THESIS

EFFECT OF ZINC SOURCE AND DOSE AND CHROMIUM SUPPLEMENTATION ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FEEDLOT CATTLE

Submitted by

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In partial fulfilment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2018

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ABSTRACT

EFFECT OF ZINC SOURCE AND DOSE AND CHROMIUM SUPPLEMENTATION ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FEEDLOT CATTLE

Four-hundred cross-bred steers were utilized in a randomized complete block design to investigate the effect of supplemental Zn source and concentration and Cr supplementation on performance and carcass characteristics of feedlot steers fed a steam-flaked corn-based finishing diet. Steers were blocked by initial BW with in cattle source and randomly assigned within block to 1 of 5 treatments (10 steers/pen; n = 8 pens per treatment). Prior to the initiation of the experiment, trace mineral supplement sources were analyzed for Zn and Cr. Zinc and Cr concentrations of the Zn sources were used to balance all dietary treatments to obtain correct Zn and Cr experimental doses. Treatments consisted of: 1) 90 mg Zn/kg DM from $ZnSO_4$ and 0.25 mg Cr/kg DM from Cr propionate (90ZS+Cr); 2) 30 mg Zn/kg DM from Zn hydroxychloride and 0.25 mg Cr/kg DM from Cr propionate (30ZH+Cr); 3) 90 mg Zn/kg DM from Zn hydroxychloride and 0.25 mg Cr/kg DM from Cr propionate (90ZH+Cr); 4) 60 mg Zn/kg DM from $ZnSO_4$ and 30 mg Zn/kg DM from Zn methionine (90ZSM); and 5) 90 mg Zn/kg DM from Zn hydroxychloride (90ZH). Steers were individually weighed on d-2 and on two consecutive days at the end of the experiment. Initial liver biopsies were obtained from all steers during processing. Equal numbers of pen replicates per treatment were transported to a commercial abattoir on d 162, 176, and 211 and slaughtered; individual carcass data and final liver samples were collected. Total finishing dietary Zn and Cr concentrations were 118.4, 58.2, 114.2, 123.0, and 108.2 mg Zn/kg DM and 0.711,0. 647, 0.731, 0.767 and 0.521 mg Cr/kg DM, for treatments

1 to 5, respectively. There were no treatment main effects for any response variables measured. However, treatment was a significant source of variation for certain response variables when single degree of contrasts (unprotected F-test) were performed. Steers receiving 90ZH+Cr had greater final BW ($P \leq 0.04$) and ADG ($P \leq 0.03$) when compared to steers receiving 90ZH. Additionally, hot carcass weight was 8.5 kg greater ($P \leq 0.03$) for 90ZH+Cr compared to 90ZH supplemented steers. Steers receiving 90ZH+Cr had greater longissimus muscle area when compared to steers receiving 90ZSM. Dry matter intake, G:F, final liver Zn concentrations, and all other carcass parameters (dressing percentage, marbling score, yield grade, subcutaneous adipose tissue depth, and KPH) were similar across treatments. These data indicate, that under the conditions of this experiment, Zn source and concentration had no impact on live performance, liver mineral Zn concentrations, and carcass characteristics. Supplemental Cr in diets containing 90 mg of supplemental Zn/kg DM may improve final BW, ADG, and hot carcass weights.

ACKNOWLEDGEMENTS

I am strong because I had to be,
I am smarter because of my mistakes,
I am happier because I have known sadness,
and I am wiser because I have learned.

-Unknown

"I didn't really know what I wanted to do, but
I knew the woman I wanted to become."

-Diane von Furstenburg

To know now that I have accomplished this milestone, is to realize I am that much closer to being the woman I want to be. Agriculture is our greatest pursuit and be a small part of this industry is a gift. I stand on the backs of giants, striving to be worthy of their knowledge, while looking to the future of the industry. I am and will continue to be eternally grateful for every person past and present who believed I could accomplish whatever I set my mind to. Because of those individuals who gave me a chance to learn, I am now a part of the next generation of Agriculturists.

They say it takes a village to raise a child, and a village indeed it took. To my parents, I will never be able to repay you for the opportunities I have been gifted, a lifetime will never be enough. To my sisters, each of you are uniquely part of me every day, I wouldn't trade the world for the craziness that ensues every time we congregate under one roof. To my graduate mentors, I will never be able to repay the kindness and guidance I have received, thank you for letting me make mistakes and allowing me to grow from them. Finally, to my best friend, you taught me more than I could have ever imagined about what a partner in life should be, let the journey continue.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I – LITERATURE REVIEW

Minerals are essential nutrients that are commonly supplemented in the diets of livestock species. They are divided into two categories: macro and micro minerals or "trace" minerals. Macrominerals are required at gram concentrations, as compared to trace minerals which are required at milligram or microgram concentrations (NASEM, 2016). Macrominerals consist of calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), chlorine (Cl), and sulfur (S) (NASEM, 2016). Trace minerals consist of chromium (Cr), cobalt (Co), copper (Cu), iodine (I) manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn) (NASEM, 2016). It has been understood for many decades that minerals play a substantial role in maintaining homeostasis and proper physiological function in livestock. Macrominerals are essential building blocks to a multitude of tissues; for example, bone, skeletal, smooth, and cardiac muscle, and nervous tissue. Macrominerals also aid in metabolic function by preserving acid-base balance, osmotic pressure, membrane electric potential, and nerve transmission (NASEM, 2016). Trace minerals are found in very low concentrations in the body and most commonly observed to be vital components in maintaining enzyme activity and hormone function.

It is common practice to supplement trace minerals to receiving diets in excess of the recommended concentrations to account for decreased feed intake of weaned calves arriving to the feed yard. However, the majority of trace minerals are poorly absorbed and are excreted from the animal and, in turn, end up in the environment and potentially contaminate soil and ground water sources in the areas where livestock are concentrated, as well as areas where animal waste is applied. Currently, phosphorus (P) is the only supplemented mineral that is subject to

oversight under the Natural Resources Conservation Service, for levels deemed acceptable in soil, groundwater, and runoff. It is more than likely that in the future, regulatory limits will be set for other minerals based on excretory losses and not on solely animal need. To minimize excretory losses of metals, sources providing better utilization to the animal must be found. In order to determine animal utilization, criteria of mineral adequacy and availability must be established using immunological, hormonal, and biochemical indices. This literature review will focus on the trace minerals of chromium (Cr) and zinc (Zn). The physiological functions, absorption capabilities, intercellular transportation, storage, excretion, and deficiency and toxicity signs for these elements will be discussed. Furthermore, a literary review of peer reviewed experimentation in the areas of Cr and Zn supplementation in the bovine species.

CHROMIUM

Chromium (Cr) is a metallic element derived from chromite ore (Cr_2O_3 FeO). Chromium (III) is the most stable of the four commonly observed oxidized states of Cr (McDowell, 2003). Chromium is most commonly used in the production of stainless steel (Fe-Cr alloys) as well as chrome, leather tanning, pigment manufacturing and wood preservations (Spears, 2017). In July of 2009, a regulatory discretion letter issued by the FDA CVM permitted commercial use of chromium in the form of Cr propionate (Kemin Industries Des Moines, Iowa) at a maximum inclusion rate of 0.5 mg Cr/kg DM in cattle diets. To date no documented cases of Cr deficiency or toxicity in beef cattle production have been reported. Currently, no Cr requirement has been established in beef cattle production.

Chromium is present in the soil at various concentrations in both Cr (III) and Cr (IV) forms, and depending on soil conditions Cr (IV) may reduce to Cr (III), or Cr (III) can be oxidized to Cr (IV) (Cary and Kubota, 1990). Plants tend to favor Cr (IV) over Cr (III)

sequestration in the root, leaf, and fruit in descending order of concentrations (Cary and Kubota, 1990). Current diets fed to beef cattle are assumed to adequately supply Cr because no Cr deficiencies have been documented (Spears, 2017). This assumption is possible provided feedstuffs processed by chopping, rolling, and pelleting, contain varying amounts of Cr either sequestered in the plant root, leaf, or fruit, or through contamination from stainless steel equipment. Spears (2017) collected samples of common feedstuffs used in cattle diets from domestic and international feed manufacturing facilities including samples of alfalfa hay, corn silage, beet pulp, corn, corn distillers dried grains with solubles, cottonseed hulls, phosphate sources, soybean hulls, and wheat; all in various stages of processing from chopped to pelleted. Phosphate sources contained on average 135 mg Cr/kg DM; cereal grains like corn, soybeans, and wheat contained 0.020, 0.077, and 0.045 mg Cr/kg DM, respectively; alfalfa hay and corn silage contained, on average, 0.522 and 0.222 mg Cr/kg DM, respectively; and finally, by-product feeds contain anywhere from 0.040 mg Cr/kg for cottonseed hulls to 1.22 mg Cr/kg for beet pulp (Spears, 2017). It is critical to note bioavailability of Cr from either plant material or Cr contamination from processing is assumed to be very low. Additionally, little research has been conducted on feedstuffs to provide a more accurate assessment of Cr bioavailability (Spears, 2017).

Chromium was initially identified as a component in Glucose Tolerance Factor (GTF) by Schwartz and Mertz in 1959. Glucose tolerance factor was described as necessary for the maintenance of normal glucose tolerance in rats (Schwartz and Mertz, 1959). Mertz went on to perform research in human subjects, observing improved glucose tolerance by Cr supplementation (Glinsmann and Mertz, 1966). Although early Cr research focused on human applications of Cr, chromic oxide (Cr_2O_3), which is considered to be indigestible, has been used

as a biomarker in swine digestibility studies (Schurch et al. 1952; Clawson, et al. 1955). The first production animal response to Cr was documented in turkey pullets, that were supplemented with 20 mg Cr/kg from chromium chloride ($CrCl_3$; Steele and Rosebrough, 1979). Samsell and Spears (1989), performed the first peer reviewed research in ruminants, evaluating blood constituents in growing lambs fed high and low fiber diets supplemented with 10 mg Cr/kg from $CrCl_3$. They evaluated the effects of $CrCl_3$ on plasma glucose, serum free fatty acids, and serum cholesterol concentrations. Samsell and Spears (1989) concluded Cr did not impact plasma glucose concentrations and serum cholesterol. However, the effects of Cr were variable and diet dependent under the conditions of the two studies conducted. To date Cr research in beef cattle has involved the use of Cr propionate, Cr picolinate, Cr methionine, Cr amino acid chelate, high Cr-yeast, Cr nicotinic acid complex, and inorganic Cr chloride ($CrCl_3$) (Spears, 2013).

According to Mertz (1993) and McDowell (2003), Cr values in biological samples reported prior to 1980 are considered to be inaccurate. Stainless steel contamination (Fe-Cr alloys) have the ability to skew results of Cr analysis, through further processing of diets in Wiley mills. Additionally, experiments prior to the 1980's, atomic absorption experiments were attempting to measure a very small signal against a large background, resulting in a linear correspondence between background absorbance and apparent Cr content of various samples (Vincent, 2016). Technological advancements after 1980 improved the accuracy and sensitivity of determining extremely low Cr concentrations in biological tissues (McDowell, 1992). Such improvements include; graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma mass spectrometry (ICP-MS; Vincent, 2016). Graphite furnace atomic absorption spectrometry works to disassociate Cr from other atoms present in the sample when injected into the furnace (Vincent, 2016). Light is passed through the hollow cathode tube,

that is element specific, through the cloud of atoms, and absorbed light from atom of interest is measured. The amount of absorbed light corresponds to a unique atom and determines the quantity of atoms in the initial sample (Vincent, 2016). An ICP-MS generates constituent atoms and ions with the use of an ICP, which allows for the ions themselves to be detected, rather than the photons of light generated by the atom in a GFAAS. The ions are then detected by their charge to mass ratio using either a quadrupole analyzer or a magnetic sector analyzer (Vincent, 2016). Inductively coupled plasma-mass spectrometry provides an improved specificity for Cr isotope detection when compared to GFAAS and has a detection limit an order of magnitude lower than a GFAAS, however cost and increased care in the operation of ICP-MS lends to the preference of a GFAAS (Vincent, 2016).

Absorption

Intestinal absorption of Cr (III) is relatively low in rats. The estimated absorption coefficient of Cr in rats is 0.4% to 2% (Ducros, 1991). Dowling et al. (1989) utilized double perfusion of rat small intestine *in situ*, with *in vitro* preparation of the intestinal lumen and its vasculature perfused simultaneously, the test dose taken up post meal was 5.90%, 5.52% transported in the vascular perfusate, and 0.38% retained by the small intestine (Dowling et al.,1989). Chen et al. (1973), utilized the inverted gut technique, as described by Sahagian et al. (1967), to determine the absorption rate of $^{51}CrCl_3$ in the presence of four chelating agents; citrate, oxalate, phytate, and ethylenediaminetetraacetic acid (EDTA). They determined in the control group that in rats, the jejunum is the location where the highest percentage of Cr is absorbed, followed by the duodenum and ileum. The mechanism for Cr absorption is not largely understood, with passive diffusion and active transport both expected to play a role in the uptake of Cr. Initially Mertz et al. (1965) and Donaldson and Barreras (1966) reported the absorption of

Cr III as a process that cannot be saturated leading to the assumption of passive diffusion along an electrochemical concentration gradient. Saturation of Cr III *in vitro* this may suggest transport proteins are involved in a facilitated transport of Cr from the lumen of the jejunum (Ducros, 1991).

Stoecker (1996) and Offenbacher et al. (1997) suggest ascorbic acid and aspirin may promote the absorption of Cr, whereas antacids and phytate in non-ruminants decrease absorption of Cr. Interactions with other metallic elements can occur, specifically Fe^{2+} and Zn^{2+}. The explanation for the interaction between Cr and Zn is demonstrated by Hahn and Evans (1975) with Zn deficient rats, where whole-body absorption and intestinal content of ^{65}Zn and ^{51}Cr where measured following oral dosing of the isotopes. When Zn ($ZnSO_4$) was administered orally with ^{51}Cr, there was a marked decrease in isotope absorption and intestinal content in rats that were Zn deficient (Hahn and Evans, 1975). It is apparent there is a similar transport mechanism utilized by Cr and Zn, though the role of this transporter in the regulation of Cr is still unclear. The transport mechanisms involved in intestinal absorption and plasma transport for Cr are not well understood, and as discussed in previously in this review active transport proteins may play a role. Therefore, the interaction of Cr and Fe is likely to occur with the Fe^{2+} transporter Ferritin and the Fe^{3+} divalent metal transporter, because of the looser specificity of these transporters (Dowling et al., 1989). Furthermore, in the blood stream Cr and Fe^{3+} compete for transferrin binding when high concentrations of Fe are present. Chelation of Cr to organic compounds like propionate may be favorable to absorption abilities of Cr, however it is unclear how different sources of dietary Cr compare to each other regarding bioavailability (Spears, 2013). However, Kegley and Spears (1995) investigated $CrCl_3$, Cr-yeast, and Cr nicotinic acid complex supplementation with a control group in an effort to determine Cr source effects on

immune response, glucose metabolism, and performance in stressed feeder calves. Although, Cr-yeast and Cr-nicotinic acid complex were observed to enhance some measured immune function indictors and no morbidity was observed, overall performance of the steers on study were not different across treatments (Kegley and Spears, 1995). Never the less, these results may support an organic source of Cr may have differing absorption abilities.

Metabolism

Absorbed Cr is assumed to be transported by transferrin in the blood stream, with the affinity of Cr to transferrin believed to be like that of Fe (Fe II; Hopkins and Schwarz, 1964). Transferrin is considered to be the primary transporter of Cr, with albumin and some degradation products also having an affinity for Cr under neutral and slightly basic pH conditions *in vitro*. However, Cr transport by transferrin has not be demonstrated *in vivo* (Vincent, 2000b). Albumin is believed to be the secondary acceptor and transporter of Cr when transferrin is bound (Ducros, 1991). Concentrations of Cr are not sequestered in high concentrations, however Cr is found in the liver, spleen, soft tissue, and bone. Chromium is excreted largely through the urine with limited quantities released through fecal matter, sweat gland excretions, and hair (McDowell, 2003).

Function

Chromium has been observed to function as a component of glucose tolerance factor (GTF). Mertz et al. (1974) hypothesized that a Cr containing protein assists in the function of insulin, likely because of Cr role as a cofactor for the activation of insulin described by Mertz, 1981. Therefore, Cr is considered an active component of GTF, although inorganic Cr (III) is considered to be less active (McDowell, 2003). Evans and Bowman (1992) findings are similar to the hypothesis of Mertz et al. (1974) where Cr may enhance the communication with insulin

and in turn acts to signal for increased uptake of glucose through the increased fluidity of the cell membrane.

The proposed biological mechanism for a Cr-insulin interaction is believed to be through a Cr-dependent oligopeptide that activates the insulin receptor tyrosine kinase (Figure 1.; Vincent, 2000b). Davis and Vincent (1997a) determined "the chromium-containing oligopeptide low molecular weight chromium-binding substance (LMWCr) does not affect the tyrosine protein kinase activity of rat adipocytic membrane fragments in the absence of insulin; however, insulin-stimulated kinase activity in the membrane fragments is increased up to 8-fold by the oligopeptide". Following this experiment, Wada and Yamamoto (1987) and later Vincent (2000a), isolated and characterized low molecular weight Cr-binding substance (LMWCr) or chromodulin. Chromodulin has been isolated from rabbit liver (Yamamoto et al., 1987), porcine kidney (Sumrall and Vincent, 1997), bovine liver (Davis and Vincent, 1997a), colostrum (Yamamoto et al., 1988), dog liver (Wada et al., 1983) and mouse and rat liver (Yamamoto et al., 1983).

Czech and Corvera (1999); Vincent (2000a), provided evidence that through the increase activity of tyrosine kinase, glucose transporter-4 is translocated to the cell membrane surface, allowing for the increased sensitivity of insulin in skeletal muscle tissue and adipose tissues. Specifically, adipocytes in rats have been reported to have an increase of glucose uptake measured by the resulting increase of CO_2 and lipid oxidation (Mertz et al., 1961; Davis and Vincent, 1997b; Vincent, 1994).

In a review, Vincent (2000b) described the conclusions of Cr homeostasis studies evaluating responses to glucose and insulin challenges as providing evidence to that chromodulin is part of an autoamplification system for insulin signaling. The autoamplification responds to an

increase in insulin concentrations during an increase in blood glucose, which triggers a conformation change of the transmembrane insulin receptor to allow the binding of chromodulin.

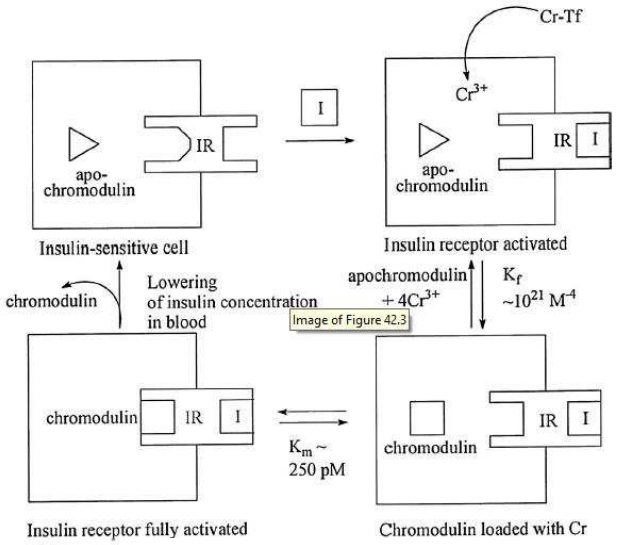

Figure 1 – Chromodulin Function

Once signaled by the binding of insulin to the α subunit of the insulin receptor, the receptor autophosphorylates the tyrosine residues to the β subunit, forming an active kinase (Saltiel, 1994). The inactive from of chromodulin is stored as apo-chromodulin, which is most often found in insulin sensitive cells (Yamamoto et al., 1989). In the event of an insulin increase the migration of chromodulin to insulin sensitive cells also occurs (Morris et al., 1993a; Morris et al., 1993b). Once insulin concentrations have returned to normal concentrations in the blood, chromodulin is eliminated from the cell, which is consistent with the increase of chromic complexes being excreted in the urine, post glucose challenge (Vincent, 2000b).

Chromium influence on Glucose Metabolism

Supplementation of Cr has been shown to influence glucose metabolism in Holstein calves (Bunting et al.,1994); Holstein heifers (Sumner et al., 2007); growing beef steers (Kegley et al., 2000; Bernhard et al., 2012a); and growing beef heifers (Spears et al., 2012). Bunting et al. (1994) utilized Cr picolinate (Cr-P; 0.37 mg CrP/kg) in a corn and cottonseed hull-based diet fed

to weaned Holstein calves (experiment 1 n=10 steers; experiment 2 n=14 heifers). Calves were subjected to glucose tolerance and insulin challenge tests (exp.1 d70-87; exp.2 d70) and performance measurements were obtained (ADG, DMI, and feed efficiency) over the duration of the experiment. No difference between the Cr-P supplemented group and control were noted for performance measurements, however both glucose tolerance and insulin challenge tests yielded higher clearance rates of glucose for Cr-P supplemented calves compared to the control group, with no influence of treatment on plasma insulin concentrations. Plasma cholesterol concentration was also measured, yielding a reduction of cholesterol concentrations in Cr-P supplemented calves, further suggesting an effect of treatment on lipid metabolism.

Sumner et al. (2007) utilized growing Holstein heifers (n=20) who were fed 0, 5, 10, or 15 mg of Cr propionate (Cr-Prop)/d in a Latin square design. A two-week adaption period was conducted for all heifers, followed by 2 weeks of treatment for 4 time periods, with two weeks between the start of a new treatment period. Measurements of individual body weights and body condition scores were obtained but did not differ among treatments. Glucose tolerance tests were performed following 14 days of treatment administration for each period in the Latin Square. Although the supplementation period was considerably shorter than other studies evaluated, insulin concentrations tended to be lower and glucose clearance rates were similar across treatments (Sumner et al., 2007). Length of supplementation and possible accumulation of Cr may have resulted in differing results across treatments. The suspected accumulation of Cr may be evident in the fourth period of treatment where insulin concentration in control animals, receiving no supplemental Cr, were less than control animals in the previous three period of treatment. Furthermore, this observation may be a result of a long-term effect of supplemental Cr on insulin sensitivity.

Kegley et al. (2000) evaluated steers supplemented with chromium-L-methionine at 0.40 or 0.80 mg Cr/kg of diet, and control animals receiving no supplemental Cr. Cattle received treatment for 22, 23, or 24 days prior to glucose tolerance and insulin challenge tests. Live performance measurements did not differ among treatments groups; however, plasma glucose concentrations were reduced in 0.40 mg supplemented steers as compared to control and 0.80 treatment groups. Similarly, glucose clearance rates were greater for Cr-L-methionine cattle when compared to cattle fed control diets. Again, length of supplementation may explain the inconsistency for glucose concentrations post glucose tolerance test in the 0.4 and 0.8 supplemented groups. In contrast, Bernhard et al. (2012a) observed inconsistent results following glucose tolerance and insulin challenge tests in steers fed a control diet or supplementation with 0.20 mg/kg of Cr from Cr-Prop for 53 days. More specifically, glucose concentrations, glucose clearance rates and insulin concentrations prior to glucose tolerance test were not different, however post insulin concentration following the glucose tolerance test tended to be greater for steers receiving supplemental Cr-Prop (Bernhard et al., 2012a). A similar trend occurred during the insulin challenge, where insulin concentrations were not different across treatments, however post challenge glucose concentrations were greater in Cr-Prop supplemented steers (Bernhard et al., 2012a). Interesting to note is the more consistent influence of Cr supplementation on non-esterified fatty acid (NEFA) concentrations, displaying lower levels prior to and following glucose tolerance tests. This suggests less dependence of Cr-Prop supplemented steers on lipid metabolism for energy (Bernhard et al., 2012a).

In an effort to isolate an ideal concentration inclusion of Cr-Prop, Spears et al. (2012) utilized thirty-six Angus and Angus x Simmental heifers in a dose titration (0, 0.47, 0.94, and 1.42 mg Cr/kg DM) experiment evaluating insulin sensitivity. Glucose tolerance tests were

performed on day 44 of the experiment. Decreased insulin concentrations were observed in Cr-Prop supplemented heifers when compared to the non-supplemented group, however no differences were noted between Cr-Prop supplemented treatments. These results suggest Cr-Prop supplementation at 0.47 mg Cr/kg DM would adequately meet the requirements of a growing beef heifer (Spears et al., 2012).

Cr influence on Immune Function

Stressed calves entering a growing and/or finishing production setting tend to have reduced intakes following weaning, transportation, and comingling with new cattle. Efforts to fortify diets that provide adequate amounts of micro minerals have yielded interesting results from a nutrient utilization standpoint, rather than traditionally measured live performance parameters. More specifically, Cr has been suggested to influence serum cortisol levels as well as overall morbidity in some of the following studies; Moonsie-Shageer and Mowat (1993) with Cr-yeast, Kegley et al. (1997) with Cr-nicotinic acid, and Bernhard et al. (2012b) with Cr-Prop.

Moonsie-Shageer and Mowat (1993) supplemented steers with Cr-yeast at 0, 0.2, 0.5 and 1.0 mg Cr/kg DM in a corn silage based diet. Collection of blood occurred at days 0, 7, 14, 21, 28 to measure blood metabolites, mineral concentrations, and immunoglobin concentrations. Live performance was measured but yielded inconsistent results; increased ADG was observed for 0.2 and 1.0 mg Cr/kg DM fed groups, DMI was increased for 0.2 and 0.5 mg Cr/kg DM fed groups, yet feed efficiency was similar across all treatments even though final body weight was greatest for Cr supplemented cattle as compared to control. Never the less, serum cortisol levels did decrease with Cr supplementation, however lower morbidity rates were observed for Cr supplemented steers (Moonsie-Shageer and Mowat, 1993). These data suggest improvement of humoral immune function, though the mechanism of enhanced immunity to Cr supplementation

is not entirely understood. Additionally, improvement of performance measurements are not consistent, warranting more research.

Kegley et al. (1997) evaluated growing Angus crossbred steers (n = 48) receiving supplemental Cr (0.04 mg Cr/kg DM from Cr-nicotinic acid complex) in a two by two factorial design, where cattle received Cr supplementation or a control diet, and cattle were transported or not transported. Chromium was supplemented for 56d prior to a stress inducing transportation, where half of each dietary treatment group was transported by truck and trailer for 343 km over 6 hours. Following transportation, steers were inoculated with infectious bovine rhinotracheitis virus (IBRV) intranasally, and continued dietary supplementation through 80 days. Cr-nicotinic acid complex fed a 0.4 mg/kg DM had no effect on immune responses measured (serum cortisol, neutrophil:lymphocyte ratio, rectal temperature, and IgG levels), yet Cr supplementation increased ADG regardless of exposure to shipping stress.

Contrastingly, Bernhard et al. (2012b) utilized cattle sourced from sale barns in Eastern Texas and transported to West Texas. Observed tendencies for linear increases in ADG, DMI, and feed efficiency for steers (n=180) supplemented with 0, 0.1, 0.2, 0.3 mg Cr-Prop/kg DM. Separately, twenty additional steers were fed 0 or 0.2 mg Cr-Prop/kg DM and were subjected a lipopolysaccharide (LPS) challenge after receiving the experimental treatments for 54d. A time x treatment interaction was observed, indicating glucose concentrations were greater and peaked earlier in Cr supplement vs. control fed steers (Bernhard et al., 2012b). Although live performance measurements and LPS challenges were performed on two separate groups of cattle, results under the conditions of the experiment still show improved performance in stressed cattle, and apparent improved immune response. It should be noted however, the measurements of immune function in this study are fewer in number as compared to previously discussed

studies, suggesting once again the need for improved understanding of the mechanisms involving Cr and immune function.

The effects of hot and humid weather conditions can result in heat stress and can negatively alter cattle performance and welfare (Zhang et al., 2014). The temperature – humidity index (THI) is a calculation used to numerically describe heat stress in cattle. Zhang et al. (2014) evaluated 24 lactating Holstein cows fed a control diet or supplemented with 3.5 mg Cr picolinate/cow daily and the effect of 3 periods of low THI, moderate THI, and high THI. Antioxidant capacity, heat shock protein 72, and cytokine responses were measured. Chromium supplementation did not affect serum glucose concentrations, whereas Cr decreased serum cholesterol concentration indicating Cr may reduce metabolic heat production by decreasing use of body fat stores in a high THI environment (Zhang et al., 2014). Furthermore, Cr increased serum concentrations of heat shock protein 72 which is likely promoted by Cr's role in anti-inflammatory responses (Zhang et al., 2014).

Cr influence on Live performance and Carcass Traits

Reports of Cr supplementation through the feedlot growing and finishing phase of production contains varied responses for average daily gain, dry matter intake, feed efficiency, final body weight. Similarly, observations from experiments including carcass traits such as hot carcass weight, dressing percentage, total fat tenderness scores, etc. are limited and varied as well.

Chang et al. (1992) supplemented ninety-six crossbred steers with 0 or 0.2 mg Cr from Cr- yeast for a total of 138 days (a 70 day growing period where cattle received corn silage based diet with either urea-corn mix or soybean meal added, followed by a 68 day finishing period on a high grain based diet). No differences among treatment were observed for ADG, DMI, feed

efficiency, dressing percentage, longissimus area, back fat, marbling score, percentage of kidney, pelvic, and heart fat. Kneeskern et al. (2016) utilized individually housed feedlot steers (n = 34) who fed to targeted back fat (1.27cm, determined by ultrasound) consuming a control diet or Cr from Cr-Prop supplemented diet containing a total amount of 3.63 Cr mg/kg and 13.47 Cr mg/kg, respectively. No differences were observed in final BW, ADG, DMI or feed efficiency; additionally, no influence of Cr supplementation was observed for HCW, back fat, KPH, or YG. Yet an increase in DP and a tendency towards larger LM area, increased marbling scores and percentage of intramuscular fat was observed for Cr supplemented steers. Furthermore, insulin sensitivity was also evaluated on d 21 and 98 of supplementation. Overall, supplementation did not affect concentrations of glucose or insulin. Similarly, Van Bibber-Krueger et al. (2016) evaluated steers blocked by weight group, light and heavy, and supplemented Cr-yeast at 3.3g/day (an average, 0.04 mg Cr/kg DM). There were no reported differences in final BW, ADG, DMI, feed efficiency, or carcass characteristics. Similar observations in varying production settings, support the points of discussed in previous sections of this review, which indicate the influence of Cr is not well understood and current measurements of live performance and carcass traits are not specific enough to detect Cr effects.

Observations of Cr supplementation with additional minerals has been evaluated in several species of livestock and laboratory animals; focus in this review is placed on Cr supplementation in combination with Zn supplementation. Limited data is able in cattle supplemented with Cr and ractopamine hydrochloride (RAC) at the end of the feeding period. Bohrer et al. (2014) utilized steers with an initial body weight of 424 ± 24 kg providing a diet with 1,000 mg of Zn propionate·steer·d^{-1} and 3 mg of Cr propionate/steer·d^{-1} with RAC, RAC only, or a control diet (20% corn silage, 20% distillers grains, 50% corn grain, and 10%

supplement) for 35 days at the end of the finishing period. No differences in live performance or carcass characteristics were observed when RAC only and Zn and Cr with RAC were compared (Bohrer et al., 2014). Additionally, Edenburn et al. (2016) also supplemented RAC with Zn (1g $Zn \cdot steer \cdot d^{-1}$ from Zn propionate) or Cr (3 mg $Cr/steer \cdot d^{-1}$ from Cr propionate) or both Zn (159.7 mg Zn/kg DM) and Cr (1.25 mg Cr/kg DM) in the diet, to steers with an initial body weight of 533 ± 94 kg for 63 days. No effect of treatment was reported for final BW, ADG, DMI, and feed efficiency, yet increased HCW in cattle supplemented with RAC and Cr was observed when compared to steers receiving RAC with Zn and Cr; all other carcass traits remained similar across all treatments (Edenburn et al., 2016). The length of supplementation, initial body weight at the beginning of supplementation, and addition of ractopamine hydrochloride to the diet with Cr or Zn or both in the diet, yield similar results to Cr alone in the diet.

The observed variation in beef cattle performance responses to Cr supplementation are unknown. Several factors such as basal dietary Cr concentrations, cattle type and breed, number of cattle evaluated in the experiment, days on feed, and analytical errors in Cr analysis could influence reported animal response to Cr supplementation. The apparent influence of Cr in lipid and glucose metabolism suggests an alteration in energy partitioning, yet current methods of live performance and carcass evaluation may not be sensitive enough to detect differences. Given the increasing discrepancies in the literature, Cr supplementation in cattle diets, in both sexes of cattle and feeding stages, warrants further investigation.

ZINC

Zinc (Zn) is primarily extracted in the form of Sphalerite or zinc sulfide (ZnS) ore, and is most commonly found in alloys such as brass. Feed grade zinc is currently available in several inorganic, organic, and hydroxychloride complexes. Evidence of Zn as an essential nutrient was

shown to be necessary for proper growth and health of laboratory animals as early as 1934 (Todd et., 1934). Induced Zn deficiencies in livestock species was first demonstrated in chicks by O'Dell and Savage (1957), followed by lambs in 1964 (Ott et al., 1964) and calves in 1967 (Mills et al., 1967).

Classic Zn deficiency manifests in most livestock species as: loss of appetite, growth depression, abnormalities of the skin and limbs, and reproductive failure (Suttle, 2010). The wide range of affected areas of the body are likely attributed to the over 300 Zn dependent enzymes (Vallee and Falchuk,1993), numerous zinc proteins (Coleman, 1992), and the necessity of Zn in over 2000 transcription factors (Beattie and Kwun, 2004; Cousins et al., 2006). Additionally, Zn can associate with cysteine and histidine residues to create zinc-finger domains in DNA binding proteins (Berg, 1990). Furthermore, Zn is also involved in gene expression, appetite control, fat absorption, and antioxidant defense.

Zinc toxicity was determined by Ott et al. (1966), where decreased weight gain in calves fed 900 mg Zn/kg for a period of 12 weeks was reported. Additionally, milk replacer fed calves receiving 500 mg Zn/L for 5 weeks exhibited no adverse effects, however calves in the same experiment receiving 700 mg Zn/L produced negative impacts on performance (decreased weight gain, lower feed intakes, and were less feed efficient) compared to their counterparts (NASEM, 2016). With this information, current toxicity concentration in the diet of beef cattle for Zn are 500 mg Zn/kg (NRC, 1980, 2005; NASEM, 2016).

Extensive research into beef cattle requirements for Zn resulted in Zn requirements for beef cattle to be set at 30 mg Zn/kg of dietary dry matter (DM). Dietary DM without additional Zn supplied in the diet is likely to contain varying amounts of Zn. Concentrations of Zn in forages, concentrates, and by-product feeds are dependent on Zn concentration in the soil,

(geological influence or application of Zn through Zn rich fertilizers). Within forages, hays are typically found to be lower in Zn as compared to silages, with legumes varying widely based on soil type. As with most cereal grains, the outer germ of the grain will sequester minerals like Zn, which logically explains the observed increase in Zn content in by-product feeds, like wheat or rice bran. Perhaps the highest containing Zn feedstuff available would that of animal origin; namely feather meal.

Absorption and Transport

Zinc is readily absorbed from the abomasum (Miller and Cragle, 1965) and across the lumen of the duodenum at about 22% to 47% maximal efficiency in calves (Underwood and Suttle, 1999). More specifically, labeled ^{65}Zn absorption has been shown in rats, described by Davies (1980), to be absorbed at rates of approximately 60% in the duodenum, 30% in the ileum, and 10% in the jejunum. Intestinal uptake of Zn occurs with Zn^{2+} crossing the brush border membrane with Zn transporters (Cousins et al., 2006). It should be noted that amount of Zn absorbed is separate from the rate at which Zn is absorbed. Specifically, in low-Zn or Zn-deficient diets the rate of Zn absorption will increase (Miller et al., 1966) and likewise diets with elevated concentrations of Zn will have homeostatic mechanisms in place to slow the amount of Zn absorbed. Homeostatic mechanisms dictate the absorption of Zn and are dependent on the stage of production of an animal (NASEM, 2016). The major regulatory proteins of enterocyte absorption of Zn are metallothionine proteins (MT). Metallothionine protein 1 and MT2 play a key role in cell detoxification and regulation of Zn homeostasis at the gastrointestinal tract level (Suhy et al, 1999; Cousins, 1996). Bremner (1993) stated Zn absorption in enterocytes can be impaired in the presence of Cd and Cu, which respectively ordered, have greater affinity to MTs than Zn. Never the less, once Zn is bound to a MT in the enterocyte, this weak complex will

serve as temporary holding for Zn. Normal enterocyte turnover can result in excretory losses of Zn; this can likely be a result of positive homeostasis that Zn was excreted without circulating systemically. If there is a homeostatic need for Zn the Zn bound to a MT will migrate to the basolateral membrane of the enterocyte, where Zn will be released to Zn transporter 1 (ZnT1) and exit the enterocyte.

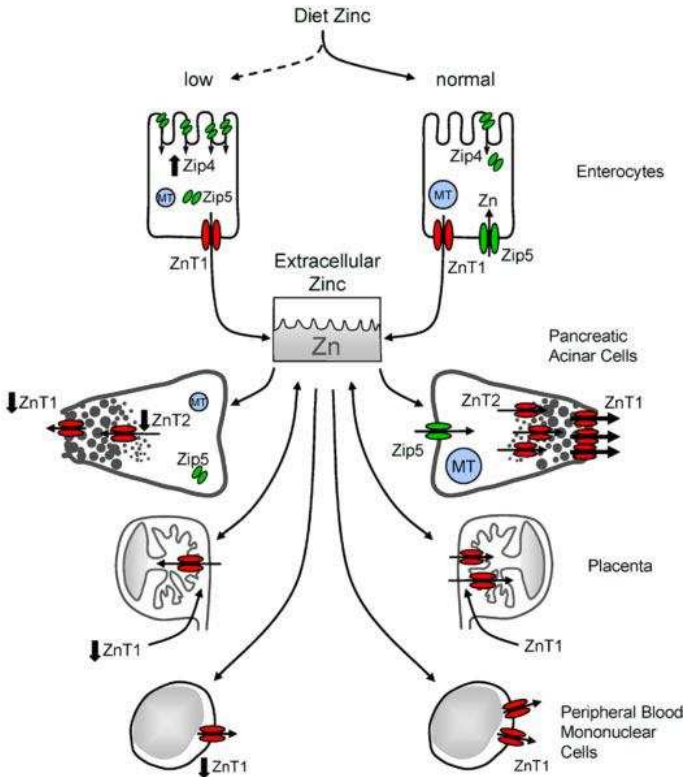

Figure 2 – Zinc Absorption and Transport

Once free Zn^{2+} is released into circulation it will bind to serum albumin (ALB) for systemic transport, this binding process mitigates Zn^{2+} ion toxicity towards erythrocytes (Handing et al., 2016). Hepatic MT synthesis is initiated by circulating Zn-ALB arriving through the portal vein branching into primarily the right lobe and extending to the left lobe of the liver, once Zn is translocated by ZnT1 into the hepatocyte Zn is partitioned to various pathways (Bremner, 1993). Approximately 30 to 40% of circulating Zn entering the liver is taken up by the

liver with the remaining concentrations are then taken up by bone, hair, the central nervous system and accumulate in pancreas and renal tissue, as well as the spleen (McDowell, 2003).

The discovery of the proteins which transport, traffic, and signal for Zn, both intracellularly and extracellularly, didn't occur until 1995 (Cousins et al., 2006). Since the discovery of Zn Transporter-1 (ZnT1) two families of transporters have since been identified; ZnT (SLC30) and Zip (SLC39) (Cousins et al., 2006). Zinc transport proteins specifically facilitate Zn efflux from cells and influx Zn into intracellular vesicles, whereas Zip proteins are involved in Zn transport both from extracellular fluid and from intracellular vesicles into the cytoplasm; both protein families are stationed at different sites within a cell (Cousins et al, 2006).

In a short review of mammalian Zn transport, trafficking, and signaling, Cousins et al. (2006) describes the location and function of specific ZnT and Zip proteins. It should be noted the exact mechanism in which these ZnT and Zip transporters function is not fully understood (Cousin et al., 2006; Hill and Link, 2009). Zinc transporter 1 is located in the plasma membrane and is largely expressed in the epithelial cells lining the esophagus, duodenum, and cecum, and ZnT5 can be found in pancreatic β cells and apical membranes (Cousins et al., 2006). Zinc transporter 4 has been identified in the large intestine, and ZnT6 has been isolated from the stomach, jejunum, cecum, colon, and rectum of mice (Yu et al., 2007). Yu et al. (2007) also isolated ZnT7 from several sections of the gastrointestinal tract of mice, noting the level of expression in the small intestine to be the greatest amongst all tissues sampled. The uptake of Zn is believed to be a facilitated process through Zip transporters that function from a concentration gradient and ATP appears to not be a requirement for this process (Hill and Link, 2007). These transporters reside in plasma membrane, with the exception of Zip7, which is located in the

Golgi apparatus (Cousins et al., 2006). Additionally, Zip transporters are uniquely constructed with the ability to translocate due to the influence of Zn availability and physiological conditions in the animal. The Zip4 concentrations will increase on the plasma membrane of the enterocyte in response to dietary restriction of Zn. Likewise, when dietary Zn is in excess, Zip4 is reduced (Dufner-Beattie et al., 2003). In hepatocytes, Zip14 expression increases during acute-phase responses, to allow for additional uptake of Zn from circulating plasma (Cousins et al., 2006).

The regulation of ZnT and Zip transporters appears to be influenced by either hormones or cytokines. More specifically, proinflammatory cytokines activate signal transducers and activators of transcription (STATs), which results in the upregulation of Zip14, whereas regulation of cell-specific expression for Zip transporters is influenced by prolactin, estrogen, and testosterone (Cousins, et al., 2006). In addition to cytokines and hormones, metallothionines (MT) have been previously reported to play an integral role in Zn regulation. More specifically, MT are relatively small proteins that can be found in the cytosol of the cell and structurally contain thiols which allow all MTs to bind to metal ions (Maret and Wedd, 2014). These proteins also have the ability to protect a cell against oxidative stress from reactive oxygen species through redox activity from thiols. It is believed that the effectiveness of MTs against oxidative stress is closely related to Zn status in the cell and could explain the increase of free-Zn in the cytosol during oxidative stress. Metallothionine 1 and MT2 are the most commonly found MTs in mammalian cells and have been demonstrated *in vivo* to be associated with Zn^{2+} (Cousins et al., 2006). In the case of increased dietary Zn, MT production will increase and bind to excess Zn, and likewise decrease production as dietary concentrations of Zn decrease.

Storage and Excretion

Storage of Zn in forms that are readily mobilized are limited. Bone provides a large storage vesicle for Zn, followed by hair and external epithelial cells; Zn bound in hair and external epithelial cells cannot release for use in other tissues. More readily available Zn is found bound to MTs; however, Zn bound to MTs in the liver, kidney, pancreas, and small intestine provide a temporary storage of Zn and can be depleted rather quickly in the occurrence of low dietary Zn (McDowell, 2003). In addition, Cu-Zn superoxide dismutase reserves in the liver may also serve as a temporary storage for Zn (McDowell, 2003).

Excretion of Zn occurs largely through endogenous fecal losses, with minimal excretion through the urine (Miller, 1970). Endogenous fecal loss contributions occur from pancreatic and biliary secretions, as well as enterocyte sluffing likely as a result of normal cell turnover (McDowell, 2003). The regulation of Zn excretion is assumed to function similarly to absorption of Zn in that dietary concentrations and local Zn status will dictate fecal losses of Zn (Miller, 1973). Therefore, low Zn diets and stage of production will influence excretion as is does with absorption (Miller et al., 1966).

Function

It is well understood that Zn is contained in several metalloenzymes and various cell types within the body. There are five extensively studied metalloenzymes; carbonic anhydrase, carboxypeptidase A, alkaline phosphatase, alcohol dehydrogenase, and cytosolic superoxide dismutase. Cytosolic superoxide dismutase contains Cu and Zn (CuZnSOD) and this enzyme plays a key role in the dismutation of superoxide radicals (Fridovich, 1978). Superoxide radicals are a group of reactive oxygen species than can result from characteristically normal oxidative cellular respiration. With the addition of CuZnSOD these reactive molecules can be using a

sequential method of oxidation and reduction of the reactive oxygen species at the metal containing center (Abreu and Cabelli, 2010). As previously mentioned, Zn is incorporated in several enzymes, in which it will provide structure to enzymes and can act as a catalyst (McDowell, 2003). Furthermore, Zn is contained in a sequence of amino acids with cysteine and histidine, to form DNA-binding proteins (McDowell, 2003). This sequence is better known by the name "Zinc fingers" for its resemblance to finger-like structures which attach to DNA to elicit gene transcription (McDowell, 2003).

Zinc in Growing and Finishing Beef Cattle

The two most recent surveys of consulting feedlot nutritionists (Vasconelos and Galyean, 2007; Samuelson et al., 2016) report average formulation concentrations of Zn to be on average 3 times the current recommendation. In the 2016 survey of consulting nutritionists (Samuelson et al., 2016) survey respondents reported preferences for utilizing a combination of organic and inorganic sources of Zinc in both receiving and finishing feedlot diets. From the 2007 to 2016 survey, Zn specifically, saw a combined mean use of 92.95 mg Zn/kg DM (Vasconcelos and Galyean, 2007) and a mean use of 109 mg Zn/kg DM and 87.3 mg Zn/kg DM for receiving diets and finishing diets, respectively (Samuelson et al, 2016). Although, reported usage concentrations from the Samuelson et al (2016) survey remain above current NASEM (2016) recommended requirements for Zn in beef cattle, the amount of Zn supplemented to finishing diets has decreased.

Inorganic and organic sources are compared frequently do to the assumption that organic bound Zn is more bioavailable to the animal. Perhaps the most accurate description of bioavailability has been written by O'Dell and Sunde (1997) "the proportion of the element consumed that is utilized for biochemical or physiologic function". Therefore, comparing

inorganic to organic bound elements seem logical from a scientific standpoint, but it can easily be manipulated commercially to alter perception of Zn bioavailability to the animal. Zinc oxide and $ZnSO_4$ have been evaluated in the literature and both sources of Zn appear to have similar bioavailability in ruminant species (NASEM, 2016). Also, Zn methionine (ZnMet) and Zn proteinate (ZnP) are commonly compared organic molecules to Zn from ZnO and $ZnSO_4$.

Reports of the effect of Zn source and Zn concentrations in the diet of growing cattle and finishing cattle have been variable. Greene et al. (1988) reported an effect of ZnMet, observing improved marbling score in Cr supplemented steers when compared to the control, but did not observe increased marbling score in steers fed ZnO. Furthermore, observed increases in external fat and percentage of kidney, pelvic, and heart in steers fed 82 mg/kg DM ZnMet as compared to the control (Greene et al., 1988). It remains unclear to the authors if the responses were due to Zn supplementation or ZnMet (Green et al.,1988).

Spears (1989) evaluated relative bioavailability of Zn in lambs and performance effects in growing heifers. Specifically, experiment 1 lambs were fed a semi-purified diet deficient in Zn to compare ZnMet and ZnO, and observations yielded no difference between treatment groups in growth or Zn plasma concentrations. Apparent absorption was similar between Zn sources, however, retention of Zn tended to be greater in lambs receiving ZnMet because of a tendency for lower urinary excretion of Zn in ZnMet supplemented lambs. Experiment 2 lambs were fed an orchard-grass hay based diet containing a total of 50 mg Zn/kg DM from ZnMet or ZnO, observed absorption and retention of Zn was similar for both treatment groups. Experiment 3 orally dosed lambs with 300 mg of Zn from ZnO or ZnMet and blood collections were taken at 0, 6, 12, and 24h post-dosing; the basal diet was a pelleted coastal bermudagrass and vitamin-mineral supplement (20 mg Zn/kg) and was fed 14d prior to experiment 3. Observations of

increased plasma Zn concentrations at 12 and 24 hrs post dose were noted for ZnMet supplemented lambs. The results from exp.3 may indicate greater absorption of Zn, slower release of Zn from ZnMet complex, or a slower clearing of Zn from plasma of lambs who received ZnMet (Spears, 1989). In the final experiment, heifers were fed corn silage based diet containing a total of 48.1 mg Zn/kg DM for 126 days with 25 mg of supplemental Zn from ZnO or ZnMet. Zinc supplementation alone increased ADG and FE for the first 56 days of the experiment, but was not sustained for the whole feeding period. Furthermore, overall growth performance did not differ between ZnO and ZnMet supplemented heifers, however, Spears (1989) noted a tendency for increase performance in heifers supplemented with ZnMet ($P <$ 0.19). Spears (1989) concluded Zn from these two sources may be metabolized differently post absorption. Additionally, ZnMet may transported to the plasma and circulated without being bound to albumin, altering its behavior in the body.

Malcolm-Callis et al. (2000) separately evaluated Zn concentration (Exp.1) at 20 (90.3 mg Zn/kg total diet), 100 (169.5 mg Zn/kg total diet), and 200 (280.3 mg Zn/kg DM total diet) milligrams of supplemental $ZnSO_4$/kg of DM and Zn source (Exp. 2) in diets supplemented with 30 mg/kg DM of Zn from $ZnSO_4$, Zinc amino acid complex, or Zinc polysaccharide complex. Serum cholesterol concentrations and fatty acid profiles were measured for in steers from exp. 1, yielding no difference in serum lipid concentrations. Observations of live performance and carcass characteristics yielded a liner decrease in DMI ($P < 0.10$) as Zn concentration increased, while all other growth performance measurements remained similar across treatments for the duration of the feeding period (Malcolm-Callis et al., 2000). The observed decrease in DMI may be linked to palatability of the diet as $ZnSO_4$ concentrations increased. However, no negative effects were observed on growth performance in steer fed the greatest concentration of Zn (200

mg/kg DM) (Malcolm-Callis et al., 2000). The dose of 200 mg Zn/kg of DM may have induced a pharmacological effect, yet in the evaluation of serum cholesterol and fatty acid profiles yielded similar results across all treatments. Perhaps the evaluation of Zn dependent enzymes in steers from experiment 1 would have provided more insight into the effect of Zn concentration. Furthermore, carcass characteristics remained similar across treatments. Malcolm-Callis et al. (2000) noted differences in fat thickness and yield in 100 mg Zn/ kg DM supplemented steers and state that the results are not of biological significance with the differences observed. Zinc source evaluation in experiment 3 yielded no differences over the entire 126 day feeding period, likewise carcass characteristics remained similar across treatments.

Reports from the previous discussed experiments may suggest increased DMI and feed efficiency during the early part of the feeding period in Zn supplemented diets. Further investigations have been conducted to evaluate immune function in beef cattle during the feedlot receiving phase of production. Specifically, Galyean et al. (1995) utilized 280 newly weaned beef steers in an experiment that supplemented differing concentrations and sources of Zn with Cu during the receiving period (28 days) and subsequently monitored these steers through the growing and finishing period where the cattle received one of three source-concentration combination treatments or a basal diet. The basal diet contained 30 mg Zn/kg from ZnO (contained in a mineral package premix); low ZnMet treatments contained the basal dose plus 35 mg Zn/kg DM from ZnMet; high $ZnSO_4$ treatment contained basal diet dose plus 70 mg Zn/kg DM from $ZnSO_4$, and high ZnMet contained basal plus 70 mg ZnMet/kg DM. During the receiving period no difference were observed among treatments for growth performance or morbidity for illness related to bovine respiratory disease (BVD). However, morbidity was decreased during the receiving and step up phase in high $ZnSO_4$ and ZnMet supplemented steers

as compared to basal diet and low ZnMet fed steers (Galyean et al., 1995). These findings may support the assumption that supplemental Zn from either source has the potential to decrease morbidity caused by BVD, though this is a weak conclusion provided only 280 steers were utilized in the experiment. Furthermore, finishing performance and carcass characteristics were not affected by Zn source-concentration treatment, however Galyean et al. (1995) note greater DMI in steers feed additional Zn as compared to basal diet fed steers. This finding is likely to be insignificant provided the diets included the premix all treatments on trial and Zn concentrations were greater than suggested dietary concentrations in all diets.

Spears and Kegley (2002) provide a more thorough evaluation of the effect of Zn source and concentration on the immune response in addition to traditional performance and carcass measurements. Basal diets consisted of 33 and 26 mg of Zn/kg of DM in the growing and finishing diets, respectively; ZnO and two sources of ZnP were added at 25 mg/kg of DM. A vaccination of infectious bovine respiratory disease (IBR) was administered to all steers on day 70 of the growing phase, antibody titers for IBR were obtained prior to vaccination and 14d and 28d post vaccination. No differences were noted across all treatments (Spears and Kegley, 2002). Additionally, *in vitro* lymphocyte blastogenesis assays were conducted to describe cell mediated immune responses in cell harvested from steers in each treatment. Results from this process were similar across treatments (Spears and Kegely, 2002). Performance and carcass characteristics remained similar for the two sources of ZnP; Zn supplementation increased ADG during the growing phase regardless of source; ZnP increased ADG and feed efficiency during the finishing period as compared to ZnO supplemented steers (Spears and Kegley, 2002). An increase in hot carcass weight and dressing percentages were noted for ZnP supplemented steers as compared to all other treatments. All other carcass characteristics (quality grade, back fat, yield grade, and

marbling) remained similar in Zn supplemented diets, but were greater than the control diet. It is interesting to compare the findings of this experiment to Spears (1989), where again the metabolism post absorption appears to have differing impacts on animal performance, however an explanation of the differences is not clear.

Salyer et al. (2004) and Nunnery et al. (2007) evaluated humoral immunity in beef heifers supplemented with differing sources of Zn and Cu. Salyer et al. (2004) evaluated newly received, lightweight heifers in two experiments where cattle were supplemented with 75 mg Zn/kg DM from $ZnSO_4$ or Zn polysaccharide complex and 10 mg Cu/kg DM from $CuSO_4$ or Cu polysaccharide complex, and heifer either received supplemental Cu and Zn or not for 35 days. In experiment 1, no differences were detected across treatments for DMI, ADG, feed efficiency or BRD induced morbidity. Yet in experiment 2 following ovalbumin vaccination, titers were great in Zn polysaccharide fed heifers when compared to $ZnSO_4$ supplemented heifers, interestingly this trend was reversed for Cu source. Moreover, Nunnery et al. (2007) supplemented beef heifers 75 mg Zn/kg DM from $ZnSO_4$, ZnMet, or Zn propionate in two experiments. Similar findings to Salyer et al. (2004) provide evidence of no economically beneficial difference between differing sources of Zn in the diet of heifers. Furthermore, Nunnery et al. (2004) observed no differences among treatment groups for ovalbumin IgG titers, nor were there any noted effects of Zn source or Zn supplementation on receiving period morbidity.

Whitman et al. (2007), Berrett et al (2015), and Caldera et al. (2017), evaluated feedlot steer performance from similar basal diets supplemented with Cu, Zn or Mn comparing source or dose or a combination. Results from the above-mentioned experiments conclude no economically beneficial differences that would result in the recommendation of excessive

supplementation and preference of source for Zn or other minerals evaluated. Economically important traits measured in traditional feedlot studies are variable, further investigations warrant experimentation into 1) post absorption of ZnMet compared to $ZnSO_4$ *in vivo*; and 2) refinement of Zn recommendations. Both areas of investigation would not only provide clarity into variations on performance and carcass parameter differences among dose and source, but could also refine feed standards to prevent excess soil and ground water contamination.

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CHAPTER II – MANUSCRIPT

INTRODUCTION

The National Academy of Science, Engineering and Medicine (NASEM) recommended concentration of Zn in beef cattle diets is 30 mg of Zn/kg DM (NASEM, 2016). However, the most recent survey of consulting feedlot nutritionists (Samuelson et al., 2016) reported the average Zn formulation for feedlot diets to be approximately 3 times the NASEM (2016) recommendations. Additionally, consultants also reported using a combination of organic and inorganic Zn sources to meet a targeted Zn concentration (Samuelson et al., 2016). The discrepancy between recommendations by NASEM (2016) and consulting nutritionists may be due to reports indicating that supplementing Zn during the finishing period may improve carcass quality and average daily gain. However, little controlled research has been conducted in this area.

Chromium was initially identified as a component in glucose tolerance factor by Schwartz and Mertz (1959). Chromium has been shown to enhance the function of insulin, alter lipid metabolism and improve immunity in beef cattle (Bernhard et al., 2012a; Bernhard et al., 2012b; Spears et al., 2012). Observations of the influence of Cr on animal performance parameters have yielded variable results. Kneeskern et al. (2016) reported increased dressing percentage and a tendency towards greater LM area in Cr-supplemented steers receiving 13.47 mg Cr/kg DM, while no other differences were observed in live performance or other carcass parameters measured. Conversely, Van Bibber-Krueger et al. (2016) supplemented heavy weight (initial BW 421 kg ± 5.76) and light weight (initial BW 384 kg ± 5.76) finishing steers with a control diet or the control diet supplemented with a mixture of yeast and Cr propionate to

provide 3.2 mg Cr/d (≈ 0.252 mg Cr/kg DM). Overall final BW, ADG and DMI were similar across treatments for both light weight and heavy weight steers. However, light weight steers supplemented with yeast and Cr propionate had greater G:F when comparted to light weight control steers whereas as treatment had no impact on G:F for heavy weight steers. Therefore, the objective of this experiment was to examine the influence of Zn source and concentration and Cr supplementation on performance and carcass characteristics in feedlot steers.

MATERIALS AND METHODS

Prior to the initiation of this experiment all animal care, handling, and procedures described herein were approved by the Colorado State University Animal Care and Use Committee (Approval # 15-6110A).

Cattle Processing

Four hundred and fifty crossbred steers (initial BW 287.0 ± 13.89 kg) were utilized from 3 separate Angus based cow herds. Each group was transported to the Colorado State University Agriculture, Research, Development, and Education Center (ARDEC) in Fort Collins, Colorado. Within 24 h of arrival, steers were individually weighed, identified with a unique ear tag, and breed type was assigned to each steer based on hair color and phenotype (red, black, or black-white face). Two groups from 2 of 3 cattle sources were processed on November 6, 2015 and 1 group of cattle from the remaining cattle source was processed on November 12, 2015. Each steer was vaccinated with the following: Presponse (Pasteurella Multocida Bacterial Extract-Mannheimia Haemolytica Toxoid, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), Pyramid 2 plus Type II BVD (Bovine Rhinotracheitis and Bovine Viral Diarrhea (Types I and II), Boehringer Ingelheim Vetmedica, Inc.), given Promectin (Ivermectin, Vedco, Inc.) and drenched with Synanthic (Oxfendazole, Boehringer Ingelheim Vetmedica, Inc.) for parasite

control, and implanted with Revalor – XS (120 mg Trenbolone Acetate and 24 mg Estradiol, Merck Animal Health, DeSoto, KS). Liver biopsies were obtained from each animal, using the technique described by Engle and Spears (2000). Following initial weighing and processing, steers were provided *ad libitum* access to long-stem grass hay and water, and were housed (10 animals per pen) overnight.

Randomization of steers in this experiment was similar to that described by (Caldera et al., 2017). Briefly, steers were ranked by BW with in cattle source and individuals whose weight was ± 2 SD from the mean BW were eliminated from further consideration for the experiment. Steers exhibiting red coat color or those found to be bulls, heifers, or displaying symptoms of illness or lameness were not enrolled in the experiment. The remaining steers were blocked by body weight and assigned a random number from 1 to 1,000 within block using the random number function in Excel 2007 (Microsoft Corporation, Redmond, WA). Steers with the lowest random numbers were eliminated from the experiment reducing the number of remaining steers to 400. The 400 eligible steers were ranked by weight within cattle source and divided into 8 weight block replicates, each one consisting of 50 steers. Within each weight block replicate, steers were ranked by weight and randomly assigned to 1 of 5 pens. By following this randomization schedule, 8 weight block replicates with 3 cattle sources, each containing 5 pens with 10 steers per pen were assembled for each of the 5 treatments in the experiment. On d -2, steers were individually weighed prior to being fed, visual ear tags identifying treatment, replicate, and animal ID within a pen replicate were inserted into the right ear of each steer, cattle were placed into their respective treatment pens, and the experiment initiated (December 10th, 2015).

Pens were checked daily to ensure that the cattle were in the correct pen assigned, that all cattle had *ad libitum* access to feed and water, and that all gates were secure. In addition, all cattle were monitored daily for illness and lameness. Steers exhibiting symptoms of distress were removed from the pen and rectal body temperatures were recorded. Steers with rectal body temperatures greater than 39.4°C were treated utilizing the appropriate treatment protocols and immediately returned to their home pen. If illness or lameness persisted in a specific steer, the steer was removed from the experiment. If a steer was removed from the experiment, the steer was weighed, the feed in the feed bunk was weighed and placed back into the feed bunk, a feed sample was obtained for DM determination, and the feed delivery was adjusted accordingly for that pen the next day.

Diets

Steers were fed a steam-flaked corn based finishing diet (Table 1). Steers were fed a series of step up diets to adjust to the finishing diet. Diet changes during the step-up program were simultaneous across all treatments, and cattle reached the finishing diet by d 36 of the experiment. Finishing diets were formulated to meet or exceed NRC (2000) requirements for growing and finishing beef cattle. Nutrient composition of the basal dietary ingredients is shown in Table 1a. Finishing diets were formulated to contain approximately 13.5% crude protein, 3.5% crude protein equivalent from non-protein nitrogen, 3.5% added fat, 0.70% calcium, 0.36% phosphorus, 0.70% potassium, 0.25% magnesium, 35.0 mg of monensin/kg DM (Rumensin 90, Elanco Animal Health, Greenfield, IN) and 10.6 mg tylosin phosphate/kg DM (Tylan 100, Elanco Animal Health, Greenfield, IN). Vitamins A and E were included in the diets at 2,200 IU/kg of DM and 40 IU/kg of DM, respectively. The above-mentioned ingredients were added to the ration in a liquid supplement. Ractopamine hydrochloride (Optaflexx 45, Elanco Animal

Health, Greenfield, IN) was fed at a feeding rate of 400 mg · steer^{-1} · d^{-1} for 28 d to replicate 1, 30 d to replicates 2, 4, and 6, and 40d to replicates 3, 5, 7, and 8. Diets were delivered once daily in the morning (0900h) in amounts to allow cattle *ad libitum* access to feed for a 24 h period. During inclement weather or when excessive amounts of feed remained in the feed bunk, feed was removed from the feed bunks, weighed and subsampled. Subsamples were analyzed for DM and used to calculate the DM weight of the orts for a given period. This value was then subtracted from the total DM delivered to a given pen of cattle to calculate DMI for a given period. Weekly samples of each treatment diet were obtained and stored at -20°C. At the end of each month, weekly treatment samples were subsampled and composited into monthly composites for use in diet analysis.

Dietary treatments consisted of: 1) 90 mg Zn/kg DM from $ZnSO_4$ and 0.25 mg Cr/kg DM from Cr propionate (KemTRACE Cr Kemin Industries Inc., Des Moines, IA) (90ZS+Cr); 2) 30 mg Zn/kg DM from Zn hydroxychloride (IntelliBond Z Micronutrients USA LLC, Indianapolis, IN) 0.25 mg Cr/kg DM from Cr propionate (30ZH+Cr); 3) 90 mg Zn/kg DM from Zn hydroxychloride and 0.25 mg Cr/kg DM from Cr propionate (90ZH+Cr); 4) 60 mg Zn/kg DM from $ZnSO_4$and 30 mg Zn/kg DM from Zn methionine (ZinMet Global Animal Products, Amarillo, TX) (90ZSM); 5) 90 mg Zn/kg DM from Zn hydroxychloride (90ZH). Trace mineral test articles appropriate for each treatment were formulated and pelleted separately. All test articles were analyzed for mineral content prior to the initiation of the experiment. Chromium propionate (0.25 mg Cr/kg DM) was added to treatments 1-3 to balance for the analyzed Cr concentration in the Zn methionine (ZinMet Global Animal Products, Amarillo, TX). Chromium propionate was not added to treatment 5 which served as a negative control for Cr. The analyzed Cr and Zn concentrations for the total mixed rations for each finishing diet are shown in Table 1.

Water samples were obtained at the main water supply line to the ARDEC feedlot facility and sent to an established laboratory (SDK Labs, Hutchinson, KS) for routine water quality analysis (pH = 7.37±0.16; total hardness = 771.3±51.3 mg/L; electrical conductivity = 1611±97.3 µs/cm; sulfate = 391.0±27.6 mg/L; sodium = 62.1±8.7 mg/L; chloride = 27.2±3.7mg/L; total dissolved solids = 981.0±62.8 mg/L).

Weighing and Carcass Data Collection

Steers were weighed individually on d -2, d 70, d 119, and 2 consecutive days prior to slaughter. Equal numbers of pen replicates per treatment were transported to a commercial abattoir on d 162, d 176, and d 211 for slaughter. Carcass data was collected by Diamond T Livestock Services Inc., Yuma, CO and liver samples were collected by Center for Meat Safety & Quality personnel at Colorado State University. Hot carcass weights were determined at the time of slaughter and carcasses were chilled for approximately 36 h before carcass data were obtained. Carcass data collected included dressing percentage (DP), LM area, adjusted subcutaneous adipose tissue thickness (USDA, 1989), KPH, marbling score, quality grade, and calculated USDA yield grade (YG). Liver samples were collected (approximately 200 g wet weight) on the day of slaughter from the left lobe of each liver after being inspected by USDA personnel. Each sample was placed into numbered Whirl Pak bags corresponding to carcass order, placed on ice, and transported to the Colorado State University Nutrition Laboratory and stored at -20°C until analyzed for trace mineral concentrations. Only liver samples obtained at the time of slaughter that corresponded to the same steer biopsied during initial processing were analyzed for trace mineral concentrations.

Analytical Procedures

Total mixed ration monthly composite samples were sent to the Department of Animal Science and Interdepartmental Nutrition Program, North Carolina State University, Raleigh for Cr analysis. Handling and analysis procedures for Cr are outlined in Lloyd et al. (2010). Total mixed ration monthly composite samples were sent to SDK Laboratories, Hutchinson, KS for Zn analysis. Liver tissue samples for Cu, Mn, and Zn analysis were allowed to thaw at room temperature. Subsamples were obtained from the innermost portion of the liver sample and placed in pre-weighed acid washed crucibles to be dried at 60°C for 24 h. After drying, samples were weighed and ashed at 600°C for 12 h. The ashed liver samples were re-suspended in 5 mL of 1.2*N* HCl and analyzed for Cu, Mn, and Zn concentrations using an inductively coupled plasma-atomic emission spectrometer (ICP-AES). Samples were diluted in distilled H_2O to fit within a linear range of a standard curve generated by linear regression of known TM concentrations. Multielement analysis was then carried out by simultaneous/sequential ICP-AES analysis with cross flow nebulization (Caldera et al, 2017).

Statistical Analysis

Feedlot performance, liver trace mineral concentrations, and continuous carcass data were analyzed on a pen mean basis as a randomized block design using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Treatment was included in the model as a fixed classification effect, and weight block pen replicate was included in the model as a random effect. Covariates of pen initial BW, number of animals used in the pen average, source of cattle, and days on feed were used in the analysis of all performance and carcass response variables. Liver mineral concentrations were analyzed using initial mineral concentration as a covariate in the analysis of final mineral concentration. There were two missing pen observations for liver mineral analysis.

Outlier tests performed on all data, no outliers were removed from the data set. A type three ANOVA table was constructed using the Kenward-Roger method of computing denominator degrees of freedom. Backwards elimination with AIC criteria was used to remove nonsignificant ($P \geq 0.10$) covariates from the model. The effect of treatment was determined significant at $P < 0.05$ and tendencies of the effect of treatment were noted at $P \leq 0.10$. Single degree of freedom contrasts was used to separate means. Contrasts were: 1) 90ZS+Cr vs. 90ZH+Cr; 2) 30ZH+Cr vs. 90ZH+Cr; 3) 90ZH+Cr vs. 90ZSM; and 4) 90ZH+Cr vs. 90ZH.

RESULTS AND DISCUSSION

Feedlot Performance

The effect of Zn source and dose and Cr supplementation on feedlot cattle performance are presented in Table 2. The main effect of treatment was not a significant source of variation for any of the response variables measured in this experiment. However, single degree of freedom contrasts (unprotected F-tests) were a significant source of variation for certain response variables. Zinc dose (30ZH+Cr vs. 90ZH+Cr) had no impact on final BW, ADG, DMI, or G:F. The observations of Zn dose effect on live animal performance in the current study tend to align with previous literature suggesting supplementation of Zn greater than NASEM (2016) recommended concentrations would not improve live animal performance. Increasing supplemental Zn from 30 to 70 mg/kg DM in growing and finishing steer diets tended ($P = 0.10$) to increase DMI but did not impact ADG or G:F (Galyean et al., 1995). Malcolm-Callis et al. (2000) reported no differences in ADG or G:F for steers during a 112 d finishing period with added Zn (as $ZnSO_4$) concentrations of 20, 100, or 200 mg/kg of dietary DM. However, DMI decreased (linear, $P = 0.10$) slightly as Zn concentration increased. Spears and Kegley (2002) reported that Zn supplementation (25 mg Zn/kg DM) did not affect performance of finishing

steers relative to those fed the control diet (analyzed 26 mg Zn/kg DM). In contrast, supplementing 75 mg Zn/kg DM to a control diet that analyzed 50.5 mg Zn/kg DM, tended to improve ADG ($P = 0.11$) and G:F ($P = 0.06$) in finishing heifers (Nunnery et al., 2007).

Zn source (90ZS+Cr vs. 90ZH+Cr or 90ZH+Cr vs. 90ZSM+Cr) had no impact on final BW, ADG, DMI, or G:F. Samuelson et al. (2016) reported consulting feedlot nutritionists using a combination of organic and inorganic Zn sources. However, impacts of Zn source vary in the When Zn sources (inorganic vs. organic) were compared at concentrations numerically greater than the recommended concentration of Zn (30 mg of Zn/kg DM; NASEM, 2016), there were no improvements in finishing steer DMI or G:F (Greene et al., 1988; Malcolm-Callis et al., 2000). Moreover, diets containing varying concentrations and sources of Zn had no impact on final BW, ADG, DMI or G:F of finishing steers (Galyean et al., 1995; Berrett et al., 2015; Caldera et al., 2017).

Chromium supplementation to steers receiving 90 ZH (90ZH+Cr) had greater final BW ($P \leq 0.04$) and overall ADG ($P \leq 0.03$) compared to steers receiving 90ZH without Cr addition to the diet (Table 2). Observations of positive improvements to ADG in stress calves have been shown by Chang and Mowat 1992; Moonsie-Shageer and Mowat, 1993; Kegley et al., 1997. Additionally, Bernhard et al. (2012b) supplemented steers with 0, 0.1, 0.2, or 0.3 mg Cr/kg DM from Cr propionate and reported a linear increase in ADG and tendency for a linear increase in BW, resulting in linear increase G:F for the duration of the feeding period (56 d). Observations in longer fed steers receiving supplemental Cr to the basal diet vary in the current literature. Van Bibber-Krueger et al. (2016) fed heavy weight (initial BW 421 kg ± 5.76) and light weight (initial BW 384 kg ± 5.76) finishing steers a control diet or the control diet supplemented with a mixture of yeast and Cr propionate to provide 3.2 mg Cr/d (≈ 0.252 mg Cr/kg DM). Overall final

BW, ADG and DMI were similar across treatments for both light weight and heavy weight steers. However, light weight steers supplemented with a mixture of yeast and Cr propionate had greater G:F when comparted to light weight control steers, whereas as treatment had no impact on G:F for heavy weight steers. In contrast, Kneeskern et al. (2016) observed no differences in final BW, ADG, DMI or feed efficiency in individually housed feedlot steers (n = 34) fed to a targeted back fat of 1.27 cm (determined by ultrasound). These steers were fed a control diet containing no supplemental Cr (basal concentration 3.63 mg Cr/kg DM) or Cr supplemented at 3 mg of Cr·steer·d^{-1} from Cr propionate, totaling 13.47 mg Cr/kg DM.

Experiments examining the influence of simultaneous supplementation of Zn and Cr on feedlot cattle performance and carcass characteristics are limited. Zinc has been reported to enhance protein deposition in mammals (Lundholm et al., 1981; Engle et al., 1997; Cousins, 1998). Furthermore, Zn supplementation to finishing cattle diets improved quality grade, marbling score, and increased subcutaneous fat thickness in beef cattle (Malcolm-Callis et al., 2000; Spears and Kegley, 2002). Chromium was initially identified as a component in glucose tolerance factor by Schwartz and Mertz (1959) and has been reported to enhance the function of insulin, alter lipid metabolism, and improve immunity in cattle (Sumner et al., 2007; Bernhard et al., 2012a; Bernhard et al., 2012b; Spears et al., 2012). Therefore, Bohrer et al. (2014) hypothesized that Zn and Cr supplementation to steers receiving ractopamine hydrochloride (RAC) (Optaflexx, Elanco Animal Health, Greenfield, IN) for 35 d would improve the effect of RAC on weight gain (muscle protein accretion) and promote intramuscular lipid deposition. In contrast to the findings of the current experiment, Bohrer et al. (2014) reported no difference in final BW, DMI, and ADG in feedlot steers receiving either a control finishing diet (no supplemental Zn, or Cr; basal diet contained 49.4 mg Zn/kg DM and 0.96 mg Cr/kg DM) or a

finishing diet supplemented with Zn propionate and Cr propionate (total dietary Zn and Cr concentrations 159.7 mg Zn/kg DM and 1.25 mg Cr/kg DM) with RAC supplementation (300 mg · steer^{-1} · d^{-1}) for 35d. Moreover, Edenburn et al. (2016), in a similar experiment, reported addition of Zn (≈ 74.1 mg of supplemental Zn/kg DM; total dietary Zn ≈ 99.0 mg of Zn/kg DM), Cr (≈ 0.222 mg of supplemental Cr/kg DM; total dietary Cr ≈ 1.14 mg Cr/kg DM), or Zn and Cr to cattle receiving RAC (400 mg · steer^{-1} · d^{-1}) had no impact on growth performance parameters in finishing beef cattle.

Several factors such as basal diet concentrations of Zn and Cr, cattle type and breed, days on feed, initial trace mineral status of the animal, disease status, mineral antagonists, stress, environmental factors, and analytical errors in Cr analysis could influence reported animal response to Zn and/or Cr supplementation. For example, the reported diet concentrations of Zn in the Zn supplemented diets in Bohrer et al. (2014) and Edenburn et al. (2016) experiments are comparable to the current experiment. However, Bohrer et al. (2014) and Edenburn et al. (2016) reported dietary Cr concentrations that were 0.526 mg Cr/kg DM and 0.426 mg Cr/kg DM higher than reported in the current experiment. Where Cr supplementation was evaluated independently, concentrations of Cr from the diets of Kneeskern et al. (2016) and Van Bibber-Krueger et al. (2016) varied substantially from 13.47 mg Cr/kg DM to 0.252 mg Cr/kg DM, respectively. Differences in Cr analysis methods are likely to account for some variation in Cr concentrations (Lloyd et al., 2010; Spears et al., 2017). In addition to analytical differences, other factors including: total days on feed (135 d Bohrer et al., 2014; ≈ 258 d Edenburn et al., 2016), length of Zn and Cr supplementation (35 d Bohrer et al., 2014; 63 d Edenburn et al., 2016), and initial BW at initiation of the experiment (424 ± 24 kg Bohrer et al., 2014; ≈ 506.3 kg Edenburn et al., 2016)

may account for variation among results when comparing published findings to the current experiment.

Liver Zn concentrations are presented in Table 3. Initial and final liver Zn concentrations were similar across treatments and were above concentrations considered to be deficient (25 mg of Zn per kg of DM; Mills, 1987; Puls, 1994). Engle et al. (1997) reported no effect of Zn source on liver Zn concentrations from marginally deficient heifer calves receiving 23 mg Zn/kg DM from Zn methionine, Zn lysine, or $ZnSO_4$. In contrast, Wright and Spears, (2004) observed greater ($P < 0.05$) liver Zn concentrations in Holstein calves receiving $ZnSO_4$ (156.8 mg Zn/kg liver DM) than those receiving Zn proteinate (133.3 mg Zn/kg liver DM) following 98d of supplementation where calves received a control diet or 20 mg supplemental Zn/kg DM from $ZnSO_4$, Zn proteinate, or Zn methionine. However, Zn source had no impact on liver Zn concentrations over the entire experiment totaling 112d.

Carcass Characteristics

The effect of Zn source and dose and Cr supplementation on carcass characteristics are presented in Table 4. Carcass characteristics (DP, YG, marbling score, subcutaneous adipose tissue depth, and LM area) were not affected by Zn dose (30ZH+Cr vs. 90ZH+Cr), Zn source (90ZS+Cr vs. 90ZH+Cr or 90ZH+Cr vs. 90ZSM+Cr), or Cr supplementation (90ZH+Cr vs. 90ZH) in the diet. However, single degree of freedom contrasts (unprotected f-tests) showed Zinc source was a source of variation in results of LM area. Specifically, treatments 90ZH+Cr compared to 90ZSM increased LM area ($P < 0.01$) and 90ZS+Cr compared to 90ZH+Cr tended to increase LM area ($P < 0.08$). These observations are not supported in the current study by other carcass characteristics or live performance response variables for Zn source. Overall, evaluation of Zn source and dose is varied in the literature. Malcolm-Callis et al. (2000)

observed increases in subcutaneous fat thickness among Zn sources ($ZnSO_4$ 0.98 cm; Zn amino acid complex 1.12 cm; Zn polysaccharide complex 1.18 cm, $P < 0.10$). These researchers also observed a quadratic response in fat thickness ($P < 0.05$) with the greatest increase from 100 mg Zn/kg DM and YG increased quadratically in Zn supplemented steers, whereas KPH tended in increase in steers supplemented with $ZnSO_4$. Spears and Kegley, (2002) reported increased HCW in Zn proteinate vs. Zn oxide supplemented steers. Nunnery et al. (2007) reported heifers supplemented with $ZnSO_4$ (82 mg Zn/kg DM) had a lower percentage of KPH than heifers receiving Zn methionine (90.9 mg Zn/kg DM), and Zn propionate (71.3 mg Zn/kg DM).

In the current experiment, steer receiving 90ZH+Cr had a greater HCW ($P < 0.03$) compared to 90ZH supplemented steers, resulting in an additional 8.5 kg of carcass weight from Cr supplementation. The influence of Cr supplementation on carcass characteristics is feedlot steers are variable. Chang et al. (1992) and Van Bibber-Krueger et al. (2016) reported no difference in carcass measurements among steers fed Cr-yeast. In contrast, Kneeskern et al. (2016) observed no influence of Cr supplementation on HCW, subcutaneous adipose tissue depth, KPH, or YG, but reported an increase in DP and a tendency towards greater LM area. Observations in the current experiment of increased final BW and ADG appear to have been captured in HCW. Although not measured in the current experiment, an alteration in energy metabolism may provide evidence for increased HCW, as well as observed increases in final BW and ADG. Bernhard et al. (2012a) evaluated feedlot steers fed a control diet or Cr supplementation (0.20 mg Cr/kg DM), observing lower concentrations of non-esterified fatty acid concentrations pre-and post-glucose infusion suggesting an alteration of lipid metabolism. Additionally, Spears et al. (2012) supplemented Cr propionate to growing heifers (0, 0.47, 0.94, and 1.42 mg Cr/kg DM) and reported a decrease in plasma insulin concentrations in Cr-

supplemented heifers compared to non-supplemented heifers. The effect of RAC (400 mg · steer^{-1} · d^{-1}) supplementation has been reported to increase HCW in steers supplemented with RAC compared to non-supplemented steers (Arp et al., 2014). The use of RAC in all treatments of the current experiment may provide additional variation for the observed increased HCW in 90ZH+Cr versus 90ZH supplemented steers, when compared to experiments where treatments included non-RAC supplemented steers fed a control diet or Cr supplemented diet. However, the results of Bohrer et al. (2014) indicate no effect on HCW from Zn and Cr supplementation with RAC in the diet at 300 mg · steer^{-1} · d^{-1}. Yet, Edenburn et al. (2016) observed increased HCW ($P < 0.09$) in cattle supplemented with RAC and Cr when compared to steers receiving RAC with Zn and Cr. Moreover, the observed results of the present experiment seem to align with the tendency for increased HCW as a result of Cr supplementation observed by Edenburn et al. (2016).

CONCLUSIONS

These data indicate that under the conditions of this experiment Zn source and concentration had no impact on live animal performance or carcass characteristics. Furthermore, Cr addition to diets supplemented with 90 mg ZnI/kg DM may improve final BW, ADG, and HCW in steers.

Table 1. Dry matter ingredient composition of the finishing diet.

Ingredient	90ZS+Cr[a]	30ZH+Cr[b]	90ZH+Cr[c]	90ZSM[d]	90ZH[e]
Corn silage, %	13.9	13.9	13.9	13.9	13.9
Steam-flaked corn, %	77.1	77.1	77.1	77.1	77.1
Liquid Suppl., %	4.2	4.2	4.2	4.2	4.2
Pellet Suppl., %	4.8	4.8	4.8	4.8	4.8
Chemical Analysis[f]					
DM, %	72.5	72.8	71.9	72.3	72.5
CP, %	13.12	13.10	13.04	13.14	13.18
NPN, %	3.22	3.22	3.22	3.22	3.22
ADF, %	6.21	6.17	6.24	6.22	6.18
NDF, %	12.41	12.74	12.24	12.32	12.29
Ether extract, %	3.80	3.62	3.71	3.64	3.68
NEg, Mcal/kg	1.44	1.40	1.42	1.41	1.41
NEm, Mcal/kg	2.0	1.98	2.0	1.98	1.99
Calcium, %	0.68	0.67	0.63	0.66	0.67
Phosphorus, %	0.30	0.30	0.33	0.30	0.32
Sulfur, %	0.20	0.19	0.21	0.22	0.20
Chromium, mg/kg	0.711	0.647	0.731	0.767	0.512
Copper, mg/kg	15.0	14.5	14.9	15.4	14.7
Manganese, mg/kg	37.9	38.3	38.6	40.0	38.3
Zinc, mg/kg	118.4	58.2	114.2	123.0	108.2

[a]Treatment 1: 90 mg of Zn/kg DM from $ZnSO_4$ and 0.25 mg Cr/kg DM from chromium propionate.
[b]Treatment 2: 30 mg of Zn/kg DM from IntelliBond Z and0.25 mg Cr/kg DM from chromium propionate.
[c]Treatment 3: 90 mg of Zn/kg DM from IntelliBond Z and 0.25 mg Cr/kg DM from chromium propionate.
[d]Treatment 4: 60 mg of Zn/kg DM from $ZnSO_4$ and 30 mg of Zn/kg DM from Zn Methionine.
[e]Treatment 5: 90 mg of Zn/kg DM from IntelliBond Z.
[f]Chemical analysis was performed on individual ingredients and nutrient composition was calculated based on the proportion of each ingredient in the diet.

Table 2. Effect of zinc source and dose and chromium supplementation on performance of feedlot steers.

Item	Treatment					SEM	P <	Contrasts, P<			
	90ZS+Cr[a]	30ZH+Cr[b]	90ZH+Cr[c]	90ZSM[d]	90ZH[e]			90ZS+Cr[a] vs 90ZH+Cr[c]	30ZH+Cr[b] vs 90ZH+Cr[c]	90ZH+Cr[c] vs 90ZSM[d]	90ZH+Cr[c] vs 90ZH[e]
Initial # of animals	80	80	80	80	80	---	---	---	---	---	---
Final # of animals	76	74	75	77	70	---	---	---	---	---	---
Initial body weight, kg	278.7	273.3	275.1	275.3	274.6	9.19	0.99	0.78	0.89	0.99	0.97
Final body weight, kg	583.2	581.2	588.4	580.7	575.3	4.25	0.30	0.41	0.23	0.20	0.04
Average daily gain, $kg \cdot hd^{-1} \cdot d^{-1}$	1.61	1.60	1.64	1.60	1.57	0.02	0.26	0.32	0.20	0.18	0.03
Dry matter intake, $kg \cdot hd^{-1} \cdot d^{-1}$	9.17	9.09	9.19	8.90	8.90	0.13	0.35	0.93	0.57	0.12	0.13
Gain:Feed (G:F)	0.176	0.177	0.180	0.180	0.177	0.003	0.80	0.39	0.52	0.89	0.57

[a]Treatment 1: 90 mg of Zn/kg DM from $ZnSO_4$ and 0.25 mg Cr/kg DM from chromium propionate.
[b]Treatment 2: 30 mg of Zn/kg DM from IntelliBond Z and 0.25 mg Cr/kg DM from chromium propionate.
[c]Treatment 3: 90 mg of Zn/kg DM from IntelliBond Z and 0.25 mg Cr/kg DM from chromium propionate.
[d]Treatment 4: 60 mg of Zn/kg DM from $ZnSO_4$ and 30 mg of Zn/kg DM from Zn Methionine.
[e]Treatment 5: 90 mg of Zn/kg DM from IntelliBond Z.

Table 3. Effect of zinc source and dose and chromium supplementation on liver mineral status of Zinc.

	Treatment							Contrasts, P<		
Item	90ZS+Cr[a]	30ZI+Cr[b]	90ZI+Cr[c]	90ZM[d]	90ZI[e]	SEM	P <	90ZS+Cr[a] vs 90ZI+Cr[c]	30ZI+Cr[b] vs 90ZI+Cr[c]	90ZI+Cr[c] vs 90ZM[d]	90ZI+Cr[c] vs 90ZI[e]
Initial Zinc mg/kg DM	97.8	97.7	97.4	99.1	92.2	3.51	0.69	0.95	0.95	0.75	0.30
Final Zinc mg/kg DM	165.3	131.9	134.9	143.3	128.6	12.20	0.27	0.09	0.86	0.61	0.74

[a] Treatment 1: 90 mg of Zn/kg DM from $ZnSO_4$ and 0.25 mg Cr/kg DM from chromium propionate
[b] Treatment 2: 30 mg of Zn/kg DM from IntelliBond Z and 0.25 mg Cr/kg DM from chromium propionate
[c] Treatment 3: 90 mg of Zn/kg DM from IntelliBond Z and 0.25 mg Cr/kg DM from chromium propionate
[d] Treatment 4: 60 mg of Zn/kg DM from $ZnSO_4$ and 30 mg of Zn/kg DM from Zn Methionine
[e] Treatment 5: 90 mg of Zn/kg DM from IntelliBond Z
[f] Initial Zn concentration were used as a covariate for statistical analysis.

Table 4. Effect of zinc source and dose and chromium supplementation on carcass characteristics of feedlot steers.

Item	Treatment					SEM	P <	Contrasts, P <			
	90ZS+Cr[a]	30ZH+Cr[b]	90ZH+Cr[c]	90ZSM[d]	90ZH[e]			90ZS+Cr[a] vs 90ZH+Cr[c]	30ZH+Cr[b] vs 90ZH+Cr[c]	90ZH+Cr[c] vs 90ZSM[d]	90ZH+Cr[c] vs 90ZH [e]
Initial # of animals	80	80	80	80	80	---	---	---	---	---	---
Final # of animals	76	74	75	77	70	---	---	---	---	---	---
Hot Carcass Weight, kg	362.5	361.8	367.8	362.5	359.3	2.67	0.25	0.19	0.12	0.16	0.03
Dressing Percentage, %[f]	62.1	62.2	62.5	62.2	62.4	0.22	0.69	0.22	0.30	0.37	0.74
Marbling Score[g]	397.1	395.4	399.7	407.5	383.2	10.69	0.61	0.87	0.77	0.59	0.27
Subcutaneous adipose tissue thickness, cm	1.39	1.34	1.38	1.41	1.31	0.05	0.60	0.80	0.59	0.59	0.31
Longissimus muscle area, cm^2	80.4	80.8	82.1	79.8	81.0	0.64	0.15	0.08	0.15	0.01	0.22
Yield grade, %	2.83	2.80	2.82	2.87	2.69	0.08	0.58	0.98	0.86	0.63	0.24

[a]Treatment 1: 90 mg of Zn/kg DM from $ZnSO_4$ and 0.25 mg Cr/kg DM from chromium propionate.
[b]Treatment 2: 30 mg of Zn/kg DM from IntelliBond Z and 0.25 mg Cr/kg DM from chromium propionate.
[c]Treatment 3: 90 mg of Zn/kg DM from IntelliBond Z and 0.25 mg Cr/kg DM from chromium propionate.
[d]Treatment 4: 60 mg of Zn/kg DM from $ZnSO_4$ and 30 mg of Zn/kg DM from Zn Methionine.
[e]Treatment 5: 90 mg of Zn/kg DM from IntelliBond Z.
[f]Dressing Percentage calculated from shrunk final body weight.
[g]Marbling score; 300 = Slight^0, 400 = Small^0, 500 = Modest^0.
[h]USDA numerical yield grade 1, 2, 3, 4, or 5 calculated as percentage of the pen, adjusted to 100%.

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