supplemental Zn, may have resulted in greater fecal Zn excretion for the SUPZN-LF steers. Decreased NDF digestibility in LF could negatively impact Zn absorption if ruminally solubilized Zn binds to undegraded fiber, while increased NDF digestibility in HF may have allowed for more solubilization and absorption with less undegraded fiber available to bind Zn. Collectively, possible depression of phytate degradation and NDF digestibility in higher concentrate diets could partially explain the interaction observed within SUPZN to increase Zn fecal excretion and decrease retention in LF (mg/d), as well as the decrease in Zn apparent absorption in steers consuming the LF diet.

The present study utilized both inorganic and amino acid complexed Zn sources in the SUPZN treatment. Recent work suggests that ruminally soluble sources of Zn may negatively affect fiber digestion (Garg et al., 2008; Faulkner et al., 2017; VanValin et al., 2018); however, fiber digestion was unaffected by SUPZN in the present study. As mentioned previously, fiber provided by the FIBER diets was highly digestible, which may

not have been the case in previous studies displaying depressed fiber digestibility with the addition of Zn, potentially resulting in greater undegraded fiber available to bind ruminally solubilized Zn. Supranutritional Zn increased DM and OM digestibility relative to CON; this is in contrast to the results of Carmichael et al. (2018), where no differences in DM or OM digestibility were noted due to SUPZN in finishing cattle.

Diet differences exist between the two experiments (Carmichael et al., 2018), with greater corn inclusion in the diet of the comparison study and greater fiber in the present study. In previous work ruminal fluid Zn concentrations of 50 μg/mL decreased cellulose digestion when compared to control (Eryavuz and Dehority, 2009); however, the authors conceded this concentration was approximately 2000 mg Zn/kg DM, four times the maximum tolerable level suggested for beef cattle (NASEM, 2016). Consequently, supplying SUPZN, dietary Zn concentrations well below those previously shown to inhibit cellulose degradation, could result in a positive impact on fiber digestion by supplying adequate Zn concentrations for microbial function. However, this was not examined in the present study and is beyond the scope of this paper. Zinc and fiber have a complex relationship within the rumen and more research should be conducted on the effect of supplemental Zn concentration and source on diet digestibility in ruminants. It is necessary to achieve a thorough understanding of this interaction while aspiring toward optimal supplemental Zn concentrations.

Apparent absorption of Zn tended to be decreased due to Zn supplementation in the present study, and closely follows previous reports where increasing dietary Zn concentration decreases Zn apparent absorption (Weigand and Kirchgessner, 1979; Mohanna and Nys, 1999; VanValin et al., 2018). These results are in contrast to more recent research with heavy-weight finishing steers (570 ± 5.6 kg; Carmichael et al., 2018), where increasing Zn

concentrations exerted no effects on Zn coefficient of absorption. Steers receiving SUPZN in the present study had greater Zn retention (mg/d) in accordance with previous studies (Weigand and Kirchgessner, 1979; Carmichael et al., 2018), and supports that increasing dietary Zn concentrations will increase Zn retained. Coefficients of Zn absorption in this study (averaging 17.2% across all treatments) were similar to those previously reported in growing steers [16.0%, Pogge et al., 2014a; 9.9%, Pogge et al., 2014b; 10.0% ($ZnSO_4$), Shaeffer et al., 2017].

Previous studies have shown a positive relationship between Zn and N retention (Oberleas and Prasad, 1969; Greeley et al., 1980; Carmichael et al., 2018). In contrast to previous work in heavy weight finishing steers (Carmichael et al., 2018), N digestibility tended to increase but N retention did not increase in SUPZN. The late stage finishing steers utilized by Carmichael et al. (2018) retained a dramatically larger proportion of ingested N (42.2%) compared with the growing steers in the present study (23.0%), reflecting an increased N need to support heavier BW. The influence of Zn supplementation on N retention in feedlot cattle across varying stages of growth remains to be fully elucidated.

Dietary fiber effects on N metabolism were also noted in the present study and portray a positive effect of the HF diet. Lesser N degradation can result from concentrate addition (Lapierre and Lobley, 2001), and similar to the present study, increased dietary concentrate inclusion has been shown to increase urinary N excretion decreasing available N for recycling to the rumen (Huntington et al., 1996). Increased N digestibility due to HF in this study is in contrast to previous research, where increased roughage decreased protein degradation in dairy cows (Balch, 1950). Additionally, Faulkner and Weiss (2017) saw lesser N retention in a diet with a similar amount of corn silage (44%) when compared to a by-product diet. However, previous research established a decrease of N lost to the environment

when corn silage was fed to dairy cattle (Dhiman and Satter, 1997; Kume et al., 2004; Kume et al., 2008b; Kume et al., 2008a), suggesting a more efficient utilization of dietary crude protein. Work conducted by Beckman and Weiss (2005) in lactating dairy cattle resulted in a linear increase in N retention with increasing NDF:starch ratio while in situ techniques evaluating differing forage-to-concentrate ratios saw decreasing protein degradation with lesser forage-to-concentrate ratios (Devant et al., 2001). Additionally, as pH decreases protein degradation decreases, and collectively protein degradation decreases when substrate is provided by concentrate rather than forage (Bach et al., 1984). Further research should be conducted to increase understanding of N utilization in beef steers fed corn silage-based diets.

Moisture content of HF vs. LF may explain the lesser water intake by HF during the collection period (41.0 vs. 47.0% DM, respectively). Nevertheless, a positive correlation was detected between daily DMI (kg/d) and water intake (L/d; $r = 0.48$, $P = 0.007$). Water intake in this study was also positively correlated to urine excretion (L/d; $r = 0.42$, $P = 0.02$). Urinary water and N excretion have previously been shown to possess a positive relationship in dairy cattle (Murphy, 1992; Kojima et al., 2005) and similarly this study exhibited a tendency for reduced urine output coupled with decreased urinary N excretion in HF. Total tract absorption of N has been hypothesized to cause reductions in urinary N through increasing hindgut fermentation in dairy cattle by increasing ammonia absorption in the colon (Faulkner and Weiss, 2017). Further work is needed to establish the impact of higher roughage diets on urinary N excretion in beef steers. Unexpectedly, water intake was greater in CON vs. SUPZN. To the authors knowledge this is the first study to report lesser water intake with increased supplemental Zn concentrations in beef cattle and further investigation to define mechanisms involved will be required.

A complex interrelationship among dietary minerals exists. Copper absorption remained unchanged due to SUPZN, similar to previous work in finishing cattle (Carmichael et al., 2018); however, contrary to the study in finishing cattle, liver Cu was lesser in SUPZN. Increasing dietary Zn (90 – 180 mg Zn/kg DM) in cattle fed for 86 d numerically decreased liver Cu (Genther-Schroeder et al., 2016b). Lesser liver Cu along with greater amounts of Cu bound to Metallothionein have been observed in sheep due to high concentrations of dietary Zn (Bremner et al., 1976). High dietary concentrations of Zn can increase Metallothionein in tissues which may bind Cu and render it unavailable for utilization in the body (Oestreicher and Cousins, 1985). Regardless of numerical decreases in Cu absorption and retention, liver Cu status was highly adequate (Kincaid, 2000) in both CON and SUPZN steers. Awareness of the Cu and Zn antagonism remains important as trace mineral requirements of beef steers continue to be refined.

Opportunity remains to further improve Zn requirement recommendations in beef steers. Dietary fiber content and fiber digestibility may influence trace mineral and N metabolism by beef steers and warrants further investigation of diet type and trace mineral supplementation strategy. Increasing dietary concentrations of Zn increases the amount of retained Zn, regardless of changes in coefficient of absorption. Additionally, the recognition of antagonistic dietary constituents and the resulting impact on trace mineral availability in the rumen will be important to understand optimal utilization of trace minerals by beef cattle.

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Table 1. Diet ingredient composition and nutrient content during metabolism period (% DM basis)

	HF[1]		LF[1]	
Ingredient	CON[2]	SUPZN[2]	CON[2]	SUPZN[2]
Dry matter	41	41	47	47
Cracked corn	22	22	42	42
Modified distillers grains	22	22	22	22
Corn silage	40	40	26	26
Dried distillers grains[3]	5	5	5	5
Hay	6	6	-	-
Micronutrients and carrier[4]	5	5	5	5
Calculated composition				
NEm, Mcal/kg	1.79	1.79	1.92	1.92
NEg, Mcal/kg	1.28	1.28	1.39	1.39
Analyzed components[5]				
Crude protein	15.3	15.3	15.1	15.1
NDF	35.3	36.2	24.7	22.4
ADF	17.0	18.6	10.5	9.2
Cu, mg/kg DM	17	18	17	16
Fe, mg/kg DM	168	164	129	127
Mn, mg/kg DM	36	40	33	32
Zn, mg/kg DM	36	165	36	142

[1] HF (~35% NDF of DM); LF (~25% NDF of DM).

[2]Control (CON) received no supplemental Zn (36 mg Zn/kg DM); Supranutritional Zn (SUPZN) diet received formulated Zn inclusion of 120 mg Zn/kg DM [CON + 60 mg Zn/kg DM as $ZnSO_4$ and 60 mg Zn/kg DM as Availa-Zn (Zinpro Corporation, Eden Prairie, MN)].

[3]Dried distillers grains alone or as carrier for SUPZN premix.

[4]Basal includes dried distillers grains with solubles as carrier and micronutrients to provide to total diet (DM basis); limestone (1.4%), Rumensin (0.0135%), urea (0.3%), and salt (0.31%). Trace minerals and vitamins provided per kilogram of total diet DM: 0.15 mg Co (cobalt carbonate), 10 mg Cu (copper sulfate), 20 mg Mn (manganese sulfate). 0.1 mg Se (sodium selenite), 0.5 mg I (calcium iodate), Vitamin A 2,200 IU [Rovimix A 1000 (1,000 kIU/g), DSM, Parsippany, NJ], and Vitamin E 25 IU [Rovimix E50 (50 kIU/g), DSM, Parsippany, NJ].

[5]Sulfur calculated as 0.25% of the diet with inclusion of modified distillers grains and dried distillers grains; S analysis on feedstuffs conducted by Dairyland Laboratories (Arcadia, WI).

Table 2. Dietary Zn influence on 60 d performance preceding metabolism period in beef steers.

	ZNTRT[1]			
Item	CON[2]	SUPZN[2]	SEM	*P*-value
Steers (*n*)	16	15		
Dry matter intake[3], kg/d	8.8	8.8	0.12	0.96
Initial BW[4], kg	310	307	6.0	0.65
d 60 BW[4], kg	410	412	10.3	0.62
ADG[4], kg	1.69	1.73	0.171	0.62
G:F[4]	0.194	0.198	0.0119	0.66

[1]ZNTRT (mineral supplementation strategy). All steers received the high fiber diet (HF; ~35% NDF) during 60 d preceding metabolism period.
[2]CON (no supplemental Zn; analyzed 36 mg Zn/kg DM); SUPZN [Con + 60 mg Zn/kg as $ZnSO_4$ + 60 mg Zn/kg as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN).
[3]Daily dry matter intake based on repeated measures analysis (no week x ZNTRT effect; $P = 0.55$).
[4]A 4% pencil shrink was applied to all body weights (BW), including average daily gain (ADG) and G:F calculations.

