EVALUATION OF ORGANIC TRACE MINERAL SUPPLEMENTATION IN GROWING HORSES AND FINISHING BEEF CATTLE

A Dissertation

by

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ABSTRACT

Complexed trace minerals (CTM) improve health and performance in multiple species, but species challenges differ. Objectives herein address species-specific challenges to 1.) Evaluate effects of supplemental Zn amino acid complex (ZnAA) during finishing phase on live performance, carcass characteristics and liver abscesses, with or without ractopamine hydrochloride (RAC) in beef steers and 2.) Understand CTM (Zn, Cu, Mn, Co) role in joint homeostasis in horses.

The first objective was facilitated through pooling related finishing data from 9 studies (285 pens) across US and Canada between 2001-2016 using three statistical analyses. The initial analysis evaluated 3,840 crossbred steers. Results suggest ZnAA in the finishing period augments RAC and improves performance without negatively affecting carcass quality. The second analysis separated effects of ZnAA and RAC using all data compiled. ZnAAxRAC describes reduced liver abscess (LA) occurrence/severity, but no ZnAA×RAC interactions for live animal performance, carcass characteristics, or carcass-adjusted performance were observed. Independently and together ZnAA and RAC improved growth performance and carcass characteristics without affecting dry matter intake, marbling, or back fat thickness. The third analysis divided pens into ZnAA levels: 0 ppm (NO), 30-54 ppm (LOW), and ≥60 ppm (HIGH). These data demonstrate positive linear response for growth and carcass characteristics. A quadratic tendency for carcass G:F suggests LOW is most efficient, and reduces LA occurrence/severity.

The second objective used sixteen weanling Quarter Horses for 56-d measuring effects of CTM vs. inorganic minerals (Zn, Cu, Mn, Co) on intra-articular inflammation and cartilage

metabolism following an acute inflammatory insult. Horses receiving CTM had a more robust immune response and increased collagen degradation, but increased aggrecan synthesis. Acute joint inflammation altered synovial fluid TM, and dietary source affected the degree of inflammatory response.

Together these data suggest utilization of organic CTM has positive effects in beef cattle and horses.

DEDICATION

I would like to dedicate this dissertation and my academic achievements to Dr. Josie Coverdale. You left us too soon, but the lessons, knowledge, and work ethic you helped to instill in me will never be forgotten. I have no doubt that your memory and impact will persevere through the many lives you touched, including mine. I will continue to work relentlessly to make you proud.

Thank you for being my role model and sharing your passion for research and teaching. You inspired me and helped to send me down this path of finding my passion. Thank you for believing in me even when I didn't believe in myself. Thank you for expecting the best from me and for holding me accountable. Thank you for becoming part of my family and allowing me to be part of yours. Thank you for crafting connections and relationships for me with people that will endure time. Thank you for never letting me quit. Thank you for making graduate school fun.

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NOMENCLATURE

ADF	Acid detergent fiber
ADG	Average Daily Gain
BCS	Body condition score
BFT	12^{th} rib back fat thickness
BL	Body length
BW	Body weight
C2C	Collagenase cleavage neopeptide
CF	Crude Fiber
CP	Crude protein
CPII	Carboxypropetide of type II collagen
CTM	Metal amino acid complexed trace minerals
CS846	Chondroitin sulfate 846 epitope
Cu	Copper
d	day
DM	Dry matter
DMI	Dry matter intake
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron
G:F	Gain-to-feed ration
h	Hour
HG	Heart girth

HH	Hip height
HR	Heart rate
IMF	Intramuscular fat
LPS	Lipopolysaccharide
LRS	Lactated ringer solution
RR	Respiration rate
t	Time
HCW	Hot carcass weight
KPH	Kidney, pelvic, heart fat
Mn	Manganese
Mo	Molybdenum
MARB	Marbling score
NDF	Neutral detergent fiber
NRC	National Research Council
PGE_2	Prostaglandin E_2
RAC	Ractopamine hydrochloride
REA	Ribeye area
RF	Rump fat
S	Sulfur
Se	Selenium
SF	Synovial fluid
SEM	Standard error of mean
TM	Trace mineral

WH	Wither height
YG	Yield grade
Zn	Zinc
ZnAA	Zinc amino acid complex

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CHAPTER I

INTRODUCTION

Inorganic elements, minerals, represent only a small fraction of an animal's diet; however, inclusion and supplementation of appropriate levels are critical to development and health. Compared to other nutrients such as protein, carbohydrates, and fats, minerals are inorganic and cannot be synthesized by living organisms. Minerals are found in varying amounts and proportions in all tissues and feedstuffs. Mineral content of animal feedstuffs is determined by calculating the remaining ash following ignition of organic matter (McDowell, 2003). Classification of minerals are divided into two distinct categories, macro and micro minerals (trace minerals), which differ by amount required by the animal. Macro minerals are required in greater amounts (percentage of diet or g/kg). Comparatively, trace minerals are required (ppm, ppb, or mg/kg diet) in minute amounts.

Mineral essentiality and toxicity was not described until the middle of the nineteenth century (Underwood, 1981; McDowell, 2003). An observation in 1874 by Forster concluded that the minerals found in the ash of animal tissues were needed for normal biological function, leading to the desire to establish dietary mineral requirements (McDowell, 2003). Requirements for specific minerals, especially trace minerals (TM), in livestock nutrition were discovered later. For example, in grazing cattle, the necessity of Cu and Zn were not recognized until the 20th century, initially recognized in the 1930s and 1960s, respectively (McDowell, 2003). Deficiencies of both were discovered in grazing cattle, and manifested themselves as health problems, which included wasting disease (loss of appetite, rapid weight loss, unthriftiness)

(deficient in Cu) and parakeratosis (Zn deficient). Deficiencies in Cu, Co, and Fe were identified in Florida, when grazing cattle exhibited a condition identified as "salt sick" which was later also recognized as wasting disease (Becker et al. 1965).

The resultant understanding of TM research has delineated their critical role in maintaining animal health and normal physiological function. Functions of TM range from structural components of tissues to metabolic regulation (Suttle, 2010), with their primary roles as components of metalloenzymes or as catalysts for enzyme systems. Generally, TM functions are classified into structural, physiological, catalytic, and hormonal or regulatory (McDowell, 2003). Ten trace minerals have been described as essential in mg or μg amounts for cattle and horses and including chromium (Cr), cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn). An established dietary requirement has been determined for 7 of the 10 in cattle and horses.

Continual challenges in beef cattle production are improving performance and while combating liver abscess prevalence in grain fed cattle. Therefore, in Chapters III and IV, the impact of Zn amino acid complex on performance, composition of growth, and liver abscess frequency in finishing cattle is investigated. In horses, maintaining joint integrity and health is a prominent challenge, especially in young performance horses. Inflammation and cartilage degradation are considered important features in the development of joint disease and the early retirement of young equine athletes. Evaluation of cartilage turnover is difficult to measure objectively in an experimental setting, as cartilage responds to both growth and exercise. However, inducing predictable levels of inflammation may provide valuable insight into potential dietary strategies, such as organic trace minerals, that can be utilized to mitigate intra-articular inflammation in young horses. In consequence, Chapter VI investigates how Co, Cu,

Mn, and Zn source impact TM role in joint inflammation and homeostasis in growing horses. Chapter VII provides insight on neonatal foal concentrations of cartilage metabolism biomarkers to improve our foundational understanding of the biomarkers used in Chapter V. Lastly, Chapter VII summarizes the findings and provides direction for future research directions in each species.

CHAPTER II

LITERATURE REVIEW

Complex interactions between minerals and animal production and management have made defining optimal inclusion levels of TM requirements challenging. Furthermore, mineral balance studies have not been completed in all age or classes of horses and cattle, thus some requirements are estimated based on previous amounts fed without negative effects or deficiency symptoms observed or based on recommendations in other species. Moreover, the inconspicuousness and overlap of deficiency symptoms across minerals makes pinpointing the cause of deficiencies difficult. Mineral interactions in the gastrointestinal (GI) tract with other feed components and/or minerals, including absorption competition alter TM status (Suttle, 2010) and TM availability, especially in ruminants (Spears, 2003). As a result, established dietary requirements for TM are based on preventing of deficiency symptoms and negative interactions in healthy, non-stressed animals (Table 2.1; NRC 2007, NASEM, 2016).

A multitude of factors influence or alter an animal's response to mineral supplementation, which includes duration, concentration, and mineral source. Furthermore, physiological status, absence or presence of dietary antagonists, and environmental factors including stress, all affect mineral metabolism and the animals' response to mineral supplementation (Suttle, 2010). Cattle and horses are exposed to and experience stressors such as, disease and transit stress throughout their life (Carroll and Forsberg, 2007; Padalino, 2015). Decreased feed intake and body weight loss (Hutcheson and Cole, 1986) associated with stress, suggests greater concentrations of TM may be required in the diet to maintain adequate mineral

intake and status; however, the current standards (NRC, 2007; NAEMS, 2016) for both species do not recommend changing the TM requirement during stress. Despite this, in order to confidently prevent deficiencies, TM supplementation beyond requirements of TM in feedlot and equine diets is commonplace (Vasconcelos and Galyean, 2007; Samuelson et al., 2016).

Table 2. 1 Trace Mineral Requirements and Maximum Tolerable Concentrations

	Cattle		Horses	
Mineral mg/kg DM	Growing & Finishing[1]	Maximum Tolerable Concentration[2]	Growing[3]	Maximum Tolerable Concentration[2]
Co	0.15	25	0.05	25
Cu	10	40	10	250
Mn	20	1,000	40	400
Se	0.10	5	0.10	5
Zn	30	500	40	500

[1]NAEMS, 2016
[2]NRC, 2005
[3]NRC, 2007

KNOWN BIOLOGICAL FUNCTIONS OF TRACE MINERALS

Cobalt

Gastro intestinal tract (GIT) microorganisms (ruminal, cecal, and colonic) require Co for synthesis of vitamin B_{12} (cobalamin). Thus, B_{12} status in ruminants and horses is directly correlated with dietary Co concentrations. Known physiological actions of Co are in the form of B_{12}; therefore, a secondary result to inadequate dietary Co is a B_{12} deficiency. As a result, Co is essential, but ruminants and horses are not dependent on dietary vitamin B_{12}. Nonruminants, like

pigs, have lower true Co requirements, and instead, a larger B_{12} requirement because they do not benefit from the utilization of B_{12} synthesized in the hindgut or rumen.

In the form of vitamin B_{12}, Co is interrelated with Fe and Cu in hematopoiesis or blood cell formation. Additionally, vitamin B_{12} dependent enzymes are involved in the transfer or synthesis of single carbon units (McDowell, 2003). For example, the enzyme responsible for activation of methylmalonyl CoA mutase is B_{12} dependent. This enzyme catalyzes the transfer of single-carbon units and is essential in the metabolism of propionate to succinate, prior to entering the Krebs cycle (McDowell, 2003; NRC, 2016).

In cattle fed high-grain finishing diets, volatile fatty acid (VFA) ratios shift towards greater propionate production. Production of VFAs through ruminal fermentation provides an estimated 60-70% of digestible energy in ruminants (Sutton, 1979; NAEMS, 2016) through three primary VFAs, acetate, propionate, and butyrate. Of these, propionate is gluconeogenic and a primary precursor to glucose in cattle (Zhang et al., 2015; Aschenbach et al., 2010; Yoest et al., 1977). Recommended levels of Co in cattle increased from 0.10 ppm (NRC, 1996; NRC, 2000) to 0.15 ppm in the most recent edition of the NRC (2016). Although Co requirements remained unchanged when high-concentrate diets were consumed (MacPherson and Chalmers, 1985), it has been reported that high concentrate diets increase microbial synthesis of B_{12} analogs (Halpin et al., 1984). Although active in bacteria, B_{12} analogs appear inert in animal tissue (Bigger et al., 1976); accordingly, evaluating B_{12} status in bovine serum concentrations is potentially misleading due to analog presence (Halpin et al., 1984). In horses, there have been limited studies conducted to determine Co requirements for horses. Although recent data has shown that Co has no effect on VFA production *in vitro* when evaluating alfalfa and smooth bromegrass (Fehlburg et al., 2019).

Absorption of Co in the form of vitamin B_{12} occurs in the terminal ileum of the small intestine and involves cobalamin transport proteins: haptocorrin, intrinsic factor, and transcobalamin II (Neilsen et al., 2012). Since majority of vitamin B_{12} synthesis occurs in the hindgut of horses, benefitting from microbial synthesis of B_{12} through small intestine absorption is unlikely; however, B_{12} absorption in the hindgut has been supported (NRC, 2007; Salminen 1975; Stillions et al. 1971).

Excretion of Co and vitamin B_{12} is predominately through the feces (Smith and Marston, 1970). Deficiency of Co is actually a vitamin B_{12} deficiency with early signs being decreased appetite and growth or weight loss (Smith, 1987). In horses, no documented cases of Co or vitamin B_{12} deficiency have been reported. Filmer (1933) reported horses surviving on Co deficient pastures that had low enough concentrations of Co to kill ruminants confined to them. The equine requirement of 0.1 ppm Co is based on prevention of deficiency in sheep and cattle.

Copper

Copper is involved in a multitude of distinct biological process in the body, including enzyme activation. Secondary to Zn in total number of enzymes activated, Cu serves as an indispensable catalytic co-factor for metalloenzymes involved in cellular respiration, protection from oxidants, ion transport, connective tissue development and tissue pigmentation (McDowell, 2003). Involvement of Cu in oxidative metabolism is driven by cytochrome c oxidase, in which Cu is a constituent of the enzyme (Malatesta et al., 1995). Energy generation in all tissues requires cytochrome c oxidase activity to catalyze final electron transport in the electron transport chain to produce adenosine triphosphate (ATP; Malatesta et al., 1995). Involvement of Cu in the removal of harmful oxidants, such as, the conversion of superoxide radical to hydrogen peroxide is achieved through activity of Cu-Zn superoxide dismutase. Copper is also a

component of a ferroxidase that oxidizes ferrous Fe to ferric Fe (Patel et al., 2002). Pigmentation and connective tissue development are two processes dependent on Cu. Tyrosinase ties Cu to its involvement with pigmentation as it catalyzes the production of melanin and other pigments from tyrosine. Lastly, lysyl oxidase facilitates the final enzymatic reaction resulting in the cross-linking of collagens and elastins (Kagan and Track, 1991).

Enterocyte transporters for Cu, include CTR1 (apical membrane) and ATP7A (basolateral membrane; Nishito and Kambe, 2018; Nose et al., 2010). Intracellular metallothioneins, synthesized in enterocytes, are in involved in Zn and Cu regulation. Consequently, metallothionein is one of the biggest deterrents of Cu absorption due to its Cu binding abilities. Once bound, the Cu is prevented from serosal transfer. Metallothionein synthesis is upregulated by Cu and Zn; therefore, Zn can indirectly inhibit Cu absorption and create deficiency. Once absorbed into the circulation, Cu is quickly transported and deposited mainly in the liver, an appreciable Cu storage location. Transport of Cu in the portal vein is primarily facilitated by albumin and α_2-macroglobulin (Cousins and Liuzzi, 2018).

Mobilized Cu is transported to the tissues via ceruloplasmin. Cerulopalsmin is produced by the liver and contains approximately 95% of circulating Cu. Ceruloplasmin functions predominately as a Cu transport protein (Hsieh and Frieden, 1975); however, it is multifunctional and has demonstrated antioxidant activity (Goldstein et al., 1979). Active excretion of Cu is predominately in bile (Underwood, 1977).

Signs of Cu deficiency include: anemia, decreased growth, depigmentation, cardiac failure, fragile bones, diarrhea, and delayed or depressed estrus (Suttle, 2010, NAESM, 2016). Notable Cu deficiency in horses is linked to bone and limb deformities (Van Weeren et al., 2003; Hurtig et al., 1993). Prevalence of osteochondrosis (OC) in foals increases in cases of

hypocupremia (Bridges et al., 1984). In cattle Cu deficiency is a common issue in rangeland cattle, due to poor absorption in mature cattle, environmental factors or induced by antagonistic actions of S and/or Mo (NAEMS, 2016). Lack of hair pigmentation is most often the earliest clinical sign of Cu deficiency in cattle.

Manganese

Physiological functions of Mn, like other trace minerals, are enzyme activation and as a component of metalloenzymes. Arginase, pyruvate carboxylase, and manganese-superoxide dismutase are the Mn-containing enzymes and a number of enzymes are activated by Mn including: hydrolases, kinases, decarboxylases, and transferases. As a result, Mn is required for normal carbohydrate and lipid metabolism. Synthesis of proteoglycans relies on glycosyltransferases requiring Mn for activation; therefore, bone and cartilage development are dependent on adequate Mn.

Absorption of Mn occurs along the small intestine and competes for transport with Fe and Co. Exact mechanism of absorption remains largely unknown. Once absorbed, transferrin transports newly absorbed Mn in the plasma to the liver, where it is taken up by liver transferrin receptors (Suttle 2010; Davidsson et al., 1989). Although Mn is widely distributed throughout the body, it is found in low concentrations with no appreciable stores. Greatest concentrations are found in the skeleton; however, this reserve is not readily mobilized in cases of deficiency.

Similar to Zn and Cu, bilinary excretion is the predominate excretory method for endogenous losses of Mn. Thus, fecal Mn concentrations represent both endogenous and dietary losses. The requirement for Mn is greater for optimal reproductive performance than for growing cattle (Hansen et al., 2006a,b).

Zinc

Currently, there are 300 known Zn dependent metalloenzymes in the body and an even greater number of functional Zn proteins. Roles of Zn are divided into catalytic, structural, and regulatory (Livingston, 2015). Examples of Zn metalloenzymes include carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase, DNA polymerase, protein chain elongation factor, and Cu-Zn superoxide dismutase. Collectively, these enzymes are required for metabolism of carbohydrates, fat, and protein and clearance of reactive oxygen species (Hambidge et al., 1986). As the second most abundant micromineral behind Fe, Zn is distributed throughout the body. Although, it is predominately found intracellular, bound to proteins with greatest concentrations located in skeletal muscle and bone. Interestingly, there is no dedicated store of Zn, with only a small pool accounting for about 10% of intracellular Zn in the liver and other tissues. Plasma Zn is bound loosely to albumin and accounts for about 0.1% of total body Zn with rapid exchange between tissue storage and plasma (Miller et al., 1994).

Enterocyte transporters for Zn are ZIP4 (apical membrane) and ZNT1 (basolateral membrane; Nishito and Kambe, 2018; McMahon and Cousins, 1998). The active saturable process of Zn absorption is directly related to the physiological need and occurs in the duodenum; thus, absorption and homeostatic mechanisms of Zn are tightly regulated (Cousins and Liuzzi, 2018). Regulation of Zn absorption is controlled by ZIP4 and metallothionein. The ZIP4 protein expression on the apical membrane regulates the uptake of Zn from the intestinal lumen and metallothionein binds excess Zn in the enterocyte, preventing entrance into portal circulation (Cousins and Liuzzi, 2018). The constitutive expression of ZNT1 under variable Zn intakes in enterocytes, particularly in the duodenum, contributes to controlling Zn supply

(Cousins and Liuzzi, 2018). Similar to Cu, Zn enters the portal circulation and binds to albumin and α_2-macroglobulin for transportation to peripheral tissues (Cousins and Liuzzi, 2018).

Zinc deficiency can be induced by high concentrations of Cu, Ca, and Fe, due to competition. Decreased performance, poor growth and reduced appetite are early effects of Zn deficiency in ruminants and horses (Harrington et al., 1973). Marginal Zn deficiency manifests as muscle degeneration and loss of muscle mass, which coincides with a reduction in the immune response that may lead to an increased risk of secondary infections (Goswami et al., 2005). Most notable clinical sign of clinical Zn deficiency is parakeratosis in cattle and horses (Harrington et al., 1973; Miller, 1970).

TRACE MINERAL SOURCES AND INTERACTIONS

Concentrations and bioavailability of TM in forages and concentrate feedstuffs vary substantially by region and depends on soil characteristics, plant growth, climate conditions, water availability, and fertilization practices (Fig. 2.1; Suttle, 2010; Greene, 2000). Additionally, processing methods further affects TM concentrations in by-product feedstuffs. Beyond TM concentrations, mineral availability is affected by diet formulation and nutritive value. For example, increasing crude protein (CP) and neutral detergent fiber (NDF) content negatively affect TM solubility and absorption of TM in ruminants. As a result, relying on naturally occurring minerals found in forage and cereal grains may not prevent deficiencies in all production settings due to poor bioavailability, mineral-mineral interactions, and ruminal interactions. Overcoming variability and insufficient TM concentrations through supplementation of TM has become an industry standard for cattle and horses. Traditional feeding programs supplement inorganic TM sources in sulfate or oxide forms. Some data

suggests these forms are more susceptible to dietary and environmental antagonisms (Genther and Hansen, 2014; Spears, 2003). As a result, supplementing with alternative organic TM sources has become increasingly more popular.

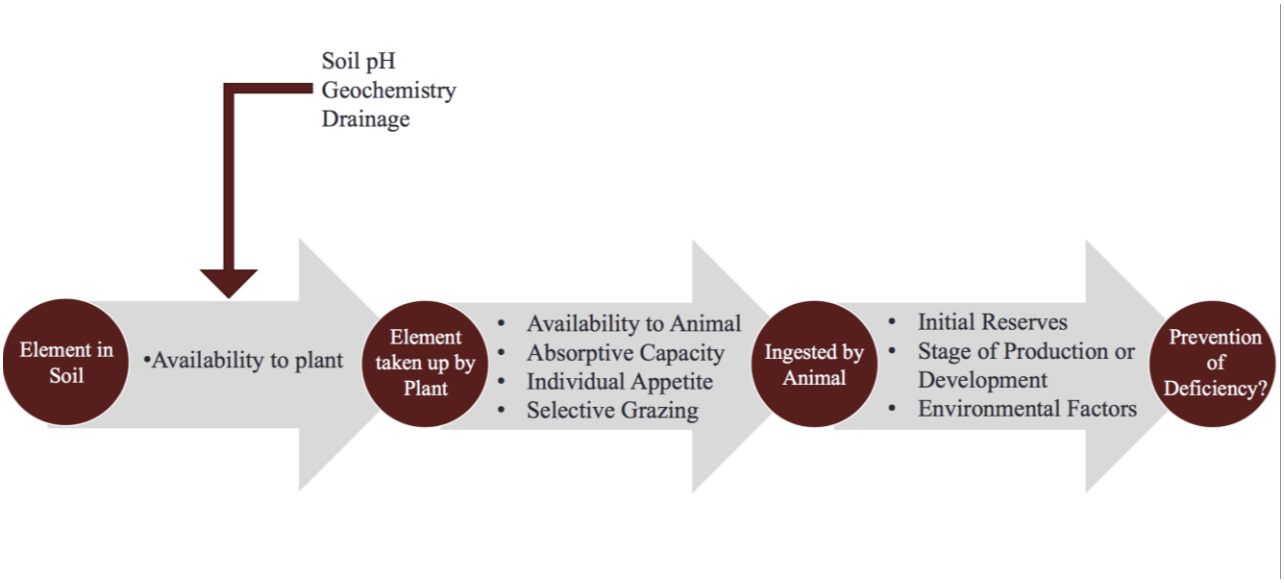

Figure 2.1 Factors affecting availability of minerals to grazing animals (adapted from Suttle, 2010)

Interactions occur pre and post-intestinal absorption and, in some cases, negatively affect TM status; thus, studying the effects of one mineral can be both difficult and misleading. Additionally, rumen environment creates favorable conditions for mineral-mineral interactions, resulting in decreased TM availability. Notable interactions are Cu and Fe, Mn and Fe, Cu and Zn, and the four-way interaction of Cu, Mn, Zn, and S. These interactions create complex challenges for achieving effective TM absorption and creating an optimum mineral status in an animal. Similarly, the horse, being a hind-gut fermenter, is also susceptible to antagonisms between Zn and phytate (Schryver et al., 1980). Absorption of Zn is impaired by Cu and

Cadmium (Cd) and reduced by phytate, Ca+Phytate, fiber, P and Cr. Cu and Zn antagonisms. For example, intemperate Zn supply can impede intestinal absorption, hepatic accumulation and placental transfer of Cu; resulting in clinical and biochemical manifestation of Cu deficiency (Breamer and Beattie, 1995). Therefore, these interactions must be considered when formulating rations.

Reports of improved bioavailability, health, and potential improvement in performance characteristics have led to increased utilization of organic sources. Organic minerals are often referred to as chelated minerals (Spears, 1996). A chelate is a chemical compound containing a ligand, typically organic or carbon containing that is bound to a central metal atom at two or more points. Chelated TM used in animal diets are covalently bound to an amino acid or a proteinate complex. The majority of research indicates that chelated minerals are more bioavailable when antagonists are present or when special circumstances exist, but overall, research has failed to produce consistent or sustained advantages for organic minerals (Suttle, 2010). Multiple types of organic TM exist on the market today; metal amino acid complexs, specific metal amino acid complexes, metal amino acid chelate, metal polysaccharide complex, metal proteinate, and metal propionate (Table 2.1). However, inorganic versus organic TM (collectively) tend to be the primary topic discussed rather than the individual effects of each type of organic TM.

Table 2.2 AAFCO[1] Feed Ingredient Definitions for Organic Mineral Products (adapted from NRC, 2007)

Product	Definition
Metal amino acid complex	Product resulting from complexing of a soluble metal salt with amino acid(s) ("Copper, amino acid complex")
Metal (specific amino acid) complex	Product resulting from complexing a soluble metal salt with a specific amino acid ("Copper lysine complex")
Metal amino acid chelate	Product resulting from the reaction of a metal ion from a stable metal salt with amino acids with a mole ratio of one metal to one to three moles of amino acids to form coordinate covalent bonds and heterocyclic ring(s) ("Copper amino acid chelate")
Metal polysaccharide complex	Product resulting from complexing of a soluble salt with a polysaccharide solution ("Copper polysaccharide complex")
Metal proteinate	Product resulting from a reaction of a soluble salt with amino acids and/or partially hydrolyzed protein ("Copper proteinate")
Metal proprionate	Product resulting from a reaction of a metal salt with propionic acid ("Zinc propionate")

[1]Association of American Feed Control Officials, 2005.

BIOAVAILABILITY OF TRACE MINERALS

Cobalt

Efficiency of microbial conversion of Co to cobalamin is affected by dietary supply and potentially, Co source and diet type (Suttle, 2010). Little data comparing forms in terms of vitamin B_{12} availability has been reported (NRC, 2016). Reported values of dietary Co conversion to B_{12} in the rumen range from 3 to 13% of Co intake in beef cattle (NRC, 2016; Smith, 1987); however, no known conversion rates are available for the horse (NRC, 2007).

Copper

Cu requirements vary in ruminants depending on dietary concentrations of other TM components, especially sulfur (S) and Mo, due to formation of insoluble compounds, thiomolybdates (Suttle and Underwood, 1999; Spears, 2003). Overall absorption of Cu in ruminants falls in the range of <1.0-10.0%, which is low compared to non-ruminants (Spears, 2003), a result of interactions created by the rumen. A number of studies have evaluated various organic forms of Cu (Nockels et al., 1993; Ward et al., 1993; Kegley and Spears, 1994; Spears, 2003). In cattle, the relative bioavailability of Cu from Cu-lysine is generally similar to cupric sulfate (Kegley and Spears, 1994; Ward et al., 1993). However, under stressful circumstances, Nockels et al. (1993) reported apparent absorption of Cu from Cu-lysine to be 53% greater in stress induced calves, compared to cupric sulfate. Authors also observed improved Cu retention during the repletion period, which was attributed to either increased absorption or decreased endogenous losses.

In horses, investigation of the relationship between Cu and Mo on Cu absorption has not been studied extensively. Nonetheless, the negative effect of Mo on Cu absorption in horses is considered minimal (Ricker et al. 1999). In contrast, dietary Zn is antognistic towards Cu absorption in horses. Few controlled studies evaluating Cu absorption rates in horses have been conducted. Schyver et al. (1987) reported absorptions of Cu ranging from 24 to 48%. Recently, studies have reported similar absorptive rates (include these here) (Hudson et al., 2001; Pagen and Jackson, 1991), resulting in the NRC requirement being based on a 35% absorption rate and estimated endogenous losses (0.068 mg/kg BW; Pagen, 1994). Wagner et al. (2005) reported no differences in percentage of Cu absorbed from oxide, sulfate and an organic-chelate (proteinate), 5.3, 6.55, and 2.76 %, respectively. However, absorption rates observed in this study were lower

than previously reported work and authors suggested previous mineral status might have influenced results.

Manganese

Reported absorption of Mn from ruminant diets is 1% or less (Spears 2003). Henry et al. (1992) reported that the relative bioavailability of manganese from Mn methionine in ruminants was 120% of that present in the sulfate form. In horses supplemented with Mn-oxide or combination of Mn-oxide and Mn-methionine, Siciliano et al. (2001) reported no changes in liver Mn concentrations due to source. True digestibility of Mn in mature horses has been reported at 28.5% (Pagen 1994).

Zinc

The impact of supplementing metal amino acid complexes in animal production has been investigated in multiple species, with Zn being the most extensively evaluated. When exposed to ruminal microorganisms, Zn methionine is degraded less when compared to inorganic sources (Heinrichs and Conrad, 1983). Ruminal soluble concentrations of Zn were greater in steers fed Zn methionine compared to steers supplemented with a similar concentration of Zn from sulfate or oxide sources (Ward et al., 1992). Data suggests that ZnAA complexes form less insoluble complexes making them more available to the animal (Spears, 1996). Furthermore, in human (enterocytes and caco-2) cells, ZnAAs were protected from inhibitory effects caused by phytic acid and folic acid. Demonstrating, the potential advantage of ZnAAs lies in their ability to utilize less saturable pathways for uptake (Sauer et al., 2017). Star et al. (2012) measured linear response of tibia Zn content in broilers, and reported an increased biological value of ZnAA when compared to Zn sulfate reference source (1.64 vs. 1.00, respectively).

TRACE MINERAL SUPPLEMENTATION

Finishing Cattle

Conventional U.S. beef production utilizes growth-promoting technologies such as ionophores, steroid implants, and β-adrenergic agonists (β-AA; Wileman et al., 2009). These technologies result in average daily gain (ADG), live body weight (BW) and hot carcass weight (HCW) improvements over natural systems (Winterholler et al., 2008). Vasconcelos and Galyean (2007) indicated that regardless of NRC recommendations in production settings, most cattle in feedyards receive total dietary concentrations of TM greater than NRC requirements. The role of TM in several antioxidant and growth-related processes suggest beef cattle may benefit from additional TM supplementation to optimize growth and health performance (Genther, 2016). Growth-enhancing technologies, like β-AA are potentially affecting trace mineral requirements; however, this has not been extensively studied.

β-adrenergic agonists

Beef cattle production in the U.S. has continually increased in production efficiency resulting in more beef produced from a smaller cattle inventory (Capper, 2011). Increases in production are often attributed to improved genetics, health, animal management techniques, and application of technologies. Among the technologies are those specifically targeted at growth enhancement, used in beef production since the mid-1950s. According to Johnson et al. (2013), roughly 90% of feedlot cattle in the U.S. will receive some type of growth enhancing technology in their lifetime. As previously mentioned, types of growth enhancing technologies include anabolic steroid implants, in-feed ionophores, in-feed hormones and β-AA (Capper and Hayes, 2012). Since 2003, β-AA have been approved for use in beef cattle production. Currently, two β-AA are approved by the FDA for use in beef cattle: ractopomine hydrochloride (RAC) and

zilpaterol hydrochloride (Johnson et al., 2013). Although, after reports in 2013 of increased lameness in slaughter cattle fed zilpaterol hydrochloride, its utilization in feedlot cattle has decreased. However, in response to this RAC use has increased markedly. Administration of β-AA occurs during the last 20 or 28 days of the finishing period to overcome decreases feed efficiency and increases in adipose accumulation associated with age. Supplementation with β-agonists improves ADG, *longissimus dorsi* muscle area (LM), yield grade, carcass transfer, hot carcass weight and feed to gain efficiency (Johnson et al., 2013). Consequently, improving opportunity for economic gains in cattle production.

Several indirect and direct mechanisms for β-agonists are proposed at the cellular level; however, exact mode of action for muscle hypertrophy and depressed adipose accretion in beef cattle remains elusive (Johnson et. al., 2014; Reed and Mersmann, 1991). Classified as phenetholamine compounds, β-agonists mimic endogenous catecholamines: epinephrine and norepinephrine. Both of which are naturally occurring in all animals including humans (Mersmann, 1998). β-agonists receptors are part of the G protein-coupled receptor (GPCR) superfamily and are found on virtually all mammalian tissues (Stiles et. al., 1984; Mersmann, 1998; Johnson et al., 2014). Further classification divides β-agonist receptors into subtypes, β_1, β_2, and β_3 (Johnson et al., 2014; Yang and McElliot, 1989). Universal distribution of β-adrenergic receptors throughout the mammalian cell types provides the setting for complex mechanisms of action depending on cell types and the distribution of the agonist to specific cell types (Mersmann, 1998).

If β-agonist indiscriminately activate receptors, negative effects in cardiac and hepatic tissues are distinctly possible; therefore, receptor subtype distinction is valuable for application. The β_2 receptor is most frequently associated with muscle growth stimulation; demonstrated

thorough *in vitro* and *in vivo* studies with clenbuterol. Clenbuterol is one of the most powerful β-agonists and has a high affinity for the β_2 receptor (Yang and McElliot, 1989). Induced hypertrophy appears to be specific to striated muscle (Reeds and Mersmann, 1991) suggesting mechanisms controlling protein turnover in striated muscle are different from other tissues. Furthermore, Johnson et al. (2014) reported an average of 96 % to 99 % of β-AR mRNA detected in bovine LM was from the β_2 receptor.

Yang and McElliot (1989) proposed several direct and indirect mechanisms may be responsible for the observed effects of β-agonist, although none are definitive and have contradictory findings nested within them. However, all proposed mechanisms of β-AA begin with stimulation of the β-agonist receptor and the downstream elevation of cyclic AMP (cAMP) concentrations (Johnson et al., 2014; Mersmann, 1998). Challiss et al. (1988) reported a threefold increase in cAMP concentrations in resting muscle of rats treated with isoprenaline, a β-agonist. Elevated cAMP results in activation of protein kinase A leading to phosphorylation of hormones such as hormone sensitive lipase, enhancing lipolysis, while inhibiting *de novo* lipogenisis through deactivation of acetyl-CoA carboxylase (Anderson et al., 2010).

Zinc and ractopamine

Investigation of Zn supplementation in finishing cattle has resulted in variable responses, often attributed to source and duration of Zn supplementation. Although, recent data supports supplementation of Zn, beyond the requirement, to both swine and beef cattle supplemented with RAC, with increases in feed efficiency and ADG compared to RAC alone (Genther-Schroeder et al. 2016a, b; Paulk et al., 2012). Moreover, steers fed both RAC and additional Zn had increased final BW and HCW (Genther-Schroeder et al. 2016a,b) suggesting a potential additive effect for Zn and RAC. As previously mentioned, the action of RAC is dependent on cAMP;

thus one hypothesis is ZnAA supplementation alters responses to RAC by inhibiting phosphodiesterase (PDE) activity, an enzyme responsible for the breakdown of cAMP (Percival et al., 1997). Although, the diminishing response to RAC from 28 to 42 d (Bittner et al., 2015; Edenburn et al., 2014), which has been linked to PDE, remained unaltered with ZnAA supplementation (Genther-Schroeder et al., 2016b), suggesting mechanistic limitations to ZnAA ability to augment the RAC response.

Supplementing Zn-proprionate yielded no additional performance improvements over RAC supplementation alone in finishing steers (Budde et al., 2019). In pigs, inclusion of supplemental Zn from ZnO in diets containing RAC tended to increase G:F linearly and loin weight (quadratic) with increasing ZnO concentrations (Paulk et al., 2015), indicating that other Zn sources have an effect in other species. Moreover, Paulk et al. (2015) reported pigs supplemented with 50 mg/kg of Zn from ZnAA and RAC (1.27 kg) tended to have increased ADG compared to pigs receiving RAC only (1.20 kg).

Varying levels of Zn supplementation have likely contributed to inconsistency in observed responses. Previously a linear growth response in beef steers fed increasing ZnAA when supplemented with RAC (Genther-Schroeder et al. 2016) up to 90 mg/kg DM was observed. Additional work by Genther-Schroeder et al. (2018) evaluated a range of ZnAA supplementation rates (1 to 8 times documented requirement) to identify a plateau to the response. They reported increasing Zn between 5 to 8 times the recommended rate had minimal effect on growth performance or carcass characteristics; however, trends toward decreased meat quality with increasing Zn were observed. Although 60 ppm of supplemental ZnAA has been recommended (Genther-Schroeder et al., 2016a) other studies have seen no effect at 60 ppm (Genther-Schroeder et al., 2018); therefore, finding an optimal supplementation range would be

beneficial to the producer's bottom line and prevent decreases in meat quality.

Liver abscesses

The primary cause of liver condemnation in harvested feedlot cattle in the United States is liver abscesses. Brown and Lawrence (2010) reported an 18.1% incidence rate of liver abnormalities, which represents a major economic loss to the beef processing industry. Considering from April 7 to May 5, 2018, approximately 2,489,000 head of steers and heifers have been harvested in the U.S (cattlefax.com), with an estimated loss of $3 to the packer for a condemned liver. In fact, losses due to liver abscesses are significant to feedyards and packers, with estimated annual losses of $15.8 million to the U.S. beef industry alone (Brown and Lawrence, 2010). Furthermore, presence of liver abscesses are associated negatively with previous performance in feedyard cattle manifested as reductions in feed intake, G:F, ADG, HCW, and carcass quality (Reinhardt and Hubbert, 2014). Ultimately these result in reduced carcass value and further contributing to economic loss.

Since 1991 incidence of liver condemnations have increased by 11.6%. Liver condemnations at slaughter are primarily due to liver abscesses (Nagaraja and Lechtenberg, 2007; Brown and Lawrence, 2010; Reinhardt and Hubbert, 2015). A decrease in the percentage of carcasses with liver abscesses was observed from between 2005 and 2011, (13.9 and 4.8%, respectively; NBQA-2011); however liver condemnations increased almost 10% since 2011 (NBQA-2016; Table 1.3). This increase in prevalence further demonstrates the need to identify avenues to decrease LA occurrence and prevent unnecessary economic losses resulting from performance reduction and liver condemnation.

Table 2.3 National Beef Quality Audit reported percentages of liver condemnations in steers and heifers; adapted from NBQA Executive Summary, 2016

NBQA[1] Year	1991	1995	2000	2005	2011	2016
Percentage of Liver Condemnations	19.2	22.2	30.3	24.7	20.9	30.8

[1]National Beef Quality Audit

Low ruminal pH (< 5.6) disrupts the ruminal epithelial and has been associated with a varity of health concerns in ruminants, including liver abscesses (Nagaraja and Chengappa, 1998). Furthermore, a disruption in the epithelial compromises the barrier function, causing ruminal ulcers of which incidence has been correlated with liver abscess formation (Smith, 1944; Jensen et al., 1954; Rezac et al., 2014; Reinhardt and Hubbert, 2014). Although Weiser et al. (1966) found no correlation between ruminal ulcers and liver abscesses; damage to the ruminal epithelium is thought to be the primary factor predisposing cattle to developing liver abscesses. Epithelial damage creates opportunity for pathogenic bacteria to exit the rumen, enter portal circulation and travel to the liver, allowing for bacteria colonization of the bacteria (i.e. abscess formation; Nagaraja and Chengappa, 1998). However, an exact mechanism and pathway has yet to be fully elucidated (Reinhardt and Hubbert, 2014).

The most commonly isolated bacterial pathogen from liver abscesses is *Fusobacterium necrophorum* (Berg and Scanlan, 1982); however, micro floras of liver abscesses tend to include secondary bacteria with *Trueperella pyogenes* being the most frequently found (Reinhardt and Hubbert, 2015; Nagaraja et al., 1999). Although *F. necrophorum* is commonly found in the rumen, it has been reported to increase 10-fold when cattle transition from a roughage-based diet

to a more energetically dense, grain-based, diet (Tan et al., 1994). Increases in fermentation rates cause acid accumulation, which in excessive amounts causes perforation of the ruminal epithelium. This creates the pathway for the invading pathogenic bacteria (Reinhardt and Hubbert, 2014). Greater levels of *F. necrophorum* resulting from high-grain, low-roughage diets create favorable circumstances in feedlot cattle for liver abscess formation.

Liver abscesses are primarily controlled in feedlot cattle through addition of antibiotics to the diet. U.S. feedyards have previously used bacitracin methylene disalicylate, chlortetracycline, oxytetracycline, tylosin, and virginiamycin; however, tylosin is the most effective and most commonly used (Galyean and Rivera, 2003; Nagaraja and Chengappa, 1998). Nagaraja and Chengappa (1998) reported liver abscess decreases of 75% with the inclusion of tylosin in commercial-scale studies. Feeding tylosin, reduces ruminal population of *F. necrophorum* by 80 to 90% (Nagaraja et al., 1999). However, the continuous feeding of tylosin does not result in eradication of liver abscesses, as Elanco (2014) reported liver abscess occurrence in 12 to 18% of tylosin fed feedlot cattle.

In addition to the overall negative economic effects and increased occurrence of liver abscesses, the recent implementation of the Veterinary Feed Directive (VFD; Effective January 1, 2017) and the push for decreased antibiotic usage, has led to increased interest in the prevention and control of liver abscess occurrence using alternative antibiotic free methods.

One potential method is dietary supplementation of complexed TM. Zinc has demonstrated a protective role for the airway epithelium against free radicals and other harmful agents, with important implications for asthma and other inflammatory diseases where the epithelial barrier is vulnerable and compromised (Truong-Tran et al., 2003; Roscioli et al., 2013). Being the first line of defense, the epithelial barrier in the respiratory and GI tract is continually

exposed to environmental or luminal challenges, thus turnover in epithelial cells is high. Rapid fermentation decreases pH in fed cattle contributing to impairment of barrier function (Aschenbach et al., 2011).

Micronutrients, such as Zn, enhance barrier integrity of the gut. In human intestinal epithelial cells, Zn alters the tight junction composition and positively modified barrier function (Wang et al., 2013). Furthermore, supplemental ZnAA alleviates negative effects of heat stress on intestinal integrity in pigs (Pearce et al., 2015; Fernandez et al. 2013). An additional necrotic condition affecting cattle and resulting from *F. necrophoreum* colonization is foot rot, a significant cause of severe lameness in dairy and beef cattle. Preventatives for foot rot include Zn and cattle fed Zn methionine at 216 mg/d had reduced incidence of foot rot by 55% (Brazle, 1993). Moreover, the inclusion of organic trace minerals (including ZnAA) led to static or reduced prevalence of digital dermatitis lesions in feedlot (Kulow et al., 2017) and dairy (Gomez et al., 2014) cattle. Although these studies supplemented multiple organic minerals and the exact mechanism for this reduction has yet to be fully elucidated, Zn's role in maintenance of skin integrity, stabilization of membranes and activation of the cell-mediated immune system (Miller et al., 1988) provides a potential link. Therefore, Zn supplementation could have the potential to protect the ruminal epithelial cell lining under conditions caused by high-grain diets.

EQUINE

Equine Joint Disease

Joint disease or osteoarthritis (OA) is one of the most significant causes of lameness in horses and often leads to early retirement and decreased athletic function in equine athletes (Todhunter and Lust, 1990). As a result, opportunities to prevent cartilage degradation and improve equine longevity and performance later in life are of current interest. Adaptation of

bone and soft tissue to mechanical loading occurs during early growth and exercise. However, repeated trauma and stress exposure during adaptation has potential to cause over production of inflammatory mediators. Inflammation allows for leukocyte diffusion across the synovial membrane to mitigate damage. In initial stages of injury, the inflammatory process is designed to promote healing; however, prolonged inflammation causes cartilage degradation (Palmer and Bertone, 1994).

Composition of Articular Cartilage

Synovial joints are anatomically designed for motion and load transfer between bones (Todhunter, 1996) and comprised of cartilaginous, osseous, synovial, and fibrous tissues (Pool, 1996). Joint tissues are frequently exposed to biomechanical stresses involved in performance and training; however, involved tissues are incapable of complete repair, with the exception of bone (Shapiro et al., 1993)

Two layers form the articular joint capsule, each of which contribute to the integrity of the joint in a unique way. The two layers are the fibrous joint capsule and the synovial membrane (Palmer and Bertone, 1994). The fibrous joint capsule utilizes a network of elastic fibers and connective tissue to stabilize the joint (Palmer and Bertone, 1994). The synovial membrane is highly vascularized and therefore responsible for providing nutrients and nourishment to the chondrocytes through the synovial fluid (Sellam and Berenbaum, 2010). The combination of ultrafiltrate plasma and synoviocytes secretions determines synovial fluid composition (Todhunter, 1996) making it ideal for evaluating cartilage turnover, as it is in closest contact with all relevant tissues.

Sitting between long bones, synovial joints include a thin protective layer of hyaline articular cartilage is located at the end of each bone (Todhunter, 1996). This layer provides a

lubricated frictionless surface which facilitates fluid motion (Kheir and Shaw, 2009). Normal, healthy, adult cartilage is approximately 70% water, and composition on a dry weight basis is as follows: 50% collagen, 35% proteoglycans, 10% glycoproteins, 3% mineral, 1% lipid, and 1-12% chondrocytes (Todhunter, 1996). The collagen component is primarily (85-90%) type II collagen providing tensile strength in the joint.

The avasular and aneural nature of hyaline cartilage prevents full repair or replacement after cartilage loss or damage (Kheir and Shaw, 2009). As a result, damaged hyaline cartilage is often replaced with fibrocartilage (Freemont and Hoyland, 2006). Fibrocartilage is mechanically and biochemically inferior because of increased type I collagen content (Freemont and Hoyland, 2006; Furukawa et al., 1980).

Although only representative of a small percentage of cartilage composition, the chondrocytes play a vital role in maintaining articular cartilage. Each chondrocyte is located in the lacunae, where it secretes and regulates the extracellular matrix (ECM). Proteoglycans comprising the ECM are hyaluronan, chondroitin sulfate, and keratin sulfate. Chondroitin sulfate and keratin sulfate are positively charged sugars that attach to hyaluronan and attract water into the joint; providing compressive strength. Chondrocytes are avascular; therefore, they receive nutrients though diffusion. Diffusion of fluid in and out of the cartilage is facilitated by joint use. Overall cartilage matrix turnover is slower compared to proteoglycan turnover. Each component of articular cartilage contributes in maintaining cartilage homeostasis and proper joint function (Lotz and Loeser, 2012).

Role of trace minerals in joint function

Trace minerals are required for normal maintenance and turnover of bone and connective tissue (Hostetler et al., 2003, Richards et al., 2010). Homeostatic maintenance of the joint space

requires Cu, Mn, and Zn for the synthesis of both collagen fibrils and components of the extracellular matrix, as well as, regulates and promotes normal turnover. The integrated relationship between Cu and Zn is demonstrated at the joint level through the complementary role of Zn and Cu enzymes in regulating ECM composition. A Zn deficiency leads to decreased rates of collagen and keratin synthesis, resulting in a variety of defects, including bone abnormalities and decreased tissue strength (Richards et al., 2010; Underwood and Suttle, 1999). Collagen turnover rates are also believed to decline in cases of inadequate dietary Zn, due to the dependency of Zn for activation of collagenases/matrix metalloproteinases (Pardo and Selman, 2005; Starcher et al., 1980).

As a result, skeletal abnormalities are a notable outcome of a severe Cu deficiency. The predominate role of Cu in bone and cartilage metabolism is through the Cu-dependent enzyme, lysyl oxidase. Lysyl oxidase enables the addition of a hydroxyly group onto specific lysine residues in collagen. Subsequently, cross linkage of collagen subunits results in mature protein formation, increasing collagen strength (Rucker et al., 1998). This is facilitated with catalytic assistance of Cu. Subsequently, formed cross linkages increase structural rigidity and elasticity of cartilage and elastins; hence, lysyl oxidase maintains a key role in the maturation and repair of connective tissues (Kagen and Li, 2003). Thus Cu contributes to skin, bone, tendon and intestinal strength through collagen crosslinking (Richards et al., 2010). Consequently, collagen in Cu deficient animals would be weak and likely unable to withstand the normal mechanical stresses (O'Dell et al., 1961), due to decreased collagen synthesis and turnover (Richards et al., 2010). Furthermore, research in poultry has demonstrated the strong correlation between bone breaking strength and collagen crosslinking (Rath et al., 1999).

Beyond the roles of Zn and Cu in skeletal development and maintenance through their actions on collagen, another TM, Mn, is involved with proteoglycan formation of the proteoglycan matrix in cartilage (Richards et al., 2010). As a result, skeletal abnormalities, such as chondrodystrophy and perosis, are associated with Mn deficiencies (Suttle, 2010). Glycosyltransferase requires Mn for activation and reduction in its activity due to Mn deficiency decreases synthesis of glycosaminoglycans (GAG) and oligosaccharide side chains (Suttle, 2010; Leach and Harris, 1997). Glycosaminoglycans are long unbranched polysaccharides with repeating disaccacharide units. Upon covalent bonding with proteins they form proteoglycans. The presence of proteoglycans within the joint allows for lubrication and shock absorption. McNatt et al. (1976) reported that the biological half-life of GAGs in Mn deficient cartilage is decreased. Furthermore, decreased growth and GAG synthesis has long been associated with a Mn deficiency (Bolze et al., 1985).

Although, the involvement of TM in the joint is understood, effects of dietary TM source on maintaining joint homeostasis are not fully elucidated. Increased bioavailability from CTM may increase joint tissue incorporation and facilitate a more rapid achievement of homeostasis following an inflammatory insult, potentially preventing cartilage destruction that may lead to the development of OA.

Measuring cartilage homeostasis and inflammation

Concentrations of cartilage biomarkers in the synovial fluid are affected by local inflammatory status. Initial production and release of TNF-α facilitates a cascade of inflammatory mediators that influence cartilage matrix turnover and homeostasis (van Weeren, 2016). The inflammatory cascade results in matrix metalloproteinases (MMP) production and facilitates neutrophil-mediated destruction of proteoglycans, eventually leading to PGE_2 release

from chondrocytes. Increased production and concentration of prostaglandin E_2 (PGE_2) promotes joint damage by enhancing synovial inflammation and cartilage degradation (van Weeren, 2016). In addition to being a useful indicator of inflammation, Bertone et al. (2001) identified PGE_2 as a marker for progression of equine joint disease. In fact, increases in synovial PGE_2 have been observed in a multitude of experimental models: exercise, osteochondral fragmentation, and LPS (Frisbie et al., 2008; de Grauw et al., 2009; Lucia et al., 2013).

The inflammatory response is considered healing; however, prolonged inflammation may lead to cartilage destruction. In response to prolonged inflammation, type II collagen begins to unwind or is cleaved by Zn dependent collagenases such as MMPs. Under these circumstances, normally hidden collagen epitopes are exposed (van Weeren, 2016). Upon exposure, these small peptide fragments of cartilage molecules can be taken up by the synoviocytes and enter circulation, undergo further degradation by lysosomal enzymes or be diffused into synovial fluid. Concentration of the small peptide fragments incorporated into synovial fluid and serum can be measured to detect subtle changes in cartilage homeostasis or assist in early identification of tissue damage before outward characteristics may be seen (de Grauw et al., 2009). Measuring collagen breakdown is accomplished through the collagenase-cleavage neopeptide (C2C; de Grauw et al., 2009; Lucia et al., 2013). Catabolic C2C increases in response to localized inflammation in both young and skeletally mature horses (de Grauw et al., 2009; Lucia et al., 2013; Kahn et al., 2016).

The role of Cu in collagen synthesis can be indirectly assessed through measuring type II collagen synthesis with the marker, C-propeptide of type II collagen (CPII; Billinghurst et al., 2003; Lucia et al., 2013). The CPII molecule is cleaved from procollagen during fibril formation and concentration of this peptide has been directly related to the rate of collagen synthesis.

Under inflammatory conditions, CPII has been shown to increase (de Grauw et al., 2009; Lucia et al., 2013; Kahn et al., 2016). When this marker remains elevated, it is often indicative of repair, which is increased in the presence of joint disease. This increase in synthesis is to overcome and mend damages within the collagen framework. Futhermore, the ratio of anabolic and catabolic markers allows for an overall understanding of joint homeostasis.

Aggrecan molecules are an essential component of the extra cellular matrix and due to their highly negative charge, provide the joint with compressive strength. A key glycosaminoglycan of aggrecan is chondroitin sulfate; therefore, its synthesis is dependent on the activation of glycosyltransferase by Mn. Synthesis of this novel aggrecan molecule can be measured through the CS846 epitope. Disruptions in the cartilage framework also cause shifts in GAG metabolism that further disrupt joint homeostasis. For example, in OA, synthesis is increased and concentrations of CS846 in the synovial fluid are increased (Poole et al., 1994).

Lipopolysaccharide model

Injection of lipopolysaccharide (LPS) affects metabolism of articular cartilage in horses (de Grauw et al., 2009; Lucia et al., 2011; Kahn et al., 2016). In response to LPS, tumor necrosis factor-α (TNF) is released, initiating the inflammatory cascade. Synovial fibroblasts maintain a high concentration of IL-1β and TNF receptors (Sadouk et al., 1995; Sellam and Berenbaum, 2010); therefore, these cytokines are self-stimulating through an autocrine mechanism. This autocatalytic effect stimulates, continued production of IL-1β and TNF, but also initiates production of other cytokines, ecoisinoids, and MMPs by the synoviocytes and chondrocytes (Sellam and Berenbaum, 2010).

Type II collagen makers, C2C and CPII markers typically increase in response to LPS stimulated inflammation (Lucia et al., 2011). Furthermore, de Grauw and others (2009) reported

enhanced of CS846 synthesis in response to LPS, with concentrations peaking at 24 h in mature horses. The LPS model allows for investigation of dietary factors at the joint tissue level through measuring synovial fluid biomarker concentrations in response to a single inflammatory insult (Leatherwood et al., 2016; Bradbery et al., 2018). Therefore, utilizing this established model of localized inflammation in horses (Leatherwood et al., 2016), the impact of trace mineral source on cartilage homeostasis can be measured through cartilage biomarkers present in the synovial fluid that are linked to TM status.

Complexed trace minerals and joint health

Although the role of TM in maintaining normal maintenance and turnover of bone and connective tissue is understood (Hostetler et al., 2003; Richards et al., 2010), the effect of TM source at the level of the joint has not been evaluated. Furthermore, interactions among minerals have been associated with occurrence of lameness and joint disease in dairy cattle and humans (Yazar et al., 2005; Sun et al., 2015); however, little is known of how synovial fluid TM concentrations in the horse respond to joint inflammation under inflammatory conditions or their role in the development or mitigation of joint disease. Information gained from other species such as dairy cattle and chickens, indicate CTM provides a more biologically available source of TM (Osorio et al., 2012; Nocek et al., 2006; Nockels et al., 1993). For example, in hens challenged with systemic LPS and supplemented with a Zn amino acid complex, serum interleukin-1β concentrations increased beginning at 3 h post induction but returned to baseline more rapidly post challenge when compared to hens receiving Zn sulfate (Cheng and Guo, 2004). Therefore with increased bioavailability there is potential for CTM to indirectly influence cartilage metabolism through enzyme systems that require TM cofactors for activation.

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CHAPTER III

EFFECTS OF SUPPLEMENTAL ZN-AMINO ACID COMPLEX ON PERFORMANCE AND CARCASS CHARACTERISTICS IN FINISHING STEERS FED RACTOPAMINE HYDROCHLORIDE

SYNOPSIS

Pooled analysis of two clinical field trials using approximately 1,680 and 2,160 steers in separate locations (TX and KS) was conducted. Studies were conducted in 2006 and 2011. Cattle were randomly assigned to one of three treatments: 1) **CON**, no Zinc amino acid (ZnAA) and no ractopamine hydrochloride (RAC); 2) **RAC**, no ZnAA, RAC the last 28 d; 3) **Zn360R**; 360 mg • hd^{-1}• d^{-1} ZnAA continuously in the finish diet + RAC the last 28 d. Data were analyzed using PROC MIXED of SAS with a random effect of study location. Cattle receiving both ZnAA and RAC had the highest final BW ($P < 0.01$), ADG ($P < 0.01$) and G:F ($P < 0.01$) compared to CON and RAC only cattle. Cattle in Zn360R treatment also had the highest carcass adjusted gain ($P < 0.01$) with an additional 12 kg and 5 kg, compared with CON and RAC, respectively. Largest ADG was also exhibited by Zn360R cattle (1.84 kg/d; $P < 0.01$) followed by RAC then CON, 1.81 kg/d and 1.75 kg/d, respectively. No effect of treatment on liver abscess prevalence or severity ($P \geq 0.23$) or quality grade ($P \geq 0.16$) were observed. Based on the current data, supplementation of ZnAA may augment the response of RAC and result in improved performance without negatively affecting carcass quality.

INTRODUCTION

Efficiency of beef production increases as a result of improved genetics, improved animal management, and application of production technologies. Among these technologies are β-agonist (**β-AA**), like ractopamine hydrochloride (**RAC**). Dietary inclusion of β-AA has shown to improve ADG, *longissimus dorsi* muscle area (**LM**), yield grade, hot carcass weight, and gain to feed efficiency (Bittner et al., 2014). Supplementation of RAC is approved for use in cattle during the last 28-42 d of the feeding period. Binding of RAC to a G-protein coupled receptor (**GPCR**) increases cyclic adenosine monophosphate (**cAMP**) production (Johnson et al., 2014): a major intracellular signaling molecule that initiates a cascade of physiological events. Consequently, an increase of lean muscle accretion is facilitated. Effect of β-AA on dietary requirements of micronutrients in cattle remains largely unknown.

Zinc is essential for animal growth and development (Suttle, 2010). Harris et al. (2013) found supplementing cultured bovine satellite cells with 1μM Zn concentration intensified responses to RAC through increased cAMP activity. Recent *in vivo* data demonstrates supplementation of Zn, beyond reported requirements, pigs and cattle fed RAC, resulted in increased feed efficiency and ADG compared to RAC alone (Genther-Schroeder et al. 2016a, b; Paulk et al., 2012). Steers fed both RAC and additional Zn beyond their requirement had greater final BW and HCW (Genther-Schroeder et al. 2016a, b); suggesting an additive effect for Zn and RAC. Thus, achieving optimal responses in animals fed β-AA may require fortification of the diet with available Zn. The primary objective of this work was to measure growth performance and carcass characteristics in response to additional Zn-amino acid complex (ZnAA) fed in conjunction with RAC in finishing feedlot cattle, through a pooled statistical analysis utilizing data collected from two large-scale feedlot studies.

MATERIALS AND METHODS

Two clinical field trials were conducted using 1,680 and 2,160 steers in separate locations (TX and KS), selected to represent major cattle feeding areas in the United States in 2006 and 2011. Trials were conducted in 2006 and 2011; mixed and crossbred steers were fed for 181 and 148-159 d in trails 1 and 2, respectively. A randomized complete block design was utilized for both studies. Arrival date and initial BW were predominate sorting factors for cattle pen assignment; pens were randomly allocated to treatment (n = 8 pens/trt). Number of cattle per pen ranged from 53-77 in trial 1 and 85-95 in trial 2. When required, pens and treatments were balanced for breed type and cattle source. Both studies evaluated the effect of Zn fortification of 360 $mg \cdot hd^{-1} \cdot d^{-1}$ in the form of ZnAA fed in combination with RAC. Supplementation was 200 and 320 mg RAC/d for trail 1 and 2, respectively. Treatments for the pooled analysis were as follows: (1) **CON**, no ZnAA; no RAC, (2) **RAC**, no ZnAA; RAC the last 28 d, (3) **Zn360R**; ZnAA continuously in the finish diet + RAC the last 28 d.

Cattle were observed daily for injury and disease; medical treatment was provided as required. Hospital records included date pulled, individual weight, diagnosis, treatment administration (drug, dose and duration; if applicable), and days of treatment, rectal temperature, re-pull rate and death rate. Cattle were generally maintained on a forage-based receiving diet, prior to study initiation. All steers were then transitioned to the finishing diet by d 28 through a series of step-up rations or systematic substitution of the finishing diet. Diet formulations represented ingredients commonly available at each study location; thus, dietary composition varied between studies (Table 3.2); however, both rations were based on steam-flaked corn grain. Cattle were fed twice to three times daily in amounts adequate to allow for *ad libitum* access to feed. Diets for all studies met or exceeded NRC (2000) nutrient requirements. Concentration of

ZnAA was determined throughout all studies, by routine sampling and laboratory assay.

Cattle were harvested by blocks (across all treatment groups) as they achieved appropriate market weight and condition at commercial packing plants. Data including liver abscess scores based on a scale of 0, A-, A, A+ (Brown et al., 1975), and HCW were recorded on the day of harvest. Marbling, lean color and maturity scores for determining quality grades and measures of rib fat depth, KPH fat percent and REA area for calculation of yield grades were obtained post-harvest.

Statistical Analysis

Live and carcass performance data were analyzed by ANOVA using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). Pen was the experimental unit for all data, and the model included the fixed effect of treatment and random effects of study location, block within location. Degrees of freedom used for testing significances were adjusted using the Satterthwaite correction; accounting for unbalanced data and unequal variances. Initial measurement of BW was used as a covariate in the analysis of the data. Pairwise comparisons among treatments means were evaluated using Tukey's honestly significant difference procedure and adjusted for differences in degrees of freedom based on correction for estimation significances. Data reported as least squares means ± SEM.

Carcass quality and yield grade data and liver abscess frequency data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC), same model as previously described. Data reported as estimates± SEM based on the scale of the mean or the inverse linked scale, allowing for estimates of predicted probabilities and their standard errors in logistic models. Means were calculated using the logit scale. Standard errors on the inverse linked

scale are computed by the delta method. Significance was declared at $P \leq 0.05$ and tendencies from $P = 0.06$-0.10.

Table 3. 1 Ingredient and analyzed composition of ractopamine HCL period diets (DM basis).

Ingredient %	[1]Study 1	[2]Study 2
Steam flaked corn	84.5	62.2
Wet corn gluten feed		11.8
Corn distillers grains dry		8.0
Corn silage		9.2
Alfalfa Hay	5.1	
[3]CCDS: crude glycerin blend (70:30)		4.1
Choice White Grease	4.2	
Blended animal fat		1.3
Finisher supplement	6.2	3.4
Analyzed Composition		
DM %	78.4	66.0-67.1
	% of DM	
CP	12.7	13.6-13.8
CF	4.35	
NDF		14.3-15.1
EE		5.0-5.3
Ca	0.66	0.63-0.73
P	0.31	0.41-0.43
	ppm DM basis	
Cu		10
Zn		76
Fe		110
Mn		35
Co		0.2

[1]Supplied 33 g Rumensin/ton DM and 90 mg Tylan/hd/d
[2]Supplied 34.3-34.8 g Rumensin/ton DM
[3]CCDS: Condensed corn distiller's solubles

RESULTS

Final BW ($P < 0.01$; Table 3.3) and ADG ($P < 0.01$) differed among treatments. The largest final BW and ADG were observed in Zn360R. No effect of treatment was observed for DMI (P = 0.18). Feed efficiency was affected by treatment ($P > 0.01$), with G:F being highest in Zn360R and RAC. A treatment effect was observed for HCW ($P < 0.01$; Table 3.4), with steers receiving ZnAA and RAC (Zn360R) having the highest HCW ($P < 0.01$). No differences in marbling or backfat thickness were detected ($P \geq 0.75$). A treatment effect was present for KPH accumulation ($P = 0.03$). Ribeye area was impacted by treatment ($P < 0.01$). When compared to other treatments, the lowest REA was observed in CON cattle ($P < 0.01$). Calculated yield grade and the HCW: REA ratio were unaffected by treatment ($P \geq 0.59$). Dressing percentage was affected by treatment ($P < 0.01$**)**, with highest dressing percentage observed in Zn360R steers.

Carcass gain, ADG and G:F were all effected by treatment ($P \leq 0.01$; Table 3.5), with Zn360R steers having the highest gains and conversion rate when compared to other treatments. Additionally, steers fed both ZnAA and RAC (Zn360R) exhibited greater gains and improved efficiency, compared to steers fed RAC only ($P < 0.01$). The percentage of quality grades, prime, choice, and select were not different between treatments ($P \geq 0.16$; Table 3.6). No differences were found in yield grade, 1, 3, and 5 percentages ($P \geq 0.17$). A tendency was observed for yield grade 2 ($P = 010$) with the largest percentage of YG 2 present in RAC cattle. A tendency for treatment was also present for YG 4 ($P = 0.07$) with largest percentage of YG 4 reported in CON cattle. Liver abscesses were not affected by treatments ($P \geq 0.23$; Table 3.7).

Table 3. 2 Effect of Zn amino acid-complex (ZnAA) and ractopamine hydrochloride (RAC) supplementation on growth performance, carcass characteristics, and carcass adjusted performance of finishing beef steers.

	Treatments[1]				
Variable	CON	RAC	Zn360R	SEM	*P*-value
Live performance[2]					
Final BW, kg	619[c]	628[b]	632[a]	3.2	< 0.01
ADG, kg/d	1.75[c]	1.81[b]	1.84[a]	0.1	< 0.01
DM intake, kg/d	9.36	9.34	9.47	0.77	0.18
G:F	0.187[b]	0.194[a]	0.194[a]	0.006	< 0.01
Carcass characteristics[3]					
HCW, kg	399[c]	406[b]	411[a]	2.1	< 0.01
Marbling[4]	373	371	375	259.64	0.75
Backfat thickness, cm	1.43	1.43	1.42	0.04	0.91
Internal fat (KPH) %	2.14 [ab]	2.13 [b]	2.24 [a]	0.15	0.03
REA, cm^2	36.17[b]	37.02[a]	37.35[a]	0.52	< 0.01
HCW: REA	28.04	27.88	27.96	0.24	0.68
CYG	3.15	3.10	3.12	0.06	0.59
Dress, %	64.50[b]	64.65[b]	64.99[a]	0.18	< 0.01
Carcass adjusted perfomance[5]					
Carcass Gain, kg	210[c]	217[b]	222[a]	2.10	< 0.01
Carcass ADG, kg	1.26[c]	1.31[b]	1.34[a]	0.07	< 0.01
Carcass G:F	0.136[b]	0.140[a]	0.142[a]	0.005	< 0.01

[1]Treatments: CON = no supplemental ZnAA or RAC (*n* = 16); RAC = no supplemental ZnAA, 320 or 200 mg·hd^{-1}·d^{-1}RAC (*n*=16); Zn360R= 360 ZnAA mg·hd^{-1}·d^{-1} and 320 [1] or 200 mg·hd^{-1}·d^{-1}RAC (*n*=16)

[2]A 4% pencil shrink was applied to all live BW measures, which were used in the calculations of ADG and GF; Day 0 body weights were used as a covariate in analysis.

[3]Day 0 body weights were used as a covariate in analysis

[4]Degrees of marbling: 300 = Slight, 400 = Small, 500 = Modest, etc.

[5]Caclulations based on Tatum et al., 2012; d 0 BW were used to calculate initial HCW; d 0 BW was used as a covariate in analysis

[abc]Denotes differences between treatments, calculated by Tukey's Honest Significant Difference; significance declared at $P \leq 0.05$

Table 3. 3 Effects of Zn amino acid-complex (ZnAA) and ractopamine hydrochloride (RAC) supplementation on quality and yield grades of finishing beef steers.

	Treatments[1]				
Variable	CON	RAC	Zn360R	SE	*P*-value
Quality grade[2]					
Prime	0.5	0.7	0.7	0.4	0.65
Choice	48.9	48.9	51.7	11.0	0.37
Select	47.8	47.3	45.7	10.5	0.63
Standard	2.1	2.2	1.2	1.0	0.16
Yield grade[2]					
1	6.7	8.6	8.5	1.0	0.17
2	31.0	35.3	33.8	3.8	0.10
3	43.1	39.7	41.6	2.7	0.25
4	16.0	13.1	12.8	3.6	0.07
5	1.8	1.9	1.9	2.0	0.95

[1]Treatments: CON = no supplemental ZnAA or RAC (*n* = 16); RAC = no supplemental ZnAA, 320 or 200 mg·hd^{-1}·d^{-1}RAC (*n*=16); Zn360R= 360 ZnAA mg·hd^{-1}·d^{-1} and 320 [1] or 200 mg·hd^{-1}·d^{-1}RAC (*n*=16)
[2]Reported values, percentage of total steers

Table 3. 4 Effects of Zn amino acid-complex (ZnAA) and ractopamine hydrochloride (RAC) supplementation on liver abscess presence and severity of finishing beef steers.

	Treatments[1]				
Abscess Severity[2]	CON	RAC	Zn360R	SEM	*P*-Value
Normal	53.7	53.4	53.4	1.3	0.75
A minus	43.4	43.6	43.7	1.1	0.83
A	4.3	4.6	2.6	1.2	0.23
A Plus	1.0	0.7	0.8	0.5	0.66

[1]Treatments: CON = no supplemental ZnAA or RAC (*n* = 16); RAC = no supplemental ZnAA, 320 or 200 mg·hd^{-1}·d^{-1}RAC (*n*=16); Zn360R= 360 ZnAA mg·hd^{-1}·d^{-1} and 320 [1] or 200 mg·hd^{-1}·d^{-1}RAC (*n*=16)
[2] Reported values, percentage of total steers

DISCUSSION

Identifying opportunities to increase lean yield and muscle mass in fed cattle is of great benefit to the cattle feeding industry. As a result, roughly 90% of feedlot cattle in the U.S. will receive some type of growth enhancing technology, including β-AA in their lifetime (Johnson et al., 2014). Based on a survey conducted by Samuelson et al. (2016), approximately 84.8% of yards included in the survey utilized a β-AA and 95.5% of nutritionists identified RAC as the most commonly used.

In the current study, the responses of RAC supplementation in non-ZnAA supplemented cattle improved live performance through increased ADG and feed efficiency over cattle receiving no RAC supplementation. This observation is in congruence with previous literature (Bittner et al., 2017; Bittner et al., 2016; Bryant et al., 2010). When compared to steers receiving 0 mg RAC, supplementation of RAC lead to 9 kg of additional live BW. Winterholler et al. (2007) reported similar results in yearling steers fed RAC daily (200 mg/d) for the final 28 d. Demonstrating an advantage of 11 kg of live weight gain over steers not receiving RAC. Furthermore, a 5.9 kg gain was observed when 300 mg RAC was fed 28 d prior to harvest (Genther-Schroeder et al., 2016b). Similarly, Bittner and others (2017) reported a tendency for increased live BW in steers fed RAC at 300 mg/d for 28 d. In contrast, some data has reported no advantage of live BW in steers fed RAC at dosages of 100 and 200 mg/d for 28 d (Bittner et al. 2016, Bryant et al., 2010). Differences in live BW are likely attributed to variable due to gut fill and environmental conditions.

Investigation of Zn supplementation in finishing cattle has reported variable responses, often attributed to source and duration of Zn supplementation. Even so, when supplementing

trace minerals, consulting feedlot nutritionist use a combination of inorganic and organic sources or organic only in finishing diets and average Zn concentration is roughly 3 times the current recommended requirement of 30 mg/kg DM (Samuelson et al., 2016). In the current study, ZnAA supplementation was provided at approximately 38 mg Zn/kg DM or 360 $mg \cdot hd^{-1} \cdot d^{-1}$ in combination with RAC.

Recent data supports supplementation of Zn, beyond the requirement, in combination with RAC in pigs and beef cattle, with increases in feed efficiency and ADG compared to RAC alone reported (Genther-Schroeder et al. 2016a, b; Paulk et al., 2012). In the current study, when fed in combination Zn and RAC, live BW and ADG were greater than cattle fed only RAC. In contrast to previous studies, no benefit over RAC alone was observed in feed efficiency; however, both treatments were improved over cattle receiving no RAC.

Steers fed both RAC and additional Zn demonstrated the largest final BW and HCW. Previous work evaluating ZnAA supplementation has resulted in similar results (Genther-Schroeder et al. 2016a, b); suggesting a potential additive effect for Zn and RAC. Paulk et al. (2015) also reported pigs supplemented with 50 mg/kg of Zn from ZnAA and RAC tended to increase ADG compared to pigs receiving RAC only.

The mode of action for RAC remains largely unknown; however, all proposed mechanisms of β-AA begin with stimulation of the β-agonist receptor and the downstream elevation of cyclic AMP (cAMP) concentrations (Johnson et al., 2014). Because RAC's dependency on cAMP concentrations, ZnAA supplementation may alter responses to RAC by inhibiting phosphodiesterase (**PDE**) activity: an enzyme responsible for the break down of cAMP (Percival et al., 1997). Hojyo et al. (2011) reported Zn deficiency causes an increase in PDE expression. Furthermore, when used together Zn and RAC increased cAMP production in

bovine satellite cells over independent supplementation (Harris, 2013). Genther-Schroeder and colleges (2016a) found that increasing ZnAA supplementation linearly increased plasma cAMP, supporting that this is a potential mechanism though which supplemental ZnAA may increase responses to RAC. However, a full understanding as to why this is seen with ZnAA but other sources need to be further investigated.

Addition of ZnAA augmented the response of RAC, shown by increased HCW, dressing percentage, and carcass adjusted gain and ADG. Increases in HCW in steers fed 360 mg • hd^{-1}• d^{-1} lead to a 12 kg gain over the CON cattle compared to a 7 kg increase cattle fed RAC alone. Furthermore, both carcass adjusted gain and ADG were greatest in cattle fed both ZnAA and RAC. Burrnet et al. (2016) suggested that Zn alters RAC response in pigs through prolonged expression of *IGF-1* and *β_1-adrenergic receptors.* Receptor expression has not been evaluated in cattle. However, ZnAA supplementation did not prevent the diminishing response of RAC from 28 to 42 d (Genther-Schroeder et al., 2016b), suggesting mechanistic limitations by which ZnAA is able to augment the RAC response.

Evaluation of other Zn sources has demonstrated variable results. Bohrer and others (2014), reported no differences in HCW between steers fed RAC and those fed RAC plus Zn and Cr propionate, when added the final 35 d of feeding before slaughter. Feedlot growth performance and carcass characteristics were not further improved by the addition of supplemental Zn and Cr propionate (1.0 g and 3 mg/d, respectively). Furthermore, Edenburn et al. (2016) reported HCW for steers fed RAC with supplemental Zn propionate were not different from control cattle. Similarly, addition of Zn sulfate at 100 mg/kg DM to feedlot heifer diets in combination with RAC for 42 days caused a decrease in ADG but did not affect other feedlot performance (Van Bibber-Krueger et al., 2017). Budde et al. (2019) reported no differences in

the live performance of cattle fed RAC due to Zn source or concentration, when evaluating hydroxychloride and methionine sources; however, no comparison was made to RAC fed cattle not receiving Zn supplementation. Furthermore, inclusion of supplemental Zn from ZnO in swine diets containing RAC tended to increase loin weight (quadratic) with increasing ZnO concentrations (Paulk et al., 2015), indicating that other Zn sources have an impact on other species. In the current study, overall supplementation of ZnAA with RAC improved live and carcass adjusted performance. This data supports that source Zn may impact both carcass characteristics and carcass-adjusted performance, when fed with RAC.

Varying levels of Zn supplementation have likely attributed to inconsistency in reports. Previous data demonstrated a linear growth response in beef steers with increasing ZnAA when supplemented with RAC (Genther-Schroeder et al. 2016) up to 90 mg/kg DM. Additional work by Genther-Schroeder et al. (2018) evaluated a range of supplementation rates (1 to 8 times documented requirement) to identify the plateau of ZnAA. Although, they found that increasing Zn between 5 to 8 times the recommended rates had minimal impact on growth performance or carcass characteristics; however, trends toward decreased meat quality with increasing Zn were observed. The current study supplements ZnAA at a much lower concentration; therefore, finding an optimal supplementation range would be beneficial to the producer's bottom line and to prevent decreases in meat quality.

Another potential hypothesis is that the Zn requirements may be altered by the presence of RAC. The current recommended NRC (2016) requirement for beef cattle is 30 mg of Zn/kg dietary DM; although this requirement should prevent deficiency in most situations, it is not considered optimal and the impact of RAC on micronutrient requirements has yet to be fully elucidated. When fed ZnAA in combination with RAC, Zn liver concentrations decreased

linearly with increasing concentrations of ZnAA, while final BW and ADG both increased linearly (Genther-Schroeder et al., 2016a), suggesting alterations in mineral status due to RAC supplementation. The ability of RAC to enhance rate of gain and deposition of carcass protein could potentially be impacting Zn requirements. Conclusions about requirements in the current study are difficult to evaluate because even the control diet had Zn concentrations above the requirement; however, further investigation is warranted.

Limited data exists on effects of RAC on amino acid requirements in beef cattle. In heifers fed diets with a higher ruminally undegradable protein as a percentage of CP showed no response to RAC (Walker et al., 2006). Furthermore, the addition of protected methionine in finishing ram lambs showed no benefits on performance or carcass characteristics (Obeidat et al., 2008). Dietary crude protein of the finishing diets for the current studies fell within or slightly above the normal range of crude protein levels in finishing rations; therefore, the impact of the contributed methionine from ZnAA is likely negligible. Additionally, Genther-Schroeder et al. (2016b) analyzed their ZnAA supplemented and non-supplemented diets and found that the amino acid profiles differences were minimal, yet still observed performance differences in finishing steers.

The pooled analysis showed inclusion of ZnAA and RAC or RAC only in finishing diets did not impact DMI ($P = 0.18$), similar to results found by Genther-Schroeder and others (2016b). Although not directly related to this study, the addition of ZnAA has been shown to linearly decrease DMI intake in the pre-RAC period for concentrations of 30 to 60/90 mg Zn per kg DM (Genther-Schroeder et al., 2016a). In the current study, pre-RAC period data was not collected and further investigation of varying ZnAA concentrations is warranted.

Both marbling and BFT were unaffected by dietary treatment; however, KPH was

increased with the addition of ZnAA. Overall changes towards lean carcass growth can be assessed in commercial slaughter data through HCW:REA (Beckett et al., 2009), which accounts for differences in muscle accretion relative to carcass fat. Branine et al. (2014) reported a decrease in HCW:REA for cattle fed 360 mg ZnAA per head per day; however they attributed the difference to higher HCW in control steers. In the current study, no differences in HCW: REA were observed. Overall carcass quality grades were minimally impacted by the addition of ZnAA and/or RAC. These data, suggest that under current conditions, the increases in performance characteristics due to ZnAA and RAC are not negligibly affecting carcass quality.

Conclusion

Under the conditions of these two large-scale studies, cattle fed diets containing both ZnAA and RAC had improved live performance by increasing ADG and larger final live BW. Improved HCW, dress, and carcass-based measures of gain were also exhibited in cattle fed both ZnAA and RAC. These data suggest that the inclusion of ZnAA and RAC in finishing diets may provide incremental improvements in steer performance over RAC alone, conveying a potential opportunity for cattle feeders to optimize performance and increase economic returns.

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CHAPTER IV

EFFECTS OF SUPPLEMENTAL ZN-AMINO ACID COMPLEX ON PERFORMANCE, CARCASS CHARACTERISTICS AND LIVER ABSCESSES IN FINISHING BEEF CATTLE: A POOLED ANALYSIS

SYNOPSIS

A pooled statistical analysis of 9 finishing studies was conducted to evaluate live and carcass adjusted performance of steers fed finishing diets with varying levels of supplemental zinc amino acid (**ZnAA**). The analysis consisted of 285 pens, representing approximately 16,192 steers, from studies in TX, KS, IA, and Alberta between 2001-2016. The analysis divided pens into one of four treatments: control (**CON,** No ZnAA or RAC; n=67), zinc AA only (**ZnAA**; n=79), ractopamine only (**RAC**; n=70), and zinc AA and ractopamine (**ZnAA + RAC**; n=69). No ZnAA × RAC interactions ($P \geq 0.23$) for live animal performance, carcass characteristics, or carcass adjusted performance were observed. Independently, ZnAA and RAC improved final BW, ADG, and hot carcass weight (HCW; $P = 0.02$). Dry matter intake, marbling, and back fat thickness were not affected by ZnAA or RAC ($P \geq 0.15$). Feed efficiency (G:F) was increased by RAC ($P < 0.01$),but there was no significant effect of ZnAA ($P = 0.24$). Supplemental ZnAA increased kidney, pelvic, heart fat (KPH; $P = 0.02$) and dressing percentage ($P = 0.01$), but no effect of RAC was observed ($P \geq 0.24$). Carcass-adjusted gain, ADG, and G:F were improved by ZnAA and RAC ($P = 0.01$). A ZnAA × RAC interaction was observed for total liver abscess (**LA**) percentage ($P = 0.02$) and A- percentage ($P = 0.01$). No interactions were observed for other levels of LA severity ($P \geq 0.17$). Independently ZnAA and RAC reduced LA percentages

of total, A-, and A+ ($P = 0.03$) versus control. A secondary analysis divided pens into one of three levels of ZnAA: no supplemental ZnAA (**NO;** *n*=137); 30-54 ppm ZnAA (**LOW**; *n*=70); and ≥60 ppm ZnAA (**HIGH**; *n*=78). Final BW and ADG linearly increased with increasing ZnAA ($P < 0.05$). Dry matter intake and feed efficiency were not significantly affected by ZnAA level ($P > 0.05$). Supplemental ZnAA linearly increased HCW ($P < 0.01$), carcass yield grade ($P < 0.05$), and KPH ($P = 0.05$). A quadratic tendency was observed for carcass G:F ($P = 0.08$), with LOW having the highest. Carcass gain and carcass ADG ($P < 0.01$) increased linearly while a quadratic effect was observed ($P = 0.02$) for total LA and A+ abscesses ($P < 0.01$), with LOW having the lowest percentage. These data suggest that inclusion of ZnAA in finishing diets may improve steer performance and reduce LA occurrence at LOW levels, conveying a potential opportunity for cattle feeders to optimize performance and increase economic returns.

INTRODUCTION

Zn plays an essential role in animal growth, development, and immune function (Suttle, 2010). Regeneration and maintenance of gut epithelial tissue also requires Zn (Alam et al., 1994). In beef cattle, the recommended feeding rate of Zn is 30 mg of Zn/kg dietary DM (NRC, 2016); although, this concentration is adequate for preventing deficiency, it may not be sufficient in all environments and scenarios: utilization of growth promoting technologies and significant stressors (i.e. shipping, heat stress, disease) may increase Zn requirements.

Zn supplementation in finishing cattle has been investigated extensively (Kegley and Spears, 2002; Bohrer et al., 2014; Edenburn et al., 2016; Genther-Schroder et al., 2016a,b; Van Bibber-Krueger et al., 2017; Genther-Schroder et al., 2018); however, reported responses have been variable, which is often attributed to source and duration of Zn supplementation. In contrast, previous research has clearly demonstrated that feeding ractopamine (**RAC**) prior to harvest, increases ADG, feed efficiency, and HCW (Bryant et al., 2010; Scramlin et al., 2010; Boler et al., 2012; Pyatt et al., 2013, Bittner et al., 2017). Recent studies have shown Zn amino acid (**ZnAA**) supplementation augments RAC responses in cattle (Genther-Schroeder et al., 2016a, b) and swine (Paulk et al., 2015). A varying response to Zn supplementation in cattle plus the aforementioned possibility of synergistic effects of Zn and RAC provides impetus for clearly defining the responses to Zn.

Accordingly, the objectives of this study were to determine how supplementation of a single source of Zn affects the performance response to RAC in finishing cattle and if level of Zn supplementation affects performance, carcass characteristics, and liver abscess prevalence in finishing cattle. Our hypotheses are supplemental ZnAA will increase responsiveness to RAC in cattle and increasing levels of ZnAA will improve finishing performance.

MATERIALS AND METHODS

Data (pen means) compiled from 9 feedlot trials, consisting of approximately 16,192 steers at study locations selected to represent the major cattle feeding areas in the United States and Canada (Table 1) were pooled for statistical analysis. Studies utilized a randomized complete block design with pen as their experimental unit. All studies utilized a corn or barley-based finishing diet (Table 2). Across all 9 studies, a total of 285 pens were utilized, those pens were divided by treatment: control (**CON,** No ZnAA or RAC; n = 67), zinc AA only (**ZnAA**; n=79), ractopamine only (**RAC**; n=70), and zinc AA and ractopamine together (**ZnAA + RAC**; n = 69). Following the initial analysis groups were divided by level of supplemental ZnAA: 0 ppm ZnAA (**NO**; *n* = 137), between 30 - 54 ppm ZnAA, (average supplemental intake of 350 $mg \cdot hd^{-1} \cdot d^{-1}$; **LOW**; *n* = 70), and ≥ 60 ppm ZnAA (average supplemental intake of 723 $mg \cdot hd^{-1} \cdot d^{-1}$; **HIGH**; n = 78). Data used included live and carcass-adjusted feedlot performance, as assessed by initial and final BW, ADG, DM intake, and feed efficiency (G:F), calculated on a deads and rejects removed basis. Additional data of interest included carcass characteristics and liver abscess scores.

In all studies cattle were harvested by blocks (across all treatment groups) as they achieved appropriate market weight and condition at commercial packing plants. Data including liver abscess scores, based on a scale of 0, A-, A, A+ (Brown et al., 1975), and hot carcass weights were recorded on the day of harvest. Marbling, lean color and maturity scores for determining quality grades and measures of rib fat depth, KPH fat percent and LM area for calculation of yield grades were also obtained post-harvest.

Statistical Analysis

Live and carcass performance data were analyzed by ANOVA using the MIXED

procedure of SAS (SAS Institute, Inc., Cary, NC). Pen was the experimental unit for all data, and the model included the fixed effect of treatment and random effects of study location, block within location. The appropriate degrees of freedom were calculated using the Satterthwaite Method, accounting for unbalanced data and unequal variances for each individual variable evaluated. Initial measurement of BW was used as a covariate in the analysis of the data. Pairwise comparisons among treatments means were evaluated using Tukey's honestly significant difference procedure and adjusted for differences in degrees of freedom based on correction for estimation significances. The first model included main effects of ZnAA and RAC and their interaction (ZnAA × RAC). The LSMEANS option was used to calculate treatment means. Lack of interactions, lead to the development of the second model, including only the effect of level of ZnAA supplementation. Orthogonal polynomial contrasts (linear and quadratic) were used to partition the treatment sums of squares. The LSMEANS option was used to calculate treatment means.

Liver abscess presence and severity data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC), same model as previously described. Data reported as estimates based on the scale of the mean or the inverse linked scale, allowing for estimates of predicted probabilities and their standard errors in logistic models. Means were calculated using the logit scale. Standard errors on the inverse linked scale are computed by the delta method. Orthogonal polynomial contrasts (linear and quadratic) were used to partition the treatment sums of squares. Significance was declared at $P \leq 0.05$ and tendencies from $P = 0.06$ - 0.10. Liver abscess data was not collected in all studies, experimental units varied from live and performance variables of interest.

Table 4. 1 Major experimental variables.

Study	Location	Year	Initial BW kg	DOF	Cattle	Pens/Trt	ZnAA [1]	RAC Rate[2]
1	TX	2001	325	152	270	5	30 | 60	--
2	KS	2002	249	215	3918	6	52 | 103	--
3	TX	2004	288	176	270	10	35	--
4	KS	2006	311	181	2015	8	42 | 85	200
5	TX	2011	344	154	2879	8	38	320
6	TX	2011	350	165	1798	10	36	300
7	AB	2011	281	253	4542	10	39	200
8	IA	2014	483	87	321	9	60	300
9	IA	2016	427	81	179	8	60 | 90	300

[1] Zinc amino acid ($mg \cdot hd^{-1} \cdot d^{-1}$)
[2] Ractopamine ($mg \cdot hd^{-1} \cdot d^{-1}$) fed last 28 d prior to harvest, except in study 7, which fed ractopamine for 35 d.

Table 4. 2 Nutrient composition of basal finishing diets for individual experiments

	Study[1]								
Item, DM basis	**1**[2]	**2**[2]	**3**[2]	**4**[2]	**5**[2]	**6**[2]	**7**[2]	**8**	**9**
DM, %		77.53		78.39	66.5	66.2	80.9		
NEm, Mcal/kg	2.07		2.23		2.18	2.16	1.94		
NEg, Mcal/kg,	1.41		1.55		1.49	1.48	1.30	1.35	1.39
CP, %	12.5	12.71	13.2	12.7	13.6	13.5	12.5	13.1	13.6
CP from NPN, %		2.58		2.59	2.2	2.4			
NDF, %					14.6	15.7	21.1	17.8	18.4
ADF, %			5.8						
Ether extract, %		7.47	6	7.59	5.3	5.2		4.5	4.85
Ash, %			4						
Ca, %	0.61	0.68	0.68	0.66	0.68	0.77	0.64		
P, %	0.3	0.31	0.3	0.31	0.43	0.43	0.37		
Mg, %			0.27		0.2	0.19			
Na, %			0.19						
K, %	0.69	0.67	0.97	0.65	0.74	0.75	0.65		
S, %			0.24		0.21	0.23			
Cl, %			0.44						
Fe, mg/kg			233		110	105		71.7	99.7
Mn, mg/kg			57		35	38		47.9	
Co, mg/kg					0.2	0.44		0.38	
Cu, mg/kg			21		10	10		17.6	18.8
Zn, mg/kg			33		76	78		60	30
Mo, mg/kg			0.61						
Salt		0.25		0.26				0.31	0.31

[1]Studies 1-6 included in liver abscess data. All studies provided rumensin to cattle at recommended feeding rates.
[2]Tylosin provided to all cattle at recommended feeding rates.

RESULTS

Zinc amino acid and ractopamine

There were no significant ZnAA × RAC interactions ($P \geq 0.23$) for live animal performance, carcass characteristics, or carcass adjusted performance (Table 3). Both final BW ($P = 0.01$) and ADG ($P = 0.02$) were greater in cattle fed ZnAA, versus those not receiving

ZnAA. An effect of RAC was also observed for final BW ($P < 0.01$) and ADG ($P < 0.01$), versus those not fed RAC. Dry matter intake was not significantly affected by ZnAA or RAC ($P \geq 0.17$). The G:F ratio was increased by RAC ($P < 0.01$); however, there was not a significant effect of ZnAA ($P = 0.24$). Hot carcass weight was improved by both ZnAA ($P < 0.01$) and RAC ($P < 0.01$). Marbling and back fat thickness were not significantly affected ($P \geq 0.15$) by supplemental ZnAA and RAC. Supplemental ZnAA increased KPH ($P = 0.02$) while no significant effect of RAC was observed ($P = 0.54$). Yield grade tended to be decreased by RAC ($P = 0.06$) and increased by ZnAA ($P = 0.11$). Dressing percentage increased in response to ZnAA ($P = 0.01$); however, RAC did not significantly affect dressing percentage ($P = 0.23$). Carcass-adjusted gain ($P < 0.01$), ADG ($P < 0.01$), and G:F ($P = 0.01$) were improved by ZnAA. Cattle receiving RAC compared to non-RAC cattle increased in carcass-adjusted gain ($P < 0.01$), ADG ($P < 0.01$), and G:F ($P < 0.01$).

A ZnAA × RAC interaction was observed for total liver abscess percentage ($P = 0.02$; Table 4), which resulted from a greater reduction in total abscesses when ZnAA and RAC were provided in combination versus just ZnAA. A similar interaction was observed for A- percentage ($P = 0.01$). No interactions were observed for percentage of abscesses scoring an A or A+ ($P \geq 0.17$). Versus CON, lower percentages were observed in both ZnAA and RAC for A+ ($P < 0.01$). There was no ZnAA × RAC interaction for A+ prevalence, when evaluated as a percentage of total abscesses ($P = 0.89$), and no effect of ZnAA ($P = 0.12$) or RAC ($P = 0.12$) was observed.

Level of zinc amino acid

Due to the lack of interactions in the initial analysis, effect of RAC was removed from the secondary analysis, which evaluated effect of ZnAA supplementation regardless of RAC

supplementation. A linear increase ($P \leq 0.05$) in final BW and ADG was observed in response to increasing ZnAA provision (Table 5). Dry matter intake and G:F were not significantly affected by ZnAA ($P \geq 0.27$). Increasing ZnAA inclusion linearly increased HCW, back fat thickness, KPH, and yield grade ($P < 0.05$). A quadratic effect was observed for dressing percentage ($P = 0.04$); however, the biological significance was small. There were no significant effects ($P \geq 0.33$) of ZnAA level on marbling or REA.

Carcass adjusted gain ($P < 0.01$) and carcass adjusted ADG ($P = 0.02$) increased linearly, in response to increasing ZnAA. A quadratic tendency was observed for carcass G:F ($P = 0.08$) with a small increase from NO to LOW and a small decrease from LOW to HIGH.

Supplementation of ZnAA resulted in quadratic decrease ($P = 0.02$; Table 6) in total liver abscess. Similarly, a quadratic decrease ($P < 0.01$) in percentage of severe (A+) abscesses with increasing ZnAA was present. When evaluating severity of abscess, a tendency for a quadratic effect was observed for more severe abscesses (percentage of A+; $P = 0.08$). No differences between treatments were observed in percentages of less severe (A- and A) abscesses $P = 0.15$ and $P = 0.88$, respectively.

Table 4. 3 Effects of supplemental zinc from zinc amino acid complex and ractopamine on performance and carcass characteristics in feedlot cattle

	Treatment					ZnAA	RAC	ZnAA×RAC
Item	Control	ZnAA only	RAC only	ZnAA+RAC	SEM	*P*-Value	*P*-Value	*P*-Value
n	67	79	70	69				
Live Performance[1]								
BW Final, kg	601.9	605.4	611.2	613.4	15.1	0.01	< 0.01	0.59
ADG, kg	1.61	1.64	1.69	1.71	0.07	0.02	< 0.01	0.92
DM intake, kg/d	9.8	9.9	9.8	9.9	0.2	0.24	0.86	0.90
G:F	0.166	0.168	0.174	0.175	0.008	0.17	< 0.01	0.59
Carcass Characteristics								
Hot carcass weight, kg	385.6	388.2	391.6	393.7	7.8	< 0.01	< 0.01	0.31
Marbling[2]	405	400	397	395	22	0.34	0.17	0.59
Back fat thickness, cm	1.17	1.20	1.16	1.17	0.06	0.15	0.43	0.51
Kidney, pelvic, heart fat	2.07	2.11	2.08	2.12	0.07	0.02	0.54	1.00
Ribeye area, cm^2	30.56	30.71	31.48	31.61	0.62	0.38	< 0.01	0.94
Carcass yield grade	3.08	3.14	3.00	3.05	0.09	0.11	0.06	0.88
Dressing %	63.90	64.15	64.10	64.19	0.58	0.01	0.24	0.24
Carcass Adj. Performance								
Carcass Gain, kg	155.4	158.9	162.4	164.5	8.0	< 0.01	< 0.01	0.33
Carcass ADG, kg	0.99	1.01	1.04	1.06	0.06	< 0.01	< 0.01	0.68
Carcass G:F, kg	0.103	0.106	0.109	0.110	0.01	0.01	< 0.01	0.24

[1]A 4% pencil shrink was applied to all live BW measures, which were used in the calculations of ADG and GF; initial body weights were used as a covariate in analysis

[2]Degrees of marbling: 300 = Slight, 400 = Small, 500 = Modest, etc.

Table 4. 4 Effects of supplemental zinc from zinc amino acid complex and ractopamine on liver abscesses prevalence and severity in feedlot cattle

	Treatment					ZnAA	RAC	ZnAA×RAC
Item	Control	ZnAA only	RAC only	ZnAA+RAC	SEM	*P*-Value	*P*-Value	*P*-Value
% Total Hd								
Total Abscess	10.75	9.19	10.17	6.37	2.19	< 0.01	< 0.01	0.02
A_minus	5.36	5.27	5.47	3.52	1.36	0.03	0.03	0.01
A	1.86	1.36	1.71	1.58	0.37	0.84	0.30	0.45
A_Plus	3.48	2.57	2.84	1.49	0.80	< 0.01	< 0.01	0.17
% Total Abscess								
A_Plus	31.17	26.23	26.88	21.69	3.71	0.12	0.12	0.89

Table 4. 5 Effects of supplemental zinc amino acid-complex concentration in feedlot cattle

	Treatments[1]				Contrast *P*-values	
Item	NO	LOW	HIGH	SE	Linear	Quadratic
n	137	70	78			
Live Performance[2]						
Final BW, kg	606.6	608.9	610.0	15.6	0.03	0.70
ADG, kg/d	1.65	1.67	1.68	0.07	0.05	0.51
DM intake, kg/d	9.8	9.8	9.9	0.2	0.27	0.87
G:F	0.171	0.172	0.172	0.008	0.47	0.38
Carcass Characteristics						
HCW, kg	388.1	391.0	390.9	8.3	< 0.01	0.15
Marbling[3]	401	399	397	22	0.33	0.92
Back fat thickness, cm	1.31	1.31	1.37	0.07	0.02	0.19
Kidney, pelvic heart, fat	2.07	2.11	2.11	0.07	0.05	0.29
Ribeye area, in^2	34.94	35.23	34.98	0.72	0.87	0.27
Carcass yield grade	3.05	3.06	3.13	0.09	0.03	0.55
Dressing %	64.00	64.23	64.11	0.59	0.23	0.04
Carcass Adj. Performance						
Carcass Gain, kg	158.9	161.7	161.6	8.4	< 0.01	0.15
Carcass ADG, kg	1.02	1.04	1.04	0.06	0.02	0.18
Carcass G:F	0.106	0.108	0.107	0.055	0.21	0.08

[1]Level of supplemented Zinpro performance minerals: No_ZnAA=0ppm, LOW <60 ppm, HIGH >/= 60 ppm, supplied by Availa Zn and/or ZINPRO Zn

[2]A 4% pencil shrink was applied to all live BW measures, which were used in the calculations of ADG and GF; Day 0 body weights were used as a covariate in analysis
[3]Degrees of marbling: 300 = Slight, 400 = Small, 500 = Modest, etc.

Table 4. 6 Effects of supplemental zinc amino acid complex concentration on liver abscess presence and severity in beef feedlot cattle

	Treatments[1]				Contrast *P*-values	
Variable	NO	LOW	HIGH	SE	Linear	Quadratic
% Total Hd						
Total Abscess	10.39	8.43	9.29	1.93	0.29	0.02
A–	5.49	4.60	4.92	1.21	0.45	0.17
A	1.64	1.71	1.53	0.34	0.71	0.68
A+	3.17	2.03	2.76	0.64	0.57	< 0.01
% Total Abscess						
A+	28.72	22.83	27.86	3.71	0.96	0.08

[1]Level of supplemented Zinpro performance minerals: No_ZnAA=0ppm, LOW <60 ppm, HIGH >/= 60 ppm, supplied by Availa Zn and/or ZINPRO Zn

DISCUSSION

As a member of the phenylethanolamine class of β-adrenergic agonist, RAC acts a repartitioning agent directing nutrients towards skeletal muscle accretion while decreasing fat deposition (Beermann, 2002; Mersmann 1998). This is demonstrated in the present study, with positive changes in HCW and carcass-adjusted parameters (ADG and G:F). Similarly, Bitner et al. (2016) reported improved steer growth performance with improved ADG, feed efficiency and HCW. In the present study, RAC supplementation rates ranged from 200-300 $mg \cdot hd^{-1} \cdot d^{-1}$ for 28 d, which agrees with the optimal feeding duration for RAC (Bittner et al., 2016; Bittner et al., 2017). Addition of RAC increased final live BW by 9.3 kg compared to control cattle. These results agree with Abney et al. (2007), who observed 9.4 kg improvement in final BW in

steers fed 200 mg·hd^{-1}·d^{-1} over those not fed RAC. Moreover, Bittner et al. (2017) reported a tendency for greater live final BW in steers fed 300 mg·hd^{-1}·d^{-1} RAC for 28 d, with an advantage of 5.8 kg over cattle fed no RAC. In contrast, Bryant et al. (2010) and Bittner et al. (2016) observed no differences in final BW when evaluating RAC at 0, 100, and 200 mg·hd^{-1}·d^{-1}. Furthermore, Haigenmaier et al. (2017) observed no final BW differences when RAC was fed at 400 mg·hd^{-1}·d^{-1}. Live weight can be influenced by a multitude of factors including, but not limited to, gut fill, intake and environmental elements; thus, differences observed in live BW may be attributed to these factors.

Recent data shows supplementation of Zn in combination with RAC in pigs and beef cattle resulted in increased feed efficiency and ADG compared to RAC alone (Genther-Schroeder et al. 2016a, b; Paulk et al., 2015). Additionally, steers fed both RAC and added Zn had increased final BW and HCW (Genther-Schroeder et al. 2016a, b) suggesting a synergistic effect or a shift in micronutrient requirement. Interestingly, inclusion of Zn in the form of Zn-proteinates has shown no added benefit when fed to finishing cattle receiving RAC (Bohrer et al., 2014; Edenburn et al. 2016). The present study similarly found no synergistic benefit of ZnAA and RAC as described by the lack of an observed interaction. In contrast to previous work, the current data set incorporated cattle with varying levels of dietary Zn; therefore, effects of supplementation of ZnAA may only be observed at specific levels of total dietary Zn.

Genther-Schroeder et al. (2016a,b) only observed increased performance with ZnAA supplemented steers that were receiving RAC; positive correlations between Zn level and N retention in beef steers have been reported (Carmichael et al. 2018). Nitrogen retention calculated as a percentage of intake was greater in cattle supplemented with 60 ppm $ZnSO_4$ + 60 ppm ZnAA (44.3%) compared to control cattle receiving no Zn supplementation (40.0%;

Carmichael et al. 2018). These data highlight Zn's role in cattle growth, which may go beyond growth induced by RAC hydrochloride. Furthermore, protein deposition has previously been linked to Zn homeostasis in mice suggesting Zn alone can positively influence growth (Giugliano and Millward, 1984; Stake et al., 1973).

Additional work by Genther-Schroeder et al. (2018) evaluated a range of supplementation rates (1 to 8 times documented requirement) to identify optimal dose response. Authors found that increasing Zn 5 to 8 times the recommended supplementation rate had marginal impact on growth performance and carcass characteristics; however, a trend towards decreased carcass quality was observed in supplemented animals. Finding an optimal supplementation range would be beneficial for maintaining meat quality and the producers' bottom line. Varying levels of Zn supplementation may explain the inconsistency of results across studies.

The statistical model for the follow-up analysis was constructed using step-wise procedures of SAS. The lack of interaction between ZnAA and RAC in the primary analysis provided necessary justification for the elimination RAC from the second analysis. This allowed further evaluation of supplementation level of ZnAA, independent of RAC supplementation. In the present study, HIGH had less than 90 ppm ZnAA with an average of 74 ppm of ZnAA, and the pre-RAC period was not evaluated independently. Results of the present analysis confirm the hypothesis that increasing levels of ZnAA supplementation to finishing cattle improves overall performance. Specifically, final BW increased linearly with increasing concentrations of ZnAA. Cattle fed the highest level of ZnAA (average 74 ppm supplemental ZnAA) weighing 3.4 kg more than control (0 ppm supplemental ZnAA). Studies included in the current analysis have an average finishing period of 161 d and demonstrated greater final BW with ZnAA

supplementation supported by the linear increase in ADG with HIGH gaining 0.03 kg/d more than CON. These data indicate that time on feed plays a significant role in ZnAA supplementation. In a similar project, from d 0 to d 86 (pre-RAC period), live d 86 BW and ADG tended to respond in a quadratic manner to increasing levels of ZnAA, with cattle receiving 60 ppm ZnAA having an advantage of 17 kg of BW and 0.18 kg ADG compared to cattle receiving no supplemental ZnAA. In a different study, weights decreased as Zn increased from 60 to 90 ppm ZnAA; however, no differences were observed in ADG or final BW between varying supplementation levels from d 86 to d 116 in cattle not receiving ractopamine and (Genther-Schroeder et al., 2016a). Comparatively, work by Genther-Schroeder et al. (2018), investigated supplementation levels from 0-150 ppm and reported no effect of ZnAA level on final BW or ADG over the 79 d feeding period. Spears and Kegley (2002) supplemented Zn at 25 ppm with percentages of 0, 10, or 15 % Zn-oxide replaced with Zn-proteinate. Regardless of source, Zn supplementation improved ADG, independent of dose. However, the inclusion of Zn proteinate tended to improve ADG in the finishing phase over Zn oxide.

DMI and G:F were not significantly affected by supplementation level of ZnAA supplementation. Previous studies evaluating supplemental Zn in multiple forms (propteinate, sulfate, oxide, AA complex) and at various levels have also reported no difference in DMI or G:F (Spears and Kegley, 2002; Nunnary et al., 2007; Edenburn et al., 2016; Van Bibber-Kruegar et al., 2017; Kegley, 2002; Van Bibber-Kruegar et al., 2017; Genther-Schroeder et al., 2018). Alternatively, changes in DMI were observed by Malcolm-Callis et al. (2000) where DMI linearly decreased with increasing Zn sulfate supplementation. Zn supplementation was supplied to steers at 20, 100, and 200 mg/kg (Malcom-Callis et al., 2000), which was greater than the 74 ppm ZnAA. In the current study, supplementation was at a lower concentration; with average

ZnAA supplementation in the HIGH was 74 ppm ZnAA. Genther-Schroeder et al. (2016a) evaluated ZnAA supplementation at 30, 60, and 90 ppm reported a tendency for a quadratic effect of added ZnAA in the pre-ractopamine feeding period (d 0 to d 86) on G:F. The current study included trials with supplementation rates of ZnAA from 30 to 103 ppm and no adverse impact on DMI or G:F was observed. The studies where differences were observed supplemented Zn at rates of 100 and 200 mg/kg, which is substantially higher than the levels included in the current analysis.

Carcass adjusted gain, carcass adjusted ADG, and carcass adjusted G:F were all affected by level of ZnAA supplementation. Futhermore, an increase in HCW of approximately 2.9 kg for LOW and HIGH was observed compared to CON cattle in the present analysis. Previous work has shown ZnAA to have a similar linear effect on HCW within RAC supplemented steers (Genther-Schroeder et al., 2016a, 2016b). Increases in skeletal muscle may be associated with the action of matrix metalloproteinases (**MMP**). Formation, remodeling, and degradation of extracellular matrix components are largely mediated by MMPs, a family of degradative enzymes (Carmeli et al., 2004). Synthesis of MMPs is regulated by cytokine and growth factor production (Milner et al., 2006), and subsequent activation of most MMP requires Zn. Within skeletal muscle, degradation of extracellular matrix components allows satellite cell migration and differentiation (Chen and Li, 2009; Wang et al., 2009). As a result, bioavailable Zn, may allow for the upregulation of MMP and satellite cell differentiation to increase skeletal muscle accretion.

The present study showed an effect of ZnAA concentration on back fat thickness with values increasing from LOW to HIGH supplementation. Furthermore, KPH increased with ZnAA inclusion; however marbling scores were not influenced by ZnAA supplementation.

Supplementing zinc methionine at 31 ppm increased marbling score and tended to increase back fat thickness in steers, while supplemental Zn oxide resulted in no differences in carcass traits (Greene et al. 1988). In contrast, when supplemented as an oxide and two forms of proteinate at 25 ppm, Zn supplementation increased quality grade and marbling score and tended to increase BFT, regardless of source (Spears and Kegley, 2002). Fat deposition was also altered in a study performed by Malcolm-Callis et al. (2010) where Zn sulfate supplementation at 20, 100, and 200 ppm of Zn resulted in a quadratic increase in backfat; however, similar to the current study, there was no effect on marbling. Together, these data raise questions on the mechanistic action of Zn on lipogenic activity in beef cattle.

Recognition of Zn's role in lipid metabolism continues to become more evident with continued investigations. Investigation has demonstrated the addition of Zn augments the conversion of glucose to lipids through insulin stimulation in mice and rats (Shisheva et al., 1992, Chen et al., 1996). Furthermore, Oh and Choi (2004) showed lipogenic activity of Zn in bovine intramuscular adipocytes through suppression of nitric oxide (**NO**). In addition to NO depression, regardless of Zn source (Zn chloride and/or Zn sulfate), Glycerol-3-phosphate dehydrogenase (**GPDH**) activity analyzed as an index of adipocyte differentiation, increased with increasing supplementation and *peroxisome proliferator-activated receptor gamma 2* (***PPARγ2***) gene expression increased 10 d after differentiation induction (Oh and Choi, 2004). The Zn finger protein (Zfp423) also plays a key role in initiating adipogenic differentiation in bovine stromal vascular cells, and is a proposed target for enhancing marbling in beef cattle (Huang et al. 2012). Alterations in BFT and KPH suggest ZnAA alters fat metabolism in the present study; however, its lack of influence on marbling shows that ZnAA may not be acting through Zfp423.

Significant economic impact results from increased prevalence of liver abscesses in cattle (Reinhardt and Hubbert, 2014). Presence of liver abscesses can reduce ADG 14% and increase G:F as much as 13% (Brink et al., 1990). In the present study, the combination of ZnAA and RAC reduced total liver abscess incidence and those of lowest severity (A-). Both RAC and ZnAA independently reduced total LA occurrence, A- and A+ occurrence; however, they worked synergistically to further reduce incidence of LA. Previous work indicates increased Zn in pig diets reduces ileal mucosal inflammation via reduced expression of IL-β while RAC- increased ileal mucosal inflammation (Paulk et al., 2015). Increased inflammation in response to RAC supplementation may be due to increases in acute phase proteins, leukocytes, and serum IL-8 concentrations (Horodagoda et al., 1999). Previous data suggests β-agonist reduce or suppress immune responses by reducing pro-inflammatory cytokine production (Izeboud et al., 1999).

Supplemental Zn may have the ability to improve epithelial cell health under increased inflammatory conditions caused by high-grain diets. Being the first line of defense, the gastrointestinal barrier is continually exposed to environmental or luminal challenges, thus turnover in epithelial cells is high. Rapid fermentation causes decreased pH and creates an acidic rumen environment in fed cattle, which contributes to impairment of barrier function (Aschenbach et al., 2011). Micronutrients, such as Zn, enhance mucosal barrier integrity. In human intestinal epithelial cells, Zn alters tight junction composition and positively modifies barrier function (Wang et al., 2013). Furthermore, supplemental ZnAA helps alleviate negative effects of heat stress on intestinal integrity in pigs (Pearce et al., 2015; Fernandez et al. 2013). Moreover, the inclusion of organic trace minerals (including ZnAA) led to static or reduced prevalence of digital dermatitis lesions in feedlot (Kulow et al., 2017) and dairy (Gomez et al., 2014) cattle. Although these studies supplemented multiple organic minerals and the exact

mechanism for this reduction has yet to be fully elucidated, Zn's role in maintenance of skin integrity, stabilization of membranes and activation of the cell-mediated immune system provide a potential explanation (Miller et al., 1988). Interestingly, level of ZnAA concentration resulted in quadratic effect where higher levels of supplementation may negate the positive effects of ZnAA on liver abscess incidence. Developing a clear understanding on the relationship between full body inflammation and liver abscess occurrence would be beneficial in understanding the mechanism by which ZnAA and RAC act synergistically to reduce LA. Overall, cattle experiencing higher levels of inflammation would likely have higher incidence of LA; therefore, reduction in full body inflammation may prevent cattle from developing LA.

Conclusion

This pooled analysis suggests that inclusion of ZnAA and RAC separately or in combination in finishing rations results in improvement of live and carcass-adjusted performance. Furthermore, feeding ZnAA and RAC in combination decreased liver abcesses. The addition of ZnAA increased BW, ADG, and HCW without influencing intake.Both HIGH and LOW ZnAA supplementation rates resulted in incremental improvements of performance response; however, liver abscess presence was lowest in the low supplementation group. Due to variability in rates of supplementation across locations, optimal concentrations were not predicted; however, adding ZnAA, with or without ractopamine, to the finishing programs may benefit producers through increases in HCW and decreased liver condemnation.

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CHAPTER V

EVALUATION OF DIETARY TRACE MINERAL SUPPLEMENTATION IN YOUNG HORSES CHALLENGED WITH LIPOPOLYSACCHARIDE

SYNOPSIS

Sixteen weanling Quarter Horses (255±22 kg) were utilized in a 56-d trial to evaluate the effects of trace mineral (TM) source on intra-articular inflammation following a single acute inflammatory insult. Horses were stratified by age, sex, and BW and then randomly assigned to TM source; concentrate formulated with Zn, Mn, Cu, and Co as inorganic sources (CON; n=8) or complexed TMs (CTM; n=8). Added TM were at iso-levels across treatments and intakes met or exceeded NRC requirements. Horses were offered 1.75% BW (as-fed) of treatment concentrate and 0.75% BW (as-fed) coastal Bermudagrass hay. Growth measurements were collected on d 0, 28, and 56 and plasma was collected bi-weekly for determination of Mn, Cu, Zn, and Co concentrations. On d 42, carpal joints were randomly assigned to receive injections of 0.5 ng lipopolysaccharide (LPS) or sterile lactated Ringer's solution (LRS; contralateral control). Synovial fluid was collected at pre-injection h 0, and 6, 12, 24, 168, and 336 h post-injection and analyzed for TM concentration, prostaglandin E_2 (PGE_2), carboxypeptide of type II collagen (CPII), collagenase cleavage neopeptide (C2C) and aggrecan chondroitin sulfate 846 epitope (CS-846). Data were analyzed using the MIXED procedure of SAS. Challenge results showed a TM source × LPS × h effect for synovial fluid Co, Cu and Se ($P < 0.05$); concentrations peaked at h 6 and decreased to h 168 in both CON and CTM-LPS knees. A delayed peak was observed

at h 12 for CTM-LRS. Peak synovial fluid Cu and Se concentrations were higher in LPS knees and Co was highest in CTM-LPS. A TM source × h interaction was observed for Zn concentrations ($P < 0.05$). Zinc concentration peaked at h 6 in CON and h 12 for CTM before decreasing through h 168. At h 168, CON had lower Zn concentration than CTM. A LPS × h interaction was observed for Mn ($P < 0.01$); synovial Mn concentration peaked at h 6 in LPS knees compared to h 24 in LRS. Synovial PGE_2, C2C, CPII, and CS846 concentrations were greater with LPS ($P \leq 0.01$), and C2C was greater ($P < 0.01$) in CTM compared to CON. Concentrations of CPII and PGE_2 were unaffected by diet. A TM source × h × LPS interaction was observed for CS846 ($P = 0.02$). Concentrations of CS-846 in CTM peaked at 12 h while CON peaked at a lower concentration at 24 h ($P < 0.05$). Data indicate sufficient intake of a complexed TM source may support a robust but transient inflammatory response following an intra-articular LPS challenge in growing horses.

INTRODUCTION

Homeostatic maintenance of articulating joints requires Cu, Mn, and Zn for turnover of collagen fibrils and they contribute to molecules contained within the extracellular matrix (Hostetler et al., 2003; Richards et al., 2010). The role of dietary TM in equine articulating joints has not been determined. However, information gained from other species such as dairy cattle and chickens, has indicated metal amino acid complexed trace minerals (CTM) provide a more biologically available source of TM. In hens challenged with systemic lipopolysaccharide (**LPS**), and supplemented with a Zn amino acid complex, serum interleukin-1β concentrations increased beginning at 3 h post induction are returned to baseline by 12 h post challenge when compared to hens receiving Zn sulfate (Cheng and Guo, 2004).

Initially, the inflammatory process is considered to be healing; however, sustained levels contribute to the development of joint disease in horses. Increased bioavailability and utilization of CTM may allow for greater incorporation of TM into articular cartilage and a more rapid achievement in homeostasis following endotoxin injection. Utilizing an intra-articular LPS challenge to induce localized inflammation and cartilage turnover in young horses, provides the ability to evaluate the impact of TM source of cartilage metabolism, joint inflammation, and measure synovial fluid TM concentrations post induction (Leatherwood et al., 2016).

Biomarkers relative to inflammation and cartilage turnover may provide valuable insight to the efficacy of CTM in reduction of joint inflammation. Synovial prostaglandin E_2 (**PGE_2**) concentration is indicative of progression of naturally occurring osteoarthritis (Bertone et al., 2001) and increases in response to intra-articular LPS. The resulting inflammation influences collagen metabolism and aggrecan synthesis by increasing catabolic collagenase cleavage neopeptide (**C2C**), anabolic carboxypropetide of type II collagen (**CPII**), and chondroitin sulfate

846 epitope (**CS-846**; de Grauw et al., 2009; Lucia et al., 2013). Therefore, objectives of this study were to compare the effects of TM source (organic versus inorganic) on joint inflammation, cartilage metabolism, and synovial fluid TM concentrations in response to an intra-articular LPS challenge in young horses.

MATERIALS AND METHODS

All care, handling, and procedures for experiment approved by the Texas A&M University Institutional Animal Care and Use Committee.

Horses and Treatments

Sixteen weanling Quarter Horses (initial BW of 255 ± 22 kg BW; n=9 colts; n=7 fillies) were used in a complete randomized design. Prior to the initiation of dietary treatments, mares and foals were maintained on a custom-pelleted concentrate that contained inorganic mineral sources (Producer's Cooperative Association, Bryan, TX). The concentrate was provided as a creep feed to foals beginning at 90 d of age. Foals were weaned at 150 ± 11 d of age and maintained on the same concentrate until the initiation of the study (233 ± 20 d of age).

Radiographs (lateral, flexed lateral and cranial-caudal views) of both radial carpal joints were performed at the Texas A&M University Large Animal Hospital (College Station, TX) prior to initiation of study. All horses considered to be radiologically normal by a licensed veterinarian were stratified by age, sex, BW, and BCS, and randomly assigned to dietary treatment. Treatment diets (Table 1) consisted of isocaloric, isonitrogenous pelleted concentrate formulated with either inorganic (**CON**; 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$ and $CoCO_3$; n = 8) or TM complexes (**CTM**; zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate; n = 8). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both

pelleted treatment diets, and daily mineral intakes met or exceeded 2007 NRC minimum requirements. All personnel involved in performing the study were blinded to dietary treatment. Composited samples of concentrate and hay were analyzed by commercial analysis for nutrient content and trace mineral concentrations (Insert analysis name, City, State).

Weanlings received their respective pelleted concentrate at 1.75% BW/d (as-fed) and all horses received 0.75% BW/d (as-fed) of coastal bermudagrass (*Cynodon dactylon*) hay: divided evenly between two feedings at 0600 and 1800. Horses were fed individually and maintained in 3 × 3 m stalls with *ad libitum* access to water. Intakes and orts were obtained and measured daily. Every 7 d, BW was obtained utilizing a calibrated digital platform scale (Bastrop Scale Inc., Bastrop, TX) and diets adjusted accordingly. All horses were allowed 8 h of free exercise in a dry lot (58.5 × 79.2 m) daily.

Growth and Performance Characteristics

Body condition score, rump fat (**RF**), wither height (**WH**), hip height (**HH**), body length (**BL**), and heart girth circumference (**HG**) were taken on d 0, 28, and 56 by the same three independent observers. Ultrasonic images were also captured of the *longissimus dorsi* muscle (**LM**) were captured by a certified technician (Designer Genes Technologies, Inc., Harrison, AR) to determine muscle area, back fat thickness (**BFT**), and intramuscular fat (**IMF**) deposition. The transducer was placed to obtain a cross-sectional image taken between the 13th and 14th as well as the 17th and 18th ribs. Subcutaneous fat thickness was measured at three-fourths the distance from the medial end of the LM; four independent images were collected laterally across the 17th and 18th ribs to estimate IMF within the LM. Four independent images were necessary to follow Annual Proficiency Testing and Certification standard format for data submission. Proper contact between transducer and horse was insured by fitting the transducer with a PIA contour pad

(Animal Ultrasound Services, Ithaca, NY) designed to conform to the curvature of the horse's back. In addition, corn oil was applied to promote acoustical contact between animal and transducer (Perkins et al., 1992). An independent laboratory interpreted all images; personnel were blinded to treatment (Designer Genes Technologies, Inc., Harrison, AR).

Table 5. 1 Nutrient composition of concentrates and hay (DM basis) fed to weanling horses.

	Concentrate		
	CON[1]	CTM[2]	Forage[3]
Dry Matter, %	89.5	91.3	89.16
Nutrient, % DM			
CP	19.3	19.6	12.51
ADF	25.9	23.7	35.51
NDF	40.0	36.1	65.99
Fat	7.7	7.9	2.26
Ca	0.94	1.26	0.32
P	0.92	1.16	0.29
Trace Minerals[4], ppm			
Zn	197.9	184.7	17.9
Cu	51.9	57.9	5.8
Mn	216.0	226.9	192.2
Co	7.1	9.5	<1.0

[1]Inorganic mineral sources ($CuSO_4$, $ZnSO_4$, and $MnSO_4$ and $CoCO_3$) – supplied for 56 d
[2]7g of 4-Plex C® (Zinpro Performance Minerals, Eden Prairie, MN) replaced a portion of inorganic added trace mineral – supplied for 56 d
[3]Costal bermudagrass, *cynodon dactylon*
[4]Total mineral content

Incorporation into Plasma and Synovial Fluid

Trace Mineral Analysis

Plasma samples for TM analysis were collected every 14 d into a 6 mL trace element K_2 EDTA, 10.8 mg, additive tube (BD Vacutainer, Franklin Lakes, NJ) prior to the morning feeding. Samples were immediately placed on ice until centrifugation at 2,000 *g* for 10 min at 4°C. After centrifugation, plasma was aliquoted into 1.5 mL micro-centrifuge tubes and stored at -80^{o}C until analysis. A certified veterinarian from the Texas A&M University Large Animal Clinic performed carpal arthrocentesis on both radial carpal joints on d 0. Horses were sedated using xylazine HCl, administered intravenously at recommended dosages. The carpal joint was aseptically aspirated using a location medial to the extensor carpi radialis tendon in the palpable depression between the radial carpal bone and the third carpal bone, to a depth of approximately 12.7 mm to avoid unnecessary contact with articular cartilage (McIlwraith and Trotter, 1996). Pooled synovial fluid (1 to 4 mL) was transferred into sterile non-additive tubes (BD Vacutainer, Franklin Lakes, NJ) and were immediately placed on ice and stored at --80^{o}C until laboratory analysis.

Intra-articular LPS Challenge

On d 42 of the study, all horses were subjected to an intra-articular LPS challenge. One radiocarpal joint was randomly selected within horse for injection with LPS while the other served as a contralateral control (injection of sterile lactated Ringer's solution; **LRS**). The use of an LRS joint was based on previous data in our laboratory that suggested repeated arthrocentesis influenced local inflammatory status in horses regardless of treatment (LRS or LPS) as evidenced by an alteration in circulating leukocytes, monocytes, or platelets (Hunt et al., 2018).

At pre-injection hour (PIH 0), the carpal joint was aseptically prepared for arthrocentesis and horses were sedated as previously described. Purified LPS derived from *Escherichia coli* O55:B5 (Sigma Aldrich, St. Louis, MO) was reconstituted and diluted in sterile lactated Ringer's solution; individual doses were 0.8 mL with a final concentration of 0.5 ng/mL. Dosage of LPS was based on previous work in our laboratory (Lucia et al., 2013; Leatherwood et al., 2016; Kahn et al. 2017). The LPS solution was inserted aseptically into the randomly selected carpal joint and LRS joints were injected with 0.8 mL of sterile lactated Ringer's solution after the withdrawal of the PIH 0 sample. Synovial fluid samples (1 to 4 mL) were obtained at PIH 0 and 6, 12, 24, 168, and 336 h post-injection). All personnel were blinded to injection.

All synovial samples were collected and transferred to sterile non-additive tubes (BD Vacutainer Blood Serum Collection Tubes; Becton-Dickinson and Company, Franklin Lakes NJ) and immediately placed on ice until aliquot into 1.5 mL micro-centrifuge tubes. Aliquots were stored at -80^{o}C until later analysis of C2C, CPII, CS846, PGE_2, and TM concentration. All horses were monitored for signs of anaphylaxis over the initial 24 h post-injection. Rectal temperature (**RT**; °C), heart rate (**HR**; beats/min), respiratory rate (**RR**; breaths/min) were recorded prior to arthrocentesis at PIH 0 and at 6, 12, and 24 h post-injection. Carpal circumference (cm) was measured at the level of the accessory carpal bone with a soft tape measure that was performed by a single individual to maintain consistency. .

Sample Analysis

Plasma and synovial fluid samples were sent to the Michigan State Diagnostics Laboratory (Lansing, MI) for TM analysis to establish TM composition. In brief, samples were diluted 20-fold with a solution containing 0.5% EDTA and Triton X-100, 1% ammonium hydroxide, 2% propanol and 20 ppb of scandium, rhodium, indium and bismuth as internal

standards. The inductively coupled plasma mass spectrometer (ICP/MS) was tuned to yield a minimum of 7500 cps sensitivity for 1 ppb yttrium (mass 89), less than 1.0% oxide level as determined by the 156/140 mass ratio and less than 2.0% double charged ions as determined by the 70/140 mass ratio (Wahlen et al., 2005). Elemental concentrations were calibrated using a 4-point linear curve of the analyte-internal standard response ratio. Standards were from Inorganic Ventures (Christiansburg, VA). In-house serum pools were used as controls.

Synovial fluid concentrations CPII, C2C, and CS-846 were measured using commercially available ELISA kits (IBEX Pharmaceuticals Inc., Montreal, QC, Canada) previously validated in horses (de Grauw et al. 2009; Lucia et al., 2013). Synovial fluid samples were analyzed in duplicate and standards were prepared according to manufacturer's recommendations with samples prepared at a 1:4 dilution for both CPII and C2C. Sample dilutions for CS-846 ranged from 1:50 to 1:1000 depending on time post-injection, to remain within detectable limits of the ELISA. . Dilutions were made with calibrator diluents provided by the kit prior to beginning the assay. Mean detectable concentrations for CPII, C2C, and CS846 were 50 ng/mL, 10 ng/mL, and 20 ng/mL, respectively. Shifts in cartilage metabolism were evaluated by the ratio of CPII to C2C with individual ratios calculated for each horse.

Synovial fluid samples were analyzed in duplicate for concentrations of PGE_2 utilizing an enzyme-linked immunoassay (R&D Systems, Minneapolis, MN), previously validated in horses (Bertone et al., 2001; de Grauw et al., 2006; Lucia et al., 2013). Samples intended for PGE_2 analysis were diluted from 1:1 to 1:10 depending on time post-injection to remain within detectable limits of the ELISA; dilutions were prepared using the calibrator diluent provided by the kit with a mean detectable dose of PGE_2 of 39 pg/mL.

Final concentrations of all markers were read using a microplate reader (Synergy H1,

Biotek, Winooski, VT) with optical density set at 450 nm. Coefficient of variation was ≤ 15% for CPII, C2C, and PGE_2 assays and ≤ 20% for CS846.

Statistical Analysis

Growth data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model contained an effect of TM source and used a random variable of horse within treatment. Plasma mineral concentration utilized the same model with an additional fixed effect of time (d) and a TM source × d interaction.

In response to the LPS challenge, statistical analysis of synovial fluid biomarker and TM concentration were analyzed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). The model contained effects of TM source, time (h), injection type (LPS), and their respective interactions. This model included a random effect of horse within treatment and h as a repeated variable. The covariate structure was utilized to specify a random effect for differences between animals within treatment, creating a correlation structure within animals that decreases with increasing amount of time between measurements (Littell et al., 1998). The model for synovial fluid TM analysis also included h 0 as a covariate. Post hoc comparison of TM source and individual time points was conducted using a paired t test. Significant differences were declared as $P \leq 0.05$ and $P \leq 0.15$ was considered a trend toward significance. Plots of residual variation were used to evaluate normal distributions for traits based on continuous variables. One biomarker (CPII) exhibited non-normal data; therefore, the data was log transformed for normalization. Outliers were identified using box plots of the residuals and removed if greater than three standard deviations from the mean. Data are reported as mean ± SEM.

RESULTS

Target intakes of 1.75% BW (as-fed) from concentrate and 0.75% BW (as-fed) from hay were achieved as all horses readily consumed their respective diets with no significant refusals across treatment groups over the 56 d trial. Weanling horses consumed energy and associated nutrients to meet or exceed requirements (NRC, 2007). Total dry matter intake throughout the 56-d study did not differ between TM source ($P = 0.89$), with intakes reported at 5.74 and 5.71 ± 0.15 for CON and CTM, respectively. Individually, concentrate and forage intakes were not different between TM sources ($P = 0.54$ and $P = 0.47$, respectively). Similarly, TM source did not affect ($P = 0.78$) final BW (285.8 and 288.8 ± 5.57kg), and both TM sources averaged 32 ± 1.96 kg gain during the 56 d as expected ($P = 0.90$), validating the diets were isocaloric and isonitrogenous.

No effect of TM source was observed in synovial biomarker concentrations during the pre-LPS dietary adaptation period; however, a TM source × day interaction was observed for CS846 ($P = 0.05$; Table 5.2). CS846 concentration increased from d 0 to d 42 in CTM horses (19,701 ± 3411, 29,717 ± 2897 ng/mL, respectively); while remaining similar from d 0 to d 42 in CON horses. An effect of day was also observed for CPII:C2C, CS846, and PGE_2. The ratio, CPII: C2C increased from d 0 to d 42 ($P = 0.04$). In contrast, PGE_2 decreased over time, regardless of TM source ($P < 0.01$).

Table 5. 2 Effect of supplemental trace mineral source on cartilage biomarkers and inflammatory markers within the synovial fluid during the dietary adaptation period.

	Dietary Treatments							
	CON[1]		CTM[2]			*P*-Value		
Synovial Fluid Variable	d 0	d 42	d 0	d 42	SEM	Mineral	d	TM source × Time
Biomarkers								
CPII[3], ng/mL	764.65	967.17	713.96	988.23	231.33	0.96	0.13	0.82
C2C[4], ng/mL	270.93	260.86	276.66	297.89	29.23	0.48	0.80	0.49
CPII:C2C	2.76	3.82	2.54	3.90	0.91	0.95	0.04	0.80
CS846[5], ng/mL	23820[a]	24803[a]	19701[a]	29717[b]	3411	0.92	0.02	0.05
PGE_2[6], pg/mL	596.85	336.37	750.68	423.02	87.90	0.13	<0.01	0.64

[1]CON: 100% inorganic mineral sources ($CuSO_4$, $ZnSO_4$, and $MnSO_4$ and $CoCO_3$), supplied for 56 d
[2]CTM: 7g of 4-Plex C® (Zinpro Corporation, Eden Prairie, MN) replaced a portion of inorganic added trace mineral, supplied for 56 d
[3]Carboxypropetide of type II collagen
[4]Collagenase cleavage neopeptide
[5]Chondroitin sulfate 846 epitope
[6]Synovial prostaglandin E_2

Growth Metrics

Growth measurements including WH, HH, HG, and BL were not significantly affected ($P \geq 0.56$) by TM source (Table 3). Similarly, no significant differences between TM sources were observed for final IMF, BFT, or rump fat ($P > 0.10$; Table 3). Changes recorded for these measurements at d 28 and d 56 were also not affected by TM source ($P > 0.10$). However, final BCS tended to be lower ($P = 0.06$; Table 3) for horses fed CTM (6.08 ± 0.16) when compared to CON (6.55 ± 0.16).

Final LM area at both measured locations was not affected by TM source ($P \geq 0.60$). No effects of TM source were observed for changes in area at the 17th and 18th rib from d-0 to d-28; all horses showed numeric increases in area from d 0 to d 28 of 0.32cm^2 (CON) and 0.72cm^2 (CTM). Overall change in area at the 17th and 18th rib was unaffected by TM source, but as expected, a positive change in area over the 56-d trial was observed in CON (+1.72 cm^2) and CTM treatments (+1.97cm^2)

Table 5. 3 Effect of supplemental trace mineral source on growth parameters and composition in young horses.

	Dietary Treatments			
Variable	CON[1]	CTM[2]	SEM	*P*-Value
BW, kg				
d 0	256.4	253.7	7.98	0.82
d 56	288.8	285.8	7.57	0.78
Wither Height, cm				
d 0	128.19	127.56	0.97	0.65
d 56	131.68	131.60	0.67	0.93
Hip Height, cm				
d 0	134.54	134.38	1.12	0.92
d 56	137.80	138.19	0.99	0.78
Heart Girth, cm				
d 0	143.25	141.67	1.97	0.56
d 56	149.52	148.13	1.82	0.60
Body Length, cm				
d 0	135.21	134.94	1.39	0.89
d 56	144.74	145.31	1.35	0.77
Body Condition Score				
d 0	5.65	5.52	0.16	0.58
d 56	6.55	6.08	0.16	0.06
Rump Fat[3]				
d 0	0.14	0.15	0.006	0.79
d 56	0.16	0.16	0.005	0.86
Intramuscular Fat, %				
d 0	3.62	3.65	0.17	0.91
d 56	3.64	3.48	0.17	0.52
Back fat thickness, 13^{th} & 14^{th} rib				
d 0	0.17	0.15	0.02	0.42
d 56	0.16	0.17	0.01	0.57
Back fat thickness, 17^{th} and 18^{th} rib				
d 0	0.14	0.14	0.01	0.74
d 56	0.14	0.14	0.01	0.81
LM area, cm^2, 13^{th} & 14^{th} rib				
d 0	10.57	10.27	0.45	0.65
d 56	11.75	12.00	0.59	0.77
LM area, cm^2,17th and 18^{th} rib				
d 0	12.01	11.63	0.52	0.61
d 56	13.73	13.60	0.28	0.60

[1]CON: 100% inorganic mineral sources ($CuSO_4$, $ZnSO_4$, and $MnSO_4$ and $CoCO_3$), supplied for 56 d

[2]CTM: 7g of 4-Plex C® (Zinpro Corporation, Eden Prairie, MN) replaced a portion of inorganic added trace mineral, supplied for 56 d
[3]Rump fat thickness measured by ultrasound (Westervelt et al., 1976)

Plasma Mineral Concentration

No significant interaction or main effects of TM source and d were observed for Mn ($P \geq 0.18$) with average concentrations of 1.73 and 1.62 ± 0.09 ng/mL for CTM and CON, respectively (data not shown). No interaction or effect of TM was observed for Cu ($P \geq 0.41$); however, an effect of d ($P < 0.01$) was present. Plasma Cu concentrations increased from d 0 to d 42 (1.07 to 1.22 ± 0.05 μg/mL; data not shown). A TM source × d interaction was present for Zn ($P = 0.02$; Fig. 5.1). Concentrations decreased in CON from d 0 to d 56. While Zn concentration increased in CTM from d 14 to d 28, before declining to levels similar to CON. A significant TM source × d interaction was observed for Co concentrations ($P < 0.01$; Fig. 5.2). Cobalt concentrations increased sharply between d 0 and 14 for all horses regardless of TM source; however, horses receiving CTM increased Co plasma concentrations more than CON, beginning at d 14 through d 56 of the study.

LPS Challenge

Clinical Assessment

During the LPS challenge, no TM source × h interactions were present for clinical parameters, including HR, RR, and RT ($P \geq 0.33$). Average beats per minute were 48.44 and 50.88 ± 1.58 ($P \geq 0.22$), for CON and CTM horses. Respiration rate was affected by h ($P < 0.01$), with an increase from PIH 0 (20.2 5± 1.58 breaths/min) to h 6 (23.5± 1.58 breaths/min), before decreasing at h 12 (15.50 ± 1.58 breaths/min) and h 24 (15.25 ± 1.58 breaths/min). An h effect was also observed with RT ($P = 0.01$). Rectal temperature increased from PIH 0 (38.03 ± 0.12 °C) to 38.40 ± 0.12 °C at h 6 before returning to baseline by h 24 (38.03 ± 0.12 °C). All

values remained within normal physiological limits throughout the 24 h period. An LPS × h interaction was observed for carpal circumference ($P < 0.01$), as the LPS injected knees rapidly increased and remained elevated to h 24, whereas the LRS knee slowly increased in circumference and returned to baseline by h 24. Carpal circumference increased over time ($P < 0.01$; data not shown), regardless of TM source.

Synovial Fluid Mineral Concentration

A TM source × LPS × h interaction was observed for Co ($P < 0.01$; Fig. 5.3). Cobalt concentration at h 6 was highest in CTM-LPS ($P < 0.01$). Additionally, at h 12, Co was higher in CTM-CON than concentrations of CON-LPS and CON-LRS ($P < 0.01$).

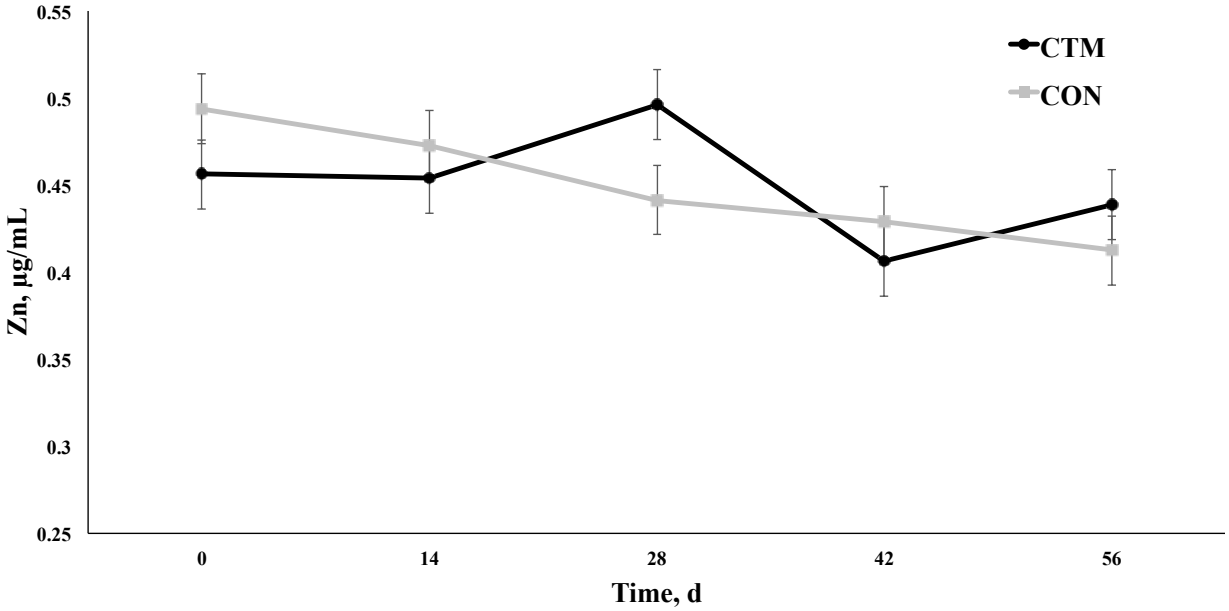
A TM source × LPS × h effect was observed for Cu ($P < 0.01$; Figure 5.4). Concentrations of Cu increased in both LPS and CON-LRS to peak concentrations by h 6 then decreased to h 168. Whereas concentration of Cu were lowest at h 6 in CTM-LRS ($P = 0.01$) before increasing more rapidly from h 6 to h 12, peaking at a lower Cu concentration than other knees before declining to h 168; concentrations at h 12 were similar to other knees. No differences were observed at h 168 or 336. Furthermore, TM source × LPS × h effect was also observed for Se ($P = 0.02$; Figure 5.5) with concentrations following the same basic pattern as Cu. Se concentrations were lowest in CTM-LRS at h 6 versus other knees ($P < 0.01$) before increasing to similar concentrations at h 12. Concentrations of Se then declined in all knees to 168, maintain similar concentrations to h 336.

No TM source × LPS × h interaction was present for Zn ($P = 0.21$; Figure 5.6); although, concentrations followed the same general pattern as Cu and Se. A TM source × h interaction was observed for Zn ($P = 0.02$). Zinc increased in concentration, peaking at h 6 in CON and h 12 for CTM before decreasing to h 168. At h 12, 24 and 168, CON had a lower concentration of Zn

than CTM ($P < 0.01$). An LPS × h interaction ($P < 0.01$) was observed for Zn; concentrations were higher in LPS knees at h 6 and 24 ($P \leq 0.01$) and tended to be higher at h 12 ($P = 0.06$).

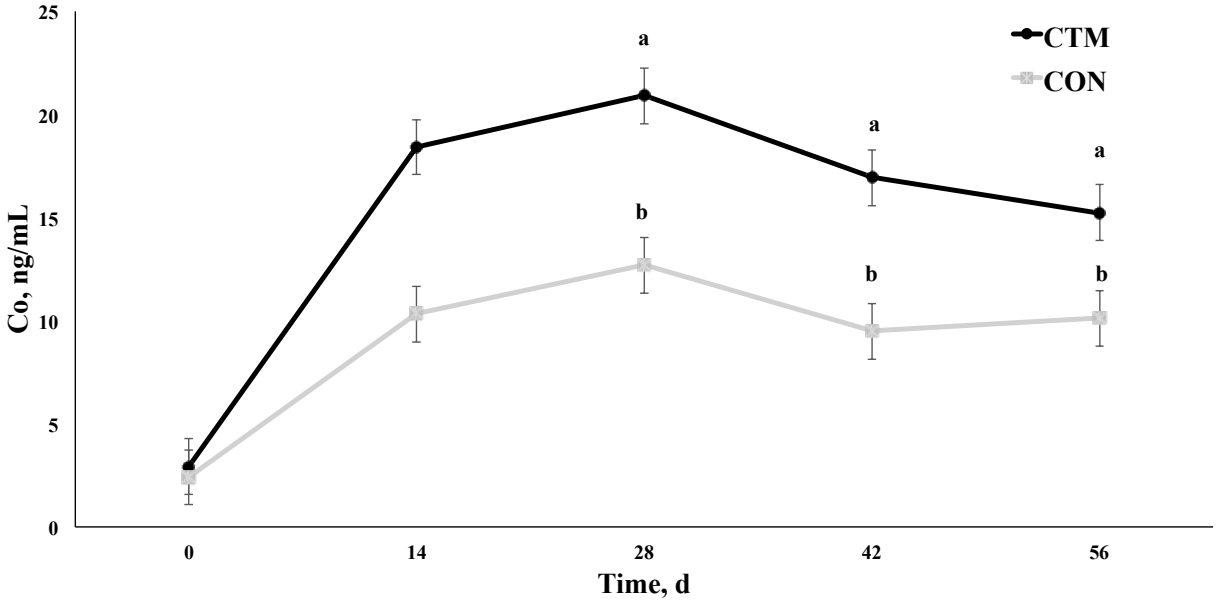
No TM source × h × LPS interaction ($P = 0.74$; Figure 5.7) was observed for Mn; however, an h × LPS interaction was present ($P < 0.01$). Manganese concentration increased in both LPS knees to h 6 in LPS knees versus LRS knees, which increased to a much higher peak at h 24 ($P < 0.01$) before decreasing to similar concentrations by h 168; concentrations of Mn was significantly higher in LRS knees than LPS at h 24 ($P < 0.01$). In response to LPS, Mn decreased ($P < 0.01$). Iron concentrations were only significantly affected by h ($P < 0.01$; data not shown).

Figure 5. 1 Mean plasma Zn concentrations over 56-day trial in weaning horses receiving a pelleted concentrate at 1.75% BW (as-fed) containing either 100% inorganic $ZnSO_4$, $CuSO_4$, and $MnSO_4$ and $CoCO_3$ (CON; *n* = 8) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (CTM; *n* = 8)[1]



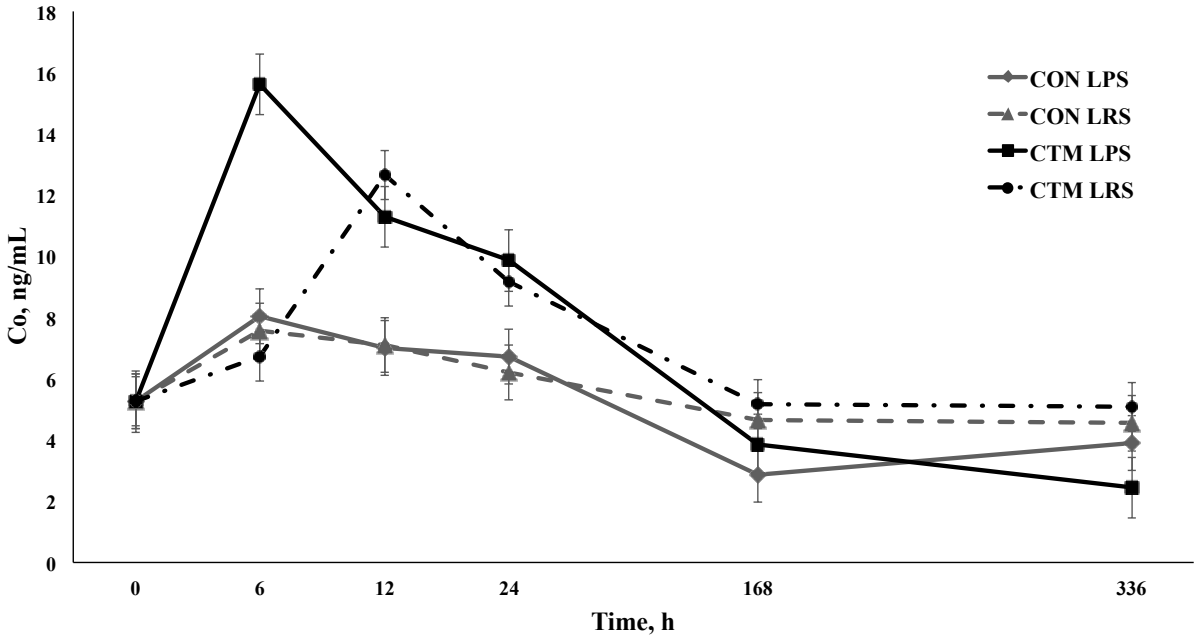
[1]Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Trace mineral source × d interaction ($P < 0.05$).

Figure 5. 2 Mean plasma Co concentrations over 56-day trial in weaning horses receiving a pelleted concentrate fed at 1.75% BW (as-fed) containing either 100% inorganic $ZnSO_4$, $CuSO_4$, and $MnSO_4$ and $CoCO_3$ (CON; *n* = 8) or trace mineral complexes Zn methionine, Mn methionine, Cu lysine, and Co glucoheptonate (CTM; *n* = 8) [1]



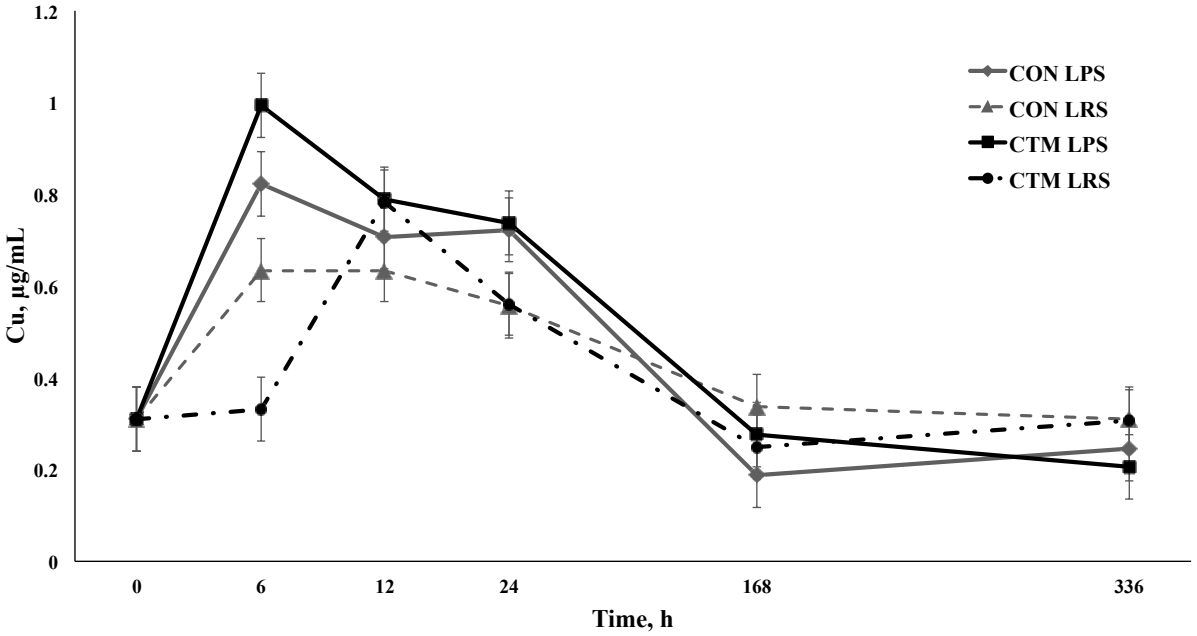
[1]Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Trace mineral source × d interaction ($P < 0.01$). [ab]Denotes differences between trace mineral sources at specific time points.

Figure 5. 3 Mean synovial fluid cobalt concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from Escherichia coli O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



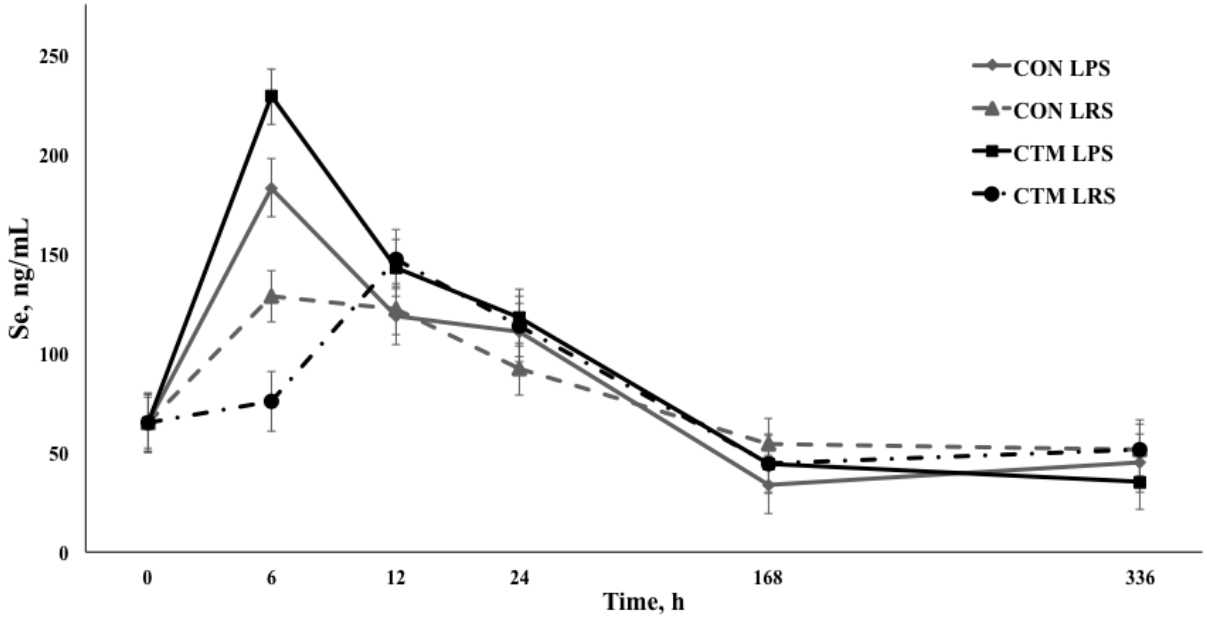
Dietary treatments consisted pelleted concentrates containing either 100% inorganic ZnSO4, CuSO4, and MnSO4 and CoCO3 (CON; n = 8) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (CTM; n = 8). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Cobalt concentrations: trace mineral source × LPS × h interaction ($P < 0.01$).

Figure 5. 4 Mean synovial fluid copper concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from Escherichia coli O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



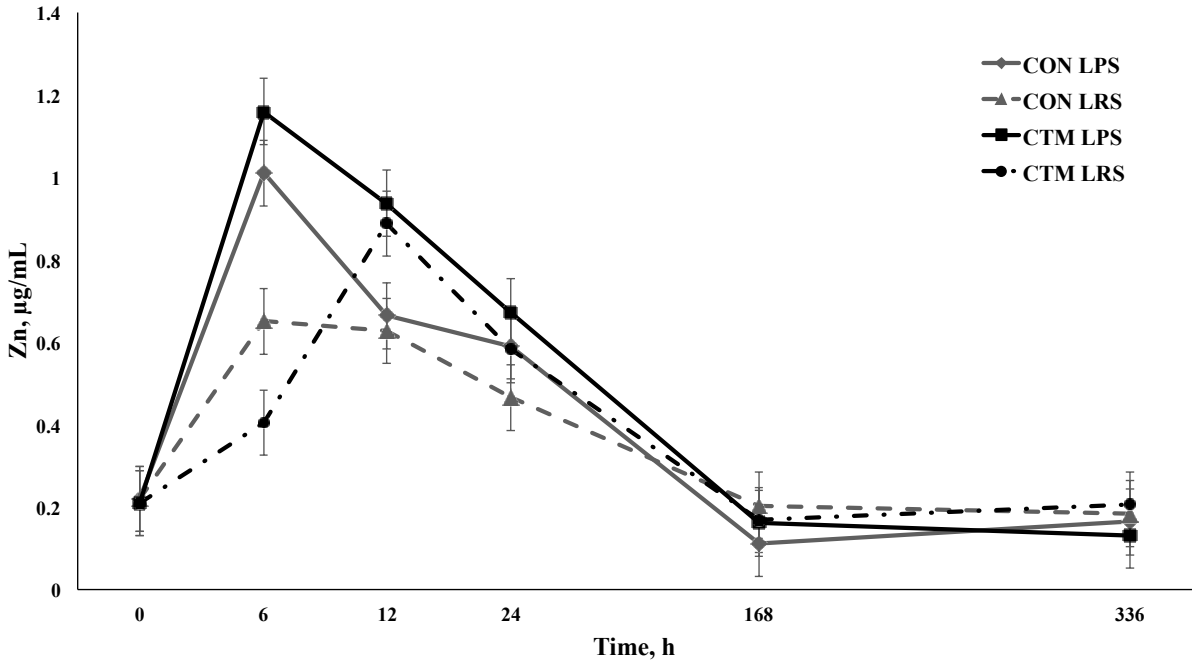
Dietary treatments consisted pelleted concentrates containing either 100% inorganic ZnSO4, CuSO4, and MnSO4 and CoCO3 (CON; n = 8) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (CTM; n = 8). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Copper concentrations: trace mineral source × LPS × h interaction (P = 0.01).

Figure 5. 5 Mean synovial fluid selenium concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from Escherichia coli O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



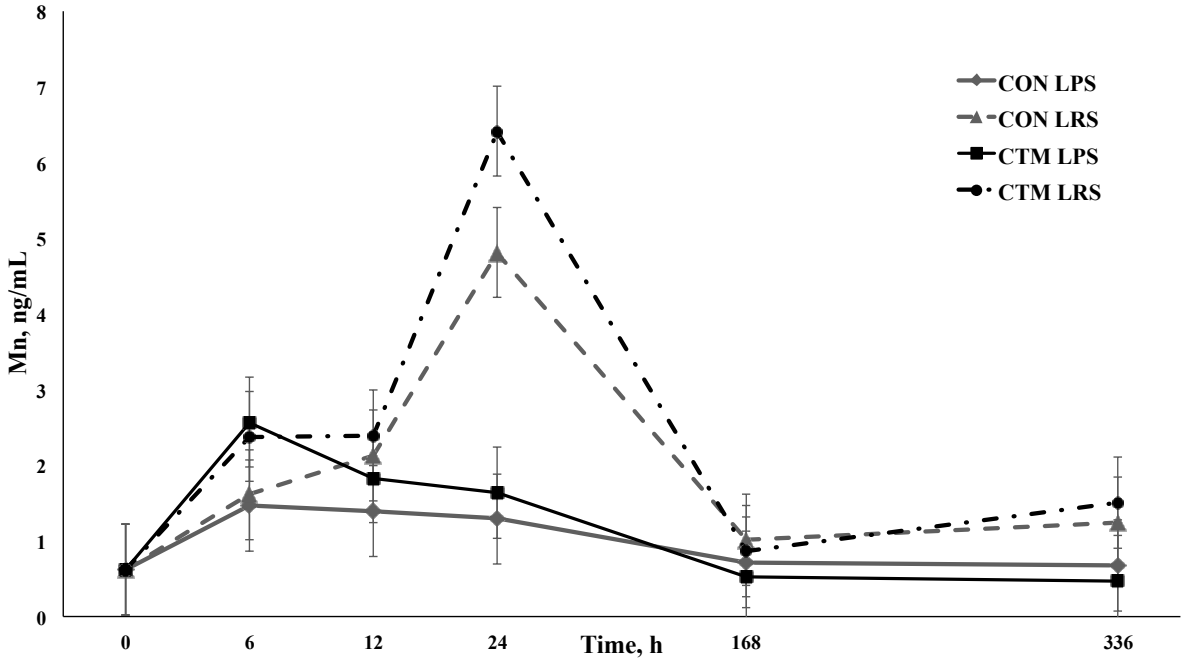
Dietary treatments consisted pelleted concentrates containing either 100% inorganic ZnSO4, CuSO4, and MnSO4 and CoCO3 (CON; n = 8) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (CTM; n = 8). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Selenium concentrations: trace mineral source × LPS × h interaction (P = 0.02).

Figure 5. 6 Mean synovial fluid zinc concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from Escherichia coli O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



Dietary treatments consisted pelleted concentrates containing either 100% inorganic ZnSO4, CuSO4, and MnSO4 and CoCO3 (CON; n = 8) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (CTM; n = 8). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Zinc concentrations: trace mineral source × LPS × h ($P = 0.21$), trace mineral source × h ($P < 0.05$), h × LPS ($P < 0.01$).

Figure 5. 7 Mean synovial fluid manganese concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from Escherichia coli O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



Dietary treatments consisted pelleted concentrates containing either 100% inorganic ZnSO4, CuSO4, and MnSO4 and CoCO3 (CON; n = 8) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (CTM; n = 8). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Manganese concentrations: trace mineral source × h × LPS interaction ($P = 0.74$), LPS × h interaction ($P < 0.01$), trace mineral source ($P = 0.35$).

Synovial Inflammation

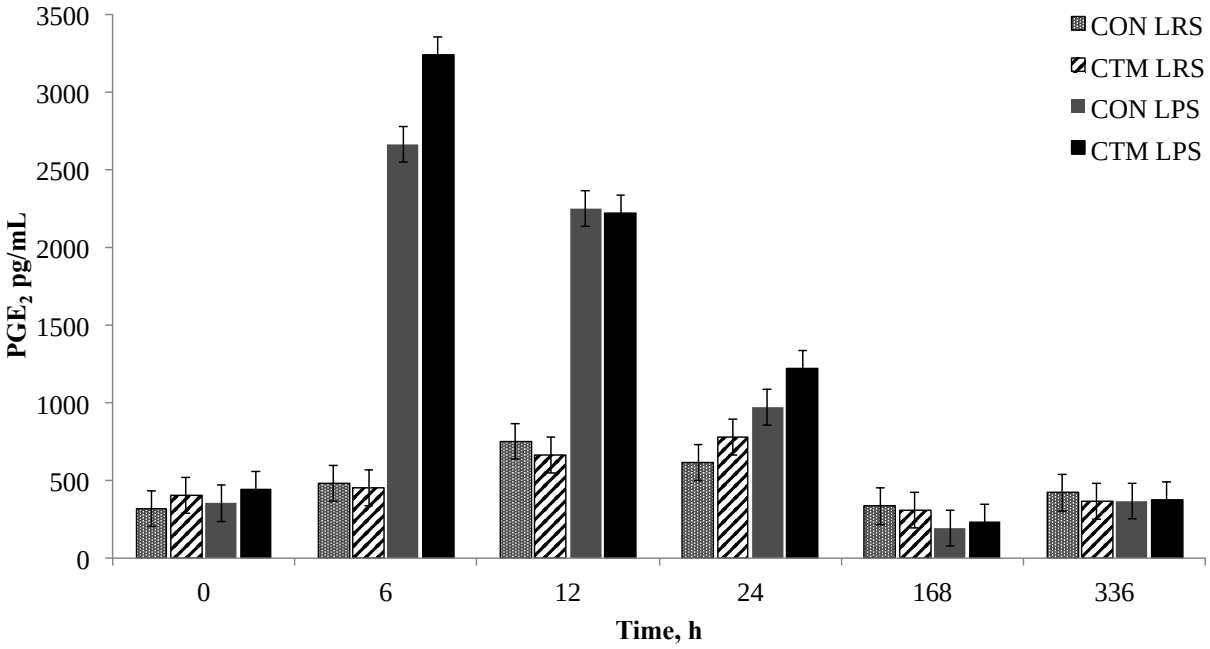
A LPS × h interaction ($P \leq 0.01$; Fig. 5.8) was observed for synovial PGE_2. Concentration of PGE_2 was greater in the LPS injected knee when compared to LRS at h 6, 12, and 24 ($P < 0.01$). Synovial PGE_2 was not affected by TM source ($P = 0.13$).

Cartilage Markers

A LPS × h interaction was present for synovial C2C ($P < 0.01$; Fig. 5.9). Concentrations in LPS knees were greater than C2C concentrations in LRS knees at h 6, 12, 24, and 168 ($P \leq 0.04$). A tendency for an interaction of TM source × LPS ($P = 0.09$) was observed with concentrations of C2C being greater in CTM-LPS compared to CON-LPS ($P < 0.01$). Synovial C2C concentrations increased ($P < 0.01$) in response to intra-articular LPS injection and an effect of h was observed ($P < 0.01$). An LPS × h interaction ($P \leq 0.01$; Fig. 5.10) was observed for anabolic CPII with LPS injection resulting in greater CPII concentrations at 6, 12 and 168 h ($P \leq 0.01$) compared to LRS. The CPII: C2C was not significantly affected by TM source ($P = 0.57$), LPS ($P = 0.11$), or h ($P = 0.19$), data not shown.

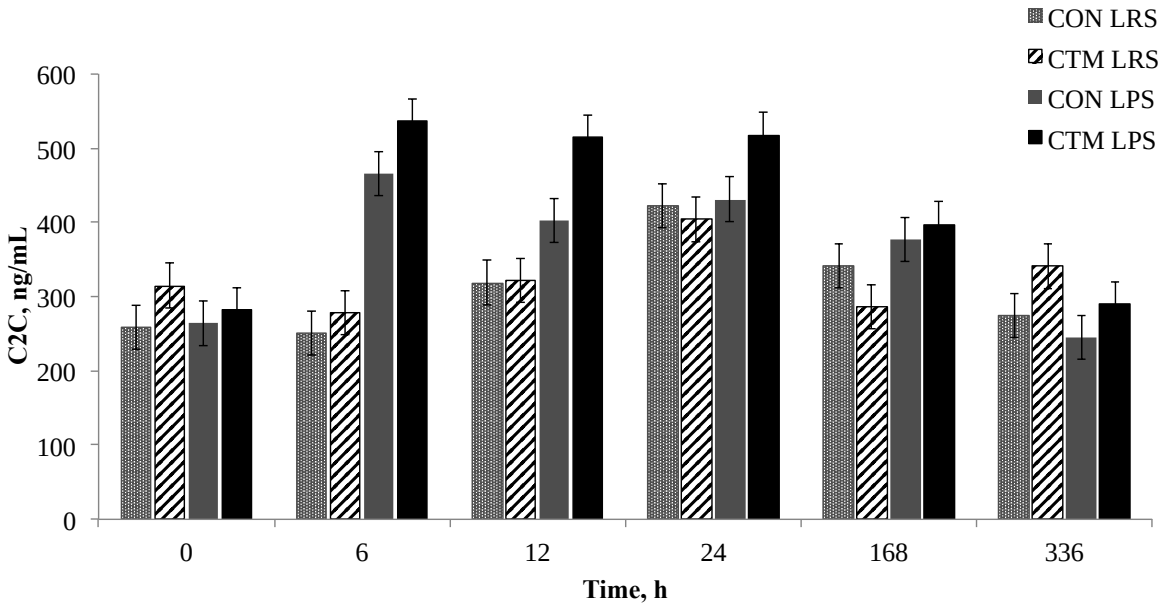
A significant interaction of TM source × LPS × h ($P = 0.02$; Fig. 5.11) was observed for synovial CS846 concentrations. Prior to application of LPS (PIH 0) there were no differences in CS846 levels; however, 6 hours after LPS injection CS846 was significantly higher in LPS knees than LRS knees with no difference between dietary mineral sources. At h 12, knees injected with LPS in CTM horses had the greatest concentration of CS846 ($P< 0.01$) followed by LPS knees in CON horses, while LRS knees remained near baseline. By h 24 LPS knees in CON horses had higher concentrations than CTM horses and h 24 LRS knees had increased above baseline but remained lower than LPS, and by h 168 differences were not detected.

Figure 5. 8 Mean synovial prostaglandin E_2 (PGE_2) concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from *Escherichia coli* O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



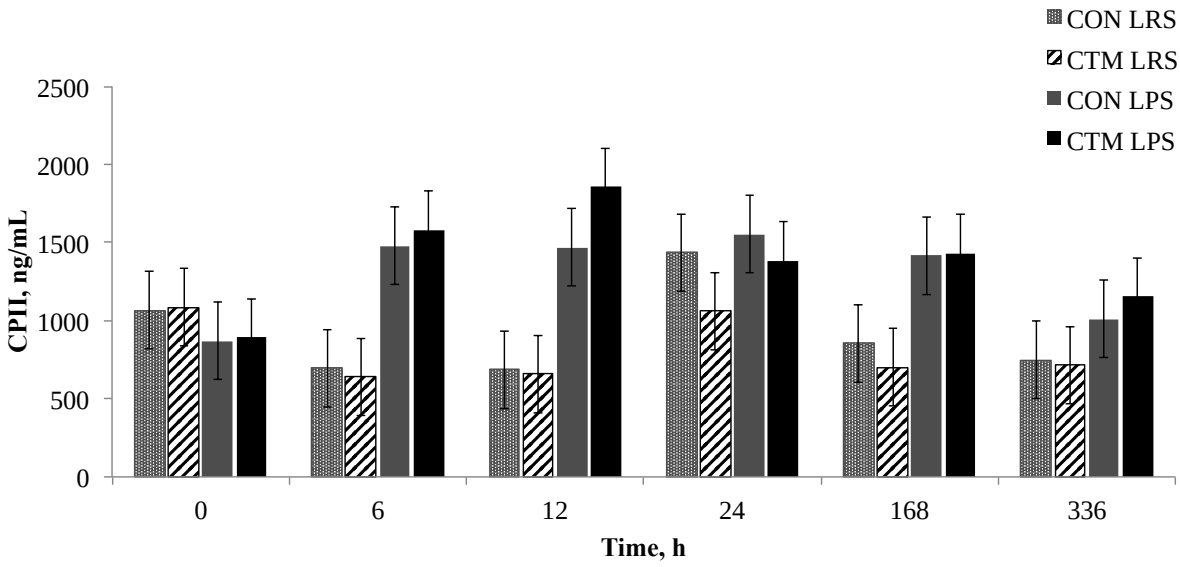
Mean synovial prostaglandin E_2 (PGE_2) concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from *Escherichia coli* O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control). Dietary treatments consisted pelleted concentrates containing either 100% inorganic $ZnSO_4$, $CuSO_4$, and $MnSO_4$ and $CoCO_3$ (CON; $n = 8$) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (CTM; $n = 8$). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Trace mineral source × LPS × h ($P = 0.48$), LPS × h interaction ($P < 0.01$). Trace mineral source ($P = 0.13$).

Figure 5. 9 Mean synovial collagenase cleavage neopeptide (C2C) concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from *Escherichia coli* O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



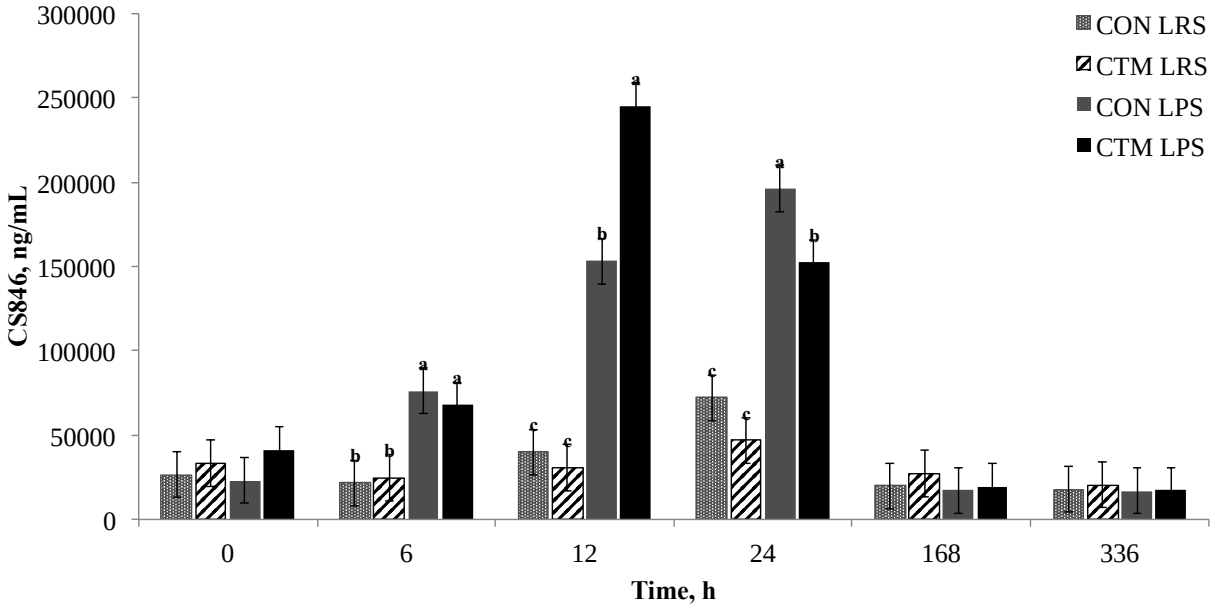
Mean synovial collagenase cleavage neopeptide (C2C) concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from *Escherichia coli* O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control). Dietary treatments consisted pelleted concentrates containing either 100% inorganic $ZnSO_4$, $CuSO_4$, and $MnSO_4$ and $CoCO_3$ (**CON**; $n = 8$) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (**CTM**; $n = 8$). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Trace mineral source × LPS × h ($P = 0.35$), LPS × h ($P < 0.01$), trace mineral source × LPS ($P = 0.09$).

Figure 5. 10 Mean synovial carboxypeptide of type II collagen (CPII) concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from *Escherichia coli* O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



Dietary treatments consisted pelleted concentrates containing either 100% inorganic $ZnSO_4$, $CuSO_4$, and $MnSO_4$ and $CoCO_3$ (**CON**; $n = 8$) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (**CTM**; $n = 8$). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Trace mineral source × LPS × h ($P = 0.99$), LPS × h ($P < 0.01$), trace mineral source ($P = 0.82$).

Figure 5. 11 Mean synovial chondroitin sulfate epitope 846 (CS846) concentrations following a 0.5 ng intra-articular lipopolysaccharide (LPS; derived from *Escherichia coli* O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (LRS; contralateral control).



Mean synovial chondroitin sulfate epitope 846 (CS846) concentrations following a 0.5 ng intra-articular lipopolysaccharide (**LPS**; derived from *Escherichia coli* O55:B5) injection at 0 to 336 h post-injection or lactated ringer's solution (**LRS**; contralateral control). Dietary treatments consisted pelleted concentrates containing either 100% inorganic $ZnSO_4$, $CuSO_4$, and $MnSO_4$ and $CoCO_3$ (**CON**; n = 8) or trace mineral complexes zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate (**CTM**; n = 8). Added levels of Zn, Mn, Cu, and Co were at iso-levels in both pelleted treatment diets, and daily mineral intakes met or exceeded NRC minimum requirements. Significant interactions: TM source × LPS × h interaction (P < 0.02) ^{abc} Denotes differences in concentration among TM sources at specific time points post intra-articular injection.

DISCUSSION

The current study examined the effect of complexed Zn, Cu, Mn, and Co source on growth and intra-articular inflammation. In contrast to our study, previous studies evaluating the impact of CTM have shown increases in growth (Genther-Schroder et al. 2016; Osorio et al., 2012). Osorio et al. (2012) reported calf wither height was positively impacted in calves fed

complexed TMs (Zn, Mn, Cu and Co) versus inorganic sources at weaning and super hutch stage. Additionally, the addition of Zn amino acid complex has been shown to increase long bone length and width in embryos and chicks (Favero et al., 2013). Limited data exists observing the effect of complexed TMs on equine growth; however, differences between TM proteinates and inorganics sources was evaluated in yearling horses fed for 112 d (Ott and Johnson, 2001). They reported no effect of source on BW, WH, HG or BL gains; however, HH gain was greater for horses receiving protienates than the inorganic supplemented horses. In the current study, as expected, over the 56-d study, all horses, regardless of diet, increased in BW, HH, WH, BL, and HG circumference.

Studies conducted previously exposing young horses to intra-articular LPS, clinical responses for HR, RR, and RT showed no signs of systemic illness (de Grauw et al., 2009; Lucia et al., 2013). Values reported remained within normal physiological ranges for horses of this age and demonstrates that the inflammatory response remained localized. Joint circumference increased regardless of TM source or intra-articular treatment (LPS vs. LRS) above baseline values at 6 h, peaked at 12 h, and began decreasing at 24 h; however, values did not return to baseline by 336 h. Circumference increased 0.61cm from h 0 to h 12. The lack of differences between joints receiving LPS versus LRS further validates the inclusion of a sham-injected knee in the LPS model to account for effects of repeated arthrocentesis. Data collected in the current study agrees with previous literature, indicating 0.5 ng LPS causes acute synovitis resulting in minor carpal circumference increases with minimal physiological changes of HR, RR, and RT (Lucia et al., 2013, Kahn et al., 2017).

Synovial fluid TM concentrations in response to an inflammatory insult has not been previously been reported in horses. The current report includes novel information and

understanding regarding concentrations of Zn, Cu, Mn, Co, and Se in the synovial fluid of horses with acute joint inflammation. Due to TM role as enzyme activators and co-factors, changes in concentrations have potential to affect other biochemical indicators. In the current study, all TMs measured varied over time, likely a result of inflammation associated with both LPS and repeated arthrocentesis. Trace minerals, Zn, Cu, Mn, and Se, were directly influenced by LPS injection. Mineral concentrations increased in the synovial fluid post injection. Concentrations of Cu, Zn, and Se responded similarly as concentrations for each mineral increased to 6 h before beginning decrease back to bassline or below baseline levels by 168 h post injection in all knees except CTM LRS which had a delayed peak at 12 h.

In human osteoarthritic patients, similar increases in synovial fluid Cu and correlation between synovial fluid Zn and Cu have been reported (Yazar et al., 2005) in response to inflammation. The increases in concentrations of Zn, Cu, and Se in response to LPS could be due to their role in combating free radical formation. Together with selenium-containing glutathione peroxidase, superoxide dismutase (SOD), are the major antioxidant defense systems against oxygen free radicals. Three isoforms of SOD exist in mammals: the cytoplasmic Cu/Zn SOD, the mitochondrial Mn SOD, and the extracellular Cu/Zn SOD (Fukai and Ushio-Fukai, 2011). However, in humans, extracellular fluids, like synovial fluid, contain limited activity of both superoxide dismutase and glutathione peroxidase (Halliwell and Gutteridge, 1990). The increase in Cu could potentially be due to an increase in the copper-containing acute phase protein, ceruloplasmin, which appears to exert antioxidant effects in human knee-joint synovial fluid (Blake et al., 1981).

In contrast to Zn, Cu, and Se, Mn concentrations had a minimal response to LPS. Peak Mn concentrations were exhibited in the CTM and CON LRS knees at 24 h, 6.41 and 4.81

ng/mL, respectively. These values are approximately 7 to 10-fold higher than reported h 0 values. The decrease in Mn in the LPS knees could potentially be attributed to cells sequestering Mn for the mitochondrial SOD or increased chondroitin sulfate synthesis in response to the LPS.

The three-way interactions observed between diet, h , and LPS for Cu and Se indicate dietary source impacted degree of response between knees. The peak concentrations were highest in the CTM LPS knee for Cu and Se, 0.99 ± 0.07 μg/mL and 228.62 ± 14.2 ng/mL, respectively; although these concentrations were not high enough to differ from CON LPS. At 6 h, the CTM LRS knee had far lower Cu and Se concentrations (0.33 ± 0.07 μg/mL and 75.6 ± 13 ng/mL) than both LPS knees and CON LRS. Concentrations of Cu and Se in CTM LRS peaked at 12 h (0.78 ± 0.07 μg/mL and 147.21 ± 13 ng/mL) that were similar levels to CTM LPS 12 h. The elevated response to inflammation from either LPS or the delayed response to repeated arthrocentesis could potentially be due to the form of Cu supplied in the diet or from the interactions of dietary from of minerals supplied with Se. Cobalt was the only mineral directly affected by diet; horses fed CTM had greater mean concentration of Co in the synovial fluid, 8.18 ± 0.5μg/mL versus 5.86 ± 0.5 μg/mL; therefore, Co glucoheptonate appears to be more bioavailable than inorganic Co. The three-way interaction observed in relation to Co concentrations also conveys that horses fed CTM had a greater response to LPS and repeated arthrocentesis than CON knees, exhibiting higher peak concentrations in the synovial fluid for both LPS (h 6) and LRS (h 12) knees than CON horses. The exact role of Co in the joint has yet to be elucidated.

Overall data indicate acute joint inflammation altered synovial fluid TM concentrations and dietary source impacted the resulting degree of response. Thus, data reported here are a preliminary exploration and do not fully explain the potential role of complex changes of TMs in

joint inflammation. However, understanding how CTM affect the physiological response of TM concentrations and the biological roles of TM within the joint further advances our knowledge of their role in joint health. These data ultimately provide a foundation for the development of further in-depth studies evaluating specific mechanisms regarding interaction networks of TM under inflammatory conditions within the joint.

Articular cartilage integrity is heavily dependent on the balance of metabolic activities (Mueller and Tuan, 2011). Presence of inflammation causes altered cartilage metabolism by decreasing anabolic and increasing catabolic activities (McIlwraith and Trotter, 1996). Although designed to promote healing, chronic exposure to inflammation can lead to articular degradation (Palmer and Bertone, 1994). Levels of cartilage biomarkers measured in the synovial fluid may be influenced by local inflammatory status. A useful indicator of inflammation and as a marker for the progression of joint disease is PGE_2 (Bertone et al., 2001). In the current study, PGE_2 concentrations were higher in LPS injected joints (1210.98 ± 37.43 pg/mL) than in LRS joints (491.11 ± 36.21 pg/mL). Concentrations of PGE_2 peaked quickly at 6 h in the LPS knee for both TM source ; however, horses receiving CTM had a more intense response (3,239 ± 133.86 pg/mL) versus CON (2,664 ± 133.91 pg/mL). Similar inflammatory responses have been reported in an acute LPS model using chickens fed Zn amino acid complex had a greater cytokine production (IL-ß) 3 h post challenge before reducing rapidly to a lower concentration than other diets at 12 h (Cheng and Guo, 2004). A similar sharp and quick rise at 6 h was reported in yearling and mature horses undergoing an intra-articular LPS challenge (Leatherwood et al., 2016 and de Grauw et al., 2009). Reported peak values for both studies were higher than peak concentration seen in the current study.

Aggrecan molecules are an essential component of the extra cellular matrix and due to their highly negative charge, are responsible for providing the joint with compressive strength. A key glycosaminoglycan of aggrecan is chondroitin sulfate; therefore, the impact of both diet and LPS on novel aggrecan molecule synthesis was measured through the CS846 epitope. A quick and transient response of aggrecan synthesis was exhibited in the presence of LPS with concentrations peaking at 12 h for CTM and 24 h CON horses. de Grauw and others (2009) also reported a fast and short-lived inflammation induced enhancement of CS846 synthesis, with concentrations peaking at 24 h in mature horses. Interestingly, the CTM horses peaked at a much higher concentration than their CON counterparts (245,290 ± 15,663 and 196,008 ± 13,653 ng/mL, respectively). In contrast, concentrations in CTM horses had started to decrease by 24 h; however, concentrations rapidly returned to baseline by 168 h for both TM source s. Multiple enzymes involved in the synthesis of chondroitin sulfate require Mn for synthesis (Leach, Jr., 1971); therefore, a potential explanation for the more rapid increase in CTM horses is the complexed Mn may be more readily available for enzyme utilization.

Inflammation can lead to articular cartilage degradation, a key feature in the development of joint disease. The primary component of articular cartilage is type II collagen; its breakdown is highly involved in the development and progression of joint disease. The destruction of cartilage results in an accumulation of breakdown products in synovial fluid. The analysis of these fragments can help elucidate the degree of cartilage turnover and potentially highlight metabolic changes (Garvican et al., 2010).

The breakdown of type II collagen has been measured using C2C (de Grauw et al., 2009). The unwinding or cleavage of collagens by collagenases exposes normally hidden epitopes and these fragments are increased with joint inflammation measured in rabbits, dogs, and horses

(Matyas et al., 2004 and Lucia et al., 2013). Multiple studies have reported peak concentrations of C2C at 24 h post injection (de Grauw et al. 2009; Lucia et al. 2013; Leatherwood et al. 2016; Kahn et al. 2017). In the current study, LPS increased C2C concentrations, although peak concentrations were exhibited at 6 h post injection for both CTM and CON (536 ± 29.85 ng/mL and 465 ± 29.85 ng/mL, respectively).

Concentrations of C2C were higher in joints of horses receiving CTM (373.51 ± 9.28 ng/mL) compared to CON (337.36 ± 9.28 ng/mL) horses. The breakdown and turnover of cartilage collagen is largely mediated by MMPs, a family of degradative enzymes that require a metal ion for activation (Garvican et al., 2010). Synthesis of MMPs is regulated by cytokine and growth factor production (Milner et al., 2006). As an established model of inflammation, an increase cytokine production can be expected when challenged with LPS. Activation of most MMP's require Zn; therefore, readily available Zn, may be allowing for the upregulation of MMPs and the inflammatory response, resulting in increased concentrations of C2C. Concentrations of C2C for CTM horses remain higher at 12 and 24 h before decreasing to baseline at 336 h (290 ± 29.85 ng/mL). However, at 336 h, the highest C2C concentrations were observed in CTM CON knee, (340.43 ± 29.85 ng/mL). Further exhibiting the need for a sham control due to results from repeated arthrocentesis.

The rate of recent collagen synthesis can be measured using CPII (de Grauw et al., 2009). This molecule is proteolytically cleaved from the procollagen strand during fibril formation and has a half-life of 16 h in synovial fluid. It has also been shown to increase in arthritic joints and in osteochondrosis (Frisbie et al., 2008). Additionally, CPII concentrations have been shown to increase in response to intra-articular LPS injection in both growing and mature horses, with variation in concentration between studies (de Grauw et al., 2009; Lucia et al., 2013;

Leatherwood et al., 2016; Kahn et al., 2017). Results from the current study are consistent in that LPS caused an increase in CPII concentrations, regardless of diet, with highest concentration at 12 h (1663.09 ± 175.45 ng/mL) when compared to CON knee that peaked later at 24 h (1248.87 ± 175.45 ng/mL).

Potential dilution effects of biomarkers could be a confounding factor in synovial fluid biomarker analysis, thus, evaluation of ratios looking at the anabolic to catabolic processes in the joint may prevent biases (de Grauw et al., 2011; te Moller and van Weeren, 2017). It also allows for observation of any metabolic shifts. Previous data has shown a shift towards synthesis in response to inflammation. This shift allows for damage within the cartilage framework to repair; however, replacement of damaged matrix may not return the joint to its original state or function (Garvican et al., 2010). In the current study the ratio of CPII to C2C was analyzed and even though an increase in C2C was observed in CTM fed horses it did not alter the ratio of type II collagen synthesis. The intra-articular LPS tended to increase the ratio due to minimal increases in anabolic processes, likely a result of damage due to inflammatory mediators.

Conclusion

In conclusion, compared to inorganic mineral sources, data suggest that sufficient intake of a complexed TM source may support a more robust pro-inflammatory immune response to an LPS challenge that was evidenced by an increase in type II collagen degradation and a more rapid rise in aggrecan synthesis. Additionally, the intra-articular LPS challenge was sufficient in inducing inflammation, cartilage turnover, and aggrecan synthesis, thus allowing for the determination of dietary impact on these synovial fluid biomarkers. Additional research is needed to fully understand the impact of TMs and their physiological role within the joint and the ability to delay the onset of joint disease in young horses.

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CHAPTER VI

EFFECTS OF MATERNAL OVERNUTRITION ON CARTILAGE METABOLISM IN THE NEONATAL FOAL

SYNOPSIS

The multifactorial origin of developmental orthopedic disease or osteochondrosis in young horses has resulted in poor understanding of the disease's etiology. Factors impacting development include but are not limited to: genetics, plane of nutrition, feeding management and exercise. However, limited information exists as to the impact of maternal nutrition on articular cartilage development. Cartilage lesions caused by osteochondrosis are associated with alterations within the extracellular matrix of articulating joints. To evaluate effects of third trimester overnutrition on cartilage metabolism in neonatal foals, 16 Quarter Horse mares (insert ±SEM BW, Age) were used in a completely randomized design. Mares with confirmed breeding dates were stratified by BW, BCS, and expected foaling date, and randomly assigned to dietary treatments, beginning on d 235 of gestation. All mares received ad libitum access to coastal bermudagrass hay (Cynodon dactylon) and 0.1% BW forage balancer daily (Empower Balance, Cargill Inc.). Control mares (CON; n=8) were fed to meet nutrient requirements during late gestation. The overfed treatment (HIGH; n=8) received an additional 40% above CON that consisted of the same diet with an additional 0.7% BW commercial concentrate (SafeChoice Mare and Foal, Cargill Inc.) daily until parturition. Maternal BW change during the experimental period for CON and HIGH mares validated the model (P=0.07), with gains of 26.4±5.5 kg and 49.2±5.5 kg, respectively. At 5-hr postpartum, foals were euthanized, and synovial fluid was

collected from both radial carpal joints via arthrocentesis. Samples were analyzed for carboxypeptide of type II collagen (CPII), collagenase cleavage neopeptide (C2C), and aggrecan chondroitin sulfate 846 epitope (CS846) concentrations. Data were analyzed using the MIXED procedure of SAS. Diet did not affect CPII or C2C concentrations ($P>0.05$). Foals maintained an average CPII:C2C ratio of 8.16 and 7.83 for CON and HIGH treatments, respectively. Synovial fluid concentration of CS846 was unaffected by diet ($P>0.05$). These data indicate that maternal overnutrition in the third trimester did not alter cartilage metabolism based on type II collagen and aggrecan synthesis within the carpal joint of neonatal foals.

INTRODