MINERAL SUPPLEMENTATION OF FEEDLOT CATTLE

by

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B.S., Colorado State University, 2007
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Four studies evaluated effects of mineral supplementation on feedlot performance, carcass characteristics and ruminal fermentation of finishing cattle. Study 1 supplemented 0 or 3.3 g/d yeast combined with Cr propionate to steers separated into light and heavy groups. No treatment × weight group interactions were observed for ADG, DMI, final BW, carcass traits, or plasma glucose of lactate concentrations ($P \geq 0.06$). A treatment × weight group interaction was observed for G:F ($P = 0.03$). In study 2, steers were supplemented 60 or 300 mg Zn/kg DM with or without zilpaterol hydrochloride (ZH). No interactions or effects of Zn or ZH were observed for IGF-1, plasma glucose, or lactate concentrations ($P > 0.05$). Plasma urea nitrogen (PUN) concentration decreased with ZH ($P < 0.01$). No interactions or effects of Zn or ZH were detected for ADG, DMI, final BW, G:F, and carcass traits were minimally affected ($P \geq 0.05$). Study 3 evaluated effects of supplementing 30 or 100 mg Zn/kg DM (30 or 100Zn) with and without ractopamine hydrochloride (RH; 200 mg/d). No interactions or effects of Zn were observed for feedlot performance or PUN ($P \geq 0.07$). Final BW, ADG, and HCW increased when heifers were fed RH ($P \leq 0.02$). Zinc × RH interactions were observed for LM area and yield grade ($P \leq 0.01$), but other carcass traits were not affected ($P \geq 0.08$). In study 4, heifers were supplemented 0, 30, 60, or 90 mg Zn/kg DM. Zinc supplementation did not affect final BW, ADG, or DMI ($P \geq 0.07$), but G:F increased linearly ($P = 0.02$). Carcass traits were not affected by Zn supplementation ($P \geq 0.07$). Effects of *in vitro* Zn titration (0, 30, 60, 60, 90, 120, or 150 mg/kg Zn) were evaluated using ground corn and soybean meal as substrate. *In vitro* fermentation was not affected by added Zn ($P \geq 0.05$). These studies suggest Cr and Zn supplementation minimally affected carcass traits, but Zn supplementation up to 60 mg/kg improved feed efficiency with minimal impact on ruminal fermentation. Supplementing increased Zn concentrations may alter fat and muscle deposition when fed with RH.

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Dedication

I dedicate this dissertation to my dad, John; my brother, Caleb; and my one and only sister, Jessa. Dad you gave me the courage and determination to follow and accomplish my dreams. Caleb you were the baby brother that was always so loving and caring no matter what, and Jessa you have always been our angel. You all may be gone but you are never forgotten. I love you all so very much.

Chapter 1 - Literature Review: Zinc in Ruminant Nutrition

INTRODUCTION

Zinc was discovered to be an essential trace mineral over a century ago being required for growth of *Aspergillus niger* (Raulin, 1869). In 1934, zinc was firmly established as an essential mineral for rat growth implicating it's essentiality for all mammals (Todd et al., 1934). Following the work with Zn supplementation in pigs and chicks in the 1950's, Legg and Spears (1960) observed zinc supplementation could reverse the effects of parakeratosis in cattle from Guyana. Zinc deficiency was further observed in calves which exhibited signs of parakeratosis, reduced weight gain, and stiffness of joints when compared to calves receiving zinc supplementation (Miller and Miller, 1962). This data revealed Zn deficiencies occur in cattle without proper supplementation, and proper supplementation can reverse deficiency symptoms. Zinc deficiencies have also been observed to cause immune dysfunction, cognitive impairment, metabolic disorders, and infertility (Jeong and Eide, 2013).

Researchers have revealed Zn to be the second most abundant micro mineral in the mammalian body, and is an essential component for over 300 enzymes and over 2000 transcription factors in microorganisms, plants, and animals (Vallee and Falchuk, 1993; Jeong and Eide, 2013). Zinc participates in cellular processes, antioxidant defenses, gene expression, and DNA stability (Reyes, 1996). Due to the vast amount of enzyme systems requiring Zn as either catalytic, co-catalytic, or participating as a structural component (Reilly, 2004), supplementation in cattle diets is essential for maintaining normal growth, normal development and function of the immune system, and metabolism (NRC, 2000). The purpose of this review is to describe some of the major aspects of zinc supplementation in ruminant diets.

THE BIOLOGY OF ZINC

Physiological Role of Zinc

Zinc is ubiquitous in biological systems throughout the body and plays important roles in normal body function and cellular metabolism. Zinc is the only metal encountered in all 6 enzyme classes established by the International Union of Biochemistry, which included oxioreductases, transferases, hydrolases, lyases, isomerases, and ligases (Vallee and Falchuk, 1993). Examples of specific enzymes utilizing Zn include carbonic anhydrase, RNA and DNA polymerase, Cu/Zn superoxide dismutase and alcohol dehydrogenase. The vast array of enzymes requiring Zn for normal function are indicative of the essentiality of Zn in macronutrient metabolism, nucleic acids, growth and development, immune function, cell division, vision, and the synthesis of microbial secondary metabolites.

Most enzymes use Zn as a catalytic ion for activation; however, there are a few enzymes that utilize Zn in purely a structural role (Berg and Shi, 1996). Zinc utilized as a catalyst is located in the center of the enzyme and is directly involved in the interaction with the substrate for making or breaking bonds (Reilly, 2004). In co-catalytic enzymes Zn can either be paired with other Zn ions (Hough et al., 1989) or other metals, such as Mg (Kim and Wyckoff, 1989) and Cu (Tainer et al., 1982) for enzyme activation. Structural Zn atoms are required to stabilize the quaternary structure of oligomeric enzymes (Vallee and Falchuk, 1993). Currently, enzymes utilizing Zn for structure stabilization consist of alcohol dehydrogenase (Vallee and Hoch, 1955), aspartate transcarbamylase (Nelbach et al., 1972), and protein kinase C (Csermely et al., 1988).

Zinc provides structural support in many proteins involved with cellular and subcellular metabolism, and aids in regulation of proteins (Reilly, 2004). It is especially important in the zinc finger motif, which is particularly important for transcription factors associated with DNA.

Zinc fingers are found in the major groove of the double helix and connect to the helix through amino acid side chains (Berg and Shi, 1996). In addition, zinc-binding units have been discovered in over 10 different classes of binding domains that function in nucleic acid binding, DNA binding, protein-protein interactions and binding of single stranded nucleic acids (Berg and Shi, 1996) indicating a wide range of Zn activity.

Zinc plays important roles in production, storage, and secretion of hormones, in addition to efficiency of receptor sites and end target responses. Insulin, testosterone, and adrenal corticosteroids are among the most renowned hormones to be affected by Zn deficiency (McDowell, 1992); however, effects are not limited to these hormones. A more complete review of Zn supplementation in relation to hormones has been summarized by Prasad (1993).

Immune function

Normal development and function of the immune system is highly dependent on Zn supplementation. Effects of Zn deficiency on immune function can be observed in Zn-deficient animals which exhibit increased susceptibility to bacterial, viral, and parasite challenges (Keen and Gershwin, 1990). All aspects of the immune system (i.e. mucosal and barrier immunity, humoral immunity, and cell mediated immunity) are affected by Zn deficiency (Keen and Gershwin, 1990), with rapid atrophy of the thymus and various influences on T-cell function being the more prominent effects (McDowell, 1992; Hambidge et al., 1986). Immune functions listed by Hambidge et al. (1986) influenced by Zn deficiency include: thymic hormone production and activity, lymphocyte function, natural killer cell function, antibody-dependent cell-mediated cytotoxicity, immunological ontogeny, neutrophil function, and lymphokine production. Zinc has also been implicated as a component of thymulin, a metallopeptidic hormone produced by thymic epithelial cells known to induce T-cell differentiation and

maturation, cytotoxicity, and production of interleukin-2 (Dardenne and Pleau, 1994; Reilly, 2004). Binding of Zn to thymulin causes a conformational change necessary for its activity. This may provide an explanation of the role of zinc on T-cell function (Dardenne and Pleau, 1994).

Orr et al. (1990) conducted a study to determine if mineral status affected responses to bovine respiratory disease (BRD), or infectious bovine rhinotracheitis virus (IBRV). The authors observed in all infected animals plasma Zn concentration decreased as a result of the disease in addition to reduced feed intake when compared to non-infected cattle. Zinc concentration of the diet for all cattle in the experiment by Orr et al. (1990) was 81.6 mg/kg, well above the recommendation for beef cattle suggesting the diet was not the cause for decreased plasma Zn concentration. The cytokine interleukin-1 (IL-1) was observed to rapidly decrease plasma Zn concentration in a dose dependent manner during an acute phase immune response when cattle were injected with 10, 33, 100, 333, or 1000 ng/kg BW IL-1 suggesting IL-1 was partially responsible for reduced plasma Zn concentration (Godson et al., 1995). Results utilizing rats injected with 0 or 5 mg IL-1 and ^{65}Zn suggest IL-1 is involved in removal of Zn from the plasma pool into the liver, bone marrow, and thymus (Cousins and Leinart, 1988) which may provide an explanation why plasma Zn decreased in immune compromised cattle. Hambidge et al. (1986) postulated the reduction in plasma Zn may enhance phagocytic cell activity and bactericidal function, whereas Keen and Gershwin (1990) suggested Zn uptake by the thymus and bone marrow may provide Zn necessary for T- and B-cell production. The reduction in plasma Zn concentration is evidence that nutrient adequacy is of vital importance especially during periods of stress due to infection.

Characteristic effects of Zn deficiency on barrier immunity consist of skin lesions, degenerative changes in enterocytes, damage to microvilli along the gastrointestinal tract, and

pulmonary function (Keen and Gershwin, 1990). Skin contains 2 to 3% Zn (Neathery et al., 1973), which has been implicated as an important aspect of wound healing, and maintaining structural integrity (McDowell, 1992). Calves fed a purified Zn deficient diet grew new skin over wound areas 2.5 weeks slower than calves fed 40 mg/kg Zn, and calves fed a restricted diet containing 40 mg/kg Zn had normal appearance in healing, but generation rate of new skin was slower compared to calves fed an *ad libitum* diet (Miller et al., 1965). Schwarz and Kirchgessner, (1975), experimentally induced Zn deficiency in adult dairy cows and observed the healing process of biopsy wounds were similar during dietary Zn depletion and repletion. The results may reflect age differences of animals suggesting calves are more susceptible to Zn deficiency compared to adult animals. An *in vitro* trial by Kaji et al. (1995) was conducted to determine if Zn promoted the repair of vascular endothelial cells after wounding. Following incubation of vascular endothelial cells in various medium, the authors concluded addition of Zn promotes the repair of wounded monolayers by potentiating repair induced by exogenous basic fibroblast growth factor. These results indicate Zn is an essential component for repairing internal barriers such as barriers in the vascular system and skin. Miller et al. (1967b) performed a study to determine the effects of Zn source and high amounts of supplemental Zn added to a practical diet on rate of wound healing in heifers with treatments consisting of 30 mg/kg Zn from Zn carbonate, 400 mg/kg Zn from Zn oxide, and 384 mg/kg Zn from Zn sulfate. The authors observed no differences in rate of wound healing among treatments suggesting increased amounts of Zn in the diet regardless of source was unnecessary to maintain maximal wound healing rate.

The review by Keen and Gershwin (1990) discuss numerous effects of Zn deficiency on immune defense mechanisms in non-ruminants; however, similar immune defense mechanisms

have not been clearly identified in cattle. Research observing the effectiveness of Zn supplementation in immune stressed cattle have shown positive results in some studies, but not in others. Galyean et al. (1999) reviewed interactions of nutrition on immune function in newly weaned and receiving cattle and suggested ration formulation for newly received cattle should take into account decreased feed intake and known nutrient deficiencies rather than include increased levels of trace minerals. Conversely, Chirase et al. (1991) reported decreased DMI, and increased mean rectal temperatures of steers supplemented with 31 mg/kg Zn (control) compared to steers supplemented with 90 mg/kg Zn from Zn methionine after cattle had been challenged with infectious bovine rhinotracheitis virus. In addition, steers supplemented with 90 mg/kg Zn from Zn methionine returned to pre-challenge DMI 5 d sooner than control calves indicating increased dietary Zn may enhance the recovery rate of morbid cattle. Heifer calves fed 17 mg/kg Zn exhibited a reduced cell mediated immune response evident by a lower skinfold swelling response, after a phytohemagglutinin (PHA) injection compared to heifers that received 40 mg/kg Zn (Engle et al., 1997). Results by Engle et al. (1997) suggested marginal Zn deficiency can affect immune response by possibly impairing lymphocyte blastogenesis. Spears et al. (1991) fed steers no supplemental Zn, Zn methionine (25 mg/kg Zn), or Zn oxide (25 mg/kg Zn), and vaccinated cattle with bovine herpesvirus-1 (BHV-1) and parainfluenza$_3$ (PI_3). Antibody titers were determined on day 0 and 14 of the experiment to determine effects of Zn source on immune response to vaccinations. The authors observed cattle supplemented with Zn tended to have higher antibody titers against BHV-1 with Zn methionine supplementation having the greatest response, but no differences were observed for antibody titers to PI_3. Results suggest some cattle may respond favorably to Zn supplementation which could be dependent on Zn status of the animal.

Effects of Zn supplementation on immune function of finishing cattle have also been analyzed. Spears and Kegley (2002) fed steers no supplemental Zn, Zn oxide, and two forms of chelated Zn with supplemental Zn treatments providing an additional 25 mg Zn/kg diet DM (basal diet contained 33 mg/kg Zn). The authors observed no improvement in antibody titers to infectious bovine rhinotracheitis following vaccination with Zn supplementation regardless of source. In addition, skinfold thickness response to intradermal injection of PHA, and in vitro lymphocyte blastogenic response to PHA and pokeweed mitogen were not affected by Zn level or source. Nunnery et al. (2007) fed 24 heifers diets containing no supplemental Zn or 75 mg/kg Zn from Zn sulfate, Zn methionine, or Zn propionate and analyzed humoral immune response by injecting 4 mL of a solution containing 4 mg of ovalbumin initially and then re-injected 14 days later. The authors observed no effect of Zn supplementation for IgG titers specific to ovalbumin when compared to non-supplemented heifers. The basal ration for these cattle contained 44.1 mg/kg Zn. The lack of a response to Zn supplementation in the previous 2 trials may be attributed to the basal rations containing dietary Zn concentrations above NRC (2000) recommendations, which would suggest cattle not receiving Zn supplementation were receiving adequate dietary Zn to maintain normal humoral and cell mediated immune function.

Growth

Growth rate is affected in all species during periods of Zn deficiency, and combined with loss of appetite are the first overt signs of deficiency (NRC, 2001). Miller and Miller (1962) conducted an experiment to assess the effects of Zn deficiency in calves fed a low-Zn diet (3.6 mg/kg Zn), diets containing 40 mg/kg supplemental Zn, or calves fed the low Zn diet until 15 weeks of age then switched to a diet containing 260 mg/kg Zn for 5 weeks. The authors observed declined weight gains by week 10 compared to calves receiving 45 mg/kg Zn, and gain remained

relatively constant in deficient calves week 10 to 20 whereas calves receiving 40 mg/kg supplemental Zn continued to gain steadily. When Zn was supplemented to the deficient calves week 15, rapid improvements in daily gains were observed. Rate of gain increased from 0 to 9.1 kg within the 1st week of supplementing deficient calves with 260 mg/kg supplemental Zn, followed by continued improvement in gain with Zn supplementation. Similar results were observed in calves fed Zn deficient, and Zn supplemented diets in a study performed by Mills et al. (1967). In addition to feeding Zn deficient calves supplemental Zn, Mills et al. (1967) switched calves fed 0.7 mg Zn/kg live weight for 6 weeks to a diet containing no supplemental Zn (1.2 mg/kg dietary Zn). The authors observed decreased live weight gain after week 7 in calves switched from high to low-Zn diet compared to calves receiving 0.3 mg Zn/kg live weight indicating the speed at which Zn deficient diets can affect growth. Reduction in growth was most likely caused by impairment of nucleic acid biosynthesis, amino acid utilization, or protein synthesis (McDowell, 1992).

ABSORPTION

Zinc absorption is a function of physiological need. As zinc content in the diet increases the amount of zinc absorbed decreases suggesting highly controlled homeostatic mechanisms (McDowell, 1992). Calves fed a zinc deficient diet then dosed with a single dose of ^{65}Zn absorbed 7% more ^{65}Zn compared to the calves fed diets containing 40 mg/kg supplemental Zn (Miller et al., 1967a). In addition, calves administered zinc deficient diets absorbed more zinc from the diet on a percent basis than calves that received 36 mg/kg supplemental zinc oxide or calves fed a practical diet (Miller et al., 1968a).

The amount of Zn absorbed by cattle varies with age and metabolic need. Miller and Cragle (1965) indicated week old calves absorbed approximately 55% of Zn in milk, whereas

calves ranging 5 to 12 months of age absorbed 20% of Zn, and mature cows only absorbed 12% of Zn; however, total dietary Zn was not reported to determine Zn status of the animal. Hansard et al. (1968) observed mature beef cattle absorbed 22% of dietary Zn when fed a diet containing 28 mg/kg Zn. The Agricultural Research Council (1980) concluded, after reviewing literature on Zn absorption, the amount absorbed by calves was about 50% of dietary Zn, growing ruminants absorbed 30% dietary Zn, and the lowest percent absorption was determined for adult ruminants at 20% absorption.

The primary site of absorption in monogastric animals is the small intestine with the duodenum and proximal part of the intestine being more active (Naveh et al., 1988) than the distal regions. Zinc absorption differs slightly in the ruminant animal. In early work investigating the site of absorption in dairy cattle, absorption in the abomasum and lower small intestine appeared to be the major sites of absorption, whereas the rumen and the upper small intestine had greater rates of secretion compared to absorption (Miller and Cragle, 1965). Hiers et al. (1968) conducted a trial utilizing calves and goats to assess endogenous secretion and reabsorption of Zn. The authors observed large amounts of radioactive Zn was secreted in the upper small intestine, rumen, and reticulum with reabsorption occurring in the abomasum and lower small intestine. In a trial comparing absorption rates in lambs, radioactivity of Zn per kg of rumen tissue was greater than radioactivity observed in abomasal, duodenal, or large intestinal (Arora et al., 1969) indicating the rumen was the primary site of absorption. The difference in results between experiments may reflect species differences as well as differences in experimental design; however, both experiments indicate the rumen is capable of absorbing Zn.

Absorption of Zn from the lumen of the digestive tract and transfer to other tissues occurs in different phases following solubilization if in the form of inorganic salt (Zn sulfate). First, Zn

must be transported from the luminal side of the mucosal cells into the cell (McDowell, 1992). Zinc not utilized for cellular function is transported through the cell to the basolateral membrane then transferred into portal circulation (Hambidge et al., 1986). Zinc in the portal blood supply is mostly bound to albumin and α_2-macroglobulin and transported to various tissues (Reyes, 1996). Zinc entering the hepatic venous supply is extracted in liver and redistributed into the blood supply to be transferred and incorporated into other tissues at differing rates (McDowell, 1992); however, specific mechanisms associated with zinc absorption have yet to be firmly elucidated.

CELLULAR TRANSPORT IN MAMMALS

The first zinc transporter was discovered in 1995 from rat kidney and was purposed to transport zinc out of cells (Palmiter and Findley, 1995). Research regarding zinc transporters has gained more interest as new scientific techniques have been developed to adequately assess transporters; however, mechanisms of action are not well characterized and information is still lacking. Recently, Ohana et al. (2004) located a Na^+/Zn^{2+} transporter in cells isolated from human embryonic kidney cell lines and corticol neurons that utilizes the Na^+ electrochemical gradient to cause an efflux of Zn from the cell. In addition, Zn transporters have been classified into 2 different transport families: the Znt proteins and the Zip family (Liuzzi and Cousins, 2004), although these transporters haven't been researched in ruminant tissue specifically, it can be assumed ruminants contain similar transporters as those found in other mammalian species.

ZnT Proteins

High intracellular Zn concentration is not well tolerated by cells and becomes toxic if mechanisms to remove excess zinc are not utilized (Vallee and Falchuk, 1993; Devergnas et al., 2004). Znt transporters main purpose is to reduce intracellular Zn concentration by promoting efflux of Zn out of the cell or into intracellular compartments (Liuzzi and Cousins, 2004). Thus

far, the Znt protein transporters consist of 10 different transporters found in mammals. The first transport protein discovered in 1995 by Palmiter and Findley was the Znt1 transporter, and has been found in a wide variety of tissues with the highest expression in the kidney and small intestine followed by adipose tissue, liver, spleen and thymus (Liuzzi et al., 2001). In a trial conducted by Devergnas et al. (2004), Znt-1 transcription levels increased in cells with increased intracellular Zn indicating increased intracellular Zn concentration results in increasing amounts of Znt-1. Results indicate Znt-1 may be part of a protection mechanism against high intracellular Zn concentration. Remaining Znt transporters (2-10) have been detected ubiquitously in various tissues analyzed with concentrations varying among tissues for each transporter. For example, Znt-3 was detected in the brain and testes of mice (Palmiter et al., 1996); whereas Znt-2 and -4 were detected in increased concentrations in the small intestine with mRNA expression being greatest in the villous cells when compared to crypt cells (Liuzzi et al., 2001). Increased concentrations of Znt-5 were detected in insulin-containing β cells in the human pancreas, and were also associated with pancreatic secretory granules (Kambe et al., 2002). Znt proteins are not only found along the plasma membrane of cells, but they also reside in subcellular organelles and may be involved with the incorporation of Zn into enzymes (Kirschke and Huang, 2003; Suzuki et al., 2005; Cousins et al., 2006). Researchers believe the mechanism of transport for many of the Znt proteins is through a Zn/H^+ or K^+ antiporter and is thus reliant on gradients of other ions to drive transportation of Zn (Eide, 2006; Guffanti et al., 2002); however this mechanism has not been clearly established in mammalian tissue.

ZIP transporters

Mammalian ZIP transporters work in opposition to Znt proteins because these proteins transport zinc either from extracellular space or from intracellular organelles into the cytoplasm

(Eide, 2006; Gaither and Eide, 2001). ZIP transporters are divided into 2 subfamilies with subfamily 1 containing fungal and plant proteins, and subfamily 2 containing insect, nematode and mammalian proteins (Guerinot, 2000). The mammalian ZIP family currently consists of 14 different transporters that have been identified in the human genome (Jeong and Eide, 2013). Whether or not the same transporters are located in the bovine genome is unknown, but is highly likely some if not all of them function in a similar manner. ZIP transporters are expressed in a wide variety of mammalian tissue, and play a large role in the physiology of the cell and live animal. Gene expression has been located in plasma membrane of cells as well as specific subcellular compartments (Huang et al., 2005; Jeong and Eide, 2013). Regulation of ZIP transporters occurs through dietary Zn concentration (Wang et al., 2004) and responds to hormonal and cytokine signaling. Liuzzi et al. (2005) performed a study utilizing mice with various genotypes to determine which Znt and ZIP proteins were expressed after LPS challenge and in mice with hypozincemia of turpentine-induced inflammation. The authors observed an up-regulation in ZIP14 by interleukin-6 indicating the acute phase response of inflammation may affect Zn status of the cell. Complete mechanisms of action for ZIP transporters have yet to be firmly established; however, Gaither and Eide (2000), through the use of a biochemical transport assay, demonstrated HCO_3^- stimulated zinc uptake by ZIP2 suggesting a Zn^{2+}/ HCO_3^- symporter may be the mechanism of action. Liu et al. (2008) conducted a trial utilizing ZIP8 cRNA-injected *Xenopus* oocyte cultures and suggested the mechanism of action for ZIP8 was also Zn^{2+}/ HCO_3^- symporter.

Metallothionein

Metallothioneins are proteins that contain 60 amino acid residues (20 Cys residues), and bind a total of 7 bivalent metal ions which are associated with Cys residues. The more common

metals associated with metallothioneins are Cd, Cu, and Zn (Kägi and Vallee, 1961; Pulido et al., 1966). Metallothioneins are found universally in all mammalian tissues (Whanger et al., 1981a), but are greatest in parenchymal cells of the liver, kidney, pancreas and intestines (Kägi and Schäffer, 1988). The exact function of metallothionein has not been definitively defined due to metallothionein containing a α and β domain, which may indicate dual physiological properties. The β domain may be the physiologically active portion of the protein, whereas the α domain may be associated with metal detoxification of the cell (Cousins et al., 2006). The most widely studied roles of metallothionein are its role in detoxification of excess levels of Cu and Zn, its role as a homeostatic mechanisms of Cu and Zn (Richards, 1989), and its ability to protect against cellular stressors such as carbon centered radicals, reactive oxygen, and nitrogen species (Cousins et al., 2006). Whanger et al. (1981b) performed a study evaluating the accumulation and depletion of Zn in cattle and sheep tissue metallothionein and observed metallothionein bound excess Zn in both cattle and sheep tissues suggesting metallothionein does aide in removal of excess Zn. Their research also indicated Zn can be transferred from metallothionein to other proteins and reutilized suggesting metabolic action. In addition, Zn was associated with metallothionein in the small and large intestine, but very little Zn was associated with metallothionein in ruminal papillae or abomasal epithelium, which the authors attributed to the potential regulatory mechanism of metallothionein on Zn absorption (Whanger et al., 1981b).

EXCRETION

The main route for excretion of Zn is through feces, which is primarily unabsorbed Zn. Miller and Cragle (1965) observed net absorption of Zn in mature cows averaged 12% and ranged from 20 to 55% in calves indicating age affected the amount of Zn absorbed, but also indicated approximately 88% of Zn consumed was unabsorbed and excreted by adult cattle.

Although calves absorbed more Zn than mature cattle the amount of unabsorbed Zn was substantial. Fecal excretion of Zn also includes a small portion of endogenous Zn secretions into the gastrointestinal tract. Most of the endogenous Zn secreted into the gastrointestinal tract consist of secretions coming from the pancreas and the bile duct (McDowell, 1992). Miller et al. (1967a) fed calves and goats diets that were either Zn deficient or contained supplemental Zn and observed animals on the Zn deficient diets excreted less Zn when compared to animals receiving Zn supplementation. These results indicate amount of Zn excreted is dependent on dietary Zn concentration and metabolic needs of the animal. Similar results have been observed in multiple species (Hambidge et al., 1986).

The amount of Zn excreted in urine is very small when compared to fecal loss and is not dependent on Zn content in the diet. Miller et al. (1968a) performed a study to assess effects of dietary Zn content on Zn metabolism. Calves received treatments that consisted of feeding purified Zn deficient diet, purified Zn added diet or practical diet containing Zn. Calves given the Zn deficient purified diet excreted less Zn in the feces compared to calves receiving Zn supplementation; however, the amount of Zn excreted in the urine was not different among treatments. These results are consistent among goats (Miller et al., 1967a), sheep (Suttle et al., 1982), and steers over 1 year of age fed a high Zn diet (Feaster et al., 1954). Exact reasoning for the small amount of Zn excreted in the ruminant urine has not been firmly established; however, Abu-Hamdan et al. (1981) indicated the amount of Zn excreted in the urine arises largely from the ultra-filterable portion of plasma Zn. Victery et al. (1981) indicated the distal part of the renal tubule was able to reabsorb approximately 95% of Zn in dogs which may give insight into what process is occurring in ruminants that results in negligible amounts of Zn excreted in the urine. Amino acids (i.e. cysteine) were observed to increase the amount of Zn excreted in the urine

which caused an increase of Zn in the unfilterable portion of plasma or by preventing reabsorption by binding of the amino acid to Zn (Abu-Hamdan et al., 1981).

ZINC HOMEOSTASIS

Ruminant animals don't have the capability to store large amounts of Zn as indicated by the rapidity in which deficiency can occur (NRC, 2001). Effective homeostatic control mechanisms must be in place to maintain Zn concentration in all tissues of the ruminant system due to the vast amount of systems that utilize Zn for normal function. In a trial performed by Mills et al. (1967), calves were fed a diet that contained 1.20 mg/kg Zn, and were given Zn supplements consisting of 0, 0.05, 0.20, 0.70 mg Zn/kg BW for 6 weeks, followed by 0.3 mg Zn/kg BW supplemented to calves that received 0.05 or 0.20 mg/kg and 0 for calves that received 0.70 mg/kg BW weeks 1 through 6. Clinical signs of severe Zn deficiency were observed for calves that received no supplementation 16 days after trial initiation, and 21 days for calves receiving 0.02 mg Zn/kg BW. Calves that had Zn removed the final 3 weeks of trial maintained BW for 1 week after depletion of Zn followed by a sharp decline thereafter. Response from severely Zn deficient calves to Zn supplementation was rapid and symptoms of Zn deficiency quickly diminished. The speed in which deficiency symptoms occur and how quickly they were alleviated are indicative of the efficiency of homeostatic mechanisms, and storage of Zn is limited. Neathery et al. (1973) performed a trial to assess the effects of feeding low and normal Zn diets on absorption and Zn contents of body tissue using mature dairy cows. Similar to results observed in calves, mature cows receiving a Zn deficient diet absorbed and retained a greater percentage of Zn and excreted less than cows fed an adequate supply of Zn indicating an efficient homeostatic control mechanism in adult ruminants.

During periods of deficiency, tissues such as liver, pancreas, and bone have a moderate decline in Zn concentration, whereas tissues including the brain and muscle have little to no change (Miller, 1970a; Neathery et al., 1973). In 24 tissues analyzed in mature dairy cows, only the cartilage of the rib and ruminal tissue contained lower Zn content in Zn deficient cows compared to cows receiving 39.5 mg/kg Zn (Neathery et al., 1973). Studies utilizing goats and calves indicated tissues such as the brain and muscle maintain relatively constant concentrations of Zn, whereas calves and goats suffering from severe deficiency had decreased Zn concentrations in hair, bone, liver, lung, kidney, spleen, pancreas, and plasma with variability existing among animals (Miller et al., 1966a; Miller et al., 1967a; Miller et al., 1968b). These results demonstrate the amount of Zn within each tissue is effectively maintained at different concentrations and Zn deficient cattle bound Zn more tightly in tissues compared to animals maintained on Zn sufficient rations.

Researchers have identified transporters that are involved in homeostasis as indicated previously, but exact homeostatic control mechanisms have not been clarified in ruminants. Ruminant control mechanisms appear to function mainly through increasing or decreasing absorption and endogenous losses primarily through fecal excretion (Miller, 1973), which coincides with Zn content in the diet and tissue metabolic need. Neathery et al. (1973) observed, as a percent of intake, cows consuming a low Zn diet (16.6 mg/kg) were able to absorb 50% more Zn compared to cows that were provided a ration containing 39.5 mg/kg Zn. In addition, cows consuming 39.5 mg/kg Zn excreted more Zn in feces than cows consuming the low Zn diet. Similarly, studies utilizing calves fed Zn deficient diets indicated Zn deficient calves could absorb up to 80% dietary Zn compared to calves fed adequate dietary Zn (Miller et al., 1967a). The amount of Zn excreted in feces of calves receiving adequate dietary Zn was consistently

higher than Zn excreted in feces of deficient calves (Miller et al., 1966a; Miller et al., 1966b). These results indicate Zn deficient animals are able to conserve more Zn through homeostatic control mechanisms by regulating absorption and excretion. In agreement, Miller et al. (1971) observed calves that received 200 or 600 mg/kg dietary Zn had higher endogenous losses of Zn compared to calves receiving 38 mg/kg Zn.

Homeostatic control mechanisms appear to efficiently regulate Zn concentration in Zn-deficient animals, and in animals receiving adequate dietary Zn; however, research with calves indicate homeostatic control mechanisms begin to break down with extremely high dietary Zn in young animals. Miller et al. (1970b) fed calves diets that contained 33, 200, or 600 mg/kg supplemental Zn and observed a very sharp increase in Zn content in the pancreas, liver, kidney, and some sections of the gastrointestinal tract when calves were fed 600 mg/kg Zn compared to the calves that received 200 mg/kg Zn. Interestingly, the heart and skeletal muscle were unaffected by dietary Zn concentration (Miller et al., 1970b) and the brain was unaffected by deficiency (Neathery et al., 1973) indicating a complete homeostatic control mechanism. The authors indicated a limited number of Zn binding sites were located on these organs and were saturated with Zn resulting the metal being tightly bound (Miller et al., 1970b). Similar results were observed by Miller et al. (1971) when calves were administered 38, 200 or 600 mg/kg dietary Zn then intravenously injected with ^{65}Zn. Kincaid et al. (1976) conducted a trial using 10 dairy cows and 10 calves fed 41 mg Zn/kg diet to cows, 42 mg Zn/kg diet to calves, or 600 mg Zn/kg diet to cows and calves and observed a similar homeostatic control breakdown in calves administered 600 mg/kg Zn indicated by a dramatic increase in Zn content of the liver, pancreas, and intracellular Zn. Zinc concentration in the liver, kidney, or intracellular Zn of adult cows was comparable between cows fed 41 or 600 mg Zn/kg diet. The results confirm the homeostatic

control mechanism is more effective in adult cattle than in calves, thus adult cattle are more likely to tolerate increased dietary Zn concentrations (Kincaid et al., 1976). Contrary to other tissues, Zn content in muscle is not affected by Zn deficiency or excessive dietary Zn intake, thus homeostatic mechanisms in muscle are highly effective at controlling Zn concentration (Miller et al., 1966a; Miller et al., 1970b; Neathery et al., 1973).

Recent advances in research have given scientists an opportunity to research mechanisms which control homeostasis. Metal-responsive transcription factor-1 (MTF-1) has been the most common transcription factor studied in mammals, and plays an important role in Zn homeostasis by activating genes associated with removal of Zn from cells and genes associated with metallothionein when intracellular Zn is in excess (Choi and Bird, 2014).The regulatory effects of Zn on MTF-1 are complex, but main actions include: Zn effects on DNA binding activity, subcellular location, and trans-activation (Choi and Bird, 2014). Metal-responsive transcription factor-1 was found to contain Zn fingers in mice and are involved in DNA recognition of the protein implicating Zn as an important metal for initiating transcription of genes associated with homeostasis (Radtke et al., 1993).

TISSUE DISTRIBUTION

Visceral Organs and Brain

Zinc content varies between organs, and can be influenced by Zn content of the diet in some tissues. Researchers have observed a large variation in Zn concentration in organs among animals given the same treatment which indicates individual animals respond differently to supplementation. Research utilizing calves indicated Zn concentration in the kidney ranged from 88.2 (Miller et al., 1971) to 118.4 (Miller et al., 1970b) µg Zn/g of dry tissue when calves were fed practical diets containing supplemental Zn concentrations near recommended values.

Neathery et al. (1973) fed mature cows diets containing 39.5 mg Zn/kg diet and observed liver Zn concentrations were similar to concentrations observed in calves (119 µg Zn/g dry tissue in mature cows) indicating liver Zn content was not affected by age. Kincaid et al. (1976) analyzed liver Zn content in mature cows fed a diet containing 41 mg Zn/kg diet compared to livers of 120-day old calves fed 42 mg/kg Zn and observed a numerical difference in liver Zn content between the different age groups (57 versus 25 mg Zn/kg fresh tissue, respectively) which may be due to differences in experimental conditions between age groups. Hambidge et al. (1986) indicated Zn is present in the nuclear, mitochondrial, and supernatant fractions of the liver with the highest concentration being found in the supernatant and microsomes.

Zinc content of the pancreas ranged from 71.9 (Miller et al., 1971) to 100.8 (Miller et al., 1970b) µg Zn/g dry tissue in calves and 114 µg Zn/g dry tissue in mature cows (Neathery et al., 1973). The kidney contained 67.1 (Miller et al., 1967b) to 92.1 (Miller et al., 1970b) µg Zn/g dry tissue in calves fed practical diets containing at least 33 mg Zn/kg diet. Neathery et al. (1973) observed the amount of Zn analyzed in the kidney of mature cows was within the range found in calves (74 µg Zn/g dry tissue). The heart contained 67.4 to 80 µg Zn/g of dry tissue in calves and mature cows (Miller et al., 1971; Miller et al., 1970b; Neathery et al., 1973), with mature cows being slightly higher in Zn content than calves. The ruminal wall contained 129.6 µg Zn/g dry tissue in calves (Miller et al., 1970b) and 140 µg Zn/g dry tissue in mature cows (Neathery et al., 1973), and the abomasum contained 33 to 46.6 µg Zn/g dry tissue. Differences in Zn concentration in the tissues listed indicate different organs require different Zn concentrations, and differences between trials may be due to differences in availability of Zn contained within the basal rations. The brain was observed to contain 53 µg Zn/g dry tissue in mature cows fed 39.5 mg Zn/kg diet (Neathery et al., 1973).

Bone

Bone is a living tissue and its composition is in constant flux with resorption and repair as bone cells maintain equilibrium with body fluids and changing structural and homeostatic demands of the body (Hidiroglou, 1980). Zinc concentration of the frontal bone, femur, humerus, rib, thoracic vertebrae, and caudal vertebrae were collected from cattle under range conditions (Blincoe and Bohman, 1966). The authors observed the caudal vertebrae contained the highest amount of Zn compared to the other bones analyzed, whereas the frontal bone tended to have the lowest Zn concentration. In a trial conducted by Neathery et al. (1973), adult cows supplemented with 39.5 mg/kg Zn contained approximately 30% of the total-body Zn in bones with Zn content varying by location. The authors observed the rib shaft contained 75 µg Zn/g dry tissue, whereas the tibia joint and tibia shaft contained 61 and 59 µg Zn/g dry tissue, respectively. Similar Zn concentrations were observed in the rib and tibia in calves supplemented with 33 mg/kg Zn (Miller et al., 1970b). In addition, Feaster et al. (1954) observed differences in ^{65}Zn accumulation in the sternal end of the rib, rib shaft, and mandible angle (45, 28 and 25 µg Zn/g fresh tissue weight, respectively) after 6 days of administration of ^{65}Zn.

Bone Zn content is not as severely affected by Zn deficiency as that observed in visceral organs. Zinc content of the rib, tibia joint, and tibia shaft remained unchanged in cows fed 16.6 mg/kg Zn compared to cows fed 39.5 mg/kg Zn (Neathery et al., 1973). Asling and Hurley (1963) stated Zn is tightly bound in mineralized bone, thus not allowing it to be readily available during periods of Zn deficiency. Miller et al. (1968a) conducted a trial utilizing calves fed Zn purified diet (2 mg/kg Zn), a Zn purified diet plus 36 mg/kg Zn, or a practical diet containing 38 mg/kg Zn and observed differences in metabolism and tissue concentration of Zn. The authors did not observe any differences in Zn content of the tibia joint and shaft in calves fed the Zn

purified diet compared to calves supplemented with Zn; however, the rib contained less Zn when calves were fed the Zn purified diet compared to supplemented calves suggesting differences in Zn mobilization between bone types. Zinc content in the bone of young animals may not be as tightly bound as that found in older animals which may result in the lower bone Zn content observed in Zn deficient calves, thus allowing Zn to be more available for metabolic processes including growth (Hidiroglou, 1980). Zinc released from bone appeared to come primarily from a rapidly turning over pool that consisted of 10-20% of the total amount of Zn in bone, thus normal bone turnover may be responsible for the release of Zn from bone (Reilly, 2004)

Feeding large amounts of supplemental Zn resulted in increased Zn content of bones in varying degrees. Miller et al. (1970b) observed no significant increase in the shaft and joint of the tibia; however, the Zn content in the rib increased when calves were supplemented with 600 mg/kg Zn. In agreement, Wright and Spears (2004) fed calves no supplemental Zn or 500 mg/kg Zn, and observed increased bone Zn content in the shaft and end of the metacarpus. Although the concentration of Zn in bone fluctuates when Zn is fed in excess it is not considered a storage site for Zn since the amount of Zn mobilized during Zn deficiency is minimal (Reilly, 2004).

Muscle

A large amount of Zn is present in muscle due to its bulk compared to others tissues (Hambidge et al., 1986). Neathery et al. (1973) indicated the dairy cow contained approximately 50% of the total-body Zn in muscle, and variable concentrations among muscles. The amount of Zn in the semitendinosus of cows and calves ranged from 75.8 (Miller et al., 1970b) to 102 (Neathery et al., 1973) µg Zn/g dry tissue. Smooth muscle contained 120 µg Zn/g dry tissue (Neathery et al., 1973) in cows supplemented with 39.5 mg/kg Zn, and the *longissimus dorsi* contained 36 mg Zn/kg of edible portion (Leheska et al., 2008) from grass fed beef

DEFICIENCY SYMPTOMS

Deficiency symptoms observed with mild Zn deficiency consist of decreased feed intake and reduced weight gain (Mayland et al., 1980; NRC, 2001). Mills et al. (1967) observed calves fed a Zn sufficient diet (0.7 mg Zn/kg live BW) then switched to a low-Zn diet (1.2 mg Zn/kg diet) exhibited decreased feed intake after 1 week of consuming the low-Zn diet. Prolonged deficiency symptoms exhibited from calves in trials fed semi purified (1.2 to 4 mg/kg Zn) without supplementation included: reduced growth of the testes with scabby wrinkled skin; listlessness; excessive salvation; inflammation of the nose and mouth with submucosa hemorrhages; rough hair coat; stiffness of the joints with swelling of the feet in front of the fetlocks; breaks in the skin around the hooves, which later developed into deep fissures; thickening and cracking of the skin around the nose, mouth, ears, and neck, alopecia, and bowing of the hind legs (Miller and Miller, 1962; Mills et al., 1967; Ott et al., 1965). Symptoms in adult dairy cattle did not appear to be as severe as those observed in calves. Schwarz and Kirchgessner (1975) fed mature cows a semi-purified diet that contained 6 mg/kg Zn and observed development of small eczemas on the skin from the hind limb just above the dew claws the second week of treatment. Prolonged Zn deficiency caused scabby dry scales covering the entire hind limb, including skin in between the hind claws. Zinc deficiency symptoms progressively worsened by development of deep fissures similar to those observed on calves. The skin lesions eventually spread to the udder and tail head and caused the animal severe discomfort; however, no limb deformities or lesions on the head, neck, and ears were observed (Schwarz and Kirchgessner, 1975). Similar symptoms were observed for adult cattle and calves; however, skin lesions on the head and neck, and skeletal deformities occurring in calves suggests Zn deficiency is more severe in young, growing animals. Zinc deficiency symptoms were corrected within a

few days after Zn repletion with almost complete recovery occurring after 5 weeks. To prevent deficiency, the National Research Council (2000) recommends beef cattle receive 30 mg Zn/kg diet, which should meet Zn requirements in most instances.

TOXIC EFFECTS

Cattle are able to tolerate large quantities of dietary Zn; however, toxic effects of Zn have been observed. Ott et al. (1966a) performed a study to determine the threshold for zinc toxicity in beef cattle by feeding diets consisting of a basal ration containing 100 mg/kg Zn and diets that supplemented 1,000, 2,000, or 3,000 mg Zn/kg diet. The authors observed a decrease in feed intake in calves fed 3,000 mg Zn/kg diet, and a linear decrease in ADG with increasing Zn suggesting 1000 mg Zn/kg diet was toxic. The authors noted no external symptoms of toxicity were observed and animals were not affected permanently by feeding high levels of Zn. Ott et al. (1966a) suggested high dietary Zn may cause metabolic alterations which can affect feed intake and utilization. In subsequent experiments, Ott et al. (1966a) determined supplementing cattle with diets containing greater than 900 mg/kg Zn caused decreased ADG and intake, but diets containing less than 500 mg Zn/kg diet did not affect performance. Mature dairy cows were fed diets containing 0, 1,000, or 2,000 mg Zn/kg diet. Cows receiving 2,000 mg Zn/kg diet demonstrated reduced feed intake and cows supplemented with 2,000 mg/kg longer than 20 weeks exhibited lower body weights compared to cows receiving 0 or 1,000 mg/kg Zn (Miller et al., 1989).

EFFECTS OF ZINC ON METABOLISM OF OTHER NUTRIENTS

High levels of Zn have been observed to cause borderline deficiencies with other minerals such as Cu and Fe (McDowell, 1992). A trial conducted by Ott et al. (1966b) utilized feeder calves that were fed diets containing 0, 0.5, 0.9, 1.3, 1.7, or 2.1 g Zn/kg of diet

supplementation for 12 weeks followed by tissue collection from a subset of animals. The authors observed high levels of Zn (> 0.9 g Zn/kg of diet) decreased liver copper and increased liver iron concentration, thus hemoglobin and packed cell volume decreased in plasma with increasing Zn content of the diet. Furthermore, liver Ca increased linearly with increasing Zn, a quadratic effect was observed for liver Mg, and linear and quadratic effects for liver Na and P concentration were observed. Confirming these results, Ott et al. (1966c) observed a linear decrease in liver Cu concentration with levels of Zn increasing 0, 0.5, 1.0, 2.0, and 4.0 g/kg Zn and liver Fe increased linearly with increasing Zn supplementation. Ivan and Grieve (1975) observed 100 mg Zn/kg diet decreased liver Cu concentration in Holstein calves. Primarily due to the negative effects of high Zn on absorption of Cu, the maximum dietary Zn concentration recommended is 500 mg/kg (NRC, 2001); however, amounts as low as 100 mg/kg can cause Cu deficiency with reduced Cu intake.

EFFECTS OF ZINC ON RUMINAL FERMENTATION AND DIGESTIBILITY

Zinc has a fundamental role in gene expression, cell development and replication, and is present in many metalloenzymes in microorganisms (Eryavuz and Dehority, 2009). Similar to mammals, it serves as part of the subunit of metalloenzymes such as DNA and RNA polymerase, alkaline phosphatase, and other various microbial enzymes (Durand and Kawashima, 1980). In addition, Zn is an important component for the stabilization of cellular structure (Dedyukhina and Eroshin, 1991). Kennedy et al. (1993) observed an increase in ruminal microbial DM in ruminal fluid from cannulated steers supplemented with 712 mg/d polysaccharide-Zn complex or 688 mg/d Zn oxide compared to ruminal fluid from non-supplemented steers, which indicated the necessity of Zn supplementation to maintain ruminal microbial DM production.

Hubbert et al. (1958) observed decreased cellulose digestion when 5 µg/mL Zn was added to fermentation liquid utilizing a washed suspension of ruminal microorganisms from steers. Martinez and Church (1970) conducted an *in vitro* trial using washed ruminal microbes from steers, and observed cellulose digestion increased with addition of 2 and 10 mg/kg Zn, but when Zn concentrations were increased to 20 and 30 mg/kg, cellulose digestion decreased 31 and 56%, respectively. To determine the changes occurring in the ruminal microbial system with Zn supplementation, Eryavuz and Dehority (2009) conducted an in vitro trial using cellulose medium and rumen inoculum from sheep. Zinc was added at rates of 5, 10, 15, 20, 25, and 50 µg Zn/mL. The authors observed decreased cellulose digestion when Zn was added at 50 µg/mL, but no difference in digestion from controls tubes and remaining Zn treatments. Interestingly, when cellulolytic and total bacterial concentrations were analyzed, control tubes contained lower concentrations of cellulolytic bacteria, and there was no difference in total bacterial counts between control and tubes with 50 µg Zn/mL. These results indicate Zn supplementation may not affect cellulose digestion by altering the bacterial population, but instead may inactivate cellulase enzymes or affect bacterial adhesion to cellulose.

Variable results have been previously reported regarding the effect of Zn supplementation on VFA and ruminal protein metabolism, and IVDMD. No information has been published regarding its effects on ruminal fermentation in feedlot cattle. Arelovich et al. (2000) incubated prairie hay for 24 h with 0, 5, 10, 15, or 20 mg/kg Zn and observed a linear decrease in IVDMD and decreased urea degradation with increasing Zn concentration. In an *in vivo* experiment, Arelovich et al. (2000) dosed Zn chloride intraruminally through ruminal cannulas at rates of 30, 250 or 470 mg Zn/kg diet. The authors observed a linear and quadratic increase in propionate production with increasing Zn concentration thus decreased A:P ratio, and

ruminal ammonia-N decreased linearly 2 h after dosing, but no further differences were observed at any other time point. Conversely, Arelovich et al. (2008) collected ruminal fluid from steers fed 0 or 430 mg Zn/kg diet DM (as Zn chloride) and analyzed VFA profiles. The authors observed no effect of Zn supplementation on VFA production or ruminal ammonia-N. Spears et al. (2004) observed decreased total VFA production with addition of 20 mg Zn/kg DM from Zn methionine and Zn glycine, but no effect with addition of Zn sulfate. Furthermore, propionate increased, and A:P ratio and butyrate decreased with addition of Zn methionine compared to control cattle, but no differences were observed with addition of Zn sulfate or Zn glycine. When valerate was analyzed, the concentration was reduced with addition of Zn methionine, Zn sulfate, and Zn glycine compared to control steers (Spears et al., 2004). Froetschel et al. (1990) conducted a trial assessing the effects of feeding 88 or 1,230 mg Zn/kg DM (as Zn sulfate) to ruminally and abomasally cannulated steers. The authors observed when feeding 1,230 mg Zn/Kg DM both dietary AA fermentation and synthesis of bacterial AA decreased, suggesting high dietary concentration of Zn may alter ruminal protein metabolism.

Digestibility responses to Zn supplementation have been variable. Daghash and Mousa (1999) observed increased OM, CP, and nitrogen-free extract apparent digestibility with addition of 50 or 100 mg Zn/kg diet as Zn sulfate to heat stressed buffalo calves, and increased crude fiber digestibility when 100 mg/kg Zn was supplemented to calves. Total tract DM digestibility was not affected by addition of 430 mg Zn/kg DM (from Zn chloride) to steers fed approximately 39% roughage and 61% concentrate (Arelovich et al., 2008). Furthermore, no differences we observed in DM digestibility when heifers were allowed free access to prairie hay and supplemented 30, 250, or 470 mg/kg Zn; however, as Zn concentration increased (0, 5, 10, 15, 20 mg/kg Zn) in the *in vitro* portion of the experiment, IVDMD decreased linearly

(Arelovich et al., 2000). The authors attributed the reduction in IVDMD to reduced cellulose digestibility with increased Zn concentration. Wang et al. (2013) assessed the effects of Zn source (Zn amino acid chelate, Zn- proteinate chelate with moderate and strong chelation strength, and Zn sulfate), and level (0, 10, or 20 µg/mL) on *in vitro* rumen fermentation that contained roughage based substrates and observed a source × level interaction for DM degradability (DMD). Supplementing Zn from 0 to 10 µg/mL did not increase DMD for the Zn sulfate treatment but increased for all organic Zn sources; however, as supplemental Zn increased from 10 to 20 µg/mL, DMD decreased for all sources indicating 20 µg/mL was excessive for the ruminal microbes.

These results suggest Zn is necessary for fermentation by ruminal microbes, but too much can decrease fermentation and digestibility. In addition, factors such as the type of diet, feed processing, Zn source, and supplying method (directly into the rumen or in feed) and *in vivo* vs. *in vitro* results can affect the microbial response to Zn supplementation (Arelovich et al., 2008), but it appears increased Zn supplementation can alter ruminal fermentation and digestion.

EFFECTS OF ZINC ON FINISHING CATTLE PERFORMANCE

Effects of Zn supplementation to finishing cattle are limited and have led to inconclusive results as to the amount of Zn required by finishing cattle to maintain adequate growth. Greene et al. (1988) concluded supplementing 360 mg/d Zn from zinc oxide or Zn methionine had no impact on DMI compared to steers receiving a basal ration containing 82 mg/kg Zn (control); whereas, ADG increased day 0 to 28 for steers supplemented zinc oxide compared to control or Zn methionine supplemented steers, but by day 85 there were no differences among treatments for ADG. In addition, fattening bulls supplemented 10 mg/kg Zn from Zn oxide, Zn proteinate, or Zn polysaccharide complex had no effect on DMI, ADG, or feed efficiency when compared to

control animals (Kessler et al., 2003). Malcolm-Callis et al. (2000) fed 20, 100 or 200 mg/kg Zn from Zn sulfate to finishing steers and observed no change in ADG, feed efficiency, or final BW during a 112-day finishing trial, but the authors did observed a linear decrease in DMI as Zn supplementation increased from 20, 100, and 200 mg/kg day 28 to 56 and DMI tended to decrease linearly day 0 to 112 with increasing Zn concentration in the diet. Spears and Kegley (2002) observed no effect for final BW with 25 mg/kg Zn supplementation from Zn oxide or Zn proteinate. No differences were detected for final BW, ADG, DMI, or feed efficiency for heifers supplemented with 75 mg/kg Zn from Zn sulfate, Zn methionine, or Zn propionate compared to control heifers receiving 50.5 mg/kg Zn in the basal ration (Nunnery et al., 2007). Multiple differences exist in previous literature including differences in Zn concentration of basal rations, differences in initial trace mineral status of study animals, and sex, which may contribute to the differences observed among research.

Effects of Zn supplementation on carcass traits have been variable. Huerta et al. (2002), Greene et al. (1988), and Nunnery et al. (2007) observed no differences in carcass traits from steers supplemented 200 mg/kg Zn (from Zn sulfate or Zn methionine), 360 mg/d Zn (from Zn oxide), or 75 mg/kg Zn (from Zn sulfate, Zn methionine, or Zn propionate), respectively. Conversely, Malcolm-Callis et al. (2000) observed a quadratic effect for 12^{th} rib s. c. fat thickness and yield grade for cattle supplemented with 20, 100, or 200 mg/kg Zn as Zn sulfate. Spears and Kegley (2002) observed an increase in marbling score and quality grade and a tendency for increased yield grade and 12^{th} rib s. c. fat thickness when steers were supplemented with 25 mg/kg Zn regardless of source compared to non-supplemented steers. The authors suggested the Zn concentration in the basal ration was slightly below NRC (2000) recommendations, which may have resulted in a greater response in carcass traits compared to

studies in which the basal ration contains adequate Zn. In addition, differences may be attributed to the animals' initial Zn status prior to starting experiments, which can potentially impact responses to Zn supplementation. Zinc is a diverse trace mineral that participates directly or indirectly in numerous enzymes within mammals, therefore making it difficult to determine the lack of congruency among research. In an in vitro trial, Oh and Choi (2004) reported an increase in lipogenic activity in bovine intramuscular adipocytes with increasing Zn concentration. Huang et al. (2012) observed the zinc finger protein Zfp423 in bovine stromal cells, which acts as a key initiator of adipogenic differentiation and may be partially responsible for increasing carcass traits related to adipose tissue.

BIOAVAILABILITY

The forms of Zn commonly fed to ruminants in the inorganic form consist of Zn oxide or Zn sulfate (Underwood and Suttle, 1999). Organic sources of Zn approved by the FDA for use in ruminants include: 1) Zn lysine complex; 2) Zn methionine complex; 3) Zn amino acid chelate; 4) Zn polysaccharide complex; and 5) Zn proteinate (AAFCO, 2014). Research regarding use of organic Zn supplementation has been inconsistent making it difficult to extrapolate whether organic sources of Zn are more beneficial for feedlot performance than inorganic. Cao et al. (2000) conducted trials to correlate laboratory results with relative bioavailability estimates of Zn using tissue Zn content following high dietary additions of Zn in chicks and lambs. Lambs received Zn sulfate, Zn proteinate, Zn amino acid chelate, and Zn methionine B with bioavailability estimates of 100, 130, 110, and 113%, respectively. Zinc proteinate was the only organic form of Zn observed to have bioavailability values greater than Zn sulfate. Bioavailability of organic sources were observed to be inversely related to solubility of Zn in pH

2 buffer solution in lambs ($r^2 = 0.91$); however, this was not related to degree of chelation (Cao et al., 2000).

Spears et al. (2004) conducted an experiment using steers to assess the effects of feeding organic (Zn glycine or methionine) or inorganic (Zn sulfate) Zn sources on Zn metabolism and ruminal VFA concentrations. The authors observed no effect of treatment on apparent absorption or retention of Zn; however, VFA concentrations were affected by Zn source. Total VFA concentrations were higher for steers fed no supplemental Zn and Zn sulfate compared to steers supplemented with Zn glycine and Zn methionine, but steers supplemented with Zn methionine had higher concentrations of propionate and lower concentrations of butyrate and valerate. Multiple studies comparing the effects of inorganic and organic sources of Zn have been conducted in finishing cattle and have observed no differences between Zn source for animal performance or carcass traits (Malcolm-Callis et al., 2000; Kessler et al., 2003; Wagner et al., 2008). Spears (1989) compared the bioavailability and metabolism of Zn from Zn oxide and Zn methionine in lambs as well as the effects on growth and performance of growing heifers. The author observed no differences in apparent absorption of Zn in lambs or any growth effects between the 2 sources of Zn in lambs and heifers; however, the results suggested Zn oxide and Zn methionine may be metabolized differently after absorption due to numerically lower rates of Zn excretion in urine of Zn methionine treated lambs and the slower rate of decline in plasma Zn after a single oral dose of Zn methionine in lambs compared to an oral dose of Zn oxide.

A few studies have revealed positive results with organic Zn supplementation compared to inorganic for finishing cattle. Steers supplemented with no Zn (control) or 360 mg/d Zn as either Zn oxide or Zn methionine exhibited improved marbling scores and a tendency for improved quality grades with Zn methionine supplementation compared to control and Zn oxide

supplemented steers (Greene et al., 1988). In the trial conducted by Spears and Kegley (2002), steers were supplemented with no supplemental Zn (control), Zn oxide, and two different forms of Zn proteinate (differing by the amount of Zn contained in the complex). The authors observed steers supplemented with either Zn proteinate treatment had increased HCW and dressed percent compared to control or Zn oxide supplemented cattle. These results may suggest an advantage to supplementing Zn in the form of organic complexes may exist, but the lack of consistent results concerning animal response to organic versus inorganic forms of Zn make it difficult to determine the benefit of supplementing finishing cattle with organic Zn sources when inorganic sources of Zn provide the necessary requirements to maintain optimal performance and health.

CONCLUSION

Zinc is an essential mineral for growth and development of mammals and for optimal activity of ruminal microorganisms. It is also required for normal development and function of the immune system and metabolism of carbohydrates, lipids, proteins, and nucleic acids. When fed in excess, Zn can alter ruminal fermentation and depress intake and ADG. Caution must be taken when formulating rations with increased concentrations of Zn primarily because of the negative consequences increased Zn supplementation has on Cu absorption.

REFERENCES

Abu-Hamdan, D. K., S. D. Migdal, R. Whitehouse, P. Rabbani, A. S. Prasad, and F. D. McDonald. 1981. Renal handling of zinc: effect of cysteine infusion. Am. J. Physiol. 241:F487-F494.

AAFCO. 2014. The Association of American Feed Control Officials.

Agricultural Research Council. 1980. The nutrient requirements of ruminant livestock. Slough, England: Commonwealth Agricultural Bureaux.

Arelovich, H. M., F. N. Owens, G. W. Horn, and J. A. Vizcarra. 2000. Effects of supplemental zinc and manganese on ruminal fermentation, forage intake, and digestion by cattle fed prairie hay and urea. J. Anim. Sci. 78:2972-2979. doi:/2000.78112972x

Arelovich, H. M., H. E. Laborde, M. I. Amela, M. B. Torrea, and M. F. Martínez. 2008. Effects of dietary addition of zinc and (or) monensin on performance, rumen fermentation and digesta kinetics in beef cattle. Span. J. Agric. Res. 6:362-372. doi:10.5424/sjar/2008063-329

Arora, S. P., E. E. Hatfield, U. S. Garrigus, T. G. Lohman, and B. B. Doane. 1969. Zinc-65 uptake by rumen tissue. J. Nutr. 97:25-28.

Asling, C. W., and L. S. Hurley. 1963. The influence of trace elements on the skeleton. Clin. Orthop. Relat. Res. 27:213-264.

Berg, J. M., and Y. Shi. 1996. The galvanization of biology: a growing appreciation for the roles of zinc. Science. 271:1081-1085.

Blincoe, C., and V. R. Bohman. 1966. Bone zinc concentration in range cattle. J. Agric. Food Chem. 14:645-646.

Cao, J., P. R., Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039-2054.

Chirase, N. K., D. P. Hutcheson, and G. B. Thompson. 1991. Feed intake, rectal temperature, and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci. 69:4137-4145.

Choi, S., and A. J. Bird. 2014. Zinc'ing sensibly: controlling zinc homeostasis at the transcriptional level. Metallomics. 6:1198-1215.

Cousins, R. J., and A. S. Leinart. 1988. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J. 2:2884-2890.

Cousins, R. J., J. P. Liuzzi, and L. A. Lichten. 2006. Mammalian zinc transport, trafficking, and signals. J. Biol. Chem. 281:24085-24089.

Csermely, P., M. Szamel, K. Resch, and J. Somogyi. 1988. Zinc can increase the activity of protein kinase C and contributes to its binding to plasma membranes in T lymphocytes. J. Biol. Chem. 263:6487-6490.

Daghash, H. A. and S. M. Mousa. 1999. Zinc sulfate supplementation to ruminant rations and its effects on digestibility in lamb; growth, rectal temperature and some blood constituents in buffalo calves under heat stress. Assiut Vet. Med. J. 40:128-146.

Dardenne M, and J-M Pleau. 1994. Interactions between zinc and thymulin. Met. Based Drugs 1:233-239.

Dedyukhina, E. G., and V. K. Eroshin. 1991. Essential metal ions in the control of microbial metabolism. Process Biochem. 26:31-37.

Devergnas, S., F. Chimienti, N. Naud, A. Pennequin, Y. Coquerel, J. Chantegrel, A. Favier, and M. Seve. 2004. Differential regulation of zinc efflux transporters ZnT-1, ZnT-5 and ZnT-7 gene expression by zinc levels: a real-time RT–PCR study. Biochem. Pharmacol. 68:699-709.

Durand, M., and R. Kawashima. 1980. Influence of minerals in rumen microbial digestion. In Digestive physiology and metabolism in ruminants Springer Netherlands. p. 375-408.

Eide, D. J. 2006. Zinc transporters and the cellular trafficking of zinc. Biochim. Biophys. Acta 1763:711-722.

Engle, T. E., C. F. Nockels, K. L. Hossner, C. V. Kimberling, R. E. Toombs, R. S. Yemm, D. L. Weaber, and A. B. Johnson. 1997. Marginal zinc deficiency affects biochemical and physiological parameters in beef heifer calves. Asian-australas. J. Anim. Sci. 10:471-477.

Eryavuz, A., and B. A. Dehority. 2009. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers in vitro. Anim. Feed Sci. Technol. 151:175–183. doi:10.1016/j.anifeedsci.2009.01.008

Feaster, J. P., S. L. Hansard, J. T. McCall, F. H. Skipper, and G. K. Davis. 1954. Absorption and tissue distribution of radiozinc in steers fed high-zinc rations. J. Anim. Sci. 13:781-788.

Froetschel, M. A., A. C. Martin, H. E. Amos, and J. J. Evans. 1990. Effects of zinc sulfate concentration and feeding frequency on ruminal protozoal numbers, fermentation patterns and amino acid passage in steers. J. Anim. Sci. 68:2874-2884. doi:/1990.6892874x

Galyean, M. L., L. J. Perino, and G. C. Duff. 1999. Interaction of cattle health/immunity and nutrition. J. Anim. Sci. 77:1120-1134.

Gaither, L. A., and D. J. Eide. 2000. Functional expression of the human hZIP2 zinc transporter. J. Biol. Chem. 27:5560-5564.

Gaither, L. A., and D. J. Eide. 2001. The human ZIP1 transporter mediates zinc uptake in human K562 erythroleukemia cells. J. Biol. Chem. 276:22258-22264.

Godson, D. L., M. E. Baca-Estrada, A. G. Van Kessel, H. P. Hughes, M. A. Morsy, J. Van Donkersgoed, R. J. Harland, D. E. Shuster, M. J. Daley, and L. A. Babiuk. 1995. Regulation of bovine acute phase responses by recombinant interleukin-1 beta. Can. J. Vet. Res. 59:249-255.

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schelling, 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J. Anim. Sci. 66:1818-1823.

Guffanti, A. A., Y. Wei, S. V. Rood, and T. A. Krulwich. 2002. An antiport mechanism for a member of the cation diffusion facilitator family: divalent cations efflux in exchange for K^+ and H^+. Mol. Microbiol. 45:145-153.

Guerinot, M. L. 2000. The ZIP family of metal transporters. Biochim. Biophys. Acta 1465:190-198.

Hambidge, K. M., C. E. Casey, and N. F. Krebs. 1986. Zinc. In: W. Mertz, editor, Trace minerals in human and animal nutrition vol. 2. Academic Press, Orlando, FL. p. 1-137.

Hansard, S. L., A. S. Mohammed, and J. W. Turner. 1968. Gestation age effects upon maternal-fetal zinc utilization in the bovine. J. Anim. Sci. 27:1097-1102.

Hidiroglou, M. 1980. Zinc, copper and manganese deficiencies and the ruminant skeleton: A review. Can. J. Anim. Sci. 60:579-590.

Hiers Jr, J. M., W. J. Miller, and D. M. Blackmon. 1968. Endogenous secretion and reabsorption of ^{65}zinc in ruminants as affected by zinc deficiency and feeding of ethylenediaminetetraacetate or cadmium. J. Dairy Sci. 51:730-736.

Hough, E., L. K. Hansen, B. Birknes, K. Jynge, S. Hansen, A. Hordvik, C. Little, E. Dodson, and Z. Derewenda. 1989. High-resolution (1.5 Å) crystal structure of phospholipase C from Bacillus cereus. Nature. 338:357-360.

Huang, L., C. P. Kirschke, Y. Zhang, and Y. Y. Yu. 2005. The ZIP7 gene (Slc39a7) encodes a zinc transporter involved in zinc homeostasis of the Golgi apparatus. J. Biol. Chem. 280:15456-15463.

Huang, Y., A. K. Das, Q.-Y. Yang, M.-J. Zhu, and M. Du. 2012. Zfp423 Promotes adipogenic differentiation of bovine stromal vascular cells. PLoS ONE, 7:e47496-e47496. doi:10.1371/journal.pone.0047496

Hubbert, F., E. Cheng, and W. Burroughs. 1958. Mineral requirement of rumen microorganisms for cellulose digestion. J. Anim. Sci. 17:559-568. doi:10.2134/jas1958.173559x

Huerta, M., R. L. Kincaid, J. D. Cronrath, J. Busboom, A. B. Johnson, and C. K. Swenson. 2002. Interaction of dietary zinc and growth implants on weight gain, carcass traits and zinc in tissues of growing beef steers and heifers. Anim. Feed Sci. Technol. 95:15-32. doi: 10.1016/s0377-8401(01)00334-0

Ivan, M., and C. M. Grieve. 1975. Effects of zinc, copper, and manganese supplementation of high-concentrate ration on digestibility, growth, and tissue content of Holstein calves. J. Dairy Sci. 58:410-415.

Jeong, J., and D. J. Eide. 2013. The SLC39 family of zinc transporters. Mol. Aspects Med. 34:612-619.

Kägi, J. H. R., and A. Schäffer. 1988. Biochemistry of metallothionein. Biochemistry 27:8509-8515.

Kägi, J. H. R., and B. L. Vallee. 1961. Metallothionein: A cadmium and zinc-containing protein from equine renal cortex II. Physicochemical properties. J. Biol. Chem. 236:2435-2442.

Kaji, T., Y. Fujiwara, S. Sakurai, C. Yamaoto, H. Kozuka, and F. Koizumi. 1995. Zinc promotes the repair of wounded monolayers of cultured vascular endothelial cells. Res. Commun. Mol. Pathol. Pharmacol. 89:189-198.

Kambe, T., H. Narita, Y. Yamaguchi-Iwai, J. Hirose, T. Amano, N. Sugiura, R. Sasaki, K. Mori, T. Iwanaga, and M. Nagao. 2002. Cloning and characterization of a novel mammalian zinc transporter, zinc transporter 5, abundantly expressed in pancreatic β cells. J. Biol. Chem. 277:19049-19055.

Keen, C. L., and M. E. Gershwin. 1990. Zinc deficiency and immune function. Annu. Rev. Nutr. 10:415-431.

Kennedy, D. W., W. M., Craig, and L. L. Southern. 1993. Ruminal distribution of zinc in steers fed a polysaccharide-zinc complex or zinc oxide. J. Anim. Sci. 71:1281-1287.

Kessler, J., I. Morel, P.-A. Dufey, A. Gutzwiller, A. Stern, and H. Geyer. 2003. Effect of organic zinc sources on performance, zinc status and carcass, meat and claw quality in fattening bulls. Livest. Prod. Sci. 81:161–171. doi:10.1016/s0301-6226(02)00262-2

Kincaid, R. L., W. J. Miller, P. R. Fowler, R. P. Gentry, D. L. Hampton, and M. W. Neathery. 1976. Effect of high dietary zinc upon zinc metabolism and intracellular distribution in cows and calves. J. Dairy Sci. 59:1580-1584.

Kim, E. E., and H. W. Wyckoff. 1989. Structure of alkaline phosphatases. Clinica chimica acta 186:175-187.

Kirschke, C. P., and L. Huang. 2003. ZnT7, a novel mammalian zinc transporter, accumulates zinc in the Golgi apparatus. J. Biol. Chem. 278:4096-4102.

Legg, S. P., and L. Spears. 1960. Zinc sulfate treatment of parakeratosis in cattle. Nature. 186:1061-1062.

Leheska, J. M., L. D. Thompson, J. C. Howe, E. Hentges, J. Boyce, J. C. Brooks, B. Shriver, L. Hoover, and M. F. Miller. 2008. Effects of conventional and grass-feeding systems on the nutrient composition of beef. J. Anim. Sci. 86:3575-3585.

Liu, Z., H. Li, M. Soleimani, K. Girijashanker, J. M. Reed, L. He, T. P. Dalton, and D. W. Nebert. 2008. Cd 2+ versus Zn 2+ uptake by the ZIP8-dependent symporter: kinetics, electrogenicity and trafficking. Biochem. Biophys. Res. Commun. 365:814-820.

Liuzzi, J. P., L. A. Lichten, S. Rivera, R. K. Blanchard, T. B. Aydemir, M. D. Knutson, T. Ganz, and R. J. Cousins. 2005. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proc. Natl. Acad. Sci. USA. 102:6843-6848.

Liuzzi, J. P., and R. J. Cousins. 2004. Mammalian zinc transporters. Annu. Rev. Nutr. 24:151-172.

Liuzzi, J. P., R. K. Blanchard, and R. J. Cousins. 2001. Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats. J. Nutr. 131:46-52.

Malcolm-Callis, K. J., G. C. Duff, S. A. Gunter, E. B. Kegley, and D.A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. J. Anim. Sci. 78:2801-2808. doi:/2000.78112801x

Martinez, A., and D. C. Church. 1970. Effect of Various Mineral Elements on Rumen Cellulose Digestion. J. Anim. Sci. 31:982-990. doi:10.2134/jas1970.315982x

Mayland, H. F., R. C. Rosenau, and A. R. Florence. 1980. Grazing cow and calf responses to zinc supplementation. J. Anim. Sci. 51:966-974.

McDowell L. R. 1992. Zinc. In: Minerals in human and animal nutrition. Academic Press, San Diego, CA. p. 265-293.

Miller, J. K., and W. J. Miller. 1962. Experimental zinc deficiency and recovery of calves. J. Nutr. 76:467-474.

Miller, W. J., J. D. Morton, W. J. Pitts, and C. M. Clifton. 1965. Effect of zinc deficiency and restricted feeding on wound healing in the bovine. Proc. Soc. Exp. Biol. Med. 118:427-430.

Miller, J. K., and R. G. Cragle. 1965. Gastrointestinal sites of absorption and endogenous secretion of zinc in dairy cattle. J. Dairy Sci. 48:370-373.

Miller, W J., D. M. Blackmon, R. P. Gentry, G. W. Powell, and H. F. Perkins. 1966a. Influence of zinc deficiency on zinc and dry matter content of ruminant tissues and on excretion of zinc. J. Dairy Sci. 49:1446-1453.

Miller, W. J., D. M. Blackmon, G. W. Powell, R. P. Gentry, and J. M. Hiers. 1966b. Effects of zinc deficiency per se and of dietary zinc level on urinary and endogenous fecal excretion of 65Zn from a single intravenous dose by ruminants. J. Nutr. 90:335-341.

Miller, W. J., D. M. Blackmon, R. P. Gentry, W. J. Pitts, and G. W. Powell. 1967a. Absorption, excretion, and retention of orally administered zinc-65 in various tissues of zinc-deficient and normal goats and calves. J. Nutr. 92:71-78.

Miller, W. J., D. M. Blackmon, J. M. Hiers Jr, P. R. Fowler, C. M. Clifton, and R. P. Gentry. 1967b. Effects of adding two forms of supplemental zinc to a practical diet on skin regeneration in Holstein heifers and evaluation of a procedure for determining rate of wound healing. J. Dairy Sci. 50:715-721.

Miller, W. J., Y. G. Martin, R. P. Gentry, and D. M. Blackmon. 1968a. ^{65}Zn and stable zinc absorption, excretion and tissue concentrations as affected by type of diet and level of zinc in normal calves. J. Nutr. 94:391-401.

Miller, W. J., G. W. Powell, D. M. Blackmon, and R. P. Gentry. 1968b. Zinc and dry matter content of tissues and feces of zinc-deficient and normal ruminants fed ethylenediaminetetraacetate and cadmium. J. Dairy Sci. 51:82-89.

Miller, W. J. 1970a. Zinc nutrition of cattle: a review. J. Dairy Sci. 53:1123-1135.

Miller, W. J., D. M. Blackmon, R. P. Gentry, and F. M. Pate. 1970b. Effects of high but nontoxic levels of zinc in practical diets on ^{65}Zn and zinc metabolism in Holstein calves. J. Nutr. 100:893-902.

Miller, W. J., E. S. Wells, R. P. Gentry, and M. W. Neathery. 1971. Endogenous zinc excretion and ^{65}Zn metabolism in Holstein calves fed intermediate to high but nontoxic zinc levels in practical diets. J. Nutr. 101:1673-1681.

Miller, W. J. 1973. Dynamics of absorption rates, endogenous excretion, tissue turnover, and homeostatic control mechanisms of zinc, cadmium, manganese, and nickel in ruminants. Fed. Proc. 32:1915-1973.

Miller, W. J., H. E. Amos, R. P. Gentry, D. M. Blackmon, R. M. Durrance, C. T. Crowe, A. S. Fielding, and M. W. Neathery. 1989. Long-term feeding of high zinc sulfate diets to lactating and gestating dairy cows. J. Dairy Sci. 72:1499-1508.

Mills, C. F., A. C. Dalgarno, R. B. Williams, and J. Quarterman. 1967. Zinc deficiency and the zinc requirements of calves and lambs. Br. J. Nutr. 21:751-68.

Naveh, Y., L. Bentur, and E. Diamond. 1988. Site of zinc absorption in dog small intestine. J. Nutr. 118:61-64.

Neathery, M. W., W. P. Miller, D. M. Blackmon, R. P. Gentry, and J. B. Jones. 1973. Absorption and tissue zinc content in lactating dairy cows as affected by low dietary zinc. J. Anim. Sci. 37:848-852.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Nunnery, G. A., J. T. Vasconcelos, C. H. Parsons, G. B. Salyer, P. J. Defoor, F. R. Valdez, and M. L. Galyean. 2007. Effects of source of supplemental zinc on performance and humoral immunity in beef heifers. J. Anim. Sci. 85:2304-2313.

Oh, Y. S., and C. B. Choi. 2004. Effects of zinc on lipogenesis of bovine intramuscular adipocytes. Asian-Australas. J. Anim. Sci. 17:1378–1382. doi:10.5713/ajas.2004.1378

Ohana, E., D. Segal, R. Palty, D. Ton-That, A. Moran, S. L. Sensi, J. H. Weiss, M. Hershfinkel, and I. Sekler. 2004. A sodium zinc exchange mechanism is mediating extrusion of zinc in mammalian cells. J. Biol. Chem. 279:4278-4284.

Orr, C. L., D. P. Hutcheson, R. B. Grainger, J. M. Cummins, and R. E. Mock. 1990. Serum copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. J. Anim. Sci. 68:2893-2900.

Ott, E. A., W. H. Smith, Martin Stob, H. E. Parker, and W. M. Beeson. 1965. Zinc deficiency syndrome in the young calf. J. Anim. Sci. 24:735-741.

Ott, E. A., W. H. Smith, R. B. Harrington, and W. M. Beeson. 1966a. Zinc toxicity in ruminants. II. Effect of high levels of dietary zinc on gains, feed consumption and feed efficiency of beef cattle. J. Anim. Sci. 25:419-423. doi:10.2134/jas1966.252419x

Ott, E. A., W. H. Smith, R. B. Harrington, H. E. Parker, and W. M. Beeson. 1966b. Zinc toxicity in ruminants. IV. Physiological changes in tissues of beef cattle. J. Anim. Sci. 25:432-438. doi:10.2134/jas1966.252432x

Ott, E. A., W. H. Smith, R. B. Harrington, M. Stob, H. E. Parker, and W. M. Beeson. 1966c. Zinc toxicity in ruminants. III. Physiological changes in tissues and alterations in rumen metabolism in lambs. J. Anim. Sci. 25:424-431. doi:10.2134/jas1966.252424x

Palmiter, R. D., and S. D. Findley. 1995. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. EMBO J. 14:639-649.

Palmiter, R. D., T. B. Cole, C. J. Quaife, and S. D. Findley. 1996. ZnT-3, a putative transporter of zinc into synaptic vesicles. Proc. Natl. Acad. Sci. USA 93:14934-14939.

Prasad A. S. 1993. Biochemistry of zinc. Plenum Press. New York, NY. p. 93-117.

Pulido, P., J. H. R. Kägi, and B. L. Vallee. 1966. Isolation and some properties of human metallothionein. Biochemistry 5:1768-1777.

Radtke, F., R. Heuchel, O. Georgiev, M. Hergersberg, M. Gariglio, Z. Dembic, and W. Schaffner. 1993. Cloned transcription factor MTF-1 activates the mouse metallothionein I promoter. EMBO J. 12:1355-1362.

Raulin J., 1869. Études cliniques sur la vegetation. Ann. Sci. Nat. Bio. Veg. Ser. 11:93.

Reilly, C. 2004 Zinc. In: The nutritional trace elements. Blackwell Publishing Ltd., Oxford, UK. p. 82-117.

Reyes, J. G. 1996. Zinc transport in mammalian cells. Am. J. Physiol. Cell Physiol. 39: C401-C410.

Richards, M. P. 1989. Recent developments in trace element metabolism and function: role of metallothionein in copper and zinc metabolism. J. Nutr. 119:1062-1070.

Schwarz, W. A., and M. Kirchgessner. 1975. Experimental zinc deficiency in lactating dairy cows. Vet. Med. Rev. 1:19-41.

Spears, J. W. 1989. Zinc Methionine for Ruminants: Relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. J. Anim. Sci, 67:835-843.

Spears, J. W., R. W. Harvey, and T. T. Brown Jr. 1991. Effects of zinc methionine and zinc oxide on performance, blood characteristics, and antibody titer response to viral vaccination in stressed feeder calves. J. Am. Vet. Med. Assoc. 199:1731-1733.

Spears, J. W., and E. B. Kegley. 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J. Anim. Sci. 80:2747-2752.

Spears, J. W., P. Schlegel, M. C. Seal, and K. E. Lloyd. 2004. Bioavailability of zinc from zinc sulfate and different organic zinc sources and their effects on ruminal volatile fatty acid proportions. Livest. Prod. Sci. 90:211–217. doi:10.1016/j.livprodsci.2004.05.001

Suttle, N. F., H. L. Davies, and A. C. Field. 1982. A model for zinc metabolism in sheep given a diet of hay. Br. J. Nutr. 47:105-112.

Suzuki, T., K. Ishihara, H. Migaki, W. Matsuura, A. Kohda, K. Okumura, M. Nagao, Y. Yamaguchi-Iwai, and T. Kambe. 2005. Zinc transporters, ZnT5 and ZnT7, are required for the activation of alkaline phosphatases, zinc-requiring enzymes that are glycosylphosphatidylinositol-anchored to the cytoplasmic membrane. J. Biol. Chem. 280:637-643.

Tainer, J. A., E. D. Getzoff, K. M. Beem, J. S. Richardson, and D. C. Richardson. 1982 Determination and analysis of the 2 Å structure of copper, zinc superoxide dismutase. J. Mol. Biol. 160:181-217.

Todd, W. R., C. A. Elvehjem, and E. B. Hart. 1934. Zinc in the nutrition of the rat. Am. J. Physiol. 77:146-156.

Underwood E. J. and N. F. Suttle. 1999 Zinc. In: The mineral nutrition of livestock. 3rd ed. CABI Publishing, New York, NY. p. 477-512.

Vallee, B L., and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. Physiol. Rev. 73:79-118.

Victery, W., J. M. Smith, and A. J. Vander. 1981. Renal tubular handling of zinc in the dog. Am. J. Physiol. 241:F532-F539.

Wagner, J. J., T. E. Engle, J. J. Wagner, J. L. Lacey, and G. Walker. 2008. The effects of zinmet brand liquid zinc methionine on feedlot performance and carcass merit in crossbred yearling steers. Prof. Anim. Sci. 24:420–429. doi:10.15232/s1080-7446(15)30887-1

Wang, F., J. Dufner-Beattie, B. Kim, M. J. Petris, G. Andrews, and D. J. Eide. 2004. Zinc-stimulated endocytosis controls activity of the mouse ZIP1 and ZIP3 zinc uptake transporters. J. Biol. Chem. 279:24631-24639.

Wang, R. L., J. G. Liang, L. Lu, L. Y. Zhang, S. F. Li, and X. G. Luo. 2013. Effect of zinc source on performance, zinc status, immune response, and rumen fermentation of lactating cows. Biol. Trace Elem. Res. 152:16–24. doi:10.1007/s12011-012-9585-4

Whanger, P. D., S. Oh, and J. T. Deagen. 1981a. Ovine and bovine metallothioneins: purification, number of species, zinc content and amino acid composition. J. Nutr. 111:1207-1215.

Whanger, P. D., S. Oh, and J. T. Deagen. 1981b. Ovine and bovine metallothioneins: accumulation and depletion of zinc in various tissues. J. Nutr. 111:1196-1206.

Wright, C. L., and J. W. Spears. 2004. Effect of zinc source and dietary level on zinc metabolism in Holstein calves. J. Dairy Sci. 87:1085-1091.

Chapter 2 - Interaction between supplemental zinc oxide and zilpaterol hydrochloride on growth performance, carcass traits, and blood metabolites in feedlot steers

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ABSTRACT

Interactive effects of supplemental Zn and zilpaterol-hydrochloride (**ZH**) were evaluated in feedlot steers (n=40; initial BW 652 kg ± 14) to determine impact on feedlot performance, blood constituents, and carcass traits. The study was conducted as a randomized complete block design with a 2 × 2 factorial treatment arrangement. Steers were blocked by BW and randomly assigned to treatments. Factors consisted of supplemental Zn (60 or 300 mg/kg diet DM), and ZH (0 or 8.33 mg/kg) in the diets. For diets supplementing 300 mg Zn/kg DM, 60 mg/kg Zn was supplemented as Zn sulfate and 240 mg/kg Zn as Zn oxide. Zilpaterol hydrochloride was fed for 21 d followed by a 3-d withdrawal. Cattle were housed in partially-covered individual feeding pens equipped with automatic waterers, fence-line feed bunks and were fed once daily for *ad libitum* intake. Plasma samples were collected d 0 and 21 to assess changes in zinc, urea nitrogen (PUN), glucose, and lactate concentrations, and serum samples were collected d 21 to assess IGF-1 concentration. On d 25 cattle were weighed, transported 450 km to a commercial abattoir for harvest, and HCW and incidence of liver abscesses were recorded. Carcass data were collected after 36 h of refrigeration. Data were analyzed as a mixed model with Zn, ZH, and Zn × ZH as fixed effects, block as a random effect, and steer as the experimental unit. No interaction or effects of Zn or ZH were observed for IGF-1 concentration, or plasma glucose and lactate concentrations ($P \geq 0.25$). No interaction between Zn and ZH was observed for PUN concentration, but PUN decreased with ZH ($P < 0.01$). There were no effects of ZH or Zn on ADG, DMI, final BW, feed efficiency, HCW, back fat, KPH, quality grade, or incidence of liver abscesses ($P > 0.05$), and tended ($P = 0.08$) to improve proportion of carcasses grading USDA Choice. Feeding ZH decreased yield grade ($P = 0.05$) and tended to increase LM area ($P = 0.07$).

In conclusion, increasing dietary concentrations of Zn does not impact response to ZH, but feeding ZH altered circulating concentrations of plasma urea nitrogen.

Key words; IGF-1, plasma urea nitrogen, steer, zilpaterol hydrochloride, zinc oxide

INTRODUCTION

Synthetic β-adrenergic agonists (**β-AA**) commonly are fed to cattle during the final phase of finishing enhance to skeletal muscle mass (for review see Mersmann, 1998), while simultaneously reducing adipose tissue deposition (Mersmann, 2002). Zilpaterol hydrochloride (**ZH**) is a β_2-AA fed finishing cattle the last 20 to 40 days prior to slaughter. Common effects of ZH supplementation on feedlot performance include improved ADG and feed efficiency (Montgomery et al., 2009; Scramlin et al., 2010). In the meta-analysis conducted by Lean et al. (2014), ZH supplementation increased HCW by 15 kg, LM area by 8 cm^2, and dressing percentage by 1.7%, whereas it decreased 12^{th} rib s. c. fat thickness by 0.11 cm. Plasma components related to nitrogen metabolism (plasma urea nitrogen) have either decreased (Parr et al., 2014) or remained unchanged (Cônsolo et al., 2015) in response to ZH; whereas, plasma constituents related carbohydrate metabolism (i.e. glucose and lactate) were unaffected by ZH supplementation (Van Bibber-Krueger et al., 2015).

Zinc is an essential component of over 300 enzymes and over 2,000 transcription factors in microorganisms, plants, and animals (Vallee and Falchuk, 1993; Jeong and Eide, 2013), some of which are involved in protein synthesis (Wu and Wu, 1987). Paulk et al. (2015) observed Zn (from Zn oxide) supplementation tended to increase G:F and loin weight when fed in conjunction with ractopamine hydrochloride. It is possible feeding increased concentrations of supplemental Zn in combination with ZH could further enhance skeletal muscle accretion and further alter blood metabolites associated with nitrogen, carbohydrate, and lipid metabolism in cattle. The

objectives of this study were to assess changes in plasma zinc and other blood constituents, and to evaluate effects on growth and carcass characteristics of finishing steers supplemented with or without ZH and 60 or 300 mg/kg diet DM supplemental Zn.

MATERIALS AND METHODS

Protocols and procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State University Beef Cattle Research Center in Manhattan, KS.

Animal Processing and Handling

Upon arrival at the Kansas State University Beef Cattle Research Center, steers were allowed *ad libitum* access to ground brome hay. Approximately 24 h after arrival, steers were assigned a numbered ear tag unique to each animal and individual BW were recorded. Steers received a topical parasiticide (Dectomax pour-on, Zoetis, Florham Park, NJ), a 5-way viral vaccine (Bovi-Shield Gold 5, Zoetis, Florham Park, NJ), and a 7-way clostridial vaccine (Ultrabac 7 Somnubac, Zoetis, Florham Park, NJ). Sixteen days after arrival, steers were implanted with Component E-S with Tylan (200 mg progesterone, 20 mg estradiol benzoate, and 29 mg tylosin tartrate, Elanco Animal Health, Greenfield, IN). Steers were re-implanted with Component TE-IS with Tylan (16 mg estradiol, 80 mg trenbolone acetate, and 29 mg tylosin tartrate, Elanco Animal Health, Greenfield, IN) 3 mo after the initial implant. Steers were housed as a group in dirt-surfaced pens prior to the experiment and fed a finishing ration consisting of 93% concentrate and 7% roughage. Approximately 4 mo after arrival, steers were weighed, placed back into their pens, and 3 d later were sorted into experimental pens and fed their respective diets. Body weights were measured prior to feeding on d 0, d 21, and prior to shipment on d 24. To minimize effects of gut fill and potential differences in initial BW, initial

carcass weights were used for performance calculations and were estimated by multiplying initial live BW by an assumed dressing percentage of 63%.

Experimental Design

The study was conducted as a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Factors consisted of: 1) diets containing 0 or 8.33 mg ZH/kg diet DM (Merck Animal Health, Millsboro, DE), and 2) diets containing 60 or 300 mg Zn/kg dietary DM (60Zn or 300Zn). Sixty milligrams Zn/kilogram diet DM was commonly supplemented to cattle at the Kansas State University Beef Cattle Research Center and is within the range recommended by feedlot consulting nutritionists (Vasconcelos and Galyean, 2007). The high supplemental Zn concentration (300 mg Zn/kg diet DM) was below the maximum tolerable level recommended by the National Research Council (NRC, 2000), but high enough to observe potential interactive effects. Both diets contained 60 mg/kg Zn from $ZnSO_4$ provided by the pre-made trace mineral premix that was included in the final experimental vitamin/mineral premix. For the 300Zn diets, an additional 240 mg/kg diet DM supplemental Zn was included as Zn oxide (Table 2.1). Zilpaterol hydrochloride was administered for 21 d followed by a 3-d withdraw prior to harvest. Forty crossbred steers (initial BW = 652 kg ± 14) were stratified by BW and randomly assigned, within strata (block), to one of 4 treatments for the 24-d feeding trial.

Blood Collection

Blood was collected from each steer via jugular venipuncture approximately 2 h prior to feeding on d 0 and d 21 using two 6-mL trace mineral-free blood collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing K_2 EDTA as anticoagulant for analysis of plasma Zn, glucose, lactate, and urea nitrogen (**PUN**) concentrations. Additionally, blood was collected using a 10-mL serum sampling tube (Vacutainer, Becton Dickinson) on d 21 for

analysis of serum IGF-1 concentration. Blood samples for plasma analyses were immediately placed on ice and centrifuged within 10 min of sampling. Samples utilized for analysis of Zn, glucose, lactate, and PUN were centrifuged at 2,494 × *g* for 20 min at room temperature to recover plasma. Plasma was transferred by pipette to 5-mL plastic tubes, capped, and frozen at -20ºC until analysis. Serum tubes were allowed to coagulate for 1 h at 4ºC, centrifuged, and serum was transferred by pipette to storage vials and frozen at -20ºC.

Housing and Diet Preparation

Steers were randomly assigned to individual, partially covered feeding pens, equipped with concrete floors, automatic waterers and fence line feed bunks (10 pens/treatment). Steers were housed in 2 barns, each containing 20 individual, concrete-surfaced pens. Each pen measured 1.5 m × 6 m. Pens were partially covered and were equipped with individual feed bunks, and water fountains were shared between adjacent pens. Feed bunks were visually inspected and allocations of feed were adjusted daily, leaving 0.23 kg/animal of residual feed before feeding the following day. Zilpaterol hydrochloride (8.33 mg/kg diet DM) was incorporated into in the feed additive premix, which was subsequently mixed into complete diets. Diets were mixed once daily and hand delivered to individual animals at 0900 h, and allowed *ad libitum* access to feed. Weights of fresh feed were recorded daily and unconsumed feed was removed from the bunk and weighed on d 21 and 24 or as needed to determine actual feed intake. A subsample of unconsumed feed was dried in a forced air oven at 55ºC for 48 h to determine dry matter content.

Harvest

Final BW (gross BW × 0.96) were determined immediately before transporting cattle 450 km to a commercial abattoir in Holcomb, KS. Hot carcass weights and incidence and severity of

liver abscesses were recorded the d of harvest. Liver abscesses were scored according to the Elanco scoring system (Liver Abscess Technical Information AI 6288, Elanco Animal Health, Greenfield, IN): 0 = no abscesses, A^- = 1 or 2 small abscesses or abscess scars, A^0 = 2 to 4 small, well-organized abscesses and A^+ = 1 or more large or active abscesses with or without adhesions). Following 36 h of refrigeration, marbling score, 12^{th} rib s. c. fat thickness, LM area, and yield grade were obtained from camera images (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany) provided by the abattoir, USDA quality grade and incidence and severity of dark cutting beef were determined by a certified USDA grader, and percentage KPH was determined by trained research personnel.

Chemical Analyses

Glucose and lactate concentrations in plasma were analyzed using the YSI 2300 STAT Plus Glucose and L-lactate Analyzer (YSI Inc., Yellow Springs, OH). Concentrations of PUN were determined according to procedure described by Marsh et al. (1965) using the Auto Analyzer II (Seal Analytical, Mequon, WI). Insulin-like glucose factor-1 was measured utilizing the IGF-1 enzyme-linked immunosorbent assay kit (Immunodiagnostic Systems Inc., Fountain Hills, AZ) and analyzed with Wallac Victor 2 1420 Multilabel Counter (PerkinElmer, Waltham, MA). Plasma samples for Zn analysis were prepared by diluting 1 mL plasma with 3 mL 0.2% lantham oxide diluent. Following dilution, samples were analyzed using an atomic absorption spectrometer (Perkin Elmer Atomic Absorption Spectrometer 3110, PerkinElmer, Waltham, MA) set at a wavelength of 214 nm.

Statistical Analyses

The study was conducted as a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Feedlot performance and blood constituents were analyzed using the

MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC). The model included fixed effects of Zn, ZH, day, and 2- and 3-way interactions. The random effect was block and the experimental unit was steer. Daily feed allocations were analyzed as repeated measures using Zn, ZH, day, and 2- and 3-way interactions as fixed effects, block as the random effect, and steers as the experimental unit. Day was included in the repeated measures statement with animal nested within treatment as the subject, and compound symmetry as the covariance structure. Hot carcass weight, dressed yield, KPH, LM area,12th rib s. c. fat, yield grade, and marbling score were analyzed using the MIXED procedure of SAS with a model statement that included fixed effects of Zn, ZH, and the interaction between Zn and ZH. The random effect was block and experimental unit was steer. Categorical data, such as liver abscess incidence and quality grade, were analyzed using the GLIMMIX procedure of SAS with fixed effects of Zn, ZH, and interaction between Zn and ZH. The random effect was block and the experimental unit was steer. Least-square means were separated using the PDIFF option for separation of means. Means were determined to be different at α level ≤ 0.05, and tendencies were declared at $0.05 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

Growth Performance

Multiple studies have documented effects of ZH and Zn supplementation for finishing cattle, but not the potential for interactive effects between ZH and Zn. We observed no Zn × ZH interactions, or effects of Zn on carcass ADG, DMI, or carcass G:F ($P \geq 0.11$; Table 2.2). In agreement, Arelovich et al. (2008) and Greene et al. (1988) concluded supplementing 430 mg Zn/kg diet or 360 mg/d Zn, respectively, had no impact on DMI, ADG, or feed efficiency. In addition, lower Zn supplementation levels ranging from 10 mg/kg DM (Kessler et al., 2003) to

75 mg/kg DM (Nunnery et al., 2007) had no effects on DMI, ADG, or feed efficiency when compared to animals fed control diets without supplemental Zn. Malcolm-Callis et al. (2000) fed Zn at 20, 100, or 200 mg/kg of diet DM as Zn sulfate to finishing steers and observed no change in ADG, feed efficiency, or final BW during a 112-day finishing trial, but the authors did observe a tendency for decreased DMI with increasing Zn concentration. Additionally, Spears and Kegley (2002) observed final BW was unaffected by supplementing Zn at 25 mg/kg DM as Zn oxide or Zn proteinate. When assessing overall feedlot performance during ZH supplementation, results from the current study suggest that high concentrations of Zn are not warranted to improve feedlot performance.

No Zn × ZH × day, Zn × ZH, or ZH × day interactions ($P \geq 0.63$; Fig. 2.1), or effects of Zn or ZH ($P \geq 0.35$) were detected for amount of feed delivered daily, but there was an effect of day ($P < 0.01$). In addition, amounts of feed consumed by steers each day declined near the end of the experimental period for steers fed 300Zn, thus resulting in Zn × day interaction ($P < 0.01$). Changes in amount of feed delivered were in response to amounts of unconsumed feed remaining in feed bunks 24 h after delivery, thus reflecting differences in feed consumption. Differences in feed allocated to cattle supplemented with 60 or 300 mg Zn/kg diet DM did not manifest until d 19 ($P > 0.10$). Day 18 thru 21 and d 24 cattle supplemented with 300 mg Zn/kg diet DM tended to consume less feed than their counterparts supplemented with 60 mg Zn/kg diet DM ($P \leq 0.08$), and on d 22 and 23, cattle supplemented 300Zn consumed less feed than cattle supplemented 60Zn ($P = 0.03$). Malcolm-Callis et al. (2000) supplemented cattle 20, 100, or 200 mg Zn/kg diet DM and observed Zn supplementation tended to decrease DMI d 0 to 112 linearly. The authors suggested increased Zn concentration as Zn sulfate may negatively influence palatability; however, it is conceivable that excess Zn adversely affects gut microbiota

or may interfere with absorption of other nutrients. Ivan and Grieve (1975) observed a decrease in liver Cu concentration when supplemental Zn was increased to 100 mg/kg, suggesting Cu absorption may be suppressed by supplementing 100 mg Zn/kg DM. In addition, increased supplemental Zn has been shown to inhibit cellulose digestion (Martinez and Church, 1970).

Zilpaterol hydrochloride supplementation had no effect on DMI ($P = 0.71$; Table 2.2), but increased carcass ADG ($P = 0.03$) resulting in a 62% improvement in carcass G:F ($P = 0.02$) with ZH supplementation. Responses to ZH supplementation in ADG, DMI, and feed efficiency have varied in previous research. Cônsolo et al. (2015) observed a 17 and 23% increase in ADG and 19 and 21% improvement in feed efficiency when ZH was fed for 20 or 30 days, respectively. Similar improvements in ADG and G:F were observed in earlier literature (Baxa et al., 2010; Montgomery et al., 2009; Parr et al., 2011). Conversely, Boyd et al. (2015) did not observe any differences in ADG, DMI, or G:F when ZH was supplemented for 21 d. In addition, Hilscher et al. (2015) observed no difference in DMI or ADG, but G:F improved when steers were supplemented ZH for 20 d compared to un-supplemented steers. Hales et al. (2014) and Scramlin et al. (2010) reported a 7 to 9% decrease in DMI with ZH supplementation for 21 or 30 d, respectively, whereas, Avendaño-Reyes et al. (2006), Montgomery et al. (2009), and Parr et al. (2011) observed no change in DMI due to ZH. Furthermore, the results of the present study are in contrast with results from work we have published previously (Van Bibber-Krueger et al., 2015) in which ZH supplementation decreased DMI, but ADG and G:F were not different between ZH supplemented steers and control steers. In the meta-analysis conducted by Lean et al. (2014), ZH supplementation increased ADG by 0.15 kg/d, final BW by 8 kg, G:F by 0.024, but decreased DMI by 0.12 kg/d. Factors such as study design, diet formulation, genetic variation

among breeds, age, sex, and environmental factors can contribute to the lack of congruency among research (Mersmann, 1998, 2002).

Carcass Characteristics

The effects of Zn and ZH on carcass characteristics are summarized in Table 2.3. There were no Zn × ZH interactions detected for any carcass traits ($P \geq 0.37$). Regardless of ZH supplementation, carcasses from cattle supplemented 300Zn tended to have a greater KPH percentage ($P = 0.09$), fewer carcass that graded Select ($P = 0.08$), and 15% more carcasses that graded Choice ($P = 0.08$) when compared to carcasses from cattle supplemented 60Zn. There were no effects of Zn supplementation for HCW, dressing percentage, percent liver abscesses, LM area, 12th rib s. c. fat thickness, USDA yield grade or marbling score ($P \geq 0.37$). In agreement, Huerta et al. (2002), Greene et al. (1988), and Nunnery et al. (2007) observed no differences in carcass traits from steers supplemented Zn at 200 mg/kg DM (from Zn sulfate or Zn methionine), 360 mg/d (from Zn oxide), or 75 mg/kg diet DM (from Zn sulfate, Zn methionine, or Zn propionate), respectively. Conversely, Malcolm-Callis et al. (2000) observed a quadratic decrease for 12th rib s. c. fat thickness and quadratic increase for yield grade in carcasses from cattle supplemented 20, 100, or 200 mg Zn/kg diet DM as Zn sulfate. Spears and Kegley (2002) observed an increase in marbling score and quality grade and a tendency for increased yield grade and 12th rib s. c. fat thickness from carcasses from steers supplemented Zn at 25 mg/kg DM, regardless of source, compared to carcasses from non-supplemented steers. The authors suggested the Zn content of the basal ration was slightly below NRC (2000) recommendations, which may have resulted in a greater response in carcass traits compared to studies in which the basal ration contained adequate Zn. In addition, differences may be attributed to initial Zn status of animals prior to starting experiments, which can potentially

impact responses to Zn supplementation. Zinc is a diverse trace mineral that participates directly or indirectly in numerous enzymes within mammals, therefore making it difficult to determine the precise cause for lack of congruence among studies. In an *in vitro* trial, Oh and Choi (2004) reported an increase in lipogenic activity in bovine intramuscular adipocytes with increasing Zn concentration. These results may partially explain the tendency for increased quality grade and percent KPH in the current study, and increased yield grade and 12th rib s. c. fat thickness observed by Malcolm-Callis et al. (2000) and Spears and Kegley (2002).

Zilpaterol hydrochloride supplementation reduced USDA yield grade ($P = 0.05$) and tended to increase LM muscle area ($P = 0.07$) compared to carcasses from cattle that were not fed ZH; however, there were no differences in dressed yield percentage, liver abscess percentage, 12th rib s. c. fat thickness, marbling score, percent KPH, and carcasses grading Select or Choice ($P \geq 0.27$) between carcasses of cattle fed diets with or without ZH. The lack of an effect on HCW and dressing percentage in the current trial was not expected and is in disagreement with previously published literature. Previous results indicate increased HCW ranged from 11 kg when ZH was fed for 20 d or 40 d (Holland et al., 2010; Plascencia et al., 2008, respectively) to 22 kg when ZH was fed for 30 d (Avendaño-Reyes et al., 2006). In the meta-analysis by Lean et al. (2014), ZH was noted to increase HCW by 15 kg and dressing percentage by 1.7%. Conversely, Cônsolo et al. (2015) observed no difference in HCW when cattle were supplemented ZH for 20 d, but after 30 d of supplementation HCW increased with ZH supplementation, while dressing percentage increased regardless of duration of supplementation. Elam et al. (2009) fed ZH for 0, 20, 30, or 40 d and observed linear increases in HCW. Cattle in the current trial were supplemented ZH for 21 d which may have been insufficiently long to detect a difference in HCW given the small number of cattle. Decreased yield grade in the

present study is consistent with results previously reported by Hales et al., (2014), Hilscher et al. (2015), and Scramlin et al. (2010) with ZH supplementation, and the 4.36 cm^2 increase in LM area in the current study is less than the average increase (8 cm^2) reported by Lean et al. (2014) though within the range of observations used in their meta-analysis.

Other than yield grade, carcass trait responses associated with adipose tissue (i.e. 12th rib s. c. fat thickness, marbling score, KPH, and quality grade) have not been consistent in carcasses from ZH-supplemented cattle compared to carcasses from un-supplemented cattle. In agreement with the current results, Van Bibber-Krueger et al. (2015) observed no difference in percent KPH, 12th rib s. c. fat thickness, marbling score, or percent of carcasses that graded Choice or Select when ZH was supplemented for 23 d, whereas Holland et al. (2010) observed fewer carcasses grading Choice and more carcasses grading Select with ZH administration following 20 d of ZH administration, regardless of withdrawal time. On the contrary, Elam et al. (2009) observed poorer marbling scores, decreased KPH, and decreased 12th rib s. c. fat thickness with ZH supplementation, which is in agreement with Vasconcelos et al. (2008). Miller et al. (2012) indicated ZH had minimal effects on adipose tissue metabolism, which in combination with genetic variation among breeds and age of cattle could contribute to the differences observed among trials for marbling score, 12th rib s. c. fat thickness, and quality grade.

Blood Metabolites

The second objective of this trial was to assess some of the underlying metabolic changes occurring with Zn and ZH supplementation. There were no Zn × ZH × day interactions for plasma glucose, plasma lactate, PUN, or plasma Zn ($P \geq 0.16$; Table 2.4).

Zinc plays an important role in the production, storage, and action of insulin (Bellia et al., 2013). Therefore, plasma glucose concentration may be affected by increased concentrations of

supplemental Zn. There were no Zn × ZH or Zn × day interactions, or effects of Zn or day for plasma glucose or lactate ($P \geq 0.16$). Arelovich et al. (2008) fed Zn at 430 mg/kg DM (Zn chloride) to beef cattle and observed no change in serum glucose concentration between steers supplemented with Zn and control steers. In agreement, no changes in serum or plasma glucose concentrations were observed when 80 and 140 mg Zn/kg DM (as Zn sulfate) were added to diets of buffalo calves (Ramulu et al., 2015) or when 50, 100, or 150 mg Zn/kg DM (as Zn sulfate) was included in diets for lambs (Wang et al., 2006). On the contrary, Daghash and Mousa (1999) observed increased serum glucose concentration due to Zn supplementation in buffalo calves under heat stress. This difference may be due to the environment in which the calves were maintained.

Glucose and lactate concentrations are important components of cellular metabolism and could potentially provide energy necessary for greater protein deposition during periods of β-agonist administration. Limited research is available comparing the effects of ZH supplementation on plasma glucose and lactate. No ZH × day interaction or effect of ZH were observed for plasma glucose or lactate ($P \geq 0.29$). In agreement with current results, Van Bibber-Krueger et al. (2015) observed no change in plasma glucose or lactate concentration between control and ZH supplemented steers after 23 d of ZH administration. These results are further supported by Cônsolo et al. (2015), who reported no change in serum glucose concentration following ZH supplementation for 30 d. Eisemann et al. (1988) observed an initial change in glucose concentration after 8 mg/d of clenbuterol was administered to steers, but after 9 d of feeding glucose concentration was not different between control and clenbuterol-supplemented animals. Plasma lactate concentrations reported previously in steers revealed an increase plasma lactate concentration with addition of cimaterol (Byrem et al., 1996) and clenbuterol (Eisemann

et al., 1988), suggesting an increase in peripheral glycolysis (Eisemann et al., 1988). Lack of congruence among results may be due to differences in beta agonist administered; however, plasma glucose concentrations are in agreement with previously reported results.

Plasma urea nitrogen can be used as an indicator of protein intake and protein degradation. Froetschel et al. (1990) indicated feeding 1,142 mg Zn/kg DM decreased ruminal amino acid digestion, decreased synthesis of bacterial amino acids, and decreased abomasal passage of total amino acids as percentage of amino acid intake. These results suggest increased concentrations of supplemental Zn may affect ruminal protein metabolism. In addition, Zn is an essential component of glutamic dehydrogenase and is required for urea synthesis (Vallee, 1959) and RNA polymerase (Vallee and Falchuk, 1993), making it essential for protein synthesis. There were no Zn × ZH or Zn × day interactions or effects of Zn on PUN concentration ($P \geq 0.25$) in the current trial. In agreement, there were no differences observed in serum urea nitrogen concentrations due to supplementation of bulls with 10 mg Zn/kg DM as Zn oxide, Zn proteinate or Zn polysaccharide (Kessler et al., 2003), or in heifers supplemented with 200 mg Zn/kg DM as Zn sulfate or Zn methionine (Huerta et al., 2002). Daghash and Mousa (1999) observed decreased serum urea nitrogen in buffalo calves receiving 50 or 100 mg Zn/kg diet compared to control calves. The reduced serum urea nitrogen concentration observed by Daghash and Mousa (1999) may be a reflection of increased Zn requirements for younger ruminants compared to the older animals in the current trial.

Zilpaterol hydrochloride has consistently increased carcass protein deposition (Leheska et al., 2009; Shook et al., 2009), and therefore, urea nitrogen concentration might be expected to decrease in response to ZH supplementation (Parr et al., 2014). A ZH × day interaction was detected for PUN concentration, with PUN concentration decreasing following ZH

supplementation. These results suggest decrease protein degradation or increased protein synthesis in response to ZH supplementation. Consistent with current results, supplementing cattle with clenbuterol (Ricks et al., 1984), cimaterol (Quirke et al., 1988), or ZH (Parr et al., 2014; Van Bibber-Krueger et al., 2015) resulted in decreased urea nitrogen concentrations. Conversely, Cônsolo et al. (2015) observed no change in serum urea nitrogen concentrations in heifers supplemented with ZH for 30 d. Differences in results may be reflective of the duration of ZH administration. It is conceivable that cattle may be more responsive to ZH supplementation early in the feeding period, but over time cells may become desensitized to ZH supplementation; however, current research suggested PUN concentration was reduced when ZH was administered for 21 d.

No Zn × ZH, or ZH × day interactions were observed ($P \geq 0.17$) for plasma Zn concentration. A Zn × day interaction was detected ($P = 0.01$), in which plasma Zn concentration was greater for cattle supplemented 300Zn compared to cattle supplemented 60Zn d 21 compared to plasma glucose concentrations d 0. Results for plasma or serum Zn concentration have been variable in previously reported literature. In agreement, Huerta et al. (2002) observed increased serum Zn concentration after supplementing heifers 200 mg Zn/kg diet DM as Zn sulfate or Zn methionine. On the contrary, supplementing 430 mg Zn/kg diet DM as Zn chloride (Arelovich et al., 2008); 20, 100, or 200 mg Zn/kg diet DM as Zn sulfate (Malcolm-Callis et al., 2000); or 360 mg Zn/d as Zn oxide or Zn methionine (Greene et al., 1988) resulted in no increase in serum Zn. Stress or disease causes rapid redistribution of Zn from extracellular tissue, thereby reducing concentrations of Zn in blood (Hambidge et al., 1986), which may contribute to differences observed for plasma or serum Zn concentrations among studies.

Insulin-like glucose factor-1 production is stimulated, in part, by growth hormone and potentially could be impacted by addition of Zn or ZH to finishing cattle diets. In particular, Zn deficiency consistently resulted in decreased circulating IGF-1 concentrations, which may reflect the role of Zn in IGF-1 induction of cell proliferation (MacDonald, 2000). Insulin-like glucose factor-1 concentrations were analyzed d 21 following ZH supplementation (Fig. 3.2). No Zn × ZH interaction was observed ($P = 0.75$), and IGF-1 concentration was not affected by Zn supplementation ($P = 0.45$). Jafarpour et al. (2015) supplemented 58, 118, or 163 mg Zn/kg diet DM as Zn methionine to sheep and observed a linear increase in plasma IGF-1 concentration as supplemental Zn increased. The difference in results may be due to different sources of Zn utilized, species, or initial trace mineral status of the animals. The current results indicate increased Zn supplementation did not influence IGF-1 concentration of finishing cattle.

Serum IGF-1 concentration was not impacted by ZH supplementation in the current trial ($P = 0.65$). Parr et al. (2014) observed no difference in IGF-1 mRNA concentrations in the LM of cattle supplemented with or without ZH for 20 d after correcting for initial IGF-1 mRNA differences between treatments prior to ZH supplementation. In agreement with current results, Dawson et al. (1993) observed no difference in plasma IGF-1 concentration from steers that received 1.5 mg/kg diet cimaterol for 23 wk. On the contrary, Beermann et al. (1987) observed decreased plasma IGF-1 levels in lambs supplemented with 10 mg cimaterol/kg diet. Differences in response to cimaterol supplementation may be due to species differences, but the current results with ZH supplementation are in agreement with Dawson et al. (1993), who suggest growth promoting effects in cattle may not be directly mediated through increased IGF-1 concentration.

In conclusion, supplementing increased concentrations of Zn in diets of finishing steers fed ZH during the final 21 days of finishing does not further enhance feedlot performance, carcass traits, or blood parameters associated with increased growth; however, increased dietary concentrations of supplemental Zn may have adverse effects on feed consumption with prolonged feeding.

REFERENCES

Arelovich H.M., H. E. Laborde, M.I. Amela, M. B. Torrea, M. F. Martínez. 2008. Effects of dietary addition of zinc and (or) monensin on performance, rumen fermentation and digesta kinetics in beef cattle. Span. J. Agric. Res. 6:362-372. doi: 10.5424/sjar/2008063-329

Avendaño-Reyes, L., V. Torres-Rodríguez, F. J. Meraz-Murillo, C. Pérez-Linares, F. Figueroa-Saavedra, and P. H. Robinson. 2006. Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84:3259–3265. doi:10.2527/jas.2006-173

Baxa, T. J., J. P. Hutcheson, M. F. Miller, J. C. Brooks, W. T. Nichols, M. N. Streeter, D. A. Yates, and B. J. Johnson. 2010. Additive effects of a steroidal implant and zilpaterol hydrochloride on feedlot performance, carcass characteristics, and skeletal muscle messenger ribonucleic acid abundance in finishing steers. J. Anim. Sci. 88:330–337. doi:10.2527/jas.2009-1797

Bellia, F., A. Pietropaolo, and G. Grasso. 2013. Formation of insulin fragments by insulin-degrading enzyme: the role of zinc (II) and cystine bridges. J. Mass Spectrom. 48:135–140. doi:10.1002/jms.3060

Beermann D. H., W. R. Butler, D. E. Hogue, V. K. Fishell, R. H. Dalrymple, C. A. Ricks, and C. G. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. J. Anim. Sci. 65:1514-1524. doi:10.2134/jas1987.6561514x

Boyd, B. M., S. D. Shackelford, K. E. Hales, T. M. Brown-Brandl, M. L. Bremer, M. L. Spangler, T. L. Wheeler, D. A. King, and G. E. Erickson. 2015. Effects of shade and feeding zilpaterol hydrochloride to finishing steers on performance, carcass quality, heat stress, mobility, and body temperature. J. Anim. Sci. 93: 5801-5811. doi:10.2527/jas.2015-9613

Byrem, T. M., D. H. Beermann, and T. F. Robinson. 1998. The beta-agonist cimaterol directly enhances chronic protein accretion in skeletal muscle. J. Anim. Sci. 76:988–998. doi:/1998.764988x

Cônsolo, N. R. B., F. D. Rodriguez, R. S. Goulart, M. O. Frasseto, V. B. Ferrari, and L. F. Silva. 2015. Zilpaterol hydrochloride improves feed efficiency and changes body composition in nonimplanted Nellore heifers. J. Anim. Sci. 93:4948-4955. doi:10.2527/jas.2015-9291

Daghash, H. A. and S. M. Mousa. 1999. Zinc sulfate supplementation to ruminant rations and its effects on digestibility in lamb; growth, rectal temperature and some blood constituents in buffalo calves under heat stress. Assiut Vet. Med. J. 40:128-146.

Dawson, J. M., J. Craigon, P. J. Buttery, and D. E. Beever. 1993. Influence of diet and β-agonist administration on plasma concentrations of growth hormone and insulin-like growth factor-1 in young steers. Br. J. Nutr. 70:93-102. doi:10.1079/bjn19930107

Eisemann, J. H., G. B. Huntington, and C. L. Ferrell. 1988. Effects of dietary clenbuterol on metabolism of the hindquarters in steers. J. Anim. Sci. 66:342–353. doi:10.2134/jas1988.662342x

Elam, N. A., J. T. Vasconcelos, G. Hilton, D. L. VanOverbeke, T. E. Lawrence, T. H. Montgomery, W. T. Nichols, M. N. Streeter, J. P. Hutchinson, D. A. Yates, and M. L. Galyean. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87:2133–2141. doi:10.2527/jas.2008-1563

Froetschel, M. A., A. C. Martin, H. E. Amos, and J. J. Evans. 1990. Effects of zinc sulfate concentration and feeding frequency on ruminal protozoal numbers, fermentation patterns and amino acid passage in steers. J. Anim. Sci. 68:2874-2884. doi:/1990.6892874x

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schelling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J. Anim. Sci. 66:1818-1823. doi:10.2134/jas1988.6671818x

Hales, K. E., S. D. Shackelford, J. E. Wells, D. A. King, M. D. Hayes, T. M. Brown-Brandl, L. A. Kuehn, H. C. Freetly, and T. L. Wheeler. 2014. Effects of feeding dry-rolled corn-based diets with and without wet distillers grains with solubles and zilpaterol hydrochloride on performance, carcass characteristics, and heat stress in finishing beef steers. J. Anim. Sci. 92:4023–4033. doi:10.2527/jas.2014-7638

Hambidge, K. M., C. E. Casey, and N. F. Krebs. 1986. Zinc. In: W. Mertz, editor, Trace minerals in human and animal nutrition vol. 2. Academic Press, Orlando, FL. p. 1-137.

Hilscher, F. H., E. M. Hussey, B. L. Nuttelman, D. B. Burken, W. A. Griffin, K. J. Vander Pol, J. P. Hutcheson, and G. E. Erickson. 2015. Impact of sorting before feeding zilpaterol hydrochloride on feedlot performance and carcass characteristics of yearling steers. J. Anim. Sci. 93:2285-2296. doi:10.2527/jas.2014-8579

Holland, B. P., C. R. Krehbiel, G. G. Hilton, M. N. Streeter, D. L. VanOverbeke, J. N. Shook, D. L. Step, L. O. Burciaga-Robules, D. R. Stein, D. A. Yates, J. P. Hutcheson, W. T. Nichols, and J. L. Montgomery. 2010. Effect of extended withdrawal of zilpaterol hydrochloride on performance and carcass traits in finishing beef steers. J. Anim. Sci. 88:338–348. doi:10.2527/jas.2009-1798

Huerta, M., R. L. Kincaid, J. D. Cronrath, J. Busboom, A. B. Johnson, and C. K. Swenson. 2002. Interaction of dietary zinc and growth implants on weight gain, carcass traits and zinc in tissues of growing beef steers and heifers. Anim. Feed Sci. Technol. 95:15-32. doi: 10.1016/s0377-8401(01)00334-0

Ivan, M., and C. M. Grieve. 1975. Effects of zinc, copper, and manganese supplementation of high-concentrate ration on digestibility, growth, and tissue content of Holstein calves. J. Dairy Sci. 58:410–415. doi:10.3168/jds.s0022-0302(75)84579-6

Jafarpour, N., M. Khorvash, H. R. Rahmani, A. Pezeshki, and M. Hosseini Ghaffari. 2015. Dose-responses of zinc-methionine supplements on growth, blood metabolites and gastrointestinal development in sheep. J. Anim. Physiol. Anim. Nutr. 99:668–675. doi:10.1111/jpn.12286

Jeong, J., and D. J. Eide. 2013. The SLC39 family of zinc transporters. Mol. Aspects Med. 34:612-619. doi:10.1016/j.mam.2012.05.011

Kessler, J., I. Morel, P. A. Dufey, A. Gutzwiller, A. Stern, and H. Geyer. 2003. Effect of organic zinc sources on performance, zinc status and carcass, meat and claw quality in fattening bulls. Livest. Prod. Sci. 81:161-171.

Lean, I. J., J. M. Thompson, and F. R. Dunshea. 2014. A meta-analysis of zilpaterol and ractopamine effects on feedlot performance, carcass traits and shear strength of meat in Cattle. PloS ONE. 9:e115904. doi: 10.1371/journal.pone.0115904

Leheska, J. M., J. L. Montgomery, C. R. Krehbiel, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. Streeter, J. R. Blanton Jr., and M. F. Miller. 2009. Dietary zilpaterol hydrochloride. II. Carcass composition and meat palatability of beef cattle. J. Anim. Sci. 87:1384–1393. doi:10.2527/jas.2008-1168

Martinez, A., and D. C. Church. 1970. Effect of various mineral elements on *in vitro* rumen cellulose digestion. J. Anim. Sci. 31:982-990. doi:10.2134/jas1970.315982x

MacDonald, R. S. 2000. The role of zinc in growth and cell proliferation. J. Nutr. 130:1500S-1508S.

Malcolm-Callis, K. J., G. C. Duff, S. A. Gunter, E. B. Kegley, and D.A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. J. Anim. Sci. 78:2801-2808. doi:/2000.78112801x

Mersmann, H. J. 1998. Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76:160–172. doi:/1998.761160x

Mersmann, H. J. 2002. Beta-adrenergic receptor modulation of adipocyte metabolism and growth. J. Anim. Sci. 80 (E. Suppl. 1):E24–E29. doi:10.2134/animalsci2002.0021881200800ES10005x

Miller, E. K., K. Y. Chung, J. P. Hutcheson, D. A. Yates, S. B. Smith, and B. J. Johnson. 2012. Zilpaterol hydrochloride alters abundance of β-adrenergic receptors in bovine muscle cells but has little effect on de novo fatty acid biosynthesis in bovine subcutaneous adipose tissue explants. J. Anim. Sci. 90:1317–1327. doi:10.2527/jas.2011-4589

Montgomery, J. L., C. R. Krehbiel, J. J. Cranston, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, D. T. Bechtol, E. Johnson, T. TerHune, and T. H. Montgomery. 2009a. Dietary zilpaterol hydrochloride. I. Feedlot performance and carcass traits of steers and heifers. J. Anim. Sci. 87:1374–1383. doi:10.2527/jas.2008-1162

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Nunnery, G. A., J. T. Vasconcelos, C. H. Parsons, G. B. Salyer, P. J. Defoor, F. R. Valdez, and M. L. Galyean. 2007. Effects of source of supplemental zinc on performance and humoral immunity in beef heifers. J. Anim. Sci. 85:2304–2313. doi:10.2527/jas.2007-0167

Oh, Y. S., and C. B. Choi. 2004. Effects of zinc on lipogenesis of bovine intramuscular adipocytes. Asian-Australas. J. Anim. Sci. 17:1378–1382. doi:10.5713/ajas.2004.1378

Parr, S. L., K. Y. Chung, M. L. Galyean, J. P. Hutcheson, Nicolas Dilorenzo, K. E. Hales, M. L. May, M. J. Quinn, D. R. Smith, and B. J. Johnson. 2011. Performance of finishing beef steers in response to anabolic implant and zilpaterol hydrochloride supplementation. J. Anim. Sci. 89:560–570. doi:10.2527/jas.2010-3101

Parr, S. L., T. R. Brown, F. R. B. Ribeiro, K. Y. Chung, J. P. Hutcheson, B. R. Blackwell, P. N. Smith, and B. J. Johnson. 2014. Biological responses of beef steers to steroidal implants and zilpaterol hydrochloride. J. Anim. Sci. 92:3348–3363. doi:10.2527/jas.2013-7221

Paulk, C. B., D. D. Burnett, M. D. Tokach, J. L. Nelssen, S. S. Dritz, J. M. DeRouchey, R. D. Goodband, G. M. Hill, K. D. Haydon, and J. M. Gonzalez. 2015. Effect of added zinc in diets with ractopamine hydrochloride on growth performance, carcass characteristics, and ileal mucosal inflammation mRNA expression of finishing pigs. J. Anim. Sci. 93:185–196. doi:10.2527/jas.2014-8286

Plascencia, A., N. G. Torrentera and R. A. Zinn. 2008. Influence of the beta-agonist, zilpaterol, on growth performance and carcass characteristics of feedlot steers. J. Anim. Vet. Adv. 7(10):1257–1260. doi: javaa.2008.1257.1260

Quirke, J. F., P. Allen, A. P. Moloney, M. Sommer, J. P. Hanrahan, W. Sheehan, and J. F. Roche. 1988. Effects of the beta-agonist cimaterol on blood metabolite and hormone concentrations, growth and carcass composition in finishing Friesian steers. J. Anim. Physiol. Anim. Nutr. 60:128–136. doi: 10.1111/j.1439-0396.1988.tb00186.x

Ramulu, S. P., D. Nagalakshmi, and M. K., Kumar. 2015. Effect of zinc supplementation on haematology and serum biochemical constituents in Murrah buffalo calves. Indian J. Anim. Res. 49:482-486. doi: 10.5958/0976-0555.2015.00095.3

Ricks, C. A., R. H. Dalrymple, P. K. Baker, and D. L. Ingle. 1984. Use of a β-agonist to alter fat and muscle deposition in steers. J. Anim. Sci. 59:1247–1255. doi:10.2134/jas1984.5951247x

Scramlin, S. M., W. J. Platter, R. A. Gomez, W. T. Choat, F. K. McKeith, and J. Killefer. 2010. Comparative effects of ractopamine hydrochloride and zilpaterol hydrochloride on growth performance, carcass traits, and longissimus tenderness of finishing steers. J. Anim. Sci. 88:1823–1829. doi:10.2527/jas.2009-2405

Shook, J. N., D. L. VanOverbeke, L. A. Kinman, C. R. Krehbiel, B. P. Holland, M. N. Streeter, D. A. Yates, and G. G. Hilton. 2009. Effects of zilpaterol hydrochloride and zilpaterol hydrochloride withdrawal time on beef carcass cutability, composition, and tenderness. J. Anim. Sci. 87:3677–3685. doi:10.2527/jas.2009-1816

Spears, J. W., and E. B. Kegley. 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J. Anim. Sci. 80:2747-2752. doi:/2002.80102747x

Vallee, B L., and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. Physiol. Rev. 73:79-118.

Vallee, B. L. 1959. Biochemistry, physiology, and pathology of zinc. Physiol. Rev. 39: 443-490.

Vasconcelos, J. T., and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. J. Anim. Sci. 85:2772–2781. doi:10.2527/jas.2007-0261

Vasconcelos, J. T., R. J. Rathmann, R. R. Reuter, J. Leibovich, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols, and M. L. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on finishing cattle performance and carcass traits. J. Anim. Sci. 86:2005–2015. doi:10.2527/jas.2008-1032

Van Bibber-Krueger, C. L., K. A. Miller, G. L. Parsons, L. K. Thompson, and J. S. Drouillard. 2015. Effects of zilpaterol hydrochloride on growth performance, blood metabolites, and fatty acid profiles of plasma and adipose tissue in finishing steers. J. Anim. Sci. 93:2419–2427. doi:10.2527/jas.2014-8771

Wang, R., X. Zhu, F. Guo, W. Zhang, and Z. Jia. 2006. Influence of different dietary levels of zinc on performance, vitamin B_{12}, and blood parameters in lambs. Int. J. Vitam. Nutr. Res. 76:353–358. doi:10.1024/0300-9831.76.6.353

Wu, F., and C. Wu. 1987. Zinc in DNA replication and transcription. Ann. Rev. Nutr. 7:251–272. doi:10.1146/annurev.nutr.7.1.251

Table 2.1. Diet composition of steers fed 0 or 8.33 mg/kg diet DM zilpaterol hydrochloride (**ZH**) and 60 (**60Zn**) or 300 (**300Zn**) mg supplemental Zn/kg diet DM for 24 d

	No ZH		ZH[1]	
Item	60Zn	300Zn	60Zn	300Zn
Ingredient, % DM basis				
Steam flaked corn	53.55	53.52	53.55	53.52
Wet corn gluten feed	35.00	35.00	35.00	35.00
Wheat straw	7.00	7.00	7.00	7.00
Feed additive premix[2]	2.16	2.16	2.16	2.16
Vitamin/mineral premix [3]	0.17	0.17	0.17	0.17
Limestone	1.82	1.82	1.82	1.82
Salt	0.30	0.30	0.30	0.30
Zinc oxide	-	0.03	-	0.03
Calculated nutrient composition[4]				
CP, %	14.0	14.0	14.0	14.0
Ca, %	0.75	0.75	0.75	0.75
P, %	0.51	0.51	0.51	0.51
K, %	0.70	0.70	0.70	0.70
Zn, mg/kg	93	333	93	333
NDF, %	22.7	22.7	22.7	22.7

[1] Zilpaterol-HCl (Merck Animal Health, Millsboro, DE) was added to the feed additive premix and fed for 21 d at 8.33 mg/kg of diet DM followed by a 3-d withdrawal prior to harvest.

[2]Formulated to provide 300 mg/d monensin and 90 mg/d tylosin (Elanco Animal Health, Greenfield, IN) in a ground corn carrier.

[3]Formulated to provide (on DM basis) the following added nutrient levels: 2,200 IU/kg vitamin A; 22 IU/kg vitamin E (alpha tocopherol acetate); 0.10 mg/kg Co (cobalt carbonate); 10 mg/kg Cu (copper sulfate); 0.6 mg/kg I (ethylenediamine dihydriodide); 60 mg/kg Mn (manganous sulfate); 0.25 mg/kg Se (sodium selenite); and 60 mg/kg Zn (Zn sulfate).

[4]Calculated from NRC values for individual ingredients.

Table 2.2. Feedlot performance over 24-d feeding period of steers fed 0 or 8.33 mg/kg zilpaterol hydrochloride (**ZH**) and supplemented 60 (**60Zn**) or 300 (**300Zn**) mg Zn/kg diet DM[1]

	No ZH		ZH			*P*-value		
Item	60Zn[2]	300Zn[2]	60Zn[2]	300Zn[2]	SEM	Zn	ZH	Zn × ZH
Initial carcass weight[3], kg	425	423	422	418	8.91	0.20	0.10	0.66
Final carcass weight, kg	437	432	444	439	10.13	0.37	0.21	0.96
DMI, kg/d	10.55	10.20	10.88	10.26	0.53	0.35	0.71	0.80
Carcass gain, kg/d	0.51	0.41	0.92	0.87	0.20	0.70	0.03	0.88
Carcass G:F	0.0426	0.0223	0.0852	0.0838	0.0223	0.61	0.02	0.66

[1]Zilparterol hydrochloride (Merck Animal Health, Millsboro, DE) was fed for 21 d followed by a 3-d withdrawal prior to harvest.

[2]The first 60 mg/kg Zn was provided as Zn sulfate and the remaining 240 mg/kg Zn in the 300Zn diet was fed as Zn oxide.

[3]Calculated as initial BW × 0.63 for all treatments.

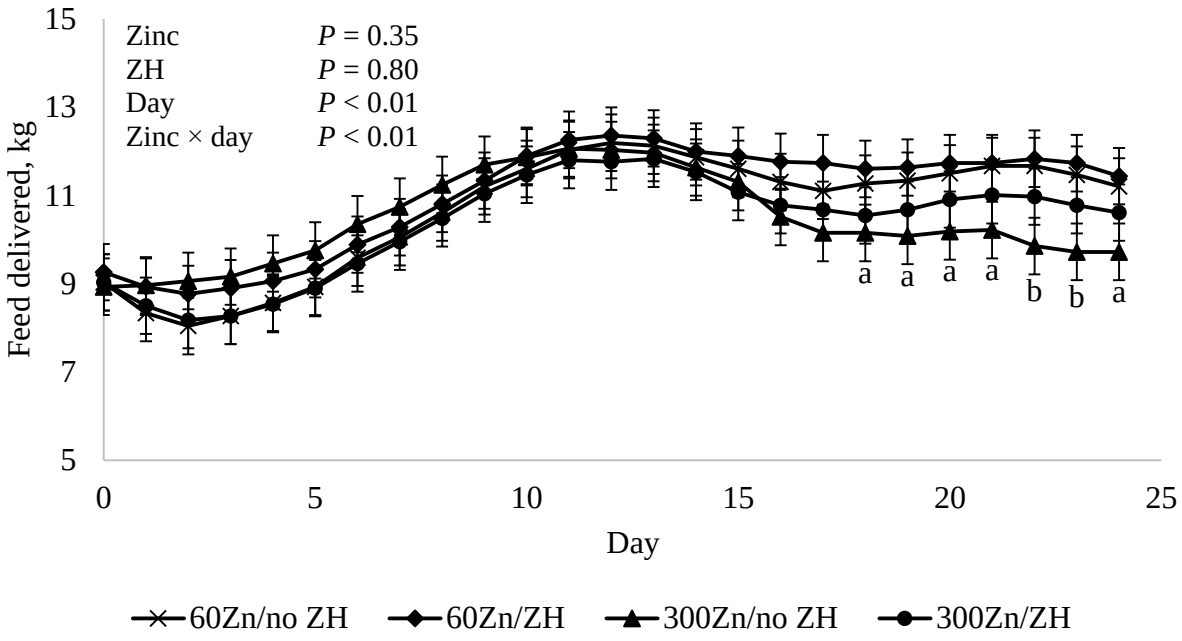

Figure 2.1 Feed delivered daily to steers fed 0 or 8.33 mg/kg zilpaterol hydrochloride (**no ZH** or **ZH**) and 60 or 300 mg Zn/kg diet DM (**60Zn** or **300Zn**). Zilpaterol hydrochloride (Merck Animal Health, Millsboro, DE) was fed for 21 d followed by a 3-d withdrawal prior to harvest. Bunks were monitored and adjusted daily so trace amounts of residual feed remained the following d. Feed delivered was recorded daily on as-fed basis then converted to DM based on ingredient DM analyses. No Zinc × ZH × day, Zinc × ZH, or ZH × day interactions ($P = 0.63$; $P = 0.67$; $P = 0.92$, respectively) were observed. [a]Within day, the average feed delivered to cattle supplemented 300 mg/kg Zn tended to decrease ($P \leq 0.08$) compared to average feed delivered to cattle supplemented with 60 mg/kg Zn regardless of ZH supplementation. [b]Within day, the average feed delivered to cattle supplemented 300 mg/kg Zn was decreased ($P \leq 0.03$) compared to average feed delivered to cattle supplemented with 60 mg/kg Zn regardless of ZH supplementation.

Table 2.3. Carcass traits of steers receiving 0 or 8.33 mg/kg zilpaterol hydrochloride (**ZH**) and 60 (**60Zn**) or 300 (**300Zn**) mg supplemental Zn/kg diet DM for a 24-d experiment[1]

	No ZH		ZH			*P*-value		
Item	60Zn[2]	300Zn[2]	60Zn[2]	300Zn[2]	SEM	Zn	ZH	Zn × ZH
Dressed yield, %	65.2	65.8	65.7	65.9	0.47	0.37	0.52	0.72
Liver abscess, %	30.0	20.0	30.0	30.0	14.81	0.72	0.72	0.72
LM area, cm^2	97.2	93.5	99.4	100.0	2.52	0.46	0.07	0.37
12^{th}-rib s. c. fat, cm	1.32	1.37	1.22	1.35	0.10	0.37	0.48	0.76
USDA yield grade	2.7	3.0	2.4	2.4	0.22	0.50	0.05	0.50
Marbling score[3]	481	492	503	523	23.8	0.52	0.27	0.85
KPH, %	1.60	1.70	1.45	1.75	0.17	0.09	0.66	0.39
Select, %	20	0	10	0	8.33	0.08	0.55	0.55
Choice, %	80	100	90	100	8.33	0.08	0.55	0.55

[1]Zilpaterol hydrochloride (Merck Animal Health, Millsboro, DE) was fed for 21 d followed by a 3-d withdrawal prior to harvest.

[2]The first 60 mg/kg Zn was provided as Zn sulfate and the remaining 240 mg/kg Zn in the 300Zn diet was fed as Zn oxide.

[3]Marbling scores were determined by a USDA grader; Small = 400 to 499; Modest = 500 to 599.

Table 2.4. Concentrations of plasma metabolites on d 0 and 21 from steers fed 0 or 8.33 mg/kg zilpaterol hydrochloride (**ZH**) and 60 (**60Zn**) or 300 (**300Zn**) mg supplemental Zn/kg diet DM[1]

	D 0				D 21				
	No ZH		ZH		No ZH		ZH		
Item	60Zn[2]	300Zn[2]	60Zn[2]	300Zn[2]	60Zn[2]	300Zn[2]	60Zn[2]	300Zn[2]	SEM
Glucose, m*M*	5.80	4.88	4.61	5.50	5.74	6.46	5.43	5.54	0.61
Lactate, m*M*	6.26	4.28	4.79	5.32	3.96	5.69	3.34	3.83	1.19
PUN[3,4,6,8], m*M*	4.72	4.82	4.51	4.40	4.21	4.61	3.47	3.33	0.22
Zinc[5,7,8], mg/L	1.32	1.40	1.45	1.39	1.46	1.81	1.53	1.67	0.07

[1]ZH (Merck Animal Health, Millsboro, DE) was fed for 21 d followed by a 3-d withdrawal prior to harvest.

[2]The first 60 mg/kg Zn was provided as Zn sulfate and the remaining 240 mg/kg Zn in the high diet was fed as Zn oxide.

[3]Plasma urea nitrogen (PUN)

[4]Zilpaterol hydrochloride × d interaction ($P = 0.03$).

[5]Zinc × day interaction ($P = 0.01$).

[6]Effect of ZH ($P < 0.01$).

[7]Effect of Zn ($P = 0.01$).

[8]Effect of d ($P < 0.01$).

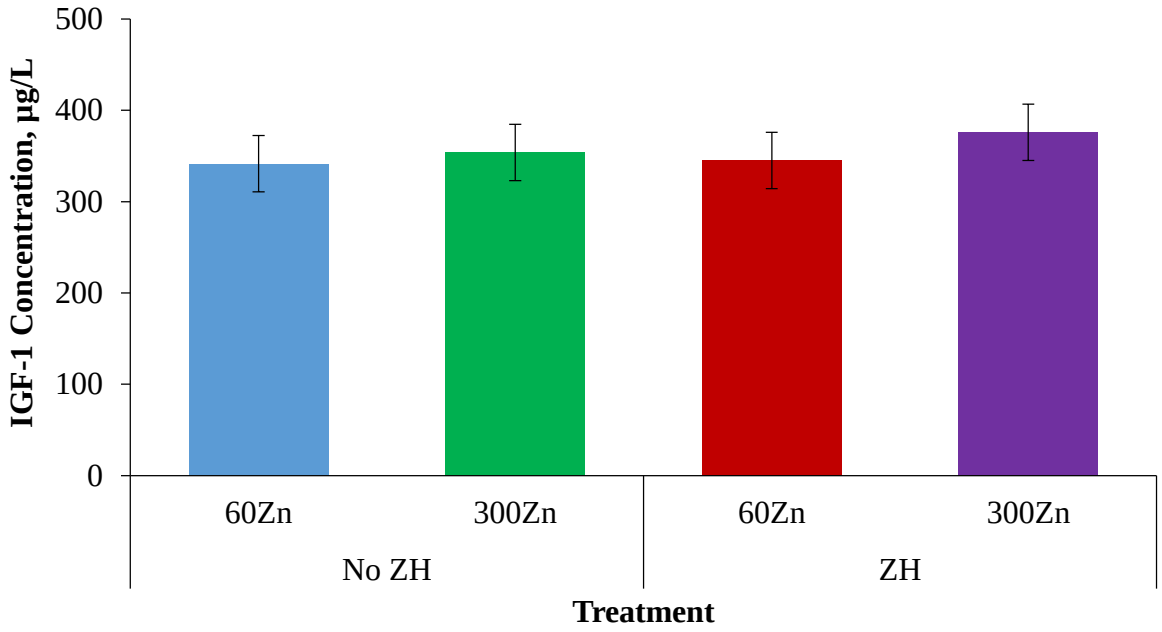

Figure 2.2. Day 21 serum IGF-1concentrations of steers fed 0 or 8.33 mg/kg zilpaterol hydrochloride (**ZH**) and 60 (**60Zn**) or 300 (**300Zn**) mg supplemental Zn/kg diet DM. The first 60 mg/kg Zn was provided as Zn sulfate and the remaining 240 mg/kg Zn in the high diet was supplemented as Zn oxide. Zilpaterol hydrochloride (Merck Animal Health, Millsboro, DE) was fed for 21 d followed by a 3-d withdrawal prior to harvest. No Zn × ZH interaction, effect of Zn or effect of ZH (P = 0.75; P = 0.45; P = 0.65, respectivley) were detected.

Chapter 3 - Interactive effects of supplemental Zn sulfate and ractopamine hydrochloride on growth performance, carcass traits, and plasma urea nitrogen in feedlot heifers

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ABSTRACT

Interactive effects of supplemental Zn and ractopamine hydrochloride (RH) were evaluated using 156 crossbred heifers (initial BW = 527 kg ± 6.61; gross BW × 0.96) to determine impact on feedlot performance, plasma urea nitrogen (**PUN**) and carcass characteristics. The study was conducted as a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Factors consisted of: 1) 30 or 100 mg supplemental Zn/kg diet DM (**30Zn** or **100Zn**) as Zn sulfate, and 2) 0 or 200 mg RH/animal daily. Heifers were blocked by BW and, assigned randomly within block to treatments. Heifers were housed in partially covered feeding pens (3 heifers/pen; 13 pens/treatment) and fed once daily *ad libitum*. RH was fed for 42 d and removed from the diet until cattle were harvested on d 43. Plasma samples were collected on d 0 and 36 to assess changes in plasma Zn and PUN. On d 43, heifers were weighed, then transported to a commercial abattoir where HCW and incidence of liver abscesses were recorded. Carcass data were collected after 32 h of refrigeration. No Zn × RH interactions were observed for plasma Zn or PUN ($P \geq 0.58$); however, there was a tendency for RH × d interaction for PUN ($P = 0.08$). Supplementing 100Zn resulted in increased plasma Zn ($P = 0.02$) compared to 30Zn. No interactions were observed for feedlot performance ($P \geq 0.24$). Final BW and ADG increased with RH supplementation ($P \leq 0.02$), but DMI was not affected ($P = 0.63$), thus feed efficiency improved ($P < 0.01$) when cattle were fed RH. Supplementing 100Zn tended to reduce ADG ($P = 0.07$), but did not affect other measures of feedlot performance ($P \geq 0.12$). Zinc × RH interactions were observed for LM area and yield grade ($P \leq 0.01$); LM area decreased and yield grade increased when cattle were supplemented 100Zn with no RH compared to other treatments. A tendency for a Zn × RH interaction was observed for dressing percentage ($P = 0.08$), but no other interactions or effects of Zn were detected for

carcass traits ($P \geq 0.11$). Supplementing RH increased HCW ($P = 0.03$), but did not affect other carcass traits ($P \geq 0.13$). In conclusion, supplemental Zn had little impact on feedlot performance or PUN concentration, but may alter muscle and fat deposition when fed in conjunction with RH.
Keywords; feedlot cattle, plasma urea nitrogen, ractopamine hydrochloride, zinc

INTRODUCTION

Synthetic β-adrenergic agonists are repartitioning compounds fed to cattle during the final phase of finishing, resulting in increased muscle tissue mass and a decrease in adipose tissue mass (Mersmann, 2002). Ractopamine hydrochloride (**RH**) is a β_1-adrenergic agonist fed to cattle the final 28 to 42 d of finishing. Improved growth rate with addition of RH to the diet is usually accompanied by improved feed efficiency (Quinn et al., 2008; Walker et al., 2006). In addition, decreased serum urea nitrogen has been observed in response to RH supplementation (Bryant et al., 2010).

Zinc is an essential mineral functioning as an important component of enzymes involved in nucleic acid, protein, and carbohydrate metabolism (NRC, 2000). Zinc is intricately involved in protein synthesis, as it is required for activation of RNA polymerase (Wu and Wu, 1987) and is an essential component of enzymes involved in the urea cycle (Vallee, 1959). Feedlot performance typically is not affected by supplementing increased Zn concentrations (Greene et al., 1988); however, increased 12th rib s. c. fat, USDA yield grade, marbling, and quality grade have been observed with increased supplemental Zn (Malcolm-Callis et al., 2000; Spears and Kegley, 2002).

In a previous experiment, feeding 0 or 300 mg Zn/kg diet DM with and without zilpaterol hydrochloride, no interactions were observed for feedlot performance, carcass characteristics, or blood metabolites (Chapter 2 in this dissertation); however, no research has documented effects

of feeding increased supplemental Zn with RH. Because RH is a β_1-agonist, it is possible interactions may exist when feeding increased Zn with RH. The objectives of this study were to evaluate growth, carcass characteristics, and plasma urea nitrogen concentrations in finishing heifers supplemented RH at 0 or 200 mg/animal daily with 30 or 100 mg supplemental Zn/kg diet DM.

MATERIALS AND METHODS

Protocols and procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State University Beef Cattle Research Center, located in Manhattan, KS during the months of February and March 2016.

Animal Processing and Housing

Approximately 9 mo prior to the study, heifers were received over a period of 30 d. Upon arrival at the Kansas State University Beef Cattle Research Center, heifers were allowed *ad libitum* access to ground brome hay and water. Newly arrived animals were processed weekly as follows: heifers were individually weighed and received a numbered ear tag unique to each animal, they were treated with a metaphylaxis (Mictotil; Elanco Animal Health, Greenfield, IN), and topical parasiticide (Permectrin; Bayer Animal Health, Shawnee, KS), and vaccinated with Bovi-Shield Gold 5 (Zoetis, Florham Park, NJ), and Ultrabac 7 Somubac (Zoetis). Approximately 1 mo after arrival, heifers were implanted with Component TE-IH with Tylan (80 mg trenbolone acetate and 8 mg estradiol; Elanco Animal Health) and were re-implanted approximately 2 months prior to study initiation with Component TE-200 with Tylan (20 mg estradiol and 200 mg trenbolone acetate; Elanco Animal Health). Heifers were fed a diet consisting of 60% steam-flaked corn, 30% wet corn gluten feed, 8% ground alfalfa hay, and 2%

vitamin/mineral premix prior to study initiation. Heifers were housed as a group in dirt surfaced pens prior to the experiment, then were sorted into experimental pens 3 d before receiving experimental diets. For the study, heifers were housed in partially covered, concrete-surfaced pens equipped with fence-line feed bunks and automatic water fountains.

Experimental Design

One hundred and fifty six crossbred heifers (initial BW = 527 kg ± 6.61; gross BW × 0.96) were used in a randomized complete block experiment with a 2 × 2 factorial arrangement of treatments. Factors consisted of: 1) 30 or 100 mg supplemental Zn/kg diet DM (**30Zn** or **100Zn**) as Zn sulfate, and 2) 0 or 200 mg RH/animal daily (Elanco Animal Health, Greenfield, IN). Heifers were individually weighed and sorted by BW into blocks (4 pens/block), then randomly assigned within block to 52 pens with 3 heifers/pen (13 pens/treatment). Pen was randomly assigned to one of 4 treatments for a 43-d feeding trial. Ractopamine hydrochloride was administered 42 d, removed from the diet, and cattle were harvested on d 43 prior to slaughter. Diet compositions are presented in Table 3.1. Body weights were measured prior to feeding experimental rations on d 0 and on the day of harvest. Body weights were multiplied by 0.96 to account for 4% shrink during shipping. Average daily gains were computed by subtracting initial shrunk live weight from shrunk final BW and dividing by days on feed. Gain efficiencies were computed by dividing ADG by DMI.

Blood Collection and Analyses

Prior to study initiation, 2 heifers/pen were randomly selected for blood collection. Blood was drawn by jugular venipuncture approximately 3 h prior to feeding d 0 and 36 using a 6 mL mineral-free blood collection tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing K2 EDTA as anticoagulant for plasma Zn analysis, and one 10 mL blood collection

tube (Vacutainer, Becton Dickinson) containing potassium oxalate and sodium fluoride as anticoagulant for PUN analysis. Blood samples were immediately placed on ice and centrifuged within 30 min of sampling. Samples were centrifuged at 2,550 × g for 10 min at room temperature to recover plasma. Plasma was transferred by pipette into 12 × 75 mm polypropylene tubes and frozen at -20°C until analysis. Plasma urea nitrogen concentrations were determined according to the procedure described by Marsh et al. (1965) using the Auto Analyzer II (Seal Analytical, Mequon, WI). Plasma samples for Zn analysis were prepared by diluting 1 mL plasma with 3 mL deionized water, then diluted samples were analyzed using an atomic absorption spectrometer (Perkin Elmer Atomic Absorption Spectrometer 3110, PerkinElmer, Waltham, MA) set at a wavelength of 214 nm.

Diet Preparation and Feeding

Feed bunks were inspected daily and amounts of feed allocated to each pen were adjusted such that only 0.23 kg/heifer of residual feed remained the following day. Ractopamine hydrochloride was premixed with ground corn, and the mixture was fed at a rate of 0.23 kg/heifer daily, supplying 200 mg RH/animal daily. Cattle receiving no RH received an equal quantity of ground corn. Feed additive premixes and vitamin/mineral mixes were weighed daily and subsequently mixed into the total mixed ration. Diets were mixed in a truck mounted mixer, then dispensed into plastic feed tubs, weighed, and hand delivered to pens at approximately 0900 h. Heifers were allowed *ad libitum* access to feed. Feed amounts delivered to pens were recorded daily on as-fed basis and unconsumed feed was removed from the bunks and weighed prior to harvest. Subsamples of unconsumed feed were dried in a forced air oven at 55°C for 48 h to determine actual feed intake.

Harvest

Final BW (gross BW × 0.96) were determined immediately before transporting cattle 451 km to a commercial abattoir in Holcomb, KS (Tyson Fresh Meats). Hot carcass weights and incidence and severity of liver abscesses were recorded the day of harvest. Liver abscesses were scored according to the Elanco scoring system (Liver Abscess Technical Information AI 6288, Elanco Animal Health, Greenfield, IN): 0 = no abscesses, A- = 1 or 2 small abscesses or abscess scars, A0 = 2 to 4 small, well-organized abscesses and A+ = 1 or more large or active abscesses determined by with or without adhesions). Following 32 h of refrigeration, marbling score, 12th rib s. c. fat thickness, LM area, and USDA yield grade were obtained from camera images (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany) provided by the abattoir, and USDA quality grade and incidence and severity of dark cutting beef were determined by a certified USDA grader.

Statistical Analyses

The study was conducted as a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Feedlot performance and non-categorical carcass data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC). The model included fixed effects of Zn, RH, and the interaction between Zn and RH, random effect was block, and the experimental unit was pen. Blood measurements were analyzed using the same model, except day and the 2- and 3- way interactions between day, Zn, and RH were included as fixed effects. Binomial carcass data were analyzed using the GLIMMIX procedure of SAS with a model that included Zn, RH, and the interaction between Zn and RH as fixed effects, block as the random effect, and pen as the experimental unit. Least-square means were separated using the PDIFF

option. Differences among means were declared significant at α level ≤ 0.05 and trends between $P > 0.05$ and $P \leq 0.10$.

RESULTS AND DISCUSSION

Feedlot Performance

Ractopamine hydrochloride is a beta agonist that has been observed to improve feed efficiency by increasing ADG with little to no effect on DMI (Walker et al., 2006), but interactive effects with Zn supplementation have not been studied. In a previous experiment, steers were supplemented 60 or 300 mg Zn/kg diet DM with or without zilpaterol hydrochloride for a 24-d study. Supplementing 300 mg Zn/kg diet DM tended to reduce feed consumption d 18 thru 24 regardless of zilpaterol hydrochloride supplementation (Chapter 2) suggesting increased concentrations on supplemental Zn may negatively affect DMI. For the current study, effects of Zn and RH on feedlot performance are presented in Table 3.2. No Zn × RH interactions were observed for feedlot performance ($P \geq 0.24$). Supplementing RH at 200 mg/animal daily for 42 d increased final BW by 8 kg ($P = 0.02$) and increased ADG by 9% ($P < 0.01$), but did not affect DMI ($P = 0.63$). This resulted in a 10% improvement in feed efficiency ($P < 0.01$). Bryant et al. (2010) observed a 0.25 kg and 0.37 kg increase in ADG and 22% and 38% improvement in feed efficiency in steers fed 200 mg/steer daily RH and heifers fed 250 mg/heifer daily RH for 28 d, respectively. In agreement with current results, no effect was observed for DMI. Scramlin et al. (2010) observed a 9 kg increase in final BW and similar improvements in ADG and feed efficiency when RH was fed at 200 mg/animal daily for 33 d. Quinn et al. (2008) indicated further improvement in performance responses in heifers when RH feeding duration was extended from 28 to 42 d while maintaining a constant RH administration rate of 200 mg/animal

daily. The current results regarding RH supplementation on feedlot performance are in the range of averages reported in the meta-analysis by Lean et al. (2014).

In the current study, increasing supplemental Zn concentration from 30 to 100 mg/kg DM tended to decrease ADG ($P = 0.07$), but did not affect final BW, DMI, or feed efficiency ($P \geq 0.12$). Similarly, Nunnery et al. (2007) observed no differences for final BW, ADG, DMI or gain efficiency when heifers were supplemented 0 or 75 mg Zn/kg diet, regardless of source. In addition, supplementing 10 mg Zn/kg diet (as Zn proteinate, Zn polysaccharide, and Zn oxide), and 25 mg Zn/kg diet DM (as Zn oxide Zn proteinate) had no effect on final BW, feed intake, ADG, or feed efficiency (Kessler et al., 2003; Spears and Kegley, 2002, respectively). Greene et al. (1988) observed increased ADG d 0 to 28 of a 112-d study when heifers were supplemented 360 mg/d Zn oxide, but daily feed intake was not affected. Conversely, Malcolm-Callis et al. (2000) observed a linear decrease in ADG from d 28 to 56 when supplementing 20, 100, or 200 mg Zn/kg DM (as Zn sulfate), but overall ADG was not affected by increased Zn supplementation. In the same trial, increasing Zn supplementation concentration tended to decrease DMI linearly, but did not affect feed efficiency. Differences observed among studies may be due to Zn content of the basal rations used as controls, the amount of supplemental Zn, or Zn source. In the current experiment, the basal ration contained 31.9 mg Zn/kg diet DM, whereas in other studies basal Zn concentration ranged from 26 to 82 mg Zn/kg diet DM, therefore making it difficult to compare effects of supplemental Zn across studies. Results from the current study suggest supplementing increased concentrations of Zn had no effects on feedlot performance when RH was included in the diet.

Carcass Characteristics

Effects of supplemental Zn and RH on carcass characteristics are presented in Table 3.3. Zinc × RH interactions were observed for LM area ($P < 0.01$) and yield grade ($P = 0.01$), in which case heifers supplemented 100Zn and no RH had decreased LM area ($P < 0.01$) and increased yield grade ($P \leq 0.04$) compared to other treatments. This effect diminished with addition of RH to the diet. Oh and Choi (2004) indicated Zn has strong lipogenic activity in cultured bovine intramuscular adipocytes and the Zn finger protein Zfp423 is a key initiator of adipogenic differentiation (Huang et al., 2012), which may partially explain the increased yield grade, but not the lack of an effect on quality grade and 12th rib fat. In the current trial, a tendency for a Zn × RH interaction for dressing percentage ($P = 0.08$) was observed, in which heifers supplemented 30Zn with RH tended to have decreased dressing percentage compared to heifers supplemented 100Zn with RH, but no other interactions were detected for carcass traits ($P \geq 0.11$).

Hot carcass weight increased approximately 4 kg with RH supplementation in the current study. In agreement, Jennings et al. (2015) and Scramlin et al. (2010) observed increased HCW when RH was supplemented at 200 mg/animal daily for 28 or 33 d. In contrast to the current results, Arp et al. (2014) and Quinn et al. (2008) did not observe differences in HCW after feeding 200 mg/animal daily RH for 28 d; however, when steers were supplemented 300 and 400 mg/animal daily RH, carcass weight increased 4 and 6 kg, respectively (Arp et al., 2014). In the current study, RH supplementation did not affect dressing percentage, incidence of liver abscesses, 12th rib s. c. fat thickness, marbling score, or quality grade ($P \geq 0.13$), indicating RH supplementation did not impact adipose tissue deposition. Bryant et al. (2010) conducted a study in which steers were supplemented RH at 0, 100, or 200 mg/animal daily for 28 d and observed

no differences in marbling score or quality grade among treatments; however, dressing percent and LM area increased with addition of 200 mg/d RH. In the same experiment, no differences were observed for dressing percent, LM area, marbling score, or quality grade in carcasses from heifers supplemented with 0 or 250 mg/d RH, suggesting RH may affect steers and heifers differently. Heifers and steers fed 200 mg/d RH had increased LM area, but no differences were observed for HCW, yield grade, marbling score, or quality grade when results were combined for both sexes (Woerner et al., 2011). Average responses to RH supplementation include 6 kg increase for HCW, 1.8 cm^2 increase for LM area, 0.003 cm decrease for 12^{th} rib fat thickness, 5 unit decrease in marbling score, and 0.28% increase for dressing percentage (Lean et al., 2014). Lack of congruence among studies may be due to differences in study design, genotype, and sex of the animals used. Results from the current trial suggest RH increased lean tissue deposition, but did not affect adipose tissue.

Zinc is an essential co-factor for enzymes involved in cell growth, cell proliferation, and protein synthesis (MacDonald, 2000). Increasing the concentration of Zn in diets of finishing cattle may impact carcass traits. In the current study, no effects of Zn supplementation were observed for incidence of liver abscesses, 12^{th} rib s. c. fat thickness, marbling score, or quality grade ($P \geq 0.16$). In agreement, Nunnery et al. (2007) observed no effects for carcass traits from steers supplemented 75 mg Zn/ kg DM (as Zn sulfate, Zn methionine, or Zn propionate), regardless of source. Huerta et al. (2002) conducted a trial that assessed effects of dietary Zn and growth implants for steers and heifers. The authors supplemented 200 mg Zn/kg diet DM as Zn sulfate or Zn methionine and observed no effect of Zn concentration or source on carcass traits from steers; however, when effects were analyzed for heifers, Zn supplementation tended to decrease fat thickness and dressing percentage, but did not affect LM area, marbling score, yield

grade, or HCW. Greene et al. (1988) observed no differences for carcass traits when steers were supplemented 360 mg/d Zn oxide, but supplementing steers with Zn methionine increased marbling score and tended to increase fat thickness and quality grade, suggesting Zn source may affect animal responses to Zn supplementation. In addition, supplementing steers with 20, 100, or 200 mg Zn/kg DM resulted in a quadratic increase in 12th rib fat thickness and yield grade, but no effect on HCW, LM area, or marbling score (Malcolm-Callis et al., 2000). Spears and Kegley (2002) supplemented 25 mg Zn/kg DM (as Zn oxide or 2 forms of Zn proteinate) and observed Zn supplementation tended to increase HCW, and 12th rib fat thickness, and increased quality grade and marbling score, regardless of source. The authors suggested carcass responses to Zn supplementation may be related to the Zn concentration of the basal diet, in which animals fed basal rations below National Research Council (2000) recommendations may have a greater response to Zn supplementation compared to cattle receiving a basal ration at or above the recommendations.

Plasma Zn and urea nitrogen

Urea nitrogen concentrations can be used as an indicator of protein metabolism, and would be expected to decrease with administration of beta agonists due to increased muscle deposition with supplementation (Parr et al., 2014). Increased concentrations of supplemental Zn have been observed to decrease postruminal passage of bacterial AA resulting in a net negative effect on total postruminal AA passage (Froetschel et al., 1990), suggesting increased concentrations of Zn may influence ruminal protein metabolism. In addition, Zn is an important co-factor for enzymes associated with the urea cycle (Vallee and Falchuk, 1993). There were no Zn × RH × day (P = 0.26), Zn × RH (P = 0.62), or Zn × day (P 0.98) interactions observed for PUN concentration in the current study (Table 3.4). There was a tendency for RH × day

interaction ($P = 0.08$), in which PUN concentration tended to decrease with RH supplementation d 36 compared to d 0. In agreement with current results, Walker et al. (2010) supplemented steers and heifers 200 mg/d RH and observed PUN concentration tended to decrease in response to RH supplementation. Similarly, Bryant et al. (2010) observed decreased serum urea nitrogen when heifers were supplemented 250 mg/d RH. These results suggest decreased protein turnover to support increased protein deposition (Bryant et al. 2010).

In the present study, increased supplemental Zn did not affect PUN concentration ($P = 0.25$). Plasma urea nitrogen concentration increased d 36 compared to d 0 ($P < 0.01$), possible indicating an increased rate of protein degradation compared to protein synthesis towards the end of finishing. These results are in agreement with Kessler et al. (2003), who observed no difference in blood urea nitrogen between bull calves fed 0 or 10 mg Zn/kg DM (as Zn oxide, Zn proteinate, or Zn polysaccharide), regardless of source. Supplementing heifers 200 mg Zn/kg DM (as Zn sulfate or Zn methionine) tended to increase serum urea nitrogen concentration compared to control heifers fed diets without supplemental Zn (Huerta et al., 2002). No effect of Zn on PUN concentration would be expected due to the lack of an effect of Zn supplementation on HCW and LM area.

Plasma or serum Zn concentrations have been widely accepted for assessing Zn status. In the current study, there were no Zn × RH × day ($P = 0.70$), Zn × RH ($P = 0.58$), Zn × day ($P = 0.24$), or RH × day ($P = 0.90$) interactions observed for plasma Zn concentration, nor an effect of RH ($P = 0.22$; Table 3.4). Plasma Zn concentration increased when heifers were supplemented 100 mg Zn/kg diet DM ($P = 0.02$). We previously fed steers diets containing 60 or 300 mg Zn/kg diet DM with and without zilpaterol hydrochloride and observed no interaction between Zn and zilpaterol hydrochloride or effect of zilpaterol hydrochloride for plasma Zn (Chapter 2 in this

dissertation). Similarly, results of the current study suggest RH supplementation does not affect plasma Zn concentration. Huerta et al. (2002) observed increased serum Zn concentration from heifers and steers supplemented 200 mg Zn/kg DM from both Zn sulfate and Zn methionine. In disagreement with current results, Malcolm-Callis et al. (2000), Nunnery et al (2007), and Spears and Kegley (2002) observed no changes in plasma or serum Zn concentrations when supplemental Zn was increased in the diets. Plasma Zn concentration can decrease when animals are sick, injured, or stressed, therefore, caution must be taken when assessing effects of Zn on plasma Zn concentrations (NRC, 2001), which may contribute to the differences observed in plasma or serum Zn concentrations among studies.

In conclusion, supplementing increased concentrations of Zn sulfate to finishing heifers had little impact on feedlot performance and PUN concentration; however, muscle and fat deposition may be altered when fed in conjunction with RH.

REFERENCES

Arp, T. S., S. T. Howard, D. R. Woerner, J. A. Scanga, D. R. McKenna, W. H. Kolath, P. L. Chapman, J. D. Tatum, and K. E. Belk. 2014. Effects of dietary ractopamine hydrochloride and zilpaterol hydrochloride supplementation on performance, carcass traits, and carcass cutability in beef steers. J. Anim. Sci. 92:836–843. doi:10.2527/jas.2013-7122

Bryant, T. C., T. E. Engle, M. L. Galyean, J. J. Wagner, J. D. Tatum, R. V. Anthony, and S. B. Laudert. 2010. Effects of ractopamine and trenbolone acetate implants with or without estradiol on growth performance, carcass characteristics, adipogenic enzyme activity, and blood metabolites in feedlot steers and heifers. J. Anim. Sci. 88:4102–4119. doi:10.2527/jas.2010-2901

Froetschel, M. A., A. C. Martin, H. E. Amos, and J. J. Evans. 1990. Effects of zinc sulfate concentration and feeding frequency on ruminal protozoal numbers, fermentation patterns and amino acid passage in steers. J. Anim. Sci. 68:2874-2884. doi:/1990.6892874x

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schelling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J. Anim. Sci. 66:1818-1823. doi:10.2134/jas1988.6671818x

Huang, Y., A. K. Das, Q.-Y. Yang, M.-J. Zhu, and M. Du. 2012. Zfp423 Promotes Adipogenic Differentiation of Bovine Stromal Vascular Cells. PLoS ONE. 7:e47496. doi:10.1371/journal.pone.0047496

Huerta, M., R. L. Kincaid, J. D. Cronrath, J. Busboom, A. B. Johnson, and C. K. Swenson. 2002. Interaction of dietary zinc and growth implants on weight gain, carcass traits and zinc in tissues of growing beef steers and heifers. Anim. Feed Sci. Technol. 95:15-32. doi: 10.1016/s0377-8401(01)00334-0

Jennings, M. A., F. R. B. Ribeiro, T. R. Young, J. T. Cribbs, B. C. Bernhard, A. D Hosford, T. L. Harris, M. J. Anderson, G. J. Vogel, J. A. Scanga, M. F. Miller, and B. J. Johnson. 2015. Ractopamine hydrochloride and estradiol–trenbolone acetate implants alter live performance and carcass components of heifers during the finishing phase. Prof. Anim. Sci. 31:543–551. doi:10.15232/pas.2014-01370

Kessler, J., I. Morel, P. A. Dufey, A. Gutzwiller, A. Stern, and H. Geyer. 2003. Effect of organic zinc sources on performance, zinc status and carcass, meat and claw quality in fattening bulls. Livest. Prod. Sci. 81:161-171.

Lean, I. J., J. M. Thompson, and F. R. Dunshea. 2014. A meta-analysis of zilpaterol and ractopamine effects on feedlot performance, carcass traits and shear strength of meat in Cattle. PloS ONE. 9:e115904. doi: 10.1371/journal.pone.0115904

Malcolm-Callis, K. J., G. C. Duff, S. A. Gunter, E. B. Kegley, and D.A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics,

and serum values in finishing beef steers. J. Anim. Sci. 78:2801-2808. doi:/2000.78112801x

MacDonald, R. S. 2000. The role of zinc in growth and cell proliferation. J. Nutr. 130:1500S-1508S.

Marsh, W. H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct methods for the determination of blood urea. Clin. Chem. 11:624–627.

Mersmann, H. J. 2002. Beta-adrenergic receptor modulation of adipocyte metabolism and growth. J. Anim. Sci. 80 (E. Suppl. 1):E24–E29. doi:10.2134/animalsci2002.0021881200800ES10005x

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Nunnery, G. A., J. T. Vasconcelos, C. H. Parsons, G. B. Salyer, P. J. Defoor, F. R. Valdez, and M. L. Galyean. 2007. Effects of source of supplemental zinc on performance and humoral immunity in beef heifers. J. Anim. Sci. 85:2304–2313. doi:10.2527/jas.2007-0167

Oh, Y. S., and C. B. Choi. 2004. Effects of zinc on lipogenesis of bovine intramuscular adipocytes. Asian-Australas. J. Anim. Sci. 17:1378–1382. doi:10.5713/ajas.2004.1378

Parr, S. L., T. R. Brown, F. R. B. Ribeiro, K. Y. Chung, J. P. Hutcheson, B. R. Blackwell, P. N. Smith, and B. J. Johnson. 2014. Biological responses of beef steers to steroidal implants and zilpaterol hydrochloride. J. Anim. Sci. 92:3348–3363. doi:10.2527/jas.2013-7221

Quinn, M. J., C. D. Reinhardt, E. R. Loe, B. E. Depenbusch, M. E. Corrigan, M. L. May, and J. S. Drouillard. 2008. The effects of ractopamine-hydrogen chloride (Optaflexx) on performance, carcass characteristics, and meat quality of finishing feedlot heifers. J. Anim. Sci. 86:902–908. doi:10.2527/jas.2007-0117

Scramlin, S. M., W. J. Platter, R. A. Gomez, W. T. Choat, F. K. McKeith, and J. Killefer. 2010. Comparative effects of ractopamine hydrochloride and zilpaterol hydrochloride on growth performance, carcass traits, and longissimus tenderness of finishing steers. J. Anim. Sci. 88:1823–1829. doi:10.2527/jas.2009-2405

Spears, J. W., and E. B. Kegley. 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J. Anim. Sci. 80:2747-2752. doi:/2002.80102747x

Vallee, B. L. 1959. Biochemistry, physiology, and pathology of zinc. Physiol. Rev. 39: 443-490.

Vallee, B L., and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. Physiol. Rev. 73:79-118.

Walker, D. K., E. C. Titgemeyer, J. S. Drouillard, E. R. Loe, B. E. Depenbusch, and A. S. Webb. 2006. Effects of ractopamine and protein source on growth performance and carcass characteristics of feedlot heifers. J. Anim. Sci. 84:2795–2800. doi:10.2527/jas.2005-614

Walker, D. K., E. C. Titgemeyer, T. J. Baxa, K. Y. Chung, D. E. Johnson, S. B. Laudert, and B. J. Johnson. 2010. Effects of ractopamine and sex on serum metabolites and skeletal muscle gene expression in finishing steers and heifers. J. Anim. Sci. 88:1349–1357. doi:10.2527/jas.2009-2409

Woerner, D. R., J. D. Tatum, T. E. Engle, K. E. Belk, and D. W. Couch. 2010. Effects of sequential implanting and ractopamine hydrochloride supplementation on carcass characteristics and longissimus muscle tenderness of calf-fed steers and heifers. J. Anim. Sci. 89:201–209. doi:10.2527/jas.2010-2857

Wu, F., and C. Wu. 1987. Zinc in DNA replication and transcription. Ann. Rev. Nutr. 7:251–272. doi:10.1146/annurev.nutr.7.1.251

Table 3.1. Diet composition of heifers fed 0 or 200 mg/animal daily ractopamine hydrochloride (**RH**) and 30 (**30Zn**) or 100 (**100Zn**) mg supplemental Zn/kg diet DM for 43 d.

	No RH		RH	
	30Zn	100Zn	30Zn	100Zn
Ingredients, % DM basis				
Steam-flaked corn	58.90	58.88	58.90	58.88
Wet corn gluten feed	30.00	30.00	30.00	30.00
Ground alfalfa hay	7.00	7.00	7.00	7.00
Vitamin/mineral premix[1]	0.11	0.11	0.11	0.11
Limestone	1.49	1.49	1.49	1.49
Salt	0.30	0.30	0.30	0.30
RH mix[2]	-	-	2.20	2.20
Ground corn	2.20	2.20	-	-
Zinc sulfate	-	0.02	-	0.02
Calculated nutrient composition[3]				
CP, %	14.08	14.08	14.08	14.08
Ca, %	0.69	0.69	0.69	0.69
P,%	0.48	0.48	0.48	0.48
NDF,%	19.03	19.03	19.03	19.03
Zn, mg/kg	61.90	131.90	61.90	131.90

[1]Formulated to provide; 300 mg/d monensin (Elanco Animal Health, Greenfield, IN); 2,200 IU/kg vitamin A; 22 IU/kg vitamin E; 10 mg/kg added Cu (Cu sulfate); 30 mg/kg added Zn (as Zn sulfate); 20 mg/kg added Mn (as Mn sulfate); 0.5 mg/kg added I (ethylenediamine dihydriodide); 0.1 mg/kg added Se (Na selenite); and 0.15 mg/kg Co (Co carbonate).

[2]Ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN) was fed 42 d and formulated to provide 200 mg/animal daily mixed in a ground corn carrier, then removed from the diet until cattle were harvested on d 43.

[3]Calculated from NRC (2000) values for individual ingredients.

Table 3.2. Feedlot performance of heifers fed 0 or 200 mg/animal daily ractopamine hydrochloride (**RH**) and supplemented 30 (**30Zn**) or 100 (**100Zn**) mg Zn/kg diet DM for 43 d[1]

	No RH		RH			*P*-value		
Item	30Zn	100Zn	30Zn	100Zn	SEM	Zn	RH	Zn × RH
Initial BW[2], kg	527	526	527	528	6.61	0.85	0.75	0.47
Final BW[2], kg	607	603	617	610	6.39	0.17	0.02	0.58
ADG, kg/d	1.85	1.80	2.10	1.91	0.06	0.07	< 0.01	0.24
DMI, kg/d	11.27	11.27	11.37	10.99	0.20	0.32	0.63	0.32
G:F	0.1636	0.1601	0.1847	0.1733	0.0053	0.12	< 0.01	0.39

[1]Ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN) was fed for 42 d, then removed from the diet until cattle were harvested on d 43.

[2]Calculated as: gross BW × 0.96.

Table 3.3. Carcass traits of heifers receiving 0 or 200 mg/animal daily ractopamine hydrochloride (**RH**) and 30 (**30Zn**) or 100 (**100Zn**) mg supplemental Zn/kg diet DM for a 43-d experiment[1,2]

	No RH		RH			*P*-value		
Item	30Zn	100Zn	30Zn	100Zn	SEM	Zn	RH	Zn × RH
HCW, kg	385	382	388	388	4.07	0.50	0.03	0.43
Dressed yield, %	63.54	63.33	62.97	63.71	0.26	0.33	0.74	0.08
Liver abscess, %	43.6	30.8	30.8	23.1	7.71	0.19	0.19	0.74
LM area, cm^2	99.9[a]	91.7[b]	97.7[a]	99.1[a]	1.55	0.03	0.07	< 0.01
12^{th}-rib fat, cm	1.39	1.57	1.45	1.33	0.09	0.78	0.32	0.11
USDA yield grade	2.3[b]	2.9[a]	2.5[b]	2.3[b]	0.14	0.14	0.14	0.01
Marbling score[3]	521	523	509	485	18.71	0.48	0.13	0.41
Prime, %	5.13	5.13	7.69	5.13	3.63	0.72	0.72	0.72
Choice, %	74.36	64.10	61.54	66.67	7.18	0.72	0.48	0.29
Select, %	10.25	23.08	15.38	20.51	6.24	0.16	0.84	0.54
Sub-Select[4], %	5.13	2.56	2.56	5.13	2.63	1.00	1.00	0.34
Dark cutters, %	5.13	5.13	12.82	2.56	4.63	0.22	0.53	0.22

[a,b]Within a row, means without a common superscript are different ($P < 0.05$).

[1]Ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN) was fed for 42 d, removed from the diet until cattle were harvested on d 43..

[2]Following 32 h of refrigeration, 12^{th} rib s. c. fat thickness, LM area, and USDA yield grade were obtained from camera images (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany) provided by the abattoir, and USDA quality grade and incidence and severity of dark cutting beef were determined by a certified USDA grader.

[3]Marbling scores were determined by camera images (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany) provided by the abattoir; Small = 400 to 499; Modest = 500 to 599.

[4]Includes carcasses that graded standard or commercial.

Table 3.4. Concentrations of plasma urea nitrogen and Zn d 0 and 36 from heifers fed 0 or 200 mg/animal daily ractopamine hydrochloride (**RH**) and 30 (**30Zn**) or 100 (**100Zn**) mg supplemental Zn/kg diet DM[1]

	D 0				D 36				
	No RH		RH		No RH		RH		
Item	30Zn	100Zn	30Zn	100Zn	30Zn	100Zn	30Zn	100Zn	SEM
PUN[2,3], m*M*	3.33	3.50	3.51	3.56	4.51	4.47	4.12	4.40	0.14
Zinc[3,4], mg/L	0.96	0.97	0.90	0.96	1.53	1.62	1.49	1.59	0.04

[1]Ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN) was fed for 42 d, then removed from the diet until cattle were harvested on d 43.

[2]Ractopamine hydrochloride × day interaction ($P = 0.08$).

[3]Effect of day ($P < 0.01$).

[4]Effect of Zn ($P = 0.02$).

Chapter 4 - Effects of supplemental zinc sulfate concentrations on growth performance and carcass characteristics of feedlot heifers, and *in vitro* ruminal fermentative activity

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ABSTRACT

Effects of supplemental Zn as Zn sulfate on feedlot performance and carcass characteristics were evaluated using 480 crossbred heifers (BW = 385 kg ± 13.08) in a randomized complete block design. Heifers were blocked by BW and randomly assigned within block to receive 0, 30, 60, or 90 mg supplemental Zn/kg diet DM. Heifers were housed in dirt-surfaced pens (20 animals/pen; 6 pens/treatment) equipped with fence-line feed bunks and automatic water fountains. Heifers were fed once daily for *ad libitum* intake. Plasma was collected days 0, 63, and 115 from 5 heifers/pen to determine plasma Zn concentrations. Heifers were transported on d 144 to a commercial abattoir where HCW and incidence of liver abscesses were recorded at harvest and carcass data were recorded after 36 h of refrigeration. Plasma Zn concentration increased ($P = 0.02$) linearly in response to increasing concentrations of dietary Zn. Final BW and ADG were not affected by supplementation ($P \geq 0.29$). Increasing dietary Zn concentrations tended to decrease (linear effect, $P = 0.07$) DMI, resulting in a linear ($P = 0.03$) and tendency for quadratic ($P = 0.12$) improvement in feed efficiency with increasing Zn concentration. No differences were detected for HCW, dressing percentage, LM area, 12^{th} rib fat, percentages of carcasses grading Select or Choice, or yield grade ($P > 0.53$). There was a tendency for a quadratic effect ($P = 0.07$) of Zn concentration on percentage of carcasses graded as USDA Prime, with percent Prime peaking at 60 mg/kg added Zn. Total carcass value was not impacted by supplemental Zn ($P = 0.25$). *In vitro* fermentations were performed using ruminal fluid cultures containing 0, 30, 60, 90, 120, 150 mg Zn/kg substrate DM to determine impact of Zn on gas production, VFA concentrations, and IVDMD. There was no Zn × time interaction or effect of Zn on *in vitro* gas production ($P \geq 0.59$). Zinc supplementation tended to reduce acetate (quadratic effect; $P = 0.07$), and decreased isovalerate (linear effect; $P = 0.05$), but did not affect

other VFA ($P \geq 0.17$) or IVDMD ($P \geq 0.20$). Overall, Zn supplementation up to 150 mg/kg substrate weight minimally affected *in vitro* fermentation. Supplementing finishing heifers up to 60 mg Zn/kg diet DM improved feed efficiency, but further supplementation to 90 mg Zn/kg diet DM may result in reduced feed efficiency.

Keywords; feedlot cattle, feed efficiency, zinc

INTRODUCTION

Zinc is a micro mineral that is an essential component of over 300 enzymes and over 2,000 transcription factors in microorganisms, plants, and animals (Vallee and Falchuk, 1993). The enzymes and protein gene expression associated with Zn affect growth; hormone production, secretion, and storage; skin and wound healing; immune function; reproduction; cell division; microbial growth; and macronutrient and nucleic acid metabolism (McDowell, 1992). Zinc has a role as a catalyst in enzymes such as RNA polymerase, alkaline phosphatase and carbonic dehydrogenase (NRC, 2001), and has a key role in formation of structural proteins during the keratinization process (Tomlinson et al. 2004) of the skin and hoof.

Research evaluating effects of Zn supplementation in the diets of finishing cattle is limited. Malcolm-Callis et al. (2000) supplemented 20, 100, or 200 mg Zn/kg as Zn sulfate to finishing steers and observed a tendency for a linear decrease in DMI with increasing Zn content, and quadratic effects for 12th rib fat thickness and yield grade. Nunnery et al. (2007) observed no effects on performance or carcass traits when 75 mg Zn/kg was supplemented to heifers. In a previous experiment using crossbred steers, feeding 300 mg Zn/kg diet DM tended to decrease feed allocated daily d 18 to 24 during a 24 day study (Chapter 2 of this dissertation), and supplementing heifers 100 mg Zn/kg DM resulted in decreased LM area and increased yield

grade (Chapter 3 of this dissertation). In addition, increased supplemental Zn has been observed to alter ruminal fermentation (Spears et al., 2004).

The NRC (2000) recommends a minimum of 30 mg Zn/kg diet to prevent growth retardation and skin abnormalities in beef cattle, but no recommendations are made specifically for finishing cattle. The objectives of this study were to evaluate feedlot performance and carcass characteristics of finishing heifers supplemented 4 concentrations of Zn as Zn sulfate and to assess changes in ruminal gas and VFA production, IVDMD and pH *in vitro*.

MATERIALS AND METHODS

Protocols and procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State University Beef Cattle Research Center in Manhattan, KS.

Experimental Design

The study was conducted using 480 crossbred yearling heifers (initial BW = 385 kg ± 13.08) in a randomized complete block design with 4 treatments. Heifers were blocked by initial BW and randomly assigned within block (3 blocks) to experimental pens, and pens were randomly assigned to treatment. Heifers were housed in 24 pens containing 20 animals/pen (6 pens/treatment). To determine if block had an effect on responses to treatment, each block contained 2 treatment replications/block. Treatments consisted of heifers supplemented with 0, 30, 60, or 90 mg Zn/kg diet DM (**0, 30, 60,** or **90Zn**). The basal ration contained approximately 32 mg Zn/kg diet DM (calculated from NRC values for individual ingredients), therefore treatments were chosen based on the minimum recommendation by the NRC (2000) of 30 mg Zn/kg diet, and was within the range of concentrations recommended by feedlot consulting nutritionists (Vasconcelos and Galyean, 2007). Previous research at the Kansas State University

Beef Cattle Research Center indicated supplementing finishing cattle with 60 mg Zn/kg diet DM as Zn sulfate plus 3 g/animal daily (approximately 96 mg total supplemental Zn/kg diet) of Zn methionine (Zinpro 120, Zinpro Corporation, Eden Prairie, MN) may reduce feed intake (L. Miranda, C. L. Van Bibber-Krueger, and J. S. Drouillard, Kansas State University, Manhattan, KS, personal communication), therefore 90 mg Zn/kg diet DM was used for the high concentration. Final finishing diet compositions are presented in Table 4.1.

Animal Processing, Housing, and Handling

On arrival at Kansas State University Beef Cattle Research Center, heifers were allowed *ad libitum* access to ground brome hay and water. Approximately one week after arrival, heifers were tagged with an ear tag that displayed a number unique to individual animals, vaccinated with Bovi-Shield Gold 5 (Zoetis, Florham Park, NJ) and Ultra-Bac 7 Somubac (Zoetis), and treated with a topical parasiticide (Permectrin; Bayer Animal Health, Shawnee, KS) and metaphylaxis (Micotil; Elanco, Greenfield, IN). Heifers received a grower ration consisting of 50% roughage and 50% concentrate for 4 mo prior to study initiation. Before beginning the trial and 2 mo thereafter, heifers were implanted with Component TE-200 with Tylan (20 mg estradiol and 200 mg trenbolone acetate implant; Elanco Animal Health). Heifers were housed in dirt surfaced pens that contained fence-line feed bunks and automatic waterers that were shared between adjacent pens, and were allowed *ad libitum* access to feed and water. Body weights were captured for each pen at 28-d intervals, and again at the end of feedlot finishing. Average daily gain was computed by subtracting initial BW from final BW, and divided by days on feed (DOF). Feed efficiencies were computed as ADG dividing by DMI.

Five heifers per pen were randomly selected at the beginning of the study and were tagged with colored tags for identification for blood collections. Blood was collected

approximately 2 h prior to feeding by jugular venipuncture d 0, 63, and 115 for determination of plasma Zn concentration using trace mineral free blood collection tubes containing K_2 EDTA as anticoagulant (BD Vacutainer, Franklin Lakes, NJ). Blood collection lasted approximately 10 min/pen with cattle returned to their pens within 20 min of sample collection. Samples were immediately placed on ice and were centrifuged within 20 min of sampling at 2,550 × *g* for 10 min at room temperature. Plasma was transferred by pipette into 5-mL plastic tubes, capped, and frozen at -20ºC for subsequent analysis.

Diet Preparation and Feeding

Heifers were transitioned to finishing diets through 3 graduated step-up diets with an 11% increase in steam-flaked corn and proportional decrease in roughage over a period of 7 d/step to allow gradual adaptation to the final high-grain diet. During the step up period, inclusion level of wet corn gluten feed, vitamin/mineral premix, and feed additive premix remained constant. Vitamin/mineral premixes were formulated to provide either 0 or 90 mg supplemental Zn/kg diet DM as zinc sulfate (**0 Zn** or **90 Zn premix**). To obtain vitamin/mineral mixes containing 30 or 60 mg supplemental Zn/kg diet DM, 0 Zn premix:90 Zn premix ratios of 66:34 or 34:66 were combined, respectively. Ractopamine hydrochloride (**RH**; Elanco Animal Health, Greenfield, IN) was mixed in a ground corn carrier and fed at a rate of 90 g/d to supply 300 mg RH/heifer daily the final 28 d prior to harvest. Melengesterol acetate (Elanco Animal Health) was removed from the feed additive premix the final 18 days on feed due to a shortage of product.

Feed additive premixes and vitamin/mineral mixes were weighed daily and subsequently mixed into total mixed rations. Diets were mixed once daily in a truck mounted mixer and delivered to cattle at approximately 0800. Feed was monitored and adjusted daily prior to

feeding so less than 0.23 kg/heifer of residual feed remained the following day. Weights of fresh feed were recorded daily, and unconsumed feed was removed from the bunk and weighed every 28 d or as needed to determine DMI. Subsamples of unconsumed feed were dried at 55°C for 48 h to determine DM content. Dry matter intake was calculated as: [(total feed offered × % DM) - (total feed refused × % DM)] / (number of animal × day).

Harvest

On d 144, final BW (gross BW × 0.96) was obtained, then heifers were transported 322 km to a commercial abattoir in Lexington, NE. On the day of harvest, HCW, and incidence and severity of liver abscesses were recorded. Liver abscesses were scored according to the Elanco scoring system (Liver Abscess Technical Information AI 6288, Elanco Animal Health, Greenfield, IN): 0 = no abscesses, A^- = 1 or 2 small abscesses or abscess scars, A^0 = 2 to 4 small, well-organized abscesses, and A^+ = 1 or more large or active abscesses with or without adhesions. After approximately 36 h of refrigeration, USDA yield grades, marbling score, 12th rib s. c. fat thickness, and LM area were collected by camera images (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany) provided by the abattoir, and quality grade and incidence of dark cutting beef were determined by a certified USDA grader. Total carcass value was calculated based on the average base price of cattle from the past 5 years obtained from the USDA (http://www.ers.usda.gov/data-products/meat-price-spreads.aspx). Premium and discount data was averaged over a 5 year period obtained from the USDA AMS report and compiled by Innovative Livestock Services, Inc. (Great Bend, KS). Carcass value was calculated as: (Average base price ($/kg) + (-) quality grade premiums or discounts + (-) yield grade and 12th rib s. c. fat thickness premiums or discounts + (-) HCW premiums or discounts) × (HCW, kg/45).

In Vitro Fermentation

Ruminal fluid was collected from a ruminally fistulated Holstein heifer consuming a diet consisting of primarily forage, approximately 10 h after feeding. Ruminal fluid was strained through 4 layers of cheese cloth, placed directly into pre-warmed insulated water jugs, and transported 2 km to the Pre-Harvest Micro Safety Laboratory on Kansas State University main campus (Manhattan, KS). Upon arrival, ruminal fluid was strained through 8 layers of cheese cloth, placed into a large separatory funnel, gassed with nitrogen, and incubated at 39°C for approximately 1 h to allow stratification into layers. Layers consisted of a top mat layer, a bottom sediment layer consisting of feed particles, and an intermediate layer consisting of microbe-rich fluid. The bottom sediment layer was discarded, and the intermediate layer was collected taking care to minimize mixing with the mat layer for *in vitro* fermentation use.

The study was conducted as a randomized complete block (replicate) design with treatments consisting of 0, 30, 60, 90, 120, 150 mg added Zn/kg substrate DM (5 replicates) as Zn sulfate heptahydrate. Zinc sulfate heptahydrate was solubilized in 10 mL deionized water equal to treatment concentrations. Three grams of substrate (90% ground corn, 10% soybean meal) was added to each fermentation flask and pre-wetted with 140 mL of McDougall's buffer (McDougall, 1948). Blank bottles (1/replicate) received no substrate, and served for baseline measurements of VFA profiles and substrate contributions of ruminal fluid. One milliliter of the Zn solution was added to the appropriate flask or an equal volume of deionized water was added to flasks serving as blanks and flasks receiving no supplemental Zn. Strained ruminal fluid (10 mL/bottle) was added and initial pH was recorded using Thermo Orion benchtop pH meter (model 230 A, Thermo Fisher Scientific Inc., Waltham, MA) while bottles were gassed briefly with nitrogen gas. Fermentation flasks were then capped with an Ankom gas pressure monitor

(Ankom Gas Production System, Ankom Technology), placed into a shaking incubator set at 39°C (New Brunswick Scientific Inc., New Brunswick, NJ), and were gently agitated continuously for 24 h. Gas pressure within each vessel was monitored and cumulative pressure was recorded in 15-min intervals.

After a 24-h fermentation period, gas pressures were converted into moles of gas (n) using the following 'ideal' gas law (Ankom Technology Corp., 2014).

$$n = p\,(V/RT)$$

In this formula, n is gas produced (mol), p is pressure (kPa), V is head-space volume in the flasks (L), T is temperature (K), and R is gas constant (8.314472 L.kPa.K-1.mol-1). Moles of gas were subsequently converted into volume of gas (mL) using Avogadro's law (Ankom Technology Corp.).

$$\text{Gas produced (mL)} = n \times 22.4 \times 1000$$

Where 22.4 is the volume occupied by 1 mole of gas at 39°C.

Final pH was recorded for each bottle using Thermo Orion benchtop pH meter (model 230 A, Thermo Fisher Scientific Inc.) after 24 h of incubation. Four milliliters of fluid contents from each bottle was combined with 1 mL of 25% (w/v) meta-phosphoric acid solution and frozen at -20°C. Remaining samples in fermentation flasks were placed into 19.0 × 12.7-cm aluminum pans and dried for 24 h at 105ºC to determine IVDMD. Calculations of percent IVDMD were as follows: [substrate weight – (dry pan weight – initial pan weight – average residue weight of blanks)]/substrate weight.

Volatile Fatty Acid Analyses

After ruminal fluid combined with meta-phosphoric acid was thawed, 2 mL of each mixture were placed into micro centrifuge tubes and centrifuged at 17,000 × *g* for 10 min.

Supernatant was removed, placed into storage vials, and stored in a freezer at -20ºC until VFA analysis. For VFA analyses, 2 mL of supernatant was pipetted into 12 × 75 gas chromatography vials. The volatile fatty acid standard from Supelco (Supelco, Inc., Bellefonte, PA) was used as the standard. The analysis was carried out using an Agilent 7890A GC (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector and a 15 m × 0.53 mm × 0.5 µm Supelco 25326 Nukol column (Supelco, Inc.) using hydrogen gas as the carrier. The initial oven temperature was 100ºC and increased 10ºC/min to a final temperature of 220ºC held for 2 min and flow rate was 5.1 mL/min with an average velocity of 45 cm/sec.

Statistical Analyses

All growth performance, carcass characteristics, and total carcass value were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC). There were no block × treatment effects when block was included as a fixed effect, therefore block was removed from the fixed effects statement and included in the random statement for analyses. Supplemental Zn concentration was the fixed effect and pen was the experimental unit. For plasma Zn concentration analysis, the statistical model was the same except day of plasma collection and the interaction between treatment and day were included as fixed effects. *In vitro* VFA production, final pH, and IVDMD were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.) using replicate as the random effect, fermentation flask as the experimental unit, and supplemental Zn concentration as the fixed effect. Gas production for *in vitro* fermentation was analyzed as a repeated measure using the MIXED procedure of SAS. The model included fixed effects of supplemental Zn concentration, time and the interaction between supplemental Zn and time, and replicate (block) as the random effect. The repeated statement included time, bottle within treatment as the subject, and compound symmetry as the covariance structure. The

Kenward-Rogers correction was applied for degrees of freedom estimation for all analyses. All variables were tested for the polynomial contrast 0Zn vs. the average of supplemental Zn concentrations, and supplemental Zn concentrations were tested for linear and quadratic responses. Least squares means (LSMEANS) were calculated for each treatment. Separations of means were determined to be significant at α level ≤ 0.05 and *P*-values falling between 0.05 and 0.15 were considered tendencies.

RESULTS AND DISCUSSION

One animal supplemented 60Zn was removed from the study due to a severe lameness. One heifer was removed from the 30Zn treatment due to death from an unknown cause. Two heifers were removed from the 0Zn group; one died due to septicemia originating from a shoulder abscess, and the other heifer died from severe interstitial emphysema in the lungs.

Plasma Zn

A linear increase in plasma Zn concentration was observed with increasing supplemental Zn ($P = 0.02$; Fig. 4.1). In addition, an effect of day was detected, in which plasma Zn concentration increased as the number of days on feed increased, regardless of supplemental Zn concentration ($P < 0.01$), but no Zn × day interaction was observed ($P = 0.99$). Previous results with regards to plasma or serum Zn concentrations have been inconclusive. In disagreement, Malcolm-Callis et al. (2000), Nunnery et al. (2007), and Spears and Kegley (2002) observed no changes in plasma or serum Zn concentration regardless of source, or supplementation concentration. In agreement with current results, Huerta et al. (2002) observed increased serum Zn concentration in steers and heifers supplemented 200 mg Zn/kg DM. The NRC (2001) indicated serum Zn concentrations within the range of 0.7 to 1.3 µg/mL is considered normal; however, stress, infection, or disease can cause a redistribution of Zn out of extracellular fluids

resulting in decreased serum Zn concentrations causing plasma or serum Zn concentrations to be an unreliable indicator of Zn status (Hambidge et al., 1986).

Feedlot Performance

Effects of supplemental Zn on feedlot performance are presented in Table 4.2. There were no effects of treatment, linear or quadratic effects of Zn, or difference between cattle receiving 0Zn and the average of Zn supplemented cattle for final BW ($P \geq 0.37$) or ADG ($P \geq 0.29$). Zinc supplementation has commonly been observed to have no effect on final BW or ADG when supplemented in increased concentrations, regardless of source (Huerta et al., 2002; Malcolm-Callis et al., 2000; Nunnery et al., 2007).

Supplementing increased concentrations of Zn tended to linearly decrease DMI ($P = 0.07$), but there was no quadratic effect of Zn ($P \geq 0.87$). Feed efficiency improved linearly with increasing supplemental Zn ($P = 0.03$) and a tendency was observed for a quadratic effect of Zn ($P = 0.12$) in G:F. Feed efficiency tended to be optimized with addition of 60Zn followed by a 3% reduction in G:F when 90Zn was supplemented. Heifers supplemented Zn, regardless of concentration, had improved feed efficiencies compared to heifers supplemented 0Zn ($P = 0.04$), suggesting finishing cattle require increased concentrations of Zn than what is recommended by NRC (2000) for beef cattle. Previous research comparing multiple concentrations of supplemental Zn are limited with regards to effects of Zn supplementation on finishing cattle performance. Malcolm-Callis et al. (2000) supplemented 20, 100, and 200 mg Zn/kg DM to finishing steers fed a diet containing approximately 70 mg/kg Zn. Similar to current results, the authors observed increased supplemental Zn tended to reduce DMI in a linear manner; however, decreased DMI did not result in improved feed efficiency. When comparing effects of no supplemental Zn to diets containing 75 mg Zn/kg DM as Zn sulfate, Zn methionine, or Zn

proteinate fed to heifers, Zn supplementation did not affect ADG, but feed efficiency tended to increase with added Zn, regardless of source (Nunnery et al., 2007). In disagreement, supplementing 25 mg Zn/kg DM (as Zn oxide or 2 forms of Zn proteinate) to steers had no effect on DMI or feed efficiency compared to cattle consuming a basal diet that contained 26 mg/kg Zn (Spears and Kegley, 2002). Supplementing 360 mg/d (as Zn oxide or Zn methionine) to steers consuming a basal ration that contained 82 mg/kg Zn had no effect on as-fed daily feed intake (Greene et al., 1988). Kessler et al. (2003) supplemented finishing bulls with 10 mg Zn/kg DM in the form of Zn oxide, Zn proteinate, or Zn polysaccharide and observed no effect of Zn supplementation for final BW, growth rate, or feed efficiency compared to bulls fed a basal ration consisting of 35 mg/kg Zn. Zinc requirements for finishing cattle are not clearly defined and little is known regarding factors that may influence Zn requirements. Increased supplemental Zn has been observed interfere with absorption of other minerals, such as copper (Ivan and Grieve, 1975). It is conceivable increasing supplemental Zn may cause unknown interactions with other trace minerals, resulting in reduced DMI. Our results suggest supplementing increased concentrations of Zn may negatively affect DMI; however, ADG was not affected, resulting in improved feed efficiency with a tendency for no further improvement supplementing above 60 mg Zn/kg diet DM.

Carcass Characteristics

Effects of Zn supplementation on carcass characteristics are presented in Table 4.3. A tendency for a treatment effect was observed for marbling score ($P = 0.08$) with carcasses from heifers supplemented 60Zn tending to have greater marbling scores compared to carcasses from heifers supplemented 90 or 30Zn ($P \leq 0.07$), but not when compared to carcasses from heifers supplemented with no Zn ($P = 0.17$). No effects of treatment, linear or quadratic effects of Zn, or

differences between 0Zn and the average of Zn treatments were observed for HCW, dressing percentage, incidence of liver abscesses, LM area, 12th rib fat thickness, overall yield grade, or total carcass value ($P \geq 0.18$). Effects of Zn on yield and quality grades are presented in Table 4.4. Tendencies for quadratic effects of Zn were observed for carcasses grading USDA Prime ($P = 0.07$) and USDA yield grade 1 and 3 ($P = 0.07$), with carcass from heifers fed 60Zn tending to have more carcasses grading Prime and fewer yield grade 1 carcasses compared to the other treatments. Zinc supplementation did not affect other yield or quality grades ($P \geq 0.22$).

In an *in vitro* system, Oh and Choi (2004) conducted a study to determine the lipogenic activity of 0, 5, 25, 50, and 100 µM Zn in bovine intramuscular adipocytes. They observed an increase in glycerol-3-phosphate dehydrogenase activity, which is an indicator of adipocyte differentiation, with the highest activity when 25 µM was included in the media suggesting a maximum concentration of added Zn before adipocyte differentiation is no longer affected. Zinc finger proteins have been observed to have major regulatory roles in the process of adipogenesis (Wei et al., 2013). At least one Zn finger protein has been isolated in bovine stromal vascular cells, and it has been implicated as a key initiator in early adipose determination (Huang et al., 2012), providing a possible explanation for the tendencies of Zn supplementation to affect marbling score, and percentage of carcasses grading Prime and yield grade one. Supplementing steers 20, 100, or 200 mg Zn/kg DM as Zn sulfate resulted in quadratic responses of Zn for 12th rib fat thickness and yield grade with carcasses from steers supplemented 100 mg Zn/kg DM having increased 12th rib fat thickness and increased yield grade compared to carcasses from steers supplemented 20 or 200 mg Zn/kg DM, but supplementation did not affect HCW, dressing percentage, LM area, or marbling score (Malcom-Callis et al., 2000). Huerta et al. (2002) observed a tendency for decreased 12th rib fat thickness and dressing percentage, but no effects

on marbling score, yield grade, or quality grade when heifers were supplemented 200 mg Zn/kg DM compared to heifers fed a diet containing 64 mg/kg Zn. In the same study, steers were supplemented the same concentration of Zn compared to steers fed a basal ration containing 84 mg/kg Zn. No differences were observed between supplemented and un-supplemented steers for any carcass trait suggesting effects of Zn supplementation may be influenced by sex (Huerta et al., 2002). Nunnery et al. (2007) observed no effect of Zn supplementation on carcass traits when heifers were supplemented 75 mg Zn/kg DM in the form of Zn sulfate, Zn methionine, or Zn propionate, regardless of source. Greene et al. (1988) observed increased 12^{th} rib fat thickness and marbling scores, and a tendency for increased quality grade when 360 mg Zn/d from Zn methionine was supplemented, but not when 360 mg Zn/d from Zn oxide was supplemented suggesting Zn source may affect carcass responses. Steers supplemented 25 mg Zn/kg DM from Zn oxide or 2 forms of Zn proteinate had increased quality grades and increased marbling scores compared to un-supplemented steers, regardless of source (Spears and Kegley, 2002). The authors speculated increasing supplementary Zn concentrations in studies that fed basal diets above the NRC (2000) recommendations had minimal impact on carcass traits, whereas a greater response to Zn supplementation was observed in studies that compared increased supplemental Zn to basal diets at or below NRC (2000) recommendations for dietary Zn. In addition, differences in study design, Zn source, Zn content in the basal diet, and supplemental Zn content make it difficult to compare effects of Zn supplementation across studies. Our study indicated Zn supplementation up to 90 mg/kg DM minimally impacted carcass traits; however, in chapter 3 of the thesis, an interaction was observed between Zn and ractopamine hydrochloride (**RH**). We observed supplementing 100 mg Zn/kg DM decreased LM area and increased yield grade, but these effects were no longer observed after supplementing heifers with RH (Chapter 3 of this

dissertation). Heifers in the current study were supplemented with ractopamine hydrochloride the final 28 d on feed, which may have obscured any effects for carcass traits that may have occurred due to feeding increased concentrations of supplemental Zn.

In vitro Fermentation

Increased concentrations of supplemental Zn have previously been observed to affect cellulose digestion (Martinez and Church, 1970), but the effects of Zn supplementation on *in vitro* fermentation containing high concentrate substrates has not been evaluated. In the current study, no treatment × time interaction ($P = 0.67$; Fig. 4.2), or effect Zn supplementation ($P = 0.59$) was observed for *in vitro* gas production. Gas production was affected by time ($P = 0.67$) with gas production increasing with increasing fermentation time. Vásquez-Armijo et al. (2011) conducted a study assessing the effects of 224.07 mg/kg added Zn with Cu, added Cu and no Zn, and 224.07 mg/kg added Zn with no Cu supplementation on *in vitro* gas production using ruminal fluid from goats fed 68% forage. Contrary to the current results, the authors observed increased gas production with addition of Zn compared to the cultures containing no Zn. The differences observed between studies could be due to the substrate used in the *in vitro* systems. In the current study no forage was included in the fermentation flasks, whereas approximately 68% forage was included in the study by Vásquez-Armijo et al. (2011). These results suggest Zn supplementation may affect fermentative gas production differently depending on the forage:concentrate ratios of the diets. Martinez and Church (1970) indicated Zn supplementation was beneficial to cellulose digestion at concentrations of 5 to 7 mg/kg added Zn, but depression of cellulose digestion occurred at concentrations above 20 mg/kg added Zn suggesting increased Zn concentrations have an inhibitory effect on fiber digestion by ruminal microbes. Our study

suggests supplementing up to 150 mg Zn/kg substrate DM does not affect *in vitro* gas production with addition of high concentrate substrate.

Effects of supplemental Zn on pH, IVDMD, and VFA concentrations are presented in Table 4.5. Including supplemental Zn in the *in vitro* fermentation flasks did not affect terminal pH or IVDMD ($P \geq 0.20$). Similar to current results, Vásquez-Armijo et al. (2011) observed no differences in IVDMD when ruminal fluid from goats was used in an *in vitro* ruminal fermentation with added Zn compared to cultures without Zn. On the contrary, Wang et al. (2013) and Kathirvelan and Balakrishnan (2008) observed decreased IVDMD when 20 µg/mL Zn from Zn sulfate and 10 mg/kg Zn from Zn chloride was added to *in vitro* cultures containing primarily forage as the substrate. Arelovich et al. (2000) observed a linear decrease in IVDMD with addition of 0, 5, 10, 15, or 20 mg/kg added Zn incubated with prairie hay as the substrate. In addition, Bateman et al. (2002) observed no differences in pH of continuous cultures with addition of 1,350 mg Zn/kg DM. Eryavuz and Dehority (2009) suggested decreased cellulose digestion with increased supplemental Zn may be due to inhibitory effects of cellulolytic enzymes produced by microorganisms rather than a direct inhibitory effect on the bacteria. This may explain the decreased IVDMD as Zn concentration increased in *in vitro* cultures containing high forage substrates. Our results suggest increased supplemental Zn to *in vitro* cultures containing 90% ground corn and 10% soybean meal did not affect IVDMD or pH.

A tendency for a quadratic decrease was observed for acetate ($P = 0.07$) concentration with addition of 60 and 90 mg Zn/kg substrate DM tending to have the lowest acetate concentrations compared to other treatments. There was no effect of added Zn on propionate concentration ($P \geq 0.17$), thus a quadratic effect of Zn was observed for A:P ratio ($P = 0.04$). A linear effect of Zn was observed for isovalerate ($P = 0.05$), in which concentration of isovalerate

decreased with increasing supplemental Zn. Concentrations of butyrate, isobutyrate, valerate, isocaproic acid, caproic acid, and heptanoic acid were not affected by supplemental Zn ($P \geq$ 0.23). Bateman et al. (2002) performed an *in vitro* fermentation study using 50:50 forage to concentrate ratio and added 0 or 1,350 mg Zn/kg DM to continuous culture fermenters. The authors observed increased acetate, and decreased butyrate concentrations with addition of Zn; however, when ruminal fluid from cows fed diets containing similar supplemental Zn concentrations were analyzed, no differences were observed for any VFA concentrations. Similarly, Zn supplementation had no effect on VFA concentrations of ruminal fluid from steers supplemented 430 mg Zn/kg DM as Zn chloride, and fed a diet consisting of 39% chopped alfalfa hay and 61% concentrate (Arelovich et al., 2008). Spears et al. (2004) supplemented steers 20 mg Zn/kg DM from Zn sulfate, Zn methionine, or Zn glycine complex (basal diet consisted of 46.4% dried citrus pulp, 39.3% cottonseed hulls, 5% corn, 7% soybean meal 2.3% mineral mix) and observed decreased valerate with addition of Zn, regardless of source; however, Zn methionine supplementation resulted in increased propionate, and decreased butyrate compared to the control and other Zn treatments. Effects of Zn supplementation on VFA concentrations appear to vary depending on diet of animals tested or substrate used for *in vitro* cultures. Our results suggest adding up to 150 mg Zn/kg substrate DM minimally impacted *in vitro* fermentation.

Overall, Zn supplementation up to 150 mg/kg substrate weight minimally affected *in vitro* fermentation, and supplementing up to 60 mg Zn/kg diet DM had the greatest improvement in feed efficiency with minimal effects on carcass characteristics; however, differences in carcass characteristics may have been masked with the addition of ractopamine hydrochloride to the diet the final 28 days on feed.

REFERENCES

Ankom Technology Corporation. 2014. Conversion of pressure to gas production. Page 44 in Ankom RF gas production system operator's manual. Rev F 8/29/14. Ankom Technology Corp. Macedon, NY.

Arelovich, H. M., F. N. Owens, G. W. Horn, and J. A. Vizcarra. 2000. Effects of supplemental zinc and manganese on ruminal fermentation, forage intake, and digestion by cattle fed prairie hay and urea. J. Anim. Sci. 78:2972–2979. doi:/2000.78112972x

Arelovich H.M., H. E. Laborde, M.I. Amela, M. B. Torrea, M. F. Martínez. 2008. Effects of dietary addition of zinc and (or) monensin on performance, rumen fermentation and digesta kinetics in beef cattle. Span. J. Agric. Res. 6:362-372. doi: 10.5424/sjar/2008063-329

Bateman, H. G., C. C. Williams, and Y. H. Chung. (2002). Effects of supplemental zinc in high quality diets on ruminal fermentation and degradation of urea in vitro and in vivo. Prof. Anim. Sci. 18:363–367. doi:10.15232/s1080-7446(15)31547-3

Eryavuz, A., and B. A. Dehority. 2009. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers in vitro. Anim. Feed Sci. Technol. 151:175–183. doi:10.1016/j.anifeedsci.2009.01.008

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schelling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J. Anim. Sci. 66:1818–1823. doi:10.2134/jas1988.6671818x

Hambidge, K. M., C. E. Casey, and N. F. Krebs. 1986. Zinc. In: W. Mertz, editor, Trace minerals in human and animal nutrition vol. 2. Academic Press, Orlando, FL. p. 1–137.

Heckman, M., 1967. Minerals in feed by atomic absorption spectrophotometry. J. Assoc. Off. Anal. Chem.. 50:45–50

Huang, Y., A. K. Das, Q.-Y. Yang, M.-J. Zhu, and M. Du. 2012. Zfp423 Promotes Adipogenic Differentiation of Bovine Stromal Vascular Cells. PLoS ONE. 7:e47496. doi:10.1371/journal.pone.0047496

Huerta, M., R. L. Kincaid, J. D. Cronrath, J. Busboom, A. B. Johnson, and C. K. Swenson. 2002. Interaction of dietary zinc and growth implants on weight gain, carcass traits and zinc in tissues of growing beef steers and heifers. Anim. Feed Sci. Technol. 95:15–32. doi: 10.1016/s0377-8401(01)00334-0

Ivan, M., and C. M. Grieve. 1975. Effects of zinc, copper, and manganese supplementation of high-concentrate ration on digestibility, growth, and tissue content of Holstein calves. J. Dairy Sci. 58:410–415. doi:10.3168/jds.s0022-0302(75)84579-6

Kathirvelan, C., and V. Balakrishnan. 2008. Effect of supplemental zinc at 10 ppm on apparent, true digestibility, microbial biomass production and exploring means to overcome ill effects in cattle. Trends Appl. Sci. Res. 3:103–108. doi:10.3923/tasr.2008.103.108

Kessler, J., I. Morel, P. A. Dufey, A. Gutzwiller, A. Stern, and H. Geyer. 2003. Effect of organic zinc sources on performance, zinc status and carcass, meat and claw quality in fattening bulls. Livest. Prod. Sci. 81:161–171.

Martinez, A., and D. C. Church. 1970. Effect of various mineral elements on *in vitro* rumen cellulose digestion. J. Anim. Sci. 31:982–990. doi:10.2134/jas1970.315982x

MacDonald, R. S. 2000. The role of zinc in growth and cell proliferation. J. Nutr. 130:1500S–1508S.

Malcolm-Callis, K. J., G. C. Duff, S. A. Gunter, E. B. Kegley, and D.A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. J. Anim. Sci. 78:2801–2808. doi:/2000.78112801x

McDowell L. R. 1992. Zinc. In: Minerals in human and animal nutrition. Academic Press, San Diego, CA. p. 265–293.

McDougall, E. I. 1948. Studies on the ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J. 43:99–109.

Murthy, G. K., U. Rhea, and J. T. Peeler. 1971. Levels of antimony, cadmium, chromium, cobalt, manganese, and zinc in institutional total diets. Environ. Sci. Technol. 5:436–442.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Nunnery, G. A., J. T. Vasconcelos, C. H. Parsons, G. B. Salyer, P. J. Defoor, F. R. Valdez, and M. L. Galyean. 2007. Effects of source of supplemental zinc on performance and humoral immunity in beef heifers. J. Anim. Sci. 85:2304–2313. doi:10.2527/jas.2007-0167

Oh, Y. S., and C. B. Choi. 2004. Effects of zinc on lipogenesis of bovine intramuscular adipocytes. Asian-Australas. J. Anim. Sci. 17:1378–1382. doi:10.5713/ajas.2004.1378

Spears, J. W., and E. B. Kegley. 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J. Anim. Sci. 80:2747–2752. doi:/2002.80102747x

Spears, J. W., P. Schlegel, M. C. Seal, and K. E. Lloyd. 2004. Bioavailability of zinc from zinc sulfate and different organic zinc sources and their effects on ruminal volatile fatty acid proportions. Livest. Prod. Sci. 90:211–217. doi:10.1016/j.livprodsci.2004.05.001

Tomlinson, D. J., C. H. Mülling, and T. M. Fakler. 2004. Invited review: formation of keratins in the bovine claw: roles of hormones, minerals, and vitamins in functional claw integrity. J. Dairy Sci. 87:797–809. doi:10.3168/jds.s0022-0302(04)73223-3

Vallee, B L., and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. Physiol. Rev. 73:79–118.

Vallee, B. L. 1959. Biochemistry, physiology, and pathology of zinc. Physiol. Rev. 39: 443–490.

Vasconcelos, J. T., and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. J. Anim. Sci. 85:2772–2781. doi:10.2527/jas.2007-0261

Vázquez-Armijo, J. F., J. J. Martínez-Tinajero, D. López, A.-F. Z. M. Salem, and R. Rojo. 2011. In vitro gas production and dry matter degradability of diets consumed by goats with or without copper and zinc supplementation. Biol. Trace Elem. Res. 144:580–587. doi:10.1007/s12011-011-9113-y

Wang, R. L., J. G. Liang, L. Lu, L. Y. Zhang, S. F. Li, and X. G. Luo. 2013. Effect of zinc source on performance, zinc status, immune response, and rumen fermentation of lactating cows. Biol. Trace Elem. Res. 152:16–24. doi:10.1007/s12011-012-9585-4

Wei, S., L. Zhang, X. Zhou, M. Du, Z. Jiang, G. J. Hausman, W. G. Bergen, L. Zan, and M. V. Dodson. 2013. Emerging roles of zinc finger proteins in regulating adipogenesis. Cell. Mol. Life Sci. 70:4569–4584. doi:10.1007/s00018-013-1395-0

Wu, F., and C. Wu. 1987. Zinc in DNA replication and transcription. Ann. Rev. Nutr. 7:251–272. doi:10.1146/annurev.nutr.7.1.251

Table 4.1. Diet composition of finishing heifers supplemented 0, 30, 60, or 90 mg Zn/kg diet DM

	Treatment, mg Zn/kg diet DM			
Item	0	30	60	90
Ingredient, % DM				
Steam flaked corn	58.90	58.90	58.90	58.90
Wet corn gluten feed	30.00	30.00	30.00	30.00
Alfalfa hay	7.00	7.00	7.00	7.00
Feed additive premix[1]	2.16	2.16	2.16	2.16
0 mg/kg Zn vitamin/mineral premix[2]	1.94	1.29	0.65	-
90 mg/kg Zn vitamin/mineral premix[2]	-	0.65	1.29	1.94
Calculated nutrient composition				
NDF, %	19.03	19.03	19.03	19.03
CP, %	14.06	14.06	14.06	14.06
Ca, %	0.70	0.70	0.70	0.70
P, %	0.48	0.48	0.48	0.48
Zn, mg/kg	31.89	61.89	91.89	121.89

[1]Formulated to provide 300 mg/day Monensin; 90 mg/day Tylan (Elanco Animal Health, Greenfield, IN); and 0.4 mg/d Melengesterol acetate (Elanco Animal Health, Greenfield, IN) in a ground corn carrier. Melengesterol acetate was removed from the diet the final 18 days on feed due to a shortage of the product. Ractopamine hydrochloride (**RH**; Elanco Animal Health, Greenfield, IN) was mixed in a ground corn carrier and fed at a rate of 0.09 kg/d to supply 300 mg RH/animal daily the final 28 d prior to harvest.

[2]Formulated to provide the following nutrient levels: added 2,200 IU/kg vitamin A; added 22 IU/kg vitamin E (alpha tocopherol acetate); added 0.10 mg/kg added Co (Co carbonate); added 10 mg/kg added Cu (Cu sulfate); added 0.5 mg/kg added I (ethylenediamine dihydriodide); added 20 mg/kg added Mn (Mn sulfate); added 0.1 mg/kg added Se (Na selenite); and 0.3% total dietary salt, and 0.7% total dietary Ca (limestone). Vitamin/mineral premixes were formulated to contain 0 or 90 mg supplemental Zn/kg diet DM (Zn sulfate) and were blended for rations formulated to contain 30 or 60 mg/kg supplemental Zn.

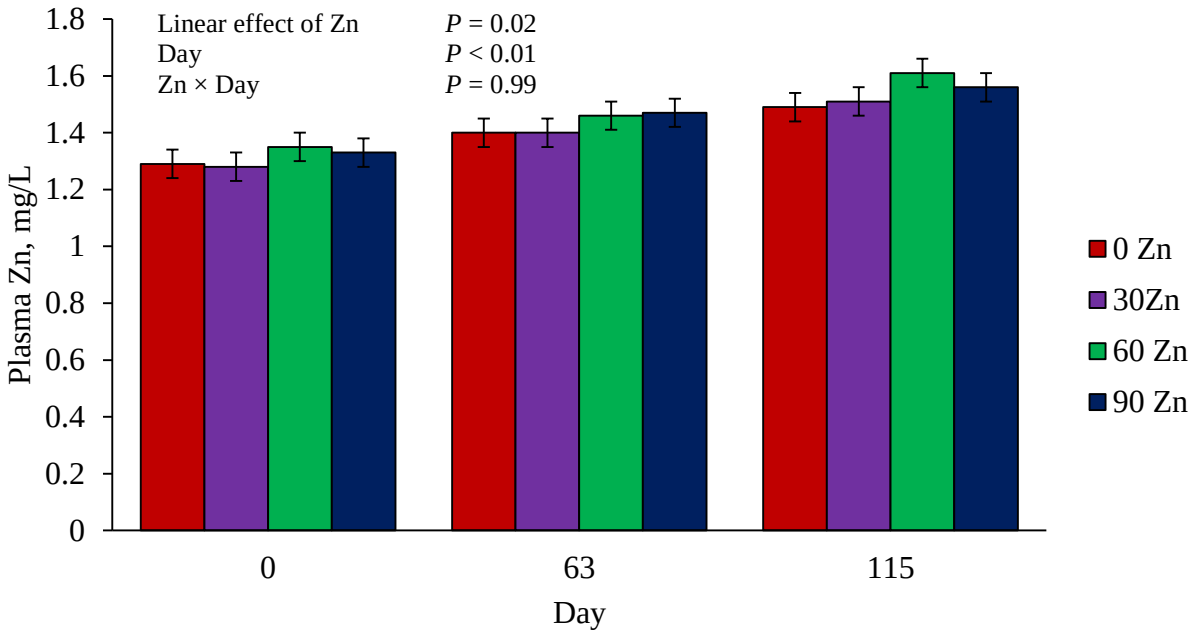

Figure 4.1. Effects of supplementing 0, 30, 60, 90 mg Zn/kg diet DM (0, 30, 60, or 90 Zn) as zinc sulfate on plasma Zn concentration of finishing heifers. Plasma was collected from the same 5 heifers/pen days 0, 63, and 115. There was no quadratic effect of Zn (P = 0.77), but there was a tendency for a difference between heifers supplemented 0 Zn vs. the average of heifers receiving added Zn (P = 0.10).

Table 4.2. Feedlot performance of finishing heifers supplemented 0, 30, 60, or 90 mg Zn/kg diet DM[1]

	Treatment, mg Zn/kg diet DM					*P*-value[2]			
Item	0	30	60	90	SEM	Trt	C1	C2	C3
n	117	118	118	120	-	-	-	-	-
Initial BW, kg	386	384	385	386	13.08	0.64	0.93	0.33	0.40
Final BW[3], kg	603	603	610	602	19.69	0.53	0.79	0.37	0.67
ADG, kg	1.51	1.52	1.56	1.51	0.05	0.52	0.77	0.29	0.57
DMI, kg	10.25	10.12	9.95	9.88	0.35	0.33	0.07	0.87	0.15
G:F	0.1470	0.1497	0.1573	0.1522	0.0024	0.03	0.04	0.12	0.04

[a,b]Within a row, means without a common superscript letter are different ($P \leq 0.03$).

[1]Zinc was included as Zn sulfate.

[2]Effect of treatment (Trt); linear effect of Zn (C1); quadratic effect of Zn (C2); 0 mg Zn/kg diet DM vs. average treatments with added zinc (C3).

[3]Gross BW × 0.96.

Table 4.3. Effects of supplementing 0, 30, 60, 90 mg Zn/kg diet DM on carcass characteristics of finishing heifers[1]

	Treatment, mg Zn/kg diet DM					*P*-value[2]			
Item	0	30	60	90	SEM	Trt	C1	C2	C3
HCW, kg	380	379	383	379	12.53	0.60	0.82	0.54	0.82
Dressed yield, %	63.01	62.86	62.81	62.99	0.26	0.80	0.89	0.34	0.54
Liver abscesses, %	11.1	6.0	7.6	10.0	3.12	0.55	0.89	0.18	0.32
LM area, cm^2	94.7	93.7	93.6	93.9	2.84	0.88	0.60	0.55	0.44
12^{th}-rib fat thickness, cm	1.45	1.40	1.47	1.40	0.08	0.53	0.68	0.87	0.55
Marbling score[3]	444	434	454	440	11.28	0.08	0.72	0.66	0.87
Total carcass value[4], $	1023	1026	1048	1033	34.86	0.32	0.25	0.39	0.29

[1]Zinc supplemented as Zn sulfate.

[2]Effect of treatment (Trt); linear effect of Zn (C1); quadratic effect of Zn (C2); 0 mg Zn/kg diet DM versus average treatments with added zinc (C3).

[3]Marbling scores were determined by camera imaging (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany); Small = 400 to 499; Modest = 500 to 599.

[4] Carcass value was calculated as: (Average base price ($/kg) + (-) quality grade premiums or discounts + (-) yield grade and 12^{th} rib s. c. fat thickness premiums or discounts + (-) HCW premiums or discounts) × (HCW, kg /45).

Table 4.4. Effects of supplementing 0, 30, 60, 90 mg Zn/kg diet DM on USDA quality and yield grades of carcasses from heifers[1]

	Treatment, mg Zn/kg diet DM					*P*-value[2]			
Item, %	0	30	60	90	SEM	Trt	C1	C2	C3
USDA Prime	1.7	2.5	5.1	0.0	1.53	0.16	0.71	0.07	0.64
USDA high Choice	18.8	15.2	17.8	20.8	4.57	0.76	0.61	0.39	0.85
USDA low Choice	46.1	49.0	51.7	48.3	4.90	0.86	0.66	0.51	0.52
USDA Select	24.8	24.8	21.2	26.7	4.77	0.80	0.91	0.51	0.90
Sub-Select[3]	6.9	6.0	2.5	4.2	2.22	0.46	0.22	0.55	0.28
Dark cutter	1.7	2.5	1.7	0.0	1.25	0.43	0.23	0.26	0.81
Overall yield grade	2.5	2.4	2.6	2.3	0.15	0.55	0.64	0.38	0.90
Yield grade 1	16.4	12.9	10.3	21.7	5.41	0.21	0.45	0.07	0.75
Yield grade 2	36.0	42.3	35.6	35.0	3.37	0.26	0.46	0.24	0.62
Yield grade 3	33.2	34.7	42.4	31.7	4.86	0.28	0.87	0.15	0.52
Yield grade 4	14.4	9.3	11.7	10.8	3.19	0.70	0.56	0.51	0.32
Yield grade 5	0.0	0.8	0.0	0.8	0.64	0.54	0.51	1.00	0.40

[1]Zinc supplemented as Zn sulfate.

[2]Effect of treatment (Trt); linear effect of Zn (C1); quadratic effect of Zn (C2); 0 mg Zn/kg diet DM versus average treatments with added zinc (C3).

[3] Includes carcasses that graded standard and commercial.

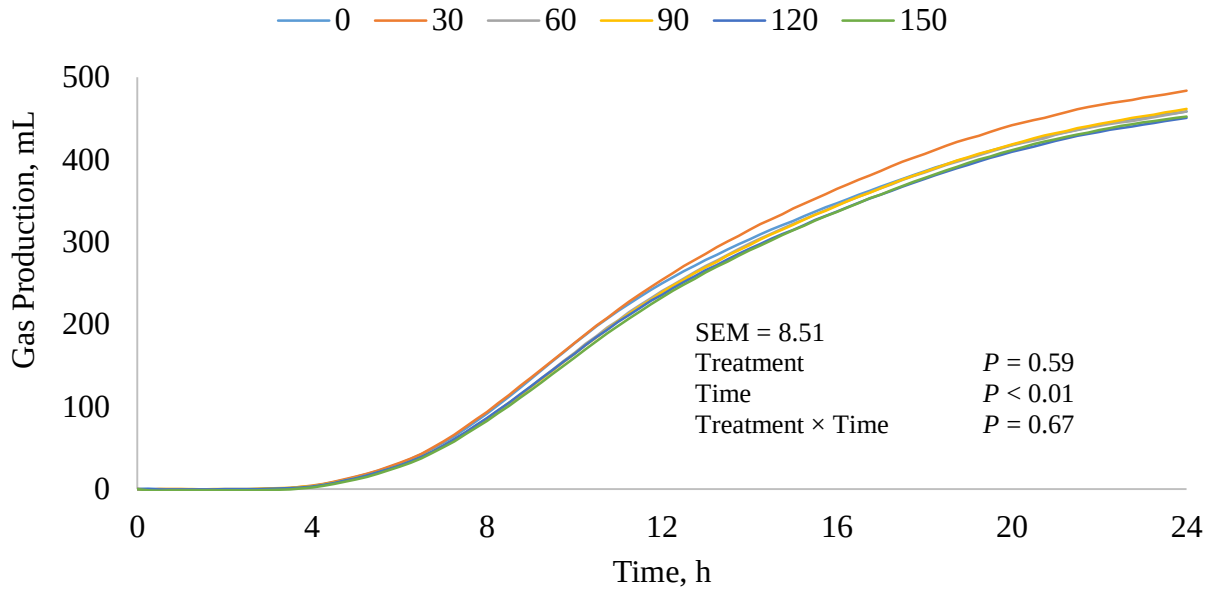

Figure 4.2. Effects of adding 0, 30, 60, 90, 120, or 150 mg Zn/kg substrate DM (0, 30, 60, 90, 120, or 150) as zinc sulfate to fermentation flasks containing 10 mL ruminal fluid, 140 mL McDougal's buffer, and 3 g substrate (90% ground corn, and 10% soybean meal) on *in vitro* gas production over 24 h fermentation period using the Ankom Gas Production System (Ankom Technology, Macedon, NY). There were no linear or quadratic effects Zn ($P = 0.69$; $P = 0.82$, respectively) or a difference between 0 mg Zn/kg substrate vs. the average of Zn treatments ($P = 0.80$).

Table 4.5. Effects of adding 0, 30, 60, 90, 120, or 150 mg Zn/kg substrate DM as zinc sulfate to fermentation flasks containing 10 mL ruminal fluid, 140 mL McDougal's buffer, and 3 g substrate[1] on pH, IVDMD, and VFA production following 24 h fermentation using Ankom Gas Production System (Ankom Technology, Macedon, NY)

	Treatment, mg Zn/kg substrate							*P*-value[2]			
Item	0	30	60	90	120	150	SEM	Trt	C1	C2	C3
pH	6.01	6.06	6.04	6.02	6.01	6.00	0.02	0.50	0.67	0.26	0.41
IVDMD, %	52.40	49.67	51.07	51.47	52.07	51.80	1.17	0.60	0.75	0.20	0.30
Volatile fatty acid, m*M*											
Acetate	36.04	34.89	33.28	33.54	35.91	34.65	1.25	0.53	0.69	0.07	0.25
Propionate	42.90	41.70	41.56	42.10	41.92	43.08	0.68	0.52	0.48	0.30	0.17
Butyrate	8.68	8.68	8.43	8.99	8.53	8.74	0.31	0.91	0.99	0.40	0.80
Isobutyrate	1.02	1.39	1.12	0.76	1.00	1.05	0.36	0.89	0.56	0.80	0.91
Isovalerate	0.093	0.068	0.054	0.048	0.025	0.081	0.032	0.43	0.05	0.90	0.12
Valerate	0.405	0.360	0.377	0.372	0.342	0.370	0.043	0.81	0.26	0.95	0.24
Isocaproic	0.027	0.010	0.009	0.003	0.012	0.056	0.025	0.52	0.58	0.56	0.44
Caproic	0.023	0.000	0.000	0.000	0.003	0.040	0.019	0.44	0.46	0.44	0.26
Heptanoic	0.035	0.013	0.010	0.007	0.022	0.070	0.031	0.48	0.68	0.43	0.43
A:P ratio	0.839	0.835	0.800	0.797	0.856	0.803	0.020	0.21	0.93	0.04	0.44

[1]Substrate used for each fermentation flask consisted of 90% corn and 10 % soybean meal.

[2]Effect of treatment (Trt); linear effect of Zn (C1); quadratic effect of Zn (C2); 0 mg Zn/kg diet DM vs. average treatments with added zinc (C3).

Chapter 5 - Effects of yeast combined with chromium propionate on growth performance and carcass quality of finishing steers[1]

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ABSTRACT

A combination of yeast and Cr propionate (**Y+Cr**) was added to the diets of crossbred finishing steers (*n* = 504; initial BW = 402 kg ± 5.76) to evaluate impact on feedlot performance and carcass traits. We hypothesized supplementation of Y+Cr would increase growth of feedlot steers. Steers with initial plasma glucose concentrations ≤ 6.0 mM were stratified by initial BW and randomly allocated, within strata, to receive 0 (**control**) or 3.3 g/d Y+Cr. Steers were further divided into heavy and light weight blocks with 6 pens/diet within each weight block. Cattle were housed in dirt-surfaced pens with 21 steers/pen and had *ad libitum* access to feed. Body weights were measured at 21-d intervals. Blood samples were collected on d 49 and 94 from a subset of steers (5/pen) for analyses of plasma glucose and lactate concentrations. At the end of the finishing phase animals were weighed and transported 450 km to an abattoir in Holcomb, Kansas. Severity of liver abscesses and HCW were collected the day of harvest, and after 36-h of refrigeration USDA yield and quality grades, LM area, and 12th rib subcutaneous fat thickness were determined. There were no treatment × time × weight block interactions ($P > 0.05$), and no treatment × block interaction for ADG, DMI, or final BW ($P \geq 0.06$), but a treatment × block interaction ($P = 0.03$) was observed for G:F, in which control-light cattle had poorer efficiency compared to other groups. Treatment × weight group interactions were observed for overall yield grade and carcasses that graded yield grade 1 ($P \leq 0.04$). Light steers supplemented with Y+Cr had decreased overall yield grade and increased percentage of carcasses grading yield grade 1 compared to their control counterparts, with differences were observed for heavy steers. No treatment interactions or effects of treatment were detected for other carcass measurements ($P \geq 0.07$). There were no treatment × weight group interactions or effects of treatment for plasma glucose or lactate concentrations on d 49 or d 94 ($P > 0.10$). Overall, yeast in combination with

Cr propionate may improve feed efficiency and decrease yield grade of light cattle, but had no effect on remaining carcass and blood constituents.

Key words: beef cattle, carcass traits, Cr propionate, glucose, yeast

INTRODUCTION

Chromium is an essential micro mineral that functions as a component of glucose tolerance factor and chromodulin, both of which potentiate actions of insulin (NRC, 2000; Pechova and Pavlata, 2007). Chromium has been implicated as a regulator of lipid and protein metabolism (Pechova and Pavlata, 2007). Enhanced lipogenesis and decreased lipolysis were observed in dairy cattle supplemented Cr propionate (McNamara and Valdez, 2005), which suggests Cr propionate might influence quality and yield grades in finishing cattle. In addition, Cr supplementation has the potential to increase amino acid uptake by muscle cells, potentially increasing total protein deposition (Pechova and Pavlata, 2007). The ability of Cr to increase glucose tolerance through effects on insulin function could improve efficiency of glucose utilization, thus leading to improved growth and efficiency (Swanson et al., 2000); however, little is known about actual requirements of Cr for finishing cattle. Some studies revealed favorable performance responses to Cr during periods of stress in ruminants. Stressed feeder calves were administered a high-Cr yeast product, which improved ADG (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993), feed efficiency (Chang and Mowat, 1992), and immune response (Moonsie-Shageer and Mowat, 1993).

Organic forms of Cr are readily absorbed, are more physiologically active, and require no dietary precursors (i.e. amino acids or niacin) for bioactivity (Mowat, 1997) compared to inorganic sources. Chromium propionate is currently the only form of supplemented Cr approved for addition to cattle diets in the United States. We hypothesized that Cr propionate, when

supplemented in combination with yeast culture, would increase gain and feed efficiency during the finishing phase. The objectives of this study were to compare feedlot performance, carcass characteristics, and plasma glucose profiles of cattle fed finishing diets with and without a combination of yeast and Cr propionate.

MATERIALS AND METHODS

Protocols and procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State University Beef Cattle Research Center located in Manhattan, KS. Trial started October 2013 and finished February 2014.

Experimental Design

The study was conducted as a randomized complete block design with 2 treatments using 504 crossbred steers (BW = 402 kg ± 5.76). Steers were from a larger population of cross-breed cattle, and were selected on the basis of initial plasma glucose concentrations; steers with plasma glucose concentrations greater than 6.0 m*M* were excluded. Steers were then blocked by initial BW (12 blocks), and randomly allocated within block to two treatments. Animals were further divided into heavy (gross BW = 421 kg ± 5.76) and light (gross BW = 384 kg ± 5.76) weight groups to determine if treatment differences existed between heavy and light animals. Steers were housed in 24 pens containing 21 steers/pen (12 pens/treatment). Treatments consisted of control, in which the steers received a basal diet (**control**), and a treatment that contained the basal diet with a combination of yeast (*Saccharomyces cerevisiae*) and Cr propionate (**Y+Cr**; Vi-Cor, Mason City, IA) added to the feed additive premix. Each treatment was represented in each weight group (i.e. control light, Y+Cr light, control heavy, and Y+Cr heavy). The Y+Cr

supplement was fed at a rate of 3.3 g/d (as-fed), which provided 3.2 mg Cr per animal daily (Table 5.1).

Animal Processing, Housing, and Handling

Upon arrival at the Kansas State University Beef Cattle Research Center, steers were allowed *ad libitum* access to ground alfalfa hay and water. Twenty-four hours after arrival of the final load of cattle, steers were tagged with an ear tag that displayed a unique number for each study animal. Before initiation of the study, steers were individually weighed and received an estradiol/trenbolone acetate implant (Component TE-200 with Tylan, Ivy Animal Health, Inc., Overland Park, KS), topical parasiticide (Dectomax; Pfizer Animal Health, New York, NY), 5-way viral vaccine (Bovishield Gold–5; Pfizer Animal Health, New York, NY), and 7-way clostridial vaccine (Ultra-Bac 7; Pfizer Animal Health, New York, NY). In addition, blood samples were obtained by jugular venipuncture for analysis of plasma glucose and lactate using 10-mL blood collection tubes containing sodium fluoride/potassium oxalate as anticoagulants (BD Vacutainer, Franklin Lakes, NJ). Samples were immediately placed onto ice and centrifuged within 20 min of sampling. Blood was centrifuged at 2,494 × *g* for 20 min and plasma was transferred by pipette into 5-mL plastic tubes and frozen at -20ºC for subsequent analysis. Steers were housed in dirt-surfaced pens that were 10.1 m × 30.5 m and of pipe construction. Watering fountains were shared between adjacent feedlot pens and steers were allowed *ad libitum* access to feed and water. Body weights were captured for each pen at 21-d intervals, and again at the end of feedlot finishing. Average daily gains were computed by subtracting previous BW from current BW for each weigh period, and divided by days on feed (**DOF**). Gain efficiencies were computed as ADG divided by DMI.

Blood was drawn by jugular venipuncture from 5 steers/pen approximately 2 h prior to feeding on d 49 and 94 to determine plasma glucose and lactate concentrations. Blood collection for each pen lasted approximately 10 min. Steers utilized for blood collection were randomly chosen at the beginning of the study, and tagged with a colored ear tag for identification. Blood sampling procedures were as previously described, and samples were stored at -20ºC until analysis. Glucose and lactate concentrations of plasma were analyzed using the YSI 2300 STAT Plus Glucose and L-lactate Analyzer (YSI Inc., Yellow Springs, OH).

Diet Preparation

Steers were transitioned to finishing diets over a period of 18 d using a series of 3 step-up diets (roughage:concentrate ratios of 46:54, 33:67, and 21:79; 6 d/step) that were formulated to allow gradual adaptation to the high-grain finishing diet. A mixture of yeast and Cr propionate (3.3 g/d) was added to the feed additive premix, which subsequently was mixed into total mixed rations. Diets were mixed once daily in a truck mounted mixer and delivered to cattle at approximately 0800. Feed intakes were visually monitored and adjusted daily so only trace amounts of residual feed remained the following day. Unconsumed feed was removed from the bunk and weighed at 21-d intervals or as needed to determine actual feed intake for each 21-d period. Subsamples of diets and unconsumed feed were dried at 55°C for 48 h to determine DM content. For each 21-d interval and for the total study period, DMI was computed as: DMI = [(total feed offered × % DM) - (total feed refused × % DM)] / (number of animal × day).

Harvest

Final BW were determined immediately before cattle were shipped on the day of harvest. Final BW was multiplied by 0.96 to account for 4% shrink during shipping. Steers were loaded onto a truck and transported 451 km to a commercial abattoir in Holcomb, Kansas. Steers in the

heavy block were shipped after 125 DOF and steers in the light block were shipped after 148 DOF. Incidence and severity of liver abscesses and HCW were recorded the day of harvest. Liver abscesses were scored according to the Elanco scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health, Greenfield, IN): 0 = no abscesses, A^- = 1 or 2 small abscesses or abscess scars, A = 2 to 4 small, well-organized abscesses and A^+ = 1 or more large or active abscesses with or without adhesions. USDA Yield grade, USDA Quality grade, marbling score, 12^{th} rib s. c. fat thickness, LM area, and incidence and severity of dark cutting beef were collected after 36 h of refrigeration from camera images (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany) provided by the abattoir; however, due to equipment failure that occurred at the abattoir, LM area was traced on tracing paper for 22 carcasses and marbling scores for these carcasses were determined by a certified USDA grader (1 carcass from control heavy group; 9 carcasses from Y+Cr light group; 12 carcasses from control light group).

Statistical Analyses

Growth data (DMI, gain, and gain efficiency) were analyzed as repeated measures using the MIXED procedure of the Statistical Analysis System (version 9.2 of SAS; Cary, NC) with a model that included feeding period, weight group, treatment, and any 2- or 3-way interactions as fixed effects. Block was random effect, pen was the experimental unit, and feeding period was the repeated measure with pen within treatment as the subject and compound symmetry as the covariance structure. Estimates were utilized to compare differences between feeding periods due to different harvest dates for light and heavy groups. Due to no significant interactions of feeding period on growth performance, feeding periods were analyzed independent of each other. Cumulative growth performance, interim measures of ADG, DMI, and G:F, plasma glucose and lactate concentrations, and non-categorical carcass traits were analyzed using the MIXED

procedure with weight group, treatment, and the interaction between weight group and treatment as fixed effects, block was the random effect, and pen was the experimental unit. The GLIMMIX procedure of SAS was used to analyze incidence and severity of liver abscesses, USDA quality grades, and USDA yield grades. Weight group, treatment, and the interaction between weight group and treatment were fixed effects, block was the random effect, and pen was the experimental unit. Treatment was tested against the residual error at the 5% level of significance. Treatment Least Squares Means (LSMeans) were calculated for each group. Pair-wise comparisons between the LSMeans of the Y+Cr group and the LSMeans of the control group were performed using the PDIFF option.

RESULTS AND DISCUSSION

Two steers were removed from the control group. One steer was removed due to an injury not related to treatment, and a urinary tract bacterial infection that resulted in death of the other steer. One steer was removed from the Y+Cr treatment group due to an injury not related to treatment. No treatment × feeding period × weight group interactions were detected for any feedlot performance parameters ($P \geq 0.36$; data not shown).

Feedlot Performance

The effects of Y+Cr supplementation on interim and overall steer performance are shown in Table 5.2. There were no interactions between treatment and weight group or an effect of Y+Cr on interim BW measurements or final BW ($P \geq 0.18$). There was an effect of weight group ($P < 0.01$) for interim BW as per study design. Similarly, Chang et al. (1992) observed no improvement in final BW when steers were supplemented with 0.2 mg/kg (diet DM) high-Cr yeast. In addition, Swanson et al. (2000) observed no differences for BW when steers were supplemented with 100, 200, or 400 µg/kg (dietary DM) high-Cr yeast. Pollard et al. (2002)

observed a decrease in final BW when steers were supplemented with 0.4 mg Cr yeast/kg dietary DM compared to steers supplemented with either 0 or 0.2 mg Cr yeast/kg dietary DM suggesting an upper tolerable limit to Cr supplemetation. Danielsson and Pehrson (1998) detected no effect on final BW when bulls were fed Cr in the form of fodder yeast. Collectively, results of these studies suggest Cr supplementation has minimal impact on live BW of cattle.

No treatment × weight group interactions were detected for DMI measured for each period or for overall DMI ($P \geq 0.28$). Supplementation with Y+Cr tended to decrease DMI day 22 to 42 and day 85 to 105 ($P \leq 0.09$); however, Y+Cr supplementation did not affect overall DMI ($P = 0.18$). Dry matter intake was affected by weight group day 1 to 21 ($P = 0.05$) and day 43 to 63 ($P = 0.03$). In agreement, Chang et al. (1992) observed no difference in DMI when growing steers were supplemented with high-Cr yeast and Pollard et al. (2002) reported no differences in DMI of feedlot steers supplemented with a Cr yeast product. In addition, overall DMI was affected by weight group ($P = 0.03$). Steers in the heavy group ate, on average, more feed per day over the entire feeding when compared to light steers. These results were expected as we assumed heavier cattle would eat more to maintain a heavier BW.

An interaction between treatment and weight group was observed for ADG. Over d 1 to 21, light steers supplemented with Y+Cr gained more than their control counter parts ($P = 0.02$), whereas no difference was observed in the heavy steers. A tendency was detected for an interaction between treatment and weight block for overall ADG ($P = 0.06$) following a similar pattern as days 1 to 21. Steers supplemented with Y+Cr day 85 to 105 had decreased ADG compared to steers fed the control diet ($P = 0.04$); however, there was no effect of Y+Cr supplementation on ADG at other feeding periods or for overall ADG ($P \geq 0.19$). Average daily gain was affected by weight group days 106 to 126 and overall ADG ($P < 0.01$), where heavy

steers gained more light steers (33% and 7%, respectively). A treatment × weight group interaction was observed for G:F ($P \leq 0.05$). Days 1 to 21 the Y+Cr steers from the light group were more efficient than control steers ($P = 0.05$) with no apparent differences in the heavy group. The control steers from the light group were the least efficient ($P = 0.03$) over the entire experimental feeding period. In contrast to our results, Chang et al. (1992) observed no response for ADG, or G:F for growing steers supplemented with 0.2 mg/kg (dietary DM) high-Cr yeast. Pollard et al. (2002) also observed no improvement in ADG or G:F with 0.2 mg/kg diet DM Cr yeast, but observed a decrease in ADG and G:F when Cr (as Cr yeast) was supplemented at 0.4 mg/kg diet DM. Average daily gain and feed efficiency of lambs were not affected by 0, 0.25, 0.35 mg/d (equivalent to approximately 0, 0.20, 0.28 mg/kg diet DM) Cr yeast supplementation (Domínguez-Vara et al., 2009). It is unclear why light cattle supplemented with Y+Cr responded more favorably to the yeast Cr-propionate combination compared to heavy steers, but it is conceivable that there were differences in Cr requirements for the two groups. Discrepancies between our results and those reported previously may reflect differences in source of Cr, bioavailability of Cr, Cr status of the animals, age of the animals, or amount of Cr in the basal diet. In addition, Cr propionate was mixed with yeast in our study, therefore making it difficult to discern if effects observed for feedlot performance were attributed to Cr propionate, yeast, or the combination.

The improvement days 1 to 21 for ADG and feed efficiency in our study may reflect the beneficial effects of Cr supplementation for stressed cattle, as this was the period steers were weighed, sorted into pens, commingled with other animals, and transitioned to high-concentrate diets. Chang and Mowat (1992) observed increased ADG and G:F the first 28-d post arrival of stressed feeder calves supplemented with Cr yeast when no antibiotic was administered.

Additionally, Bernhard et al. (2012a) conducted a study to assess effects of Cr propionate fed at 0, 0.1, 0.2, or 0.3 mg/kg dietary DM during the receiving period and observed linear improvements in ADG and G:F of steers. The current results for feedlot performance suggest steers entering the feedlot with a lighter BW may benefit from yeast combined with Cr propionate.

Carcass Characteristics

No treatment × weight group interactions or effects of treatment were observed for HCW, dressing percentage, incidence of liver abscesses, LM area, or 12th rib s. c. fat thickness ($P \geq 0.16$; Table 5.3). Differences between weight groups were observed for dressing percentage, incidence of liver abscesses, and LM area ($P \leq 0.05$), wherein carcasses from heavy steers had increased dressing percentage, increased incidence of liver abscesses, and greater LM area compared to carcasses from light steers. In addition, carcasses from heavy steers tended to have heavier HCW compared to carcasses from light steers ($P = 0.09$). No interaction between treatment and weight group was observed for marbling score ($P = 0.47$; Fig. 5.1); however, supplementing cattle with Y+Cr tended to decrease marbling score ($P = 0.10$) and carcasses from heavy steers had decreased marbling scores compared to carcasses from light steers ($P < 0.01$). Differences observed between weight groups may reflect differences in degree of finish as light steers were harvested 23 d later than heavy steers.

Effects of Y+Cr supplementation and weight groups on yield and quality grades are presented in Table 5.4. A tendency for a treatment × weight group interaction was observed for carcasses grading high choice ($P = 0.07$), which carcasses from heavy steers supplemented Y+Cr had no carcasses that graded high Choice compared to their control counterparts and no differences were observed for light steers. No interactions between treatment and weight group

were observed for remaining quality grades ($P \geq 0.43$). Fewer carcasses from heavy steers graded mid Choice and consequently more carcasses from heavy steers graded Select then carcasses from light steers ($P \leq 0.03$). Supplementing steers with Y+Cr did not affect carcasses grading Prime, mid or low Choice, Select, Standard, or carcasses classified as B-maturity ($P \geq 0.23$). A treatment × weight group interaction was observed for overall yield grade ($P = 0.02$). Carcasses from light control steers had a greater overall yield grade than their Y+Cr counterparts whereas no apparent difference was observed in carcasses from heavy steers ($P \leq 0.01$). The difference in overall yield grade was reflected in percentage of carcasses that graded yield grade 1 ($P = 0.04$), wherein a greater percentage of carcasses from light Y+Cr supplemented steers was observed compared to their control counterparts, but no difference was observed in the heavy steers for carcasses grading yield grade 1. No treatment × weight group interactions were observed for carcasses grading yield grade 2, 3, 4, or 5 ($P \geq 0.12$). Regardless of weight group, a greater percentage of carcasses from steers supplemented with Y+Cr graded yield grade 2 ($P = 0.03$) and consequently fewer carcasses from steers supplemented with Y+Cr graded yield grade 3 ($P < 0.01$) then carcasses from control steers. A greater ($P = 0.07$) percentage of carcasses from light steers graded yield grade 4 then carcasses from heavy steers reflecting differences in degree of finish between weight groups.

Reports of Cr supplementation and its effects on carcass traits have been inconsistent. Pollard et al. (2002) observed decreased marbling scores and yield grades in carcasses from steers supplemented with 0.4 mg Cr yeast/kg dietary DM compared to carcasses that came from steers supplemented with 0 or 0.2 mg Cr yeast/kg dietary DM. Carcasses from lambs supplemented with 0, 400, or 800 mg Cr yeast/kg dietary DM were observed to have decreased amounts of intramuscular fat compared to control lambs, but no other differences were observed

for remaining carcass traits (Yan et al., 2008). Chromium is essential for improving insulin binding and sensitivity (Pechova and Pavlata, 2007), and may explain the decreased marbling scores and yield grade by the ability of insulin to increase lipid utilization (Debski et al., 2004); however, not all studies demonstrated a response to Cr supplementation. Chang et al. (1992) supplemented steers with 0.2 mg Cr yeast/kg dietary DM and observed no differences on carcass characteristics between carcasses from control and Cr yeast supplemented animals. In addition, there was no difference in any of the carcass traits analyzed from bulls supplemented with fodder yeast compared to un-supplemented bulls (Danielsson and Pehrson, 1998). Differences observed may reflect differences in Cr source, Cr concentration, or physiological differences of the animals at the time of slaughter. Our results suggest supplementation with yeast combined with Cr propionate could decrease marbling score and yield grade, but other carcass traits were not affected.

Plasma Glucose and Lactate

Chromium plays a vital role in improving the effects of insulin binding as well as over-all insulin sensitivity, therefore affecting glucose clearance rate (Pechova and Pavlata, 2007) and possibly the concentration of glucose in plasma. Treatment effects for plasma glucose and lactate concentrations are summarized in Table 5.5. No treatment × weight group interactions or effects of treatment were observed for plasma glucose concentrations on d 49 or 94 ($P > 0.28$). An effect of weight group was detected d 94 ($P = 0.02$), which light steers had greater plasma glucose concentrations than heavy steers. Researchers have consistently observed no differences in either serum or plasma glucose concentrations between control and cattle supplemented with Cr nicotinic acid (Kegley and Spears, 1995; Kegley et al., 1997), Cr propionate (Bernhard et al., 2012b; Spears et al., 2012), Cr tripicolinate (Bunting et al., 1995), Cr-yeast (Kegley and Spears,

1995; Swanson et al., 2000), Cr chloride hexahydrate (Kegley and Spears, 1995), and chelated Cr (Mowat et al., 1993). In addition, plasma glucose concentrations in lambs supplemented with 0.4 or 0.8 mg/kg dietary DM Cr-yeast were not different than control lambs (Yan et al., 2008). Chromium supplementation, regardless of form administered, did not influence glucose concentrations in plasma.

Lactate is a product of glucose catabolism, is produced in cells that have high demand for glucose utilization (Kravitz, 2005), and can be converted back to glucose via the Cori Cycle (Sano et al., 1997; Nelson and Cox, 2005) if necessary. No treatment × weight group interactions or effect of treatment were observed for plasma lactate concentrations on d 49 or 94 ($P \geq 0.32$). An effect of weight group was detected d 94 ($P = 0.04$), which cattle in the light group had greater concentrations of plasma lactate than heavy steers. In agreement with our results, Sano et al. (1997) supplemented rams with 0.5 mg/kg dietary DM chelated Cr and observed no differences in plasma lactate concentration between supplemented or control lambs. It is conceivable the differences detected for weight group on day 94 for plasma glucose and lactate concentrations may be reflective of the differences in degree of finish of the light and heavy cattle. Glucose and lactate, although used in a limited amount, contribute to fatty acid synthesis (Lawrence et al., 2012). The heavy steers may have been accumulating adipose tissue at a higher rate than the light steers at the d 94 blood collection, which could explain the decline in plasma glucose and lactate concentrations. Nevertheless, Y+Cr supplementation did not affect plasma lactate concentrations.

In conclusion, yeast combined with Cr propionate improved feed efficiency of light weight finishing cattle. Yeast combined with Cr propionate decreased yield grade of light weight finishing steers, but minimally affected other carcass traits and plasma glucose and lactate

concentrations. Caution must be taken when interpreting results from this experiment because the product in this study consisted of yeast combined with Cr propionate making it difficult to ascertain whether the results were due to Cr propionate, yeast, or a combination of both.

REFERENCES

Bernhard, B. C., N. C. Burdick, W. Rounds, R. J. Rathmann, J. A. Carroll, D. N. Finck, M. A. Jennings, T. R. Young, and B. J. Johnson. 2012a. Chromium supplementation alters the performance and health of feedlot cattle during the receiving period and enhances their metabolic response to a lipopolysaccharide challenge. J. Anim. Sci. 90:3879-3888. doi:10.2527/jas.2011-4981

Bernhard, B. C., N. C. Burdick, R. J. Rathmann, J. A. Carroll, D. N. Finck, M. A. Jennings, T. R. Young, and B. J. Johnson. 2012b. Chromium supplementation alters both glucose and lipid metabolism in feedlot cattle during the receiving period. J. Anim. Sci. 90:4857-4865. doi:10.2527/jas.2011-4982

Bunting, L. D., J. M. Fernandez, D. L. Thompson, and L. L. Southern. 1994. Influence of chromium picolinate on glucose usage and metabolic criteria in growing Holstein calves. J. Anim. Sci. 72:1591-1599. doi:/1994.7261591x

Chang, X., and D. N. Mowat. 1992. Supplemental chromium for stressed and growing feeder calves. J. Anim. Sci. 70:559-565. doi:/1992.702559x

Chang, X., D. N. Mowat, and G. A. Spiers. 1992. Carcass characteristics and tissue-mineral contents of steers fed supplemental chromium. Can. J. Anim. Sci. 72: 663-669. doi:10.4141/cjas92-077

Danielsson, D-A., and B. Pehrson. 1998. Effects of chromium supplementation on the growth and carcass quality of bulls fed a grain-based diet during the finishing period. J. Vet. Med. A. 45:219-224. doi:10.1111/j.1439-0442.1998.tb00820.x

Dębski, B., W. Zalewski, M. A. Gralak, and T. Kosla. 2004. Chromium-yeast supplementation of chicken broilers in an industrial farming system. J. Trace Elem. Med. Biol. 18:47-51. doi:10.1016/j.jtemb.2004.02.003

Domínguez-Vara, I. A., S. S. González-Muñoz, J. M. Pinos-Rodríguez, J. L. Bórquez-Gastelum, R. Bárcena-Gama, G. Mendoza-Martínez, L. E. Zapata, and L. L. Landois-Palencia. 2009. Effects of feeding selenium-yeast and chromium-yeast to finishing lambs on growth, carcass characteristics, and blood hormones and metabolites. Anim. Feed Sci. Technol. 152:42-49. doi:10.1016/j.anifeedsci.2009.03.008

Kegley, E. B., and J. W. Spears. 1995. Immune response, glucose metabolism, and performance of stressed feeder calves fed inorganic or organic chromium. J. Anim. Sci. 73:2721-2726. doi:/1995.7392721x

Kegley, E. B., J. W. Spears, and T. T. Brown. 1997. Effect of shipping and chromium supplementation on performance, immune response, and disease resistance of steers. J. Anim. Sci. 75:1956-1964. doi:/1997.7571956x

Kraidees, M. S., I. A. Al-Haidary, S. I. Mufarrej, M. Y. Al-Saiady, H. M. Metwally, and M. F. Hussein. 2006. Effect of supplemental chromium levels on performance, digestibility and carcass characteristics of transport-stressed lambs. Asian-Aust. J. Anim. Sci. 22:1124-1132. doi:10.5713/ajas.2009.80385

Kravitz, L. 2005. Lactate: Not guilty as charged. IDEA Fit. J. 2:23-25.

Lawrence, T. L. J., V. R Fowler, and J. E. Novakofski, 2012. Tissues: Basic structure and growth. In: Growth of farm animals. 3rd ed. CABI, Oxfordshire, UK. p. 57-74.

McNamara, J. P., and F. Valdez. 2005. Adipose tissue metabolism and production responses to calcium propionate and chromium propionate. J. Dairy Sci. 88:2498–2507. doi:10.3168/jds.s0022-0302(05)72927-1

Moonsie-Shageer, S., and D.N. Mowat. 1993. Effect of level of supplemental chromium on performance, serum constituents, and immune status of stressed feeder calves. J. Anim. Sci. 71:232-238. doi:/1993.711232x

Mowat, D. N., X. Chang, and W. Z. Yang. 1993. Chelated chromium for stressed feeder calves. Can. J. Anim. Sci. 73:49-55. doi:10.4141/cjas93-004

Mowat, D. N. 1997. Organic chromium in animal nutrition. Chromium books, Guelph, ON, Canada.

Nelson, D. L., and M. M. Cox. 2005. Glycolysis, gluconeogenesis, and the pentose phosphate pathway. In: Lehniger priniciples of biochemistry. 4th ed. W. H. Freeman and Company, New York, NY. p. 521-559.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Pechova, A., and L. Pavlata. 2007. Chromium as an essential nutrient: a review. Vet. Med. (Praha) 52:1-18.

Pollard, G. V., C. R. Richardson, and T. P. Karnezos. 2002. Effects of supplemental organic chromium on growth, feed efficiency and carcass characteristics of feedlot steers. Anim. Feed Sci. Technol. 98:121-128. doi:10.1016/s0377-8401(02)00010-x

Sano, H., D. N. Mowat, R. O. Ball, and D. R. Trout. 1997. Effect of supplemental chromium on whole-body kinetics of glucose, lactate, and propionate in rams fed a high grain diet. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 118:117-121. doi:10.1016/s0305-0491(97)00023-0

Scanes, C. G. 2003. Origins of domestic animals and their importance to man. In: C. G. Scanes, editor, Biology of growth of domestic animals. Iowa State Press, Ames, Iowa. p. 3-19.

Spears, J. W., C. S. Whisnant, G. B. Huntington, K. E. Lloyd, R. S. Fry, K. Krafka, A. Lamptey, and J. Hyda. 2012. Chromium propionate enhances insulin sensitivity in growing cattle. J. Dairy Sci. 95:2037-2045. doi:10.3168/jds.2011-4845

Swanson, K. C., D. L. Harmon, K. A. Jacques, B. T. Larson, C. J. Richards, D. W. Bohnert, and S. J. Paton. 2000. Efficacy of chromium-yeast supplementation for growing beef steers. Anim. Feed Sci. Technol. 86:95-105. doi:10.1016/s0377-8401(00)00142-5

Yan, X., W. Zhang, J. Cheng, R. Wang, D. O. Kleemann, X. Zhu, and Z. Jia. 2008. Effects of chromium yeast on performance, insulin activity, and lipid metabolism in lambs fed different dietary protein levels. Asian-Aust. J. Anim. Sci. 21:853-86. doi:10.5713/ajas.2008.70643

Table 5.1. Composition of finishing diet fed to steers supplemented with and without yeast combined with Cr propionate

Item	% DM
Ingredient	
Steam-flaked corn	57.90
Wet corn gluten feed	30.00
Ground alfalfa	8.00
Limestone	1.46
Salt	0.30
Vitamin/mineral premix[1]	0.18
Feed additive premix[2]	2.16
Calculated nutrient composition[3]	
CP	14.1
NDF	19.4
Ca	0.70
P	0.48
K	0.70

[1]Formulated to provide the following added nutrient levels: 2,200 IU/kg vitamin A; 22 IU/kg vitamin E (alpha tocopherol acetate); 0.10 mg/kg Co (cobalt carbonate); 10 mg/kg Cu (copper sulfate); 0.6 mg/kg I (ethylenediamine dihydriodide); 60 mg/kg Mn (manganese sulfate); 0.30 mg/kg Se (sodium selenite); 60 mg/kg Zn (zinc sulfate).

[2]Formulated to provide 300 mg/d monensin and 90 mg/d tylosin (Elanco Animal Health, Greenfield, IN) in a ground corn carrier. Yeast combined with Cr propionate (Y+Cr; Vi-Cor, Mason City, IA) was added to the premix to equal a feeding rate of 3.3 g/d (as-fed) Y+Cr treatment to provide 3.2 mg Cr per animal daily .

[3]Nutrient composition was calculated based on NRC (2000) values for individual ingredients.

Table 5.2. Growth performance of steers separated into light and heavy weight groups and supplemented 0 (**Control**) or 3.3 g/d of a yeast Cr propionate combination (**Y+Cr**; Vi-Cor, Mason City, IA)[1]

	Light		Heavy			*P*-value[3]		
Item	Control	Y+Cr	Control	Y+Cr	SEM	Trt	WG	Trt × WG
Initial no. of steers	126	126	126	126	-	-	-	-
BW[4], kg								
Initial	384	383	420	420	5.76	0.91	< 0.001	0.83
21-d	459	463	491	492	4.61	0.18	< 0.01	0.34
42-d	505	507	535	538	5.50	0.51	< 0.01	1.00
63-d	551	555	579	584	5.05	0.27	< 0.01	0.87
84-d	585	590	611	616	4.93	0.19	< 0.01	0.93
105-d	616	619	646	646	5.63	0.74	< 0.01	0.75
126-d	634	635	671	669	6.08	0.86	< 0.01	0.73
148-d	655	663	-	-	6.55	0.30	-	-
Day 1-21								
No. of steers	126	126	125	126	-	-	-	-
ADG, kg/d	2.59[a]	2.87[b]	2.58[a]	2.49[a]	0.09	0.21	0.08	0.02
DMI, kg/d	12.54	12.44	12.97	12.73	0.16	0.31	0.05	0.68
G:F	0.2073[a]	0.2314[b]	0.1991[a]	0.1960[a]	0.008	0.12	0.03	0.05
Day 22-42								
No. of steers	126	126	125	126	-	-	-	-
ADG, kg/d	2.20	2.10	2.12	2.19	0.09	0.86	0.92	0.37
DMI, kg/d	12.70	12.32	13.03	12.92	0.19	0.07	0.08	0.28
G:F	0.1729	0.1703	0.1627	0.1698	0.006	0.72	0.40	0.44
Day 43-63								
No. of steers	126	126	125	126	-	-	-	-
ADG, kg/d	2.20	2.25	2.11	2.21	0.07	0.19	0.45	0.63
DMI, kg/d	12.66	12.38	13.08	13.02	0.20	0.40	0.03	0.52
G:F	0.1738	0.1820	0.1616	0.1702	0.006	0.04	0.13	0.97
Day 64-84								
No. of steers	126	126	124	126	-	-	-	-
ADG, kg/d	1.59	1.68	1.50	1.51	0.07	0.33	0.18	0.38
DMI, kg/d	13.13	12.83	13.36	13.29	0.22	0.39	0.17	0.60
G:F	0.1209	0.1308	0.1125	0.1134	0.005	0.09	0.07	0.16
Day 85-105								
No. of steers	126	126	124	126	-	-	-	-

ADG, kg/d	1.49	1.38	1.66	1.46	0.06	0.04	0.07	0.44
DMI, kg/d	13.10	12.47	13.26	12.93	0.25	0.09	0.25	0.58
G:F	0.1135	0.1110	0.1255	0.1127	0.004	0.08	0.12	0.22
	Day 106-126							
No. of steers	126	126	124	126	-	-	-	-
ADG, kg/d	0.85	0.74	1.26	1.12	0.11	0.30	< 0.01	0.92
DMI, kg/d	11.94	11.78	12.53	12.09	0.24	0.24	0.10	0.58
G:F	0.0715	0.0617	0.1000	0.0933	0.009	0.38	< 0.01	0.86
	Day 127-148							
No. of steers	126	125	-	-	-	-	-	-
ADG, kg/d	0.98	1.30	-	-	0.12	0.07	-	-
DMI, kg/d	12.16	12.02	-	-	0.24	0.68	-	-
G:F	0.0811	0.1087	-	-	0.01	0.11	-	-
Overall[5]								
ADG, kg/d	1.70	1.76	1.88	1.84	0.03	0.69	< 0.001	0.06
DMI, kg/d	12.57	12.29	13.00	12.83	0.17	0.18	0.03	0.75
G:F	0.1349[a]	0.1430[b]	0.1445[b]	0.1434[b]	0.002	0.08	0.09	0.03

[a, b]Within a row, means without a common superscript letter are different ($P \leq 0.05$).

[1]Provided 3.2 mg Cr/d.

[2]Heavy block shipped day 125 and light block shipped day 148.

[3]Effect of treatment = Trt; effect of weight group = WG.

[4]BW measurements = gross BW.

[5]From trial initiation through harvest.

Table 5.3. Carcass traits of steers separated into light and heavy weight groups and supplemented 0 (**Control**) or 3.3 g/d yeast combined with Cr propionate (**Y+Cr**; Vi-Cor, Mason City, IA)[1]

	Light		Heavy			*P*-value[2]		
Item	Control	Y+Cr	Control	Y+Cr	SEM	Trt	WG	Trt × WG
Final BW[3], kg	629	637	645	642	5.84	0.56	0.19	0.26
HCW, kg	398	400	408	407	3.75	0.77	0.09	0.61
Dressed yield, %	63.2	62.8	63.3	63.4	0.20	0.49	0.05	0.16
Liver abscesses, %	10.4	6.3	16.9	16.9	2.67	0.47	< 0.01	0.47
LM area, cm^2	88.15	90.10	93.10	93.00	0.97	0.31	< 0.01	0.27
12^{th} rib s. c. fat[4], cm	1.30	1.23	1.26	1.25	0.04	0.32	0.89	0.45

[1]Provided 3.2 mg Cr/d.

[2]Effect of treatment = Trt; effect of weight group = WG.

[3]Final BW = gross BW × 0.96.

[4]One carcass from control heavy group; 9 carcasses from Y+Cr light group steers and 11 carcasses from the Y+Cr heavy group do not have measurements for 12^{th} rib back fat due to excessive trim from equipment failure at the abattoir. One animal was condemned at slaughter from the control light group due to melanosis.

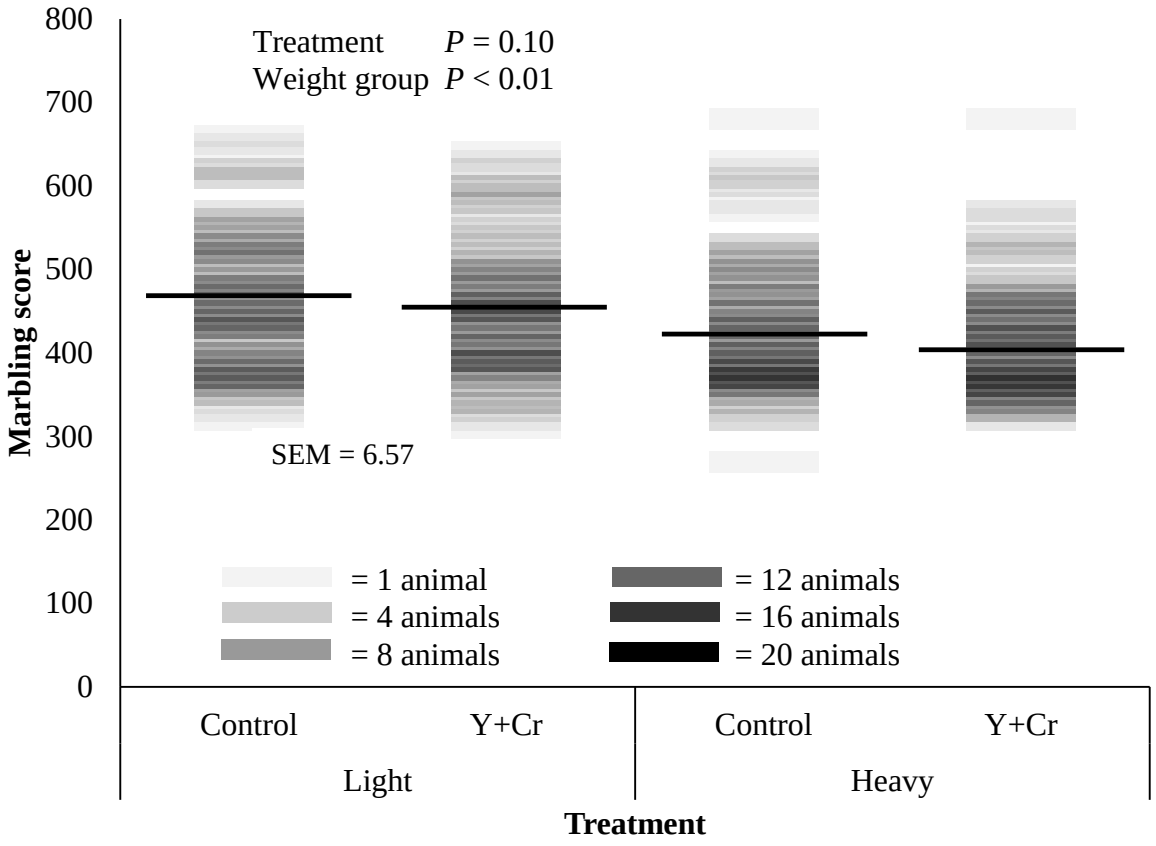

Figure 5.1. Marbling scores of carcasses from steers separated into light and heavy weight blocks and supplemented 0 (**Control**) or 3.3 g/d yeast combined with Cr propionate (**Y+Cr**; Vi-Cor, Mason City, IA), which provided 3.2 mg Cr per animal daily. Marbling scores were determined by camera imaging (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany); Trace = 200 to 299; Slight = 300 to 399; Small = 400 to 499; Modest = 500 to 599; Moderate = 600 to 699; Slightly abundant = 700 to 799. Black horizontal lines indicate treatment means. There was no interaction between treatment and weight group ($P = 0.47$).

Table 5.4. Quality and yield grades of steers separated into light and heavy weight groups and supplemented 0 (**Control**) or 3.3 g/d yeast Cr propionate combination (**Y+Cr**; Vi-Cor, Mason City, IA)[1]

	Light		Heavy			*P*-value[2]		
Item	Control	Y+Cr	Control	Y+Cr	SEM	Trt	WG	Trt × WG
USDA Prime, %	0.8	0.8	0.0	0.8	0.70	0.56	0.60	0.56
USDA high Choice, %	6.4	5.6	5.7	0.0	1.81	0.02	0.19	0.07
USDA mid Choice, %	23.3	19.1	13.0	8.9	3.51	0.23	0.02	1.00
USDA low Choice, %	44.0	44.8	42.9	44.0	5.25	0.86	0.86	0.97
USDA Select, %	25.5	27.2	36.0	43.9	5.21	0.38	0.03	0.57
USDA Standard, %	0.0	0.8	1.6	1.6	1.03	0.69	0.28	0.69
B-maturity, %	0.0	1.7	0.8	0.8	1.02	0.43	1.00	0.43
Overall yield grade	2.79[a]	2.54[b]	2.66[ab]	2.67[ab]	0.05	0.02	1.00	0.02
Yield grade 1, %	4.0[b]	10.4[a]	6.4[ab]	5.6[ab]	1.51	0.09	0.45	0.04
Yield grade 2, %	30.5	40.9	30.5	35.1	3.70	0.03	0.53	0.34
Yield grade 3, %	49.5	35.9	55.0	48.1	4.15	< 0.01	0.13	0.21
Yield grade 4, %	15.2	10.4	6.5	9.6	2.36	0.72	0.07	0.12
Yield grade 5, %	0.8	2.4	1.6	1.6	0.98	0.42	1.00	0.44

[a, b]Within a row, means without a common superscript letter are different ($P \leq 0.01$).

[1]Provided 3.2 mg Cr/d.

[2]Effect of treatment = Trt; effect of weight group = WG.

Table 5.5. Plasma glucose and lactate concentrations of steers separated into light and heavy weight group and supplemented 0 (**Control**) or 3.3 g/d yeast Cr propionate combination (**Y+Cr**; Vi-Cor, Mason City, IA)[1]

	Light		Heavy			*P*-value[2]		
Item, m*M*	Control	Y+Cr	Control	Y+Cr	SEM	Trt	WG	Trt × WG
Pretrial								
Glucose	3.92	3.90	3.83	3.90	0.08	0.78	0.59	0.58
Lactate	3.27	2.64	3.52	2.89	0.32	0.68	0.44	0.15
Day 49								
Glucose	4.45	4.80	3.99	4.43	0.35	0.28	0.26	0.89
Lactate	3.42	3.31	2.06	3.42	0.59	0.70	0.40	0.32
Day 94								
Glucose	5.08	4.94	4.32	4.44	0.23	0.98	0.02	0.59
Lactate	4.36	4.16	3.07	3.24	0.46	0.97	0.04	0.70

[1]Provided 3.2 mg Cr per animal daily.

[2]Effect of treatment = Trt; effect of weight group = WG.