Table 3. Influence of dietary Zn and fiber concentration on daily nutrient intake and urine and fecal output in beef steers during 5 d collection period[1].

	ZNTRT[2]		ZNTRT	FIBER[2]		FIBER	
Item	CON[3]	SUPZN[3]	*P*-value[5]	HF[4]	LF[4]	*P*-value[5]	SEM
Steers (*n*)	15	16		16	15		
Intake							
DM, kg/d	8.24	8.15	0.88	7.85	8.54	0.25	0.391
OM, kg/d	7.69	7.81	0.85	7.27	8.24	0.13	0.418
NDF, kg/d	2.48	2.34	0.55	2.80	2.02	0.01	0.154
ADF, kg/d	1.15	1.09	0.55	1.41	0.84	0.01	0.067
N, g/d	200.8	199.7	0.92	193.5	207.0	0.26	8.06
Water, L/d	24.5	19.6	0.03	17.8	26.3	0.01	1.47
Urine output							
L/d	8.82	7.83	0.54	6.82	9.83	0.08	1.087
N, g/d	88.7	90.2	0.86	81.1	97.8	0.07	6.17
Fecal output							
DM, kg/d	2.36	2.16	0.30	2.13	2.39	0.21	0.132
OM, kg/d	2.01	1.82	0.29	1.79	2.04	0.18	0.116
NDF, kg/d	1.00	0.92	0.32	0.98	0.94	0.58	0.057
ADF, kg/d	0.43	0.39	0.42	0.45	0.37	0.07	0.028
N, g/d	67.4	62.0	0.30	59.4	70.0	0.06	3.43

[1]Steers were adapted to metabolism crates for 10 d (d 75 to 85 of study) followed by 5 d of collection (d 85 to 90 of study).
[2]ZNTRT (mineral supplementation strategy); FIBER (dietary fiber strategy).
[3]CON (no supplemental Zn; analyzed 36 mg Zn/kg DM); SUPZN [Con + 60 mg Zn/kg as $ZnSO_4$ + 60 mg Zn/kg as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN); HF analyzed 165 mg Zn/kg DM; LF analyzed 142 mg Zn/kg DM].
[4]HF (~35% NDF of DM); LF (~25% NDF of DM).
[5]No ZNTRT × FIBER effect ($P \geq 0.17$).

Table 4. Influence of dietary Zn and fiber concentration on nutrient digestibility and nitrogen metabolism in beef steers[1].

Item	ZNTRT[2]		ZNTRT *P*-value[5]	FIBER[2]		FIBER *P*-value[5]	SEM
	CON[3]	SUPZN[3]		HF[4]	LF[4]		
Steers (*n*)	15	16		16	15		
DMD[6], %	71.5	73.6	0.02	72.9	72.2	0.37	0.53
OMD[6], %	73.9	76.7	0.02	75.4	75.2	0.86	0.73
NDFD[6], %	58.2	59.8	0.27	64.9	53.1	0.01	0.97
ADFD[6], %	61.3	62.2	0.56	68.0	55.5	0.01	1.12
N digestibility, %	66.3	68.9	0.07	69.2	66.0	0.04	0.92
N retention, g/d	45.0	47.5	0.69	52.9	39.6	0.06	4.71
N retention[7], %	22.1	23.9	0.60	27.3	18.7	0.03	2.43

[1]Steers were adapted to metabolism crates for 10 d (d 75 to 85 of study) followed by 5 d of collection (d 85 to 90 of study).
[2]ZNTRT (mineral supplementation strategy); FIBER (dietary fiber strategy).
[3]CON (no supplemental Zn; analyzed 36 mg Zn/kg DM); SUPZN [Con + 60 mg Zn/kg as $ZnSO_4$ + 60 mg Zn/kg as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN); HF analyzed 165 mg Zn/kg DM; LF analyzed 142 mg Zn/kg DM].
[4] HF (~35% NDF of DM); LF (~25% NDF of DM).
[5]No ZNTRT × FIBER effect ($P \geq 0.23$).
[6]Dry matter digestibility (DMD); Organic matter digestibility (OMD); Neutral detergent fiber digestibility (NDFD); Acid detergent fiber digestibility (ADFD).
[7]Reported as percentage of N intake.

Table 5. Influence of dietary Zn and fiber concentration on daily micro mineral intake, fecal and urine excretion, and mineral retention in milligrams per day of beef steers during 5 d collection period[1].

Item	ZNTRT[2]		ZNTRT *P*-value[5]	FIBER[2]		FIBER *P*-value[5]	SEM
	CON[3]	SUPZN[3]		HF[4]	LF[4]		
Steers (*n*)	15	16		16	15		
Mineral intake							
Cu, mg/d	142	139	0.82	140	141	0.95	8.7
Fe, mg/d	1232	1185	0.78	1322	1095	0.20	115.4
Mn, mg/d	288	293	0.82	302	279	0.36	16.7
Zn, mg/d[6]	299	1255	0.01	766	788	0.69	36.5
Fecal excretion							
Cu, mg/d	131	132	0.89	128	135	0.43	6.2
Fe, mg/d	1132	1044	0.32	1175	1001	0.07	59.5
Mn, mg/d	236	233	0.87	245	224	0.18	10.2
Zn, mg/d	238	1070	0.01	606	701	0.03	26.2
Urinary excretion							
Cu, mg/d	0.29	0.20	0.11	0.25	0.23	0.66	0.026
Fe, mg/d	5.26	4.84	0.68	5.37	4.73	0.54	0.702
Mn, mg/d	0.71	0.51	0.21	0.63	0.57	0.72	0.109
Zn, mg/d	2.66	3.33	0.27	2.71	3.27	0.35	0.388
Mineral retention							
Cu, mg/d	11	7	0.54	12	6	0.27	4.1
Fe, mg/d	89	136	0.67	141	84	0.61	74.4
Mn, mg/d	51	59	0.61	56	54	0.88	11.9
Zn, mg/d[6]	57	181	0.01	157	82	0.01	14.4

[1]Steers were adapted to metabolism crates for 10 d (d 75 to 85 of study) followed by 5 d of collection (d 85 to 90 of study).
[2]ZNTRT (mineral supplementation strategy); FIBER (dietary fiber strategy).
[3]CON (no supplemental Zn; analyzed 36 mg Zn/kg DM); SUPZN [Con + 60 mg Zn/kg as $ZnSO_4$ + 60 mg Zn/kg as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN); HF analyzed 165 mg Zn/kg DM; LF analyzed 142 mg Zn/kg DM].
[4] HF (~35% NDF of DM); LF (~25% NDF of DM).
[5] No ZNTRT × FIBER effect for Cu, Fe, or Mn ($P \geq 0.11$).
[6]For fecal Zn excretion there was a ZNTRT × FIBER effect ($P = 0.04$) where within SUPZN Zn fecal excretion was lesser in HF vs. LF (979 vs. 1161 mg/d; $P < 0.01$), while CONHF (233 mg/d) was similar to CONLF (242 mg/d; $P = 0.88$). For Zn retention there was a ZNTRT × FIBER effect ($P = 0.01$) where within SUPZN Zn retention was increased in HF vs. LF (250 vs. 113 mg/d; $P < 0.01$), while CONHF (64 mg/d) was similar to CONLF (51 mg/d; $P = 0.65$).

Table 6. Influence of dietary Zn and fiber concentration on daily micro mineral fecal and urine excretion, and mineral retention of beef steers as a percent of nutrient intake during 5 d collection period.

	ZNTRT[1]		ZNTRT	FIBER[1]		FIBER	
Item	CON[2]	SUPZN[2]	*P*-value[4]	HF[3]	LF[3]	*P*-value[4]	SEM
Steers (*n*)	15	16		16	15		
Fecal excretion							
Cu, %	93.1	95.3	0.57	91.9	96.6	0.24	2.67
Fe, %	95.1	89.4	0.41	91.5	93.0	0.83	4.74
Mn, %	83.2	80.0	0.56	81.9	81.4	0.92	3.72
Zn, %	80.2	85.6	0.06	79.4	86.4	0.02	1.70
Urinary excretion							
Cu, %	0.21	0.14	0.08	0.18	0.17	0.69	0.023
Fe, %	0.44	0.42	0.78	0.42	0.44	0.84	0.069
Mn, %	0.25	0.18	0.20	0.21	0.21	0.93	0.040
Zn, %	0.91	0.27	0.01	0.45	0.54	0.44	0.078
Apparent absorption							
Cu, %	6.9	4.7	0.57	8.1	3.4	0.24	2.67
Fe, %	4.9	10.6	0.41	8.5	7.0	0.83	4.74
Mn, %	16.8	20.0	0.56	18.1	18.7	0.92	3.72
Zn, %	19.8	14.5	0.06	20.6	13.6	0.02	1.70
Mineral retention							
Cu, %	6.6	4.5	0.59	7.9	3.2	0.24	2.70
Fe, %	4.4	10.1	0.41	8.0	6.5	0.83	4.75
Mn, %	16.5	19.7	0.56	17.8	18.4	0.92	3.72
Zn, %	18.6	14.1	0.12	20.0	12.7	0.02	1.81

[1]ZNTRT (mineral supplementation strategy); FIBER (dietary fiber strategy).
[2]CON (no supplemental Zn; analyzed 36 mg Zn/kg DM); SUPZN [Con + 60 mg Zn/kg as $ZnSO_4$ + 60 mg Zn/kg as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN); HF analyzed 165 mg Zn/kg DM; LF analyzed 142 mg Zn/kg DM].
[3]HF (~35% NDF of DM); LF (~25% NDF of DM).
[4]No ZNTRT × FIBER effect ($P \geq 0.13$).

Table 7. Dietary Zn and fiber concentration influence on liver mineral concentrations of beef steers.

Item	ZNTRT[1]		ZNTRT	FIBER[1]		FIBER	
	CON[2]	SUPZN[2]	*P*-value[4]	HF[3]	LF[3]	*P*-value[4]	SEM
Steers (*n*)	16	15		15	16		
Liver mineral, mg/kg DM[5]							
Cu	390	291	0.01	364	317	0.08	16.5
Fe	173	180	0.61	178	176	0.88	9.2
Mn	8.3	8.5	0.62	8.3	8.5	0.61	0.30
Zn	150	153	0.79	153	151	0.86	8.5

[1]ZNTRT (mineral supplementation strategy); FIBER (dietary fiber strategy).
[2]CON (no supplemental Zn; analyzed 36 mg Zn/kg DM); SUPZN [Con + 60 mg Zn/kg as $ZnSO_4$ + 60 mg Zn/kg as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN); HF analyzed 165 mg Zn/kg DM; LF analyzed 142 mg Zn/kg DM].
[3]HF (~35% NDF of DM); LF (~25% NDF of DM).
[4]No ZNTRT × FIBER effect ($P \geq 0.16$).
[5]Liver biopsies taken on d 95 following metabolism period. No ZNTRT × FIBER effect ($P \geq 0.33$).

CHAPTER 6.

THE INFLUENCE OF DIETARY ENERGY AND ZINC SOURCE AND CONCENTRATION ON PERFORMANCE, TRACE MINERAL STATUS, AND GENE EXPRESSION OF BEEF STEERS

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Abstract: The objective of this study was to determine the effects of increased supplemental Zn from differing sources on growth performance of steers fed diets differing in net energy. Angus steers (n = 72, 324 ± 2.1 kg) with Genemax gain scores of 3, 4, or 5 were blocked by BW and stratified by Genemax gain score into 12 pens of 6 steers each. Pens were randomly assigned to 1 of 3 Zn treatments (**ZNTRT**) 1) control (no supplemental Zn, analyzed 33 mg Zn/kg DM; **CON**) 2) inorganic Zn (CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$ for entire trial; **INZN**) or 3) 120 mg supplemental Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN) for first 60 d, then a blend of $ZnSO_4$ and Zn-AA complex [CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ + 60 mg supplemental Zn/kg DM as Zn-amino acid complex] for the remainder of the trial (**ZNBLD**). Two dietary energy strategies (**ENERGY**) were formulated to reach ADG rates of 1) 1.6 kg/d (**LE**) or 2) 2.0 kg/d (**HE**) utilizing a 3 × 2 factorial arrangement (12 steers/treatment). All steers were fed LE for initial 60 d growing period, then pens were randomly assigned to ENERGY treatments fed the remaining 91 d. Day 60 BW tended to be greater ($P = 0.07$) in steers receiving supplemental Zn vs. CON. Liver Cu was decreased in Zn supplemented steers vs. CON ($P = 0.02$). Liver Zn concentrations on d 56 did not differ for Zn vs. CON ($P = 0.22$) nor were there

differences due to Zn source ($P = 0.98$). There were or tended to be ZNTRT × ENERGY effects for d 67-90 ADG and G:F ($P \leq 0.01$), and d 122 BW and d 90-122 G:F ($P \leq 0.10$) driven by improved performance for ZNBLD-HE over ZNBLD-LE, while ENERGY within CON and INZN did not differ. Day 90 -122 ADG, overall ADG and overall G:F was greater ($P \leq 0.02$) and d 67-90 G:F tended to be greater ($P = 0.10$) for HE vs. LE. No ZNTRT × ENERGY or ZNTRT effects were detected for HCW, REA, BF, KPH, MS, or YG ($P \geq 0.37$) while HE increased HCW, BF, MS, and YG compared to LE ($P \leq 0.05$). During the finishing period ZNTRT × ENERGY effect was noted for plasma Zn ($P = 0.04$) where INZN-HE was greater than CON or ZNBLD HE and INZN-LE ($P \leq 0.03$) and ZNBLD-LE was greater ($P = 0.003$) and INZN-LE tended to be greater ($P = 0.06$) than CON-LE. No effect of ZNTRT, ENERGY, or the interaction was noted for SUN or plasma NEFA concentrations ($P \geq 0.15$) but a ZNTRT × ENERGY effect was seen for IGF-1 ($P = 0.04$) where CON LE was greater than CON HE and INZN HE ($P \leq 0.02$) and ZNBLD HE was greater than CON HE ($P = 0.04$). In liver, ZNTRT affected d 97 MT1A expression ($P = 0.05$) where INZN was greater than ZNBLD or CON ($P \leq 0.05$) while ZIP14 remained unaffected due to ZNTRT, BA, or the interaction ($P \geq 0.39$). Supplying greater supplemental Zn as ZNBLD during the transition period appeared to improve performance measures and may have eased transition for steers, but no final performance advantages were noted due to increased supplemental Zn, regardless of source. Additionally, liver MT1A expression differed between supplemental Zn treatment, suggesting differing post-absorptive and utilization mechanisms due to source.

KEY WORDS: zinc, dietary energy, Metallothionein, beef cattle

Introduction

The trace mineral Zn supports a variety of metabolic process in mammals as an important constituent in pathways such as DNA and protein synthesis (Cousins et al., 2006). Current NASEM (2016) recommendations for Zn (30 mg Zn/kg DM) were initially established more than 40 years ago. However, since 1977 cattle ADG has increased approximately 44% (Capper, 2011), in some degree due to growth promoting technologies. When cattle are supplemented the last 33 d on feed a 24% increase in ADG has been shown in response to the ß-adrenergic agonist ractopamine hydrochloride (Avendaño-Reyes et al., 2006). Previous work has shown that increasing supplemental Zn (as a blend of organic and inorganic sources) to well over NASEM (2016) recommendations has increased growth of ractopamine hydrochloride-fed steers, suggesting a greater need for dietary Zn when cattle experience rapid growth (Genther-Schroeder et al. 2016a,b). Follow up work by Carmichael et al. (2018) using the same dietary Zn and ß-adrenergic agonist supplementation strategies noted N retention was increased 5% by ractopamine hydrochloride, which may be expected, but was also improved 4% due to supplemental Zn. Indeed, Zn has been shown to be integral to N retention in other species, possibly due to its role in protein synthesis (Oberleas and Prasad, 1969; Somers and Underwood, 1969; Greeley et al., 1980). It is well known that increasing dietary net energy increases cattle growth rate (Kleiber, 1961; Lofgreen and Garrett, 1968) and while ractopamine hydrochloride causes a rapid increase in growth, Zn may be important to support rapid growth in cattle not receiving a ß-adrenergic agonist. Therefore, the objective of this study was to determine the effects of increased supplemental Zn on growth performance of steers fed diets differing in net energy. The hypothesis was that steers experiencing greater growth rates induced by the high energy diet would further benefit from increased supplemental Zn compared to those receiving the low energy diet.

Materials and Methods

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (8-15-8073-B).

Experimental Design

Seventy-two Angus steers (324 ± 2.1 kg) from a single source with Genemax gain scores of 3, 4, or 5, indicating a predicted genetic value belonging in the top 60% for growth potential of tested Angus cattle (Zoetis, Parsippany, NJ), were utilized in this study. Approximately 30 d before the beginning of the study, steers received a booster vaccine for Bovine Viral Diarrhea Virus type 1 and II (Bovi-Shield Gold, One Shot, Zoetis) and received a broad coverage dewormer pour-on solution dose (Dectomax, Zoetis). Steers were blocked by BW and stratified by Genemax gain score into 12 pens of 6 steers each. Pens were randomly assigned to 1 of 3 Zn treatments (**ZNTRT**) for the entirety of the trial: 1) control (no supplemental Zn, analyzed 33 mg Zn/kg DM; **CON**) 2) inorganic Zn supplementation (CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; **INZN**) or 3) blend of supplemental Zn sources (**ZNBLD**) where steers received 120 mg supplemental Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN) for the initial growing period of 60 d, then switched to a blend of $ZnSO_4$ and Zn-AA complex [CON + 60 mg supplemental Zn/kg DM $ZnSO_4$ + 60 mg supplemental Zn/kg DM as Zn-amino acid complex] for the remainder of the trial.

The trial consisted of two phases: an initial growing period where all steers received a low energy diet (**LE**) for 60 d, followed by a 91 d finishing period where half the steers transitioned to a high energy (**HE**) diet while half remained on LE. The two dietary energy (**ENERGY**) strategies were formulated to reach targeted growth rates of 1) 1.6 kg ADG (LE) or 2) 2.0 kg ADG (HE). Diet composition and analysis is shown in **Table 1**. For the initial 60

d (growing period) of the study all steers (n = 24 steers/ ZNTRT) were fed the LE diet. Beginning on d 60, 2 pens per ZNTRT (one pen from each weight block) was assigned to HE and transitioned for 7 d. Feed delivery on the first day of transition was such that steers received a similar amount of dry matter (**DM**) as the day before when all were receiving the LE diet. Adjustments in feed delivery over the rest of the transition period were made according to previous days bunk score. Following transition all steers were fed their respective treatment combination for a 91 d finishing period (n = 12 steers/treatment combination).

During the entire trial period (158 d) all steers were fitted with unique electronic identification tags and were housed in pens equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) to collect daily individual as-fed intake. Steers were fed once daily at 0800 h and provided ad libitum access to feed and water. On d 0 all steers were implanted with Component TE-IS with Tylan (80 mg trenbolone acetate, 16 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health, Greenfield, IN) and on d 90 all steers were implanted with Component TE-S with Tylan (140 mg trenbolone acetate, 24 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health). Pre-feeding BW were recorded prior to feeding on d -1, 0, 59, 60, 66, 67, 90, 122, 157 and 158, and a 4% pencil shrink was applied to all BW measurements before calculation of ADG and gain-to-feed (**G:F**).

Liver and muscle biopsies were collected on d 56, 97, and 153. Liver biopsies were collected using methods established by Engle and Spears (2000). For muscle biopsies, the area was clipped, scrubbed with betadine and 70% ethanol, and injected with 10 mL of 2% lidocaine between the 11 and 12th ribs into the Longissimus dorsi muscle. A modified Jamshidi bone marrow biopsy/aspiration needle (8 g × 10 cm needle) was inserted into the

injected area to collect muscle tissue (~0.5 g wet basis). The collected tissue was rinsed with 0.1 M phosphate buffered saline (pH 7.0) to remove any vestigial blood from the sample, placed into an acid washed container and flash frozen in liquid nitrogen. Muscle tissue was stored at -80°C until further analysis.

Blood was collected by jugular venipuncture on d 59, 90, 122, and 157 into serum, sodium heparin, K_2 EDTA Plus, and trace element K_2 EDTA blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) for analysis of plasma trace mineral (**TM**) and various blood metabolites.

At the end of the experiment (158 d) steers were shipped to a commercial abattoir (Iowa Premium, Tama, IA) for slaughter followed by a 48 h chilling period. Personnel from the Hansen laboratory, blinded to treatments, were dispatched to collect carcass data, including: hot carcass weight (**HCW**), longissimus dorsi muscle area (**REA**) 12^{th} rib fat thickness (**BF**), kidney, pelvic, and heart fat (**KPH**), and marbling score (**MS**), all of which were utilized to calculate yield grade (**YG**) and quality grade (**QG**).

Feed, Tissue, and Blood Analysis

Total mixed ration (**TMR**) samples of each diet were collected weekly, subsamples dried in a forced-air oven for 48 h at 70°C and the resulting DM value was multiplied by as-fed feed intake from the GrowSafe system to determine steer dry matter intake (**DMI**). Dry matter of TMR was determined according to AOAC (1990) procedures. Dried TMR were ground through a 2mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and stored in sealed plastic bags until nutrient analysis. Feed efficiency was calculated from the total gain and total DMI determined at each weighing interval.

Inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 7000 DV, Perkin Elmer, Waltham, MA) was used for trace mineral analysis of TMR, plasma, and liver tissue. Dried, ground, and composited TMR were acid digested prior to mineral analysis as described by Pogge et al. (2014). Liver and plasma samples were digested and analyzed for Zn and Cu according to Pogge and Hansen (2013). To verify instrument accuracy a bovine liver standard from the National Institute of Standards and Technology (Gaithersburg, MA) or serum standard (UTAK Laboratories, Inc., Valencia, CA) was utilized and yttrium (PerkinElmer, Waltham, MA) served as an internal standard to account for sample introduction variation. Serum non-esterified fatty acid (**NEFA**) concentrations were measured by colorimetric assay (HR series NEFA-HR(2) assay kit; Wako Pure Chemical Industries, Ltd., Osaka, Japan; inter-assay CV of 9.2%). Serum urea nitrogen (**SUN**) concentrations were determined by colorimetric assay (B551; TECO Diagnostics, Anaheim, CA; inter-assay CV of 9.8%). Heparinized plasma was analyzed to determine plasma insulin-like growth factor-1 (**IGF-1**) concentrations in a commercial ELISA kit (SG100; R&D Systems, Minneapolis, MN; inter-assay CV of 1.9%) shown to have 100% cross-reactivity with bovine IGF-1 (Moriel and Arthington, 2013).

Liver and muscle samples for mRNA extraction were kept frozen with liquid nitrogen while ground with a mortar and pestle. Extraction of mRNA was conducted using the Trizol (Life Technologies, Carlsbad, CA) method and the RNeasy Mini Kit (Qiagen GmbH, Qiagen Strasse 1, Hilden, Germany) per manufacturer's instructions. A Qubit 4 Fluorometer (Invitrogen, ThermoFisher Scientific, Waltham, MA) was used to quantify and measure integrity and quality of RNA. For each sample, 0.5 microgram was reverse transcribed using the Superscript III kit (ThermoFisher Scientific) per manufacturer's instructions. Complementary DNA samples were prediluted (1:10) and 2μL were combined with 20μL of

a master mix containing SYBR Green fluorescent dye (Life Technologies LTD, Warrington, UK), forward and reverse primers (Integrated DNA Technologies, Coralville, IA) and RNAse free water for gene expression analysis. Utilizing quantitative real-time PCR, samples were then analyzed for RNA abundance of 40s Ribosomal Protein 9 (Rps9), Zrt- and Irt-like protein 14 (ZIP14), and Metallothionein 1A (MT1A) within liver and muscle tissue. Primers, primer sequences, and primer conditions are listed in **Table 2**. Melting temperature during the 40 amplification cycles was 94°C and resulting cycle threshold (**Ct**) values were normalized to the abundance of RPS9. Resulting values for gene expression are ΔCt, calculated by subtracting Ct values of RPS9 from the Ct value of the gene of interest.

Statistical Analysis

Data were analyzed as a randomized complete block design. Performance, DMI, and liver TM data for the initial 60 d growing period were analyzed using the Mixed procedures of SAS (SAS Institute Inc., Cary, NC), with steer as the experimental unit (n = 24 per ZNTRT). The model included the fixed effects of ZNTRT and block. Two single degree of freedom contrasts were constructed for the growing period: 1) no supplemental Zn vs. supplemental Zn as both INZN and ZNBLD and 2) INZN vs. ZNBLD. Data collected following d 67 (finishing period) were analyzed as a 3 × 2 factorial arrangement using the Mixed procedure of SAS. The model included the fixed effects of ZNTRT, ENERGY, block, and the interaction of ZNTRT and ENERGY; steer was the experimental unit (n = 12/treatment combination) for all analyses. Finishing period plasma and IGF-1, SUN, and serum NEFA data were analyzed as repeated measures with date as the repeated effect. The compound symmetry variance structure for repeated measures was selected to achieve the lowest Akaike information criterion value for all repeated measures analysis and d 60 IGF-1 concentrations were used as a covariate for IGF-1 repeated measures. The Shapiro-Wilk test

of normality was utilized and NEFA and finishing period G:F data were normally distributed by log transformation; treatment means and SEM shown are reverse transformed. Outliers were determined using Cook's D and removed if Cook's D ≥ 0.5. Due to health reasons unrelated to treatment one steer from ZNBLD-HI was removed from analysis following d 67. Significance was declared at $P \leq 0.05$ and tendencies identified if $P \geq 0.06$ and ≤ 0.10. Tabular values reported reflect the least square means and the PDIFF statement in SAS was utilized to determine pairwise differences.

Results

Growing Period Performance

Data for the initial 60 d growing period when all steers received the LE diet are shown in **Table 3**. No differences were detected for DMI, ADG or G:F when comparing Zn vs. CON ($P \geq 0.12$) during this period, nor were there difference due to Zn source ($P \geq 0.20$). However, d 60 BW tended to be greater in steers receiving supplemental Zn vs. CON ($P = 0.07$). Liver Cu was decreased in Zn supplemented steers vs. CON ($P = 0.02$) while no differences were detected between Zn sources ($P = 0.48$). Liver Mn was not different between supplemental Zn and CON ($P = 0.16$) but was lesser in INZN when compared to ZNBLD ($P = 0.01$). Day 56 liver Zn concentrations did not differ for Zn vs. CON ($P = 0.22$) nor were there differences due to Zn source ($P = 0.98$).

Finishing Period Performance

Effects of ZNTRT and ENERGY strategies on steer performance during the finishing period are shown in **Table 4**. No ZNTRT × ENERGY effects were noted for DMI, d 67, 90 and final BW, or d 90-122, 122-158, and overall finishing period ADG, or d 122-158 or overall G:F ($P \geq 0.21$). However, there were or tended to be ZNTRT × ENERGY effects for d 67-90 ADG and G:F ($P \leq 0.01$), and d 122 BW and d 90-122 G:F ($P \leq 0.10$). Collectively,

these interactions are driven by improved performance during this time by ZNBLD-HE over ZNBLD-LE, while within CON and INZN, HE and LE did not differ for these measures. No ZNTRT effects were noted for interim or overall DMI, BW, ADG or G:F during the finishing period ($P \geq 0.15$). An ENERGY effect was detected where HE was greater than LE for d 90 - 122 ADG, overall ADG and overall G:F ($P \leq 0.02$), and tended to be greater for d 67-90 G:F ($P = 0.10$). No ENERGY effects were detected for DMI or d 67, d 90, or d 122 BW, or d 122-158 ADG and d 122-158 G:F ($P \geq 0.11$).

Carcass data are shown in **Table 5**. A ZNTRT × ENERGY effect was noted for DP ($P = 0.02$) where no effect of ENERGY was detected within cattle receiving CON or ZNBLD, but within INZN, HE tended to dress greater than LE. No ZNTRT × ENERGY or ZNTRT effects were detected for HCW, REA, BF, KPH, MS, or YG ($P \geq 0.37$). The HE diet increased HCW, BF, MS, and YG compared to LE ($P \leq 0.05$), but ENERGY had no effect on REA or KPH ($P \geq 0.63$).

Finishing Period Liver and Plasma Trace Mineral and Blood Metabolites

Plasma Cu and Zn repeated measures data are shown in **Table 6** and d 97 and 153 liver Cu, Mn, and Zn data are shown in **Table 7**. A ZNTRT × ENERGY effect was noted for plasma Zn ($P = 0.04$) where INZN HE was greater than CON or ZNBLD HE and INZN LE ($P \leq 0.03$) and ZNBLD LE was greater ($P = 0.003$) and INZN tended to be greater ($P = 0.06$) than CON LE. No interactions with time ($P \geq 0.24$) were noted for plasma Zn concentrations; however, there was an effect of time ($P = 0.0001$) where concentrations were least on d 60 (1.25 mg/L) relative to d 90, 122, and 157 (1.38, 1.33, and 1.38 mg/L, respectively). No ZNTRT × ENERGY × time, ZNTRT × time, or ZNTRT effects were noted for plasma Cu ($P \geq 0.19$). However, there was an ENERGY × time effect for plasma Cu ($P =$

0.02) where HE tended to be greater than LE on d 90 and 122 ($P \leq 0.08$) while HE and LE did not differ on d 60 and 157 ($P \geq 0.43$; **Figure 1**). No effects of ZNTRT, ENERGY, or the interaction were observed for liver Mn or Zn ($P \geq 0.11$). No ZNTRT × ENERGY or ENERGY effects were noted for liver Cu ($P \geq 0.20$). Liver Cu concentrations tended to be affected by ZNTRT ($P = 0.07$) on d 97 where ZNBLD was lesser than CON ($P = 0.04$) and on d 153 where INZN was lesser ($P = 0.04$) and ZNBLD tended to be lesser ($P = 0.06$) than CON.

No ZNTRT, ENERGY, ZNTRT × ENERGY, or interactions with time were noted for SUN or plasma NEFA concentrations ($P \geq 0.15$; **Table 6**). Serum urea nitrogen concentrations (10.2, 10.9, 10.6 and 10.8 mg/dL for d 60, 90, 122 and 157, respectively) tended to differ over time ($P = 0.10$), where d 60 was lesser than d 90 and 157 but did not differ from d 122 while d 90, 122, and 157 did not differ from each other. Plasma NEFA concentrations (232, 168, 165, and 176 mEq/L for d 60, 90, 122 and 157, respectively) differed over time ($P = 0.0001$), where d 60 was greater than all other time points. No ZNTRT × ENERGY × time effect was noted for plasma IGF-1 concentrations ($P = 0.30$). A ZNTRT × ENERGY effect was noted for plasma IGF-1 concentrations ($P = 0.04$) where CON LE was greater than CON HE and INZN HE ($P \leq 0.02$) and ZNBLD HE was greater than CON HE ($P = 0.04$; **Figure 2A**). Additionally, an ENERGY × time effect was noted for plasma IGF-1 concentrations ($P = 0.0001$) where on d 122 LE was greater than HE ($P = 0.0039$) while on d 90 and 157 ENERGY did not differ ($P \geq 0.20$). A tendency for a positive correlation was noted between plasma IGF-1 concentrations and liver MT1A mRNA expression on d 90 ($r = 0.33$; $P = 0.06$), which can be interpreted as when plasma IGF-1 concentrations increase MT1A expression decreases, but no correlation was detected on d 153 ($r = -0.12$; $P = 0.50$).

Gene Expression

Gene expression data are shown in **Table 7**. No ZNTRT × ENERGY or ENERGY effects were detected for ZIP14 or MT1A in d 97 or 153 liver or d 97 muscle ($P \geq 0.38$). In liver, ZNTRT affected d 97 MT1A expression ($P = 0.05$) indicating INZN was greater than ZNBLD or CON ($P \leq 0.05$) while ZNBLD and CON did not differ ($P = 0.73$). There were no effects of ZNTRT ($P \geq 0.39$) on liver MT1A on d 153, liver ZIP14 on d 97 and 153, or muscle MT1A or ZIP14 on d 97.

Discussion

Zinc is an integral component in multiple growth processes including DNA and protein synthesis and the growth hormone and insulin-like growth factor-1 (**IGF-1**) pathway (Clifford and MacDonald, 2000; Maret, 2013). Studies in late stage finishing steers have noted positive performance outcomes due to Zn supplementation (as Zn-amino acid complex; **Zn-AA**) above NASEM (2016) recommendations, when fed in tandem with the β-adrenergic agonist (**β-AA**) ractopamine hydrochloride (Genther-Schroeder et al., 2016a,b). It is possible that during periods of rapid growth, such as those induced by feeding β-AA or more energy dense diets, cattle require additional Zn to support protein accretion. Indeed Carmichael et al. (2018) reported Zn and N retention were strongly correlated in finishing steers. The objective of this study was to determine the impact of greater supplemental Zn concentrations, from $ZnSO_4$ alone or as a blend with Zn-AA complex, on growth performance of steers receiving diets differing in energy.

During the initial 60 d LE period, all steers exceeded expected performance and supplementation of Zn tended to increase BW during this time when compared to un-supplemented steers. Using similar supplemental Zn concentrations the growth response of steers in the 42-86 d prior to β-AA supplementation has been mixed, with no effect on BW

(Genther-Schroeder et al., 2016a) or slight increases in BW (Genther-Schroeder et al., 2016b). Growing beef heifers fed a corn-silage based diet supplemented with 25 mg Zn/kg DM as either Zn oxide or Zn methionine had increased gain and feed efficiency during the initial 56 d on trial compared to non-supplemented heifers (Spears, 1989). It was suggested the growth response due to supplemental Zn was because non-supplemented diets were marginally Zn deficient (23 mg Zn/kg DM; Spears, 1989). The CON diet in the present study analyzed 33 mg Zn/kg DM, close to the NASEM (2016) requirement of 30 mg Zn/kg DM, which may have been marginal to support rates of growth achieved early in the trial. Additionally, source of Zn did not differentially affect steer performance during the initial growing period of the present study.

The HE diet utilized in the present study was designed to elicit ADG improvements over LE-fed steers that were similar or in excess of the ADG response typically observed during ractopamine hydrochloride supplementation (Lean et al., 2014). The introduction of higher concentrate diets during transition creates many challenges for the ruminant gastrointestinal tract (Dixon and Stockdale, 1999), but improvements in ADG and G:F during the first few weeks following transition suggests steers receiving ZNBLD were better able to adapt to these changes. Zinc is involved in intestinal mucosal function and epithelial integrity (Alam et al., 1994; Rodriguez et al., 1996; Sturniolo et al., 2001). Highly fermentable diets increase the concentration of short chain fatty acids in the rumen, effectively decreasing rumen pH and challenging metabolism and intracellular homeostasis of the rumen epithelium (Penner et al., 2011). The effect of Zn supplementation on ruminant gut barrier function does not appear to have been well studied to date, but in swine supplementing 200 mg Zn/kg DM as Zn-amino acid complex improved gut integrity during a 7 d heat stress event (Sanz Fernandez et al., 2014). Further examination is needed to determine the role, if any, ZNBLD

may play in protection against gastrointestinal stress in cattle. It is interesting to note that the improvement in overall ADG in HE vs. LE steers was only 2% within unsupplemented CON, while INZN and ZNBLD increased ADG by 5 and 13%, respectively, as energy density of the diet increased. When HE was fed, dietary Zn supplied by CON (33 mg Zn/kg DM) appeared to be insufficient to support growth rates induced by HE, suggesting a greater dietary need for Zn beyond that supplied by CON.

The Zn importer ZIP14 is one mechanism allowing cellular Zn influx, and ZIP14 knock out mice exhibit stunted growth and impaired gluconeogenesis, potentially due to the role of Zn in modulation of G-coupled protein receptor signaling pathways critical to growth (Hojyo et al., 2011). The importer ZIP14 was analyzed in the current study to determine the effects of inducing growth through increasing dietary energy on expression in liver and muscle. However, increasing dietary energy did not affect ZIP14, and the lack of change in ZIP14 expression due to supplemental Zn is similar to previous reports (Lichten et al., 2009; Aydemir and Cousins, 2018).

Free Zn (not bound to a protein; Krężel and Maret, 2016) induces MT mRNA expression, but the MT protein has a greater binding affinity for Cu, resulting in Cu bound to MT in the enterocyte being lost in feces as enterocytes are sloughed off (Cousins, 1985). Though intestinal MT was not assessed, liver Cu concentrations in the present study decreased due to supplemental Zn but remained adequate (Kincaid, 2000) regardless of ZNTRT. Status of some trace minerals (e.g. Cu, Se) in cattle can be assessed according to liver concentrations; however, supplementing Zn at five times current NASEM (2016) recommendations had no effect on liver Zn concentrations, supporting the assertation that liver Zn is insufficiently sensitive to distinguish among cattle with adequate or greater status. Therefore, MT1A was examined as a potentially more sensitive measure for Zn status and

availability in ruminants. Interestingly, liver MT1A mRNA expression on d 97 was greater for INZN compared to ZNBLD and CON, and previous studies have shown an increase in hepatic MT due to increased supplemental Zn in chicks (as Zn acetate, Zn methionine, and Zn proteinate; Cao et al., 2002), Holstein calves (Zn proteinate and $ZnSO_4$; Wright and Spears, 2010) and sheep (Zn lysine; Rojas et al., 1996). However, this difference was null by d 153, suggesting possible changes in absorption and excretion after long periods of greater Zn supplementation, such as that in rats where excretion and absorption shift to maintain homeostatic Zn accretion above requirements (Weigand and Kirchgessner, 1978). Metallothionein induction by Zn is accomplished through metal transcription factor-1 (**MTF-1**) and is only induced by free Zn which indicates accumulation of Zn in the cytosol (Fukada and Kambe, 2014; Kimura and Kambe, 2016). Previous research results indicate some Zn-amino acid complexes may be absorbed through amino acid transporters (Glover and Hogstrand, 2002; Sauer et al., 2017) and since liver Zn concentrations did not differ due to ZNTRT, the Zn-AA source of Zn may be bound to an amino acid in the liver unavailable for recognition by MTF-1, while $ZnSO_4$ may exist in the liver as free Zn. Further work should be conducted to better understand differences between Zn sources in post-absorptive metabolism as well as source effects on putative Zn biomarkers in ruminants.

Plasma Zn was adequate in all treatments (Kincaid, 2000), and only INZN-HE and ZNBLD-LE had greater plasma Zn concentrations than unsupplemented CON steers. Zinc source solubility and absorption and physiological utilization can be impacted by the diet (Spears, 1989; Garg et al., 2008; Faulkner et al., 2017; VanValin et al., 2018), and may have influenced the differences in plasma Zn concentrations observed herein. Carmichael et al. (2019) found that Zn retention was greater in steers receiving a low energy, high fiber diet vs. those receiving a high concentrate diet regardless of Zn supplementation, and that

supplementing the same blend of Zn sources as those in the current study (60 mg Zn/kg DM as $ZnSO_4$ and 60 mg Zn/kg DM as Zn-AA) also increased Zn retention. Overall, if animals are not Zn deficient plasma Zn has limited use as a Zn biomarker as it does not appear to be related to whole animal Zn retention.

Steers receiving supplemental Zn exhibited limited advantages in final performance in the present study. This is in accordance with previous studies where supplementing similar concentrations of Zn as Zn oxide to finishing heifers did not improve final BW or ADG (Van Bibber-Krueger et al., 2017; Van Bibber-Krueger et al., 2019). Van Bibber-Krueger et al. (2017) similarly saw no differences in plasma urea N when 100 mg Zn/kg DM as Zn oxide was supplemented to finishing heifers. Zinc has been shown to increase N retention in finishing steers (Carmichael et al., 2018) and is integral for N utilization in other mammals (Oberleas and Prasad, 1969; Greeley et al., 1980). Furthermore, N retention has been shown to improve with β-AA supplementation and use of anabolic implants in Holstein steers, but no differences in plasma urea N were detected due to either treatment (Walker et al., 2007). Neither dietary energy or supplemental Zn affected SUN in the present study and collectively SUN do not appear to be reflective of increased N retention (protein accretion) in feedlot steers.

It is well recognized that increasing dietary energy increases growth rates of cattle (Geay, 1984; Owens et al., 1993; Owens et al., 1995). In the present study HE increased G:F, HCW, MS, BF, and YG regardless of ZNTRT when compared to LE. The anabolic hormone IGF-1 is heavily involved in growth and the maintenance of muscle mass (Estívariz and Ziegler, 1997; Maggio et al., 2013) and in the current study LE generally had greater plasma IGF-1, though effects of ZNTRT within LE were not noted. However, within HE ZNBLD had greater IGF-1 than CON with the inorganic treatment being intermediate. Previous

research has shown that IGF-1 inversely regulates Metallothionein expression, and since downregulation of MT would increase the amount of free Zn available for incorporation into proteins, it was suggested that Zn may be involved in balancing atrophy and hypertrophy in skeletal muscle (Latres et al., 2005). Zinc has been shown to play an integral role in IGF-1 activity, inhibiting tyrosine phosphatase activity and therefore increasing activity of the p70 S6 kinase, MAPK, and EGF pathways (Haase and Maret, 2003) which when induced by IGF-1 result in hypertrophy of skeletal muscle. A tendency for a positive correlation was detected between plasma IGF-1 concentration and MT1A ΔCT values on d 90 of the current study, which is interpreted to mean that when IGF-1 concentrations increase MT1A expression decreased, but this correlation was not observed on d 153. Further work should be conducted to determine the relationship between circulating IGF-1 and MT1A gene expression in ruminants.

Previous research in ruminants and humans has shown that IGF-1 concentrations will change depending on nutritional status (Estívariz and Ziegler, 1997; Maggio et al., 2013). Circulating concentrations of IGF-1 in the current study were increased in LE compared to HE on d 122, 32 d following administration of a high potency anabolic implant. Studying the effects of implant or a low-energy diet in swine, Lee et al. (2005) found that implant increased circulating IGF-1 concentrations but were unaffected by the low energy diet. It is well established that following administration of an anabolic implant circulating IGF-1 concentrations will increase in cattle (Johnson et al., 1996; Johnson et al., 1998; Walker et al., 2007). Additionally, Hutcheson et al. (1997) noted that steers receiving either estrogenic or combination implants decrease NEg requirements ~19% and increase daily protein accretion over non-implanted steers. According to the current study, it appears steers receiving LE were more receptive to the action of anabolic implants ability to increase

circulating IGF-1 concentrations, and while LE did not surpass growth induced by HE following implant on d 90, there was no advantage of HE over LE for ADG during d 122 to 158. Further research should be conducted to determine the impacts of combination anabolic implants on circulating IGF-1 concentrations in feedlot cattle receiving diets differing in energy content.

Increasing dietary energy improved performance measures in finishing cattle regardless of Zn supplementation strategy; however improvements in growth rate of HE over LE were not achieved. Collectively, increasing supplemental Zn during the growing period improved performance and ZNBLD may have eased transition for growing steers, but limited final performance advantages were noted due to increased Zn supplementation concentrations. Differences in liver gene expression of the Zn storage protein Metallothionein suggests post-absorption metabolism may differ between $ZnSO_4$ and Zn-amino acid complex and future work is needed to better understand this discrepancy between sources.

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Table 1. Diet ingredient composition and nutrient content (% DM basis).

	LE[1]			HE[1]		
Ingredient	CON[2]	INZN[2]	ZNBLD[2]	CON[2]	INZN[2]	ZNBLD[2]
Cracked corn	22.3	22.3	22.3	41.8	41.8	41.8
Corn silage	40	40	40	26.2	26.2	26.2
Modified distillers grains	22	22	22	22	22	22
Dried distillers grains[3]	5	5	5	5	5	5
Hay	5.7	5.7	5.7	0	0	0
Micronutrients and carrier[4]	5	5	5	5	5	5
Calculated components						
NEm, Mcal/kg	1.89	1.89	1.89	2.05	2.05	2.05
NEg, Mcal/kg	1.25	1.25	1.25	1.38	1.38	1.38
Analyzed components						
Crude protein, %	15.3	15.3	15.3	16.3	16.3	16.3
NDF, %	31.1	31.1	31.1	21.8	21.8	21.8
Cu	15	15	15	15	15	15
Fe	95	90	91	94	78	91
Mn	33	33	33	33	33	32
Zn	33	133	143	33	146	143

[1]LE diet (calculated to target ~1.6 kg ADG); HE diet (calculated to target ~2.0 kg ADG).

[2]Control (CON) received no supplemental Zn (diets analyzed 33 mg Zn/kg DM); Inorganic Zn (INZN) diet received 120 mg supplemental Zn/kg DM (CON + 120 mg Zn/kg DM as $ZnSO_4$); Zinc blend (ZNBLD) diet received 120 mg supplemental Zn/kg DM [CON + 60 mg Zn/kg DM as $ZnSO_4$ and 60 mg Zn/kg DM as Availa-Zn (Zinpro Corporation, Eden Prairie, MN)].

[3]Dried distillers grains alone or as carrier for INZN and ZNBLD premix

[4]Basal includes dried distillers grains with solubles as carrier and micronutrients to provide to total diet (DM basis); limestone (1.4%), Rumensin (0.0135%), urea (0.3%), and salt (0.31%). Trace minerals and vitamins provided per kilogram of total diet DM: 0.15 mg Co (cobalt carbonate), 10 mg Cu (copper sulfate), 20 mg Mn (manganese sulfate). 0.1 mg Se (sodium selenite), 0.5 mg I (calcium iodate), Vitamin A 2,200 IU [Rovimix A 1000 (1,000 kIU/g), DSM, Parsippany, NJ], and Vitamin E 25 IU [Rovimix E50 (50 kIU/g), DSM, Parsippany, NJ].

Table 2. Nucleotide sequences and conditions of primers used for qPCR amplifications.

Primer[1]	Forward (5' to 3')	Reverse (3' to 5')	Annealing Temperature (C)
Rps9	CCTCGACCAAGAGCTGAAG	CCTCCAGACCTCACGTTTGTTC	52.0
ZIP14	AGGCTCCTGCTCTACTTC	AGCGTCTCAGAGGTATAATG	56.0
MT1a	ATGGACCCGAACTGCTCCTGC	GCGCAGCAGCTGCACTTGTCCG	59.0

[1]Rps9 (40S ribosomal protein S9; Janovick-Guretzky et al., 2007); ZIP14 (Zrt- and Irt-like protein 14; Hansen et al., 2010); MT1a (Metallothionein 1a; Fry et al., 2013).

Table 3. Dietary Zn influence on initial 60 d growing period performance and liver trace mineral concentrations when all steers received the low energy diet.

Item	CON[1]	INZN[1]	ZNBLD[1]	SEM	C vs. Z[2]	ZB vs. ZS[2]
Steers (*n*)	24	24	24			
Dry matter intake, kg/d	9.11	9.19	9.25	0.237	0.70	0.84
BW[3], kg						
d 0	323	324	326	2.1	0.45	0.72
d 60	429	437	436	3.4	0.07	0.91
ADG[3], kg	1.76	1.87	1.84	0.049	0.12	0.69
Gain to feed[3]	0.196	0.201	0.205	0.0062	0.38	0.69
Liver d 56, mg/kg DM						
Cu	417	322	350	27.2	0.02	0.48
Mn	9.3	7.9	9.5	0.330	0.16	0.01
Zn	111	119	118	5.1	0.22	0.98

[1]Control (CON) received no supplemental Zn (diet analyzed 33 mg Zn/kg DM); Inorganic Zn (INZN) was CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$); Zinc blend (ZNBLD) was CON + 120 mg supplemental Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN)].

[2]Contrasts: C vs. Z = CON vs. ZNBLD and INZN; ZB vs. ZS = ZNBLD vs. INZN.

[3]All weight values include 4% pencil shrink in calculations.

Table 4. Dietary Zn and energy concentration influence on performance measures during the finishing period.

	CON		INZN		ZNBLD			*P*-value		
Item	LE	HE	LE	HE	LE	HE	SEM	ZNTRT	ENERGY	ZNTRT x ENERGY
Steers (*n*)	12	12	12	12	12	11				
Dry matter intake, kg/d										
d 67 to 90	11.0	10.9	11.3	11.2	11.1	11.3	0.32	0.61	0.94	0.91
d 90 to 122	11.1	11.1	11.4	11.6	11.3	11.2	0.32	0.43	0.85	0.86
d 122 to 158	11.5	11.1	11.5	12.1	11.7	11.5	0.42	0.50	0.91	0.47
d 67 to 158	11.2	11.0	11.4	11.6	11.4	11.3	0.33	0.48	0.96	0.82
BW, kg										
d 67	440	440	449	443	442	448	5.3	0.49	0.98	0.61
d 90	492	488	499	497	494	508	6.1	0.18	0.55	0.26
d 122	546	546	557	557	545	570	6.6	0.15	0.15	0.10
Final, d 158	604	609	614	619	605	629	8.2	0.35	0.11	0.44
ADG, kg										
d 67 to 90	2.18^{ab}	1.98^{b}	2.07^{b}	2.24^{ab}	2.14^{b}	2.54^{a}	0.093	0.02	0.11	0.01
d 90 to 122	1.70	1.81	1.84	1.87	1.61	1.92	0.078	0.34	0.02	0.21
d 122 to 158	1.61	1.76	1.58	1.72	1.67	1.60	0.104	0.88	0.40	0.49
d 67 to 158	1.81	1.85	1.82	1.93	1.79	2.02	0.059	0.43	0.01	0.30
G:F										
d 67 to 90	0.208^{ab}	0.191^{b}	0.192^{b}	0.208^{ab}	0.201^{b}	0.236^{a}	0.0095	0.04	0.10	0.01
d 90 to 122	0.151^{xy}	0.164^{xy}	0.163^{xy}	0.162^{xy}	0.143^{y}	0.172^{x}	0.0070	0.72	0.02	0.10
d 122 to 158	0.141	0.158	0.138	0.142	0.144	0.140	0.0081	0.40	0.51	0.31
d 67 to 158	0.167	0.171	0.165	0.171	0.163	0.181	0.0045	0.62	0.01	0.24

[1]Control (CON) received no supplemental Zn (diets analyzed 33 mg Zn/kg DM); Inorganic Zn (INZN) diet was CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; Zinc blend (ZNBLD) diet was CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ and 60 mg supplemental Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN)].
[2]LE: low energy diet formulated to target ~1.6 kg ADG; HE: high energy diet formulated to target ~2.0 kg ADG.
[3]All weight values include 4% pencil shrink in calculations; ADG = average daily gain; G:F= Gain to feed.
[4]Results with differing superscripts are different (a,b; $P \leq 0.05$) or tend to be different (x,y; $P \leq 0.10$).

Table 5. Dietary Zn and energy concentration influence on carcass characteristics for final 91 d finishing period.

	CON[1]		INZN[1]		ZNBLD[1]			*P*-value		
Item	LE[2]	HE[2]	LE[2]	HE[2]	LE[2]	HE[2]	SEM	ZNTRT	ENERGY	ZNTRT x ENERGY
Steers (*n*)	12	12	12	12	12	11				
Carcass characteristics										
HCW, kg	380	387	384	394	382	394	5.3	0.56	0.03	0.92
DP[3], %	62.9^{xy}	63.7^{xy}	62.4^{y}	63.8^{x}	63.2^{xy}	62.7^{xy}	0.33	0.52	0.06	0.02
REA, cm^2	85.8	84.5	87.1	87.7	85.8	89.0	1.94	0.38	0.63	0.43
Back fat, cm	1.61	1.80	1.57	1.82	1.50	1.83	0.132	0.96	0.02	0.87
KPH, %	2.2	2.2	2.0	2.1	2.1	2.1	0.09	0.48	0.67	0.81
Marbling score[4]	442	455	445	470	406	466	17.2	0.46	0.02	0.37
Yield grade	3.0	3.3	2.9	3.3	2.9	3.2	0.18	0.81	0.05	0.98

[1]Control (CON) received no supplemental Zn (diets analyzed 33 mg Zn/kg DM); Inorganic Zn (INZN) diet was CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; Zinc blend (ZNBLD) diet was CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ and 60 mg supplemental Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN)].
[2]LE: low energy diet formulated to target ~1.6 kg ADG; HE: high energy diet formulated to target ~2.0 kg ADG.
[3]DP = Dressing percentage; Means with differing superscripts tend to be different (x,y; $P \leq 0.10$).
[4]300 = slight; 400 = small; 500 = modest.

Table 6. Dietary Zn and energy concentration influence on plasma trace mineral and blood metabolites concentrations analyzed as repeated measures during the finishing period.

	CON[1]		INZN[1]		ZNBLD[1]			*P*-value		
Item[3]	LE[2]	HE[2]	LE[2]	HE[2]	LE[2]	HE[2]	SEM	ZNTRT	ENERGY	ZNTRT x ENERGY
Steers (*n*)	6	6	6	6	6	5				
Plasma										
Cu[4], mg/L	1.00	1.03	1.01	1.09	0.96	1.00	0.050	0.37	0.22	0.88
Zn[4],	1.21[c]	1.23[c]	1.34[bc]	1.49[a]	1.42[ab]	1.32[bc]	0.047	0.01	0.54	0.04
NEFA[5], mEq/L	193	185	192	178	190	165	13.2	0.67	0.15	0.79
SUN[6], mg/dL	10.1	10.7	10.9	10.9	10.6	10.7	0.70	0.75	0.71	0.89

[1]Control (CON) received no supplemental Zn (diets analyzed 33 mg Zn/kg DM); Inorganic Zn (INZN) diet was CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; Zinc blend (ZNBLD) diet was CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ and 60 mg supplemental Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN)].

[2]LE: low energy diet formulated to target ~1.6 kg ADG; HE: high energy diet formulated to target ~2.0 kg ADG.

[3]Analyzed as repeated measures utilizing the compound symmetry covariate structure to achieve the lowest Akaike information criterion value, values shown are overall repeated measures means.

[4]There were no ZNTRT × ENERGY × time or ZNTRT × time effects ($P \geq 0.19$) but an ENERGY × time interaction for plasma Cu ($P = 0.02$; Figure 1). No interactions with time were detected for plasma Zn ($P \geq 0.24$).

[5]NEFA concentrations differed over time ($P = 0.0001$) and were greater on d 60 than all other time points ($P \leq 0.0001$; 232 mEg/L) while d 90, 122, and 157 did not differ ($P \geq 0.12$; 168, 165, and 176 mEg/L, respectively).

[6] Serum urea nitrogen concentrations (10.2, 10.9, 10.6 and 10.8 mg/dL for d 60, 90, 122 and 157, respectively) tended to differ over time ($P = 0.10$), where d 60 was lesser than d 90 and 157 but did not differ from d 122 while d 90, 122, and 157 did not differ from each other.

Table 7. Dietary Zn and energy concentration influence on liver trace mineral concentrations and liver and muscle relative mRNA expression for MT1A and ZIP14.

	CON[1]		INZN[1]		ZNBLD[1]			*P*-value		
Item	LE[2]	HE[2]	LE[2]	HE[2]	LE[2]	HE[2]	SEM	ZNTRT	ENERGY	ZNTRT x ENERGY
Steers (*n*)	6	6	6	6	6	5				
Liver										
Cu, mg/kg DM										
d 97	373	342	270	345	260	292	33.3	0.07	0.37	0.30
d 153	305	277	203	267	214	260	26.9	0.07	0.22	0.20
Mn, mg/kg DM										
d 97	7.8	8.7	7.7	8.5	8.2	8.7	0.56	0.85	0.11	0.94
d 153	9.2	8.6	8.0	8.9	9.4	9.1	0.40	0.14	0.96	0.16
Zn, mg/kg DM										
d 97	104	125	117	125	110	106	7.9	0.25	0.22	0.34
d 153	106	103	113	112	116	108	4.4	0.14	0.27	0.67
MT1A[3]										
d 97	-0.743	-0.467	-1.693	-2.507	-1.213	-0.408	0.6005	0.05	0.86	0.42
d 153	-1.989	-1.103	-2.886	-1.889	-1.951	-1.223	0.6581	0.39	0.13	0.98
ZIP14[3]										
d 97	1.422	1.022	0.926	0.795	0.962	1.317	0.3065	0.50	0.82	0.47
d 153	0.783	1.285	0.750	1.145	1.458	0.8823	0.3011	0.79	0.68	0.22
Muscle[3]										
d 97										
MT1A	3.301	3.340	3.499	3.407	3.603	3.279	0.2059	0.78	0.48	0.67
ZIP14	6.575	6.562	6.388	6.693	6.409	6.562	0.2001	0.91	0.38	0.74

[1]Control (CON) received no supplemental Zn (diets analyzed 33 mg Zn/kg DM); Inorganic Zn (INZN) diet was CON + 120 mg supplemental Zn/kg DM as $ZnSO_4$; Zinc blend (ZNBLD) diet was CON + 60 mg supplemental Zn/kg DM as $ZnSO_4$ and 60 mg supplemental Zn/kg DM as Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN)].
[2]LE: low energy diet formulated to target ~1.6 kg ADG; HE: high energy diet formulated to target ~2.0 kg ADG.
[3]Relative abundance of the Metallothionein 1A (MT1A) and Zrt- and Irt-like protein 14 (ZIP14) genes were normalized with the 40S Ribosomal Protein 9 (RPS9) endogenous control by using the change in cycle threshold (ΔCT).

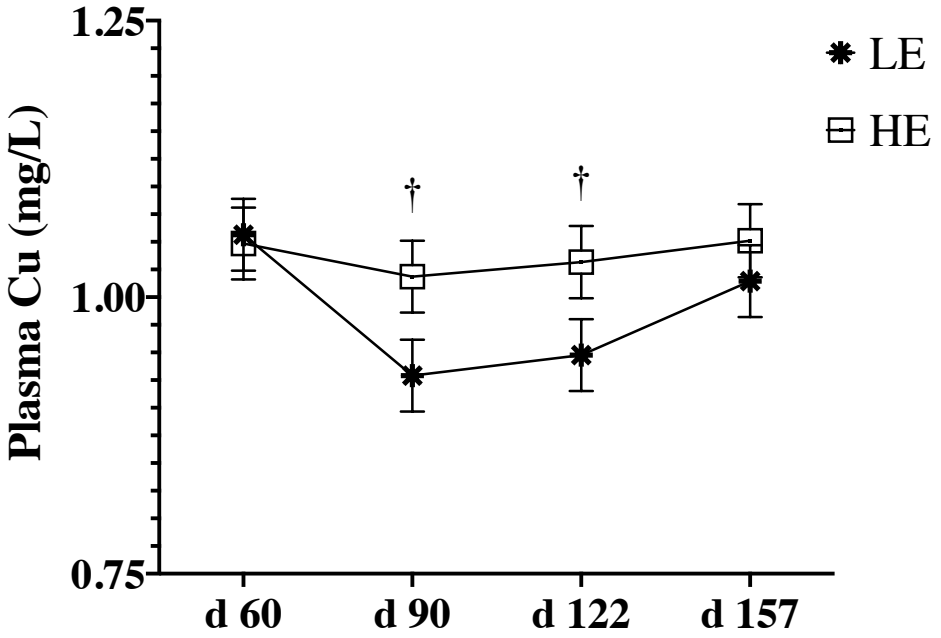

Figure 1. Effect of diets formulated to achieve either ~1.6 kg ADG (**LE**) or ~2.0 kg ADG (**HE**) on plasma Cu concentrations of steers across the finishing period (ENERGY × time; $P = 0.02$). † = indicates values tend to be different within a day ($P \leq 0.10$).

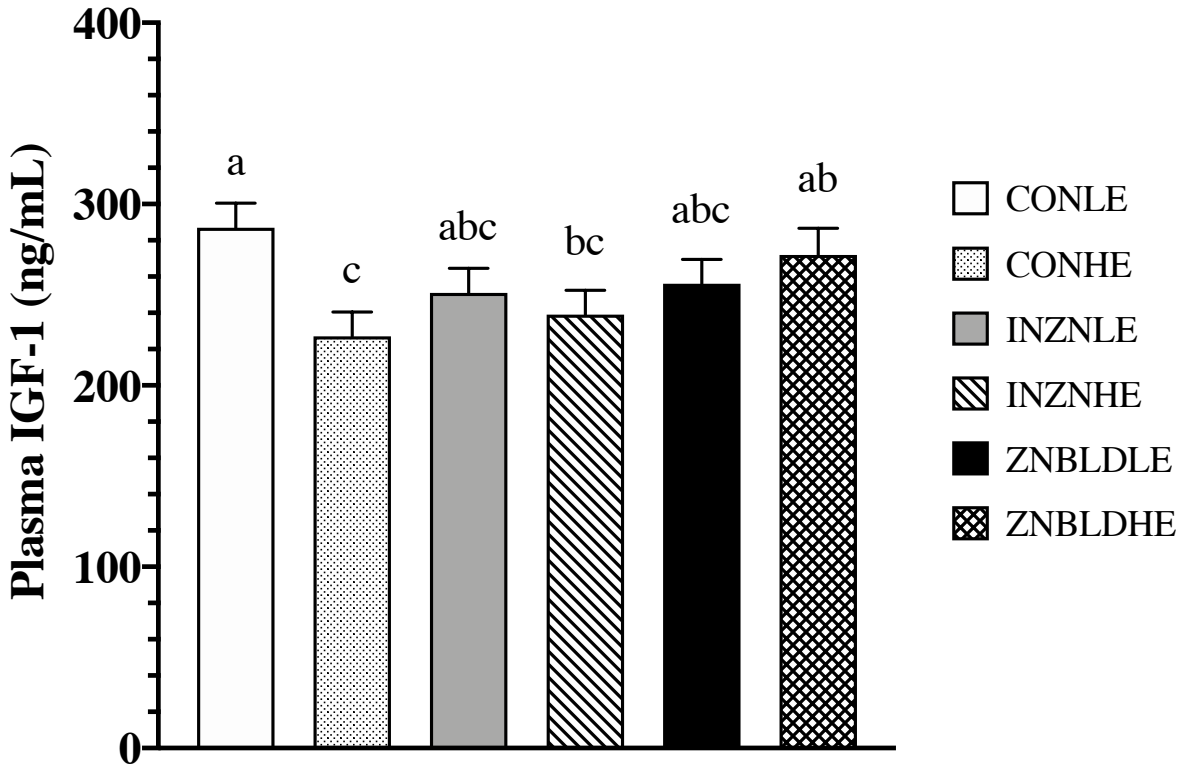

Figure 2. The effect of either no supplemental Zn (33 mg Zn/kg DM; **CON**) or 150 mg Zn/kg DM [CON + 120 mg Zn/kg DM as $ZnSO_4$ (**INZN**) or CON + 60 mg Zn/kg DM as $ZnSO_4$ and 60 mg Zn/kg DM as Availa-Zn (**ZNBLD**; Zinpro Corporation, Eden Prairie, MN)] in combination with diets formulated to achieve ~1.6 kg ADG (**LE**) or ~2.0 kg ADG (**HE**) on plasma insulin-like growth factor 1 (IGF-1) concentrations (ZNTRT × ENERGY; $P = 0.04$). Differing superscripts (a,b,c) indicate means differ ($P \leq 0.05$).

CHAPTER 7.

GENERAL CONCLUSIONS

Average daily gain over the life of feedlot cattle has improved 44% since 1977 (Capper, 2011) partially due to improved management practices, genetic enhancements, and availability of growth promoting technologies. During this same period however the recommended Zn requirements for beef cattle have not changed from 30 mg Zn/kg DM (NRC, 1984). Additionally, the trace mineral Zn is the most widely utilized metal in biological processes and is involved in growth processes such as DNA and protein synthesis and the GH/IGF-1 axis (Maret, 2013). Previous literature has suggested that the combination of Zn supplementation sources, $ZnSO_4$ and Zn-AA complex, improve performance during the period of rapid growth induced by ractopamine HCl (Genther-Schroeder et al., 2016a,b). However, the feedlot industry generally utilizes inorganic (sulfate bound) trace minerals as supplemental sources. The Zn concentrations selected for the studies completed for this dissertation stem from Genther-Schroeder et al. (2016a) where the greatest performance response during ractopamine HCl supplementation was observed with a total Zn concentration in the diet of 150 mg Zn/kg DM. In this same study a performance response due to supplemental Zn was only noted when supplementation coincided with ractopamine HCl supplementation. However, it was unclear if the performance response was due to an interaction with the β-AA ractopamine HCl or could be attributed to periods of rapid growth. Therefore, the purpose of this research dissertation was to determine if increased Zn supplementation is needed to support periods of rapid growth in beef steers. The overarching hypothesis was that rapid growth increases Zn requirements in cattle, regardless of how this growth is achieved. The work reported in this dissertation explores the interactions of Zn and rapid rates of growth induced by one of two growth models: the β-AA ractopamine

hydrochloride or differing dietary energy to imitate the rapid rates of growth typical of ractopamine HCl supplementation.

Ractopamine HCl is fed to late stage finishing steers during the last 28-42 d prior to slaughter to increase protein synthesis and decrease adipose synthesis. Growth induced by ractopamine HCl supplementation requires the utilization of dietary N and previous research has shown that Zn is integral for protein utilization from the diet (Oberleas and Prasad, 1969; Greeley et al., 1980). It was hypothesized that greater dietary Zn supplementation would increase N retention beyond that induced by ractopamine HCl supplementation and that increased growth due to ractopamine HCl supplementation would increase the body's physiological need for Zn. As reported in Chapter 3, we found that while ractopamine HCl did increase N retention, it did not increase Zn absorption, suggesting that growth induced by ractopamine HCl does not directly influence Zn homeostasis. However, we also observed an increase in N retention due to greater supplemental Zn, regardless of ractopamine HCl supplementation. This research was the first conducted to identify a positive relationship of greater dietary Zn and N retention in late stage finishing steers. It appears Zn might be important in capture of protein in cattle experiencing rapid rates of lean growth; however, in contrast to our hypothesis no interactions were noted between dietary Zn concentration and ractopamine HCl supplementation for the majority of measures assessed in this study.

Prior research by Genther-Schroeder et al. (2016a) noted linear increases in circulating cAMP, a potent second messenger induced in response to β-AA feeding, in steers receiving increasing concentrations of Zn-amino acid complex in the diet. In ZIP14 knockout mice G-protein coupled receptor signaling activity and gluconeogenesis are impaired (Hojyo et al., 2011) because Zn is unavailable to inhibit phosphodiesterase, leading to greater degradation of cAMP, resulting in less activity of the β-AR cascade. In a performance study

utilizing similar diets (cracked-corn based finishing diet; Chapter 4) analysis of liver and muscle ZIP14 mRNA expression was conducted to determine if Zn interaction with the β-AA cascade is facilitated through changes in ZIP14. In this chapter, it was hypothesized that increasing supplemental Zn may allow tissues such as muscle to accumulate greater concentrations of Zn to inhibit the enzyme responsible for cAMP degradation, phosphodiesterase, and potentiate β-AA cascade activity. However, no change in ZIP14 mRNA expression was noted in liver or muscle. In contrast, expression of the Zn sequestering protein metallothionein was differentially regulated due to Zn concentration and source during ractopamine HCl supplementation suggesting ractopamine HCl may impact utilization and sequestration of Zn. Prior research has suggested introduction of catecholamines can increase metallothionein expression in adipose tissue to increase antioxidant capacity (Beattie et al., 2000). Genther-Schroeder (2016a) noted increases in circulating inflammatory markers following 300 mg ractopamine HCl·steer^{-1}·d^{-1} administration. The influence of ractopamine hydrochloride administration may be partially explained by the previous work, where plasma Cu increased and Fe decreased following ractopamine administration, possibly indicating a function of nutritional immunity. Muscle growth however has been shown to lead to slight inflammation through myokine production, and indicates that not all inflammation is harmful inflammation, and further research should be utilized to determine this process on trace mineral plasma and tissue concentrations. It appears that while ractopamine HCl does not influence Zn absorption during supplementation it may influence Zn trafficking post-absorption.

While Chapter 5 was initially meant to serve as a study of differing dietary energy, because of differences in quality of silage fed to steers the study became focused more on the differing amounts of digestible fiber in the diet. Previous research by Eryavuz and Dehority

(2009) noted that when rumen fluid Zn concentrations were 50 μg/mL cellulose digestion was decreased; however, the authors acknowledged that to achieve these concentrations in the rumen fluid ~ 2000 mg Zn/kg DM would need to be supplemented, 4 times the maximum tolerable concentration suggested by NASEM (2016). Additionally, VanValin et al. (2018) reported greater dietary fiber digestibility when lambs received 40 mg Zn/d from Zn hydroxychloride relative to Zn methionine, suggesting dietary Zn source and not only concentrations may impact fiber digestibility. In contrast to the work of others, supranutritional Zn as a blend of $ZnSO_4$ and Zn-amino acid complex in our study actually increased DM and OM digestibility and tended to increase N digestibility. While we observed no detrimental effects of supranutritional Zn concentrations from the Zn blend on fiber digestibility, $ZnSO_4$ was not measured as a source in our metabolism study. No differences between Zn sources were noted for performance, but clearly source, inherent fiber digestibility, and amount of Zn require further research. Steers fed the high fiber (low energy) diet displayed increases in coefficient of Zn absorption and retention compared to low fiber when receiving the supranutritional blend of $ZnSO_4$ and Zn-amino acid complex. Total tract digestibility of DM was not different between low or high fiber diets, and fiber digestibility was markedly higher in steers fed the high fiber diet. Collectively, more undigested fiber in the low fiber (high energy) diet, potentially due to negative associative effects of adding corn to a diet rich in fibrous corn co-products, may have resulted in not only less Zn released from feed component cell wall fractions but also more undigested fiber available to bind solubilized supplemental Zn, resulting in lesser rates of absorption and retention of Zn compared to the high fiber diet. However, this research is a stark reminder that dietary interactions with supplemental Zn and Zn absorption may be impacted by unknown factors such as digestibility, passage rate, digesta pH, and dietary antagonists such

as phytate. Further research evaluating fiber sources differing in digestibility and ADF and NDF content should be conducted to further determine dietary interactions with Zn. Steers (Chapter 3) displayed higher than expected rates of Zn absorption and did not display the classical depression in apparent absorption of Zn when greater amounts of Zn were supplemented, suggesting there may be other influences on Zn metabolism in late stage finishing cattle, while in Chapter 5 decreases in apparent absorption of Zn with supranutritional Zn concentrations were noted. An assessment of Zn rates of absorption across varying ages of cattle and stages of feedlot production would be useful in refining future feeding recommendations.

It is well known that supplying dietary energy above maintenance is required for growth. The research completed for Chapter 7 of this dissertation illustrates that dietary energy supplied to feedlot steers will result in performance advantages during the feedlot period. Regardless, targeted difference in growth rate between LE and HE were not achieved. However, our research also elucidated a performance advantage for steers receiving the blend of $ZnSO_4$ and Zn-amino acid complex immediately following the transition period. Periods of transition between diets high in roughage to diets higher in concentrates can be challenging to the rumen as increased intakes of high concentrate diets decrease rumen pH and can damage the rumen epithelium and disrupt cellular homeostasis (Penner et al., 2011). Zinc has been shown to be involved in gut epithelium tight junction function (Alam et al., 1994; Rodriguez et al., 1996; Sturniolo et al., 2001) and prior research in swine has noted improved gut integrity following a 7 d heat stress challenge when supplemented with Zn-amino acid complex (Sanz Fernandez et al., 2014). While rumen permeability was not investigated in the research for this dissertation, advantages in performance following transition may suggest a protective mechanism of the supplemental Zn blend on rumen

health. While no final performance advantages were noted due to supplemental Zn, steers receiving the unsupplemented control diet displayed only a 2% advantage in ADG when receiving the high energy diet compared to the low energy diet. In contrast, steers receiving $ZnSO_4$ or the supplemental Zn source blend displayed more typically expected increases in ADG of 5 and 13%, respectively. This advantage appears to support our hypothesis and implies that cattle receiving diets very close to NASEM (2016) recommendations for Zn may underperform relative to their Zn supplemented counterparts when receiving greater dietary energy. However, steers supplemented Zn at current recommendations appear to support resulting growth from receiving ractopamine HCl or experiencing moderate rates of growth. According to Samuelson et al. (2016) the mean concentration supplemented by nutritionists in the feedlot industry is 109 mg Zn/kg DM. While our research shows limited final performance advantages due to supplementing Zn at greater concentrations than currently recommended, the improvements in N retention and performance following transition suggest a need for further inquiry. Additionally, further evaluation is necessary to determine impacts in regard to environmental concerns from Zn excretion.

According to our research differences between dietary Zn sources exist, which include gene expression, tissue mineral concentrations, and interim steer performance, though overall performance was unaffected in either performance trial by source of supplemental Zn. Although the Zn sequestering protein metallothionein is known to increase following Zn supplementation, our research shows it can be differentially expressed between sources fed at similar concentrations. Additionally, results from the two performance studies suggest that dietary differences may also influence the ability of Zn to impact mRNA expression of the Zn sequestering protein metallothionein. Steers fed a dry-rolled corn-based diet for 56 d (Chapter 4) had greater liver Zn concentrations for the blend of $ZnSO_4$ and Zn-

AA complex vs. inorganic source and greater liver metallothionein expression due to blend, while steers receiving a corn silage-based diet displayed no differences in liver Zn concentration but lesser metallothionein expression for the blended sources of Zn vs. inorganic. Higher concentrate diets have been shown to decrease rumen pH, and therefore increase solubility of Zn in the rumen. This solubilized Zn is thereafter available for absorption or for interactions with dietary antagonists. Regardless, the metabolism studies conducted for this dissertation only utilized the blend of $ZnSO_4$ and Zn-amino acid complex so direct comparisons regarding absorption between the inorganic supplemental Zn and supplemental Zn blend are difficult to make. Additionally, while supplemental Zn increased plasma Zn concentrations, liver Zn concentrations did not change or appeared to be influenced by source rather than Zn dietary concentration. Supplementing Zn at five times the current requirements did not change liver concentrations and determining a biomarker may be an elusive task. Further research into biomarkers for Zn status may need to be on a more molecular scale.

Currently recommended Zn supplementation concentrations appear to be adequate in feedlot steers receiving the β-adrenergic agonist ractopamine HCl or experiencing moderate rates of growth. Contrary to our hypothesis, our research suggests that supplementing Zn at greater concentrations than what is currently recommended may not be necessary under these circumstances. However, steers receiving the higher energy diets seem to increase performance when supplemental Zn is fed at greater concentrations beyond those steers receiving dietary Zn marginally above current recommendations. This suggests they were more capable of utilizing energy supplied by the diet for growth. Although no interactions on performance of beef steers were noted between supplemental Zn concentrations and the β-agonist ractopamine hydrochloride, ractopamine appears to influence the Zn sequestering

activity of metallothionein and further research is warranted regarding source influences on metallothionein expression and Zn trafficking following administration of ractopamine HCl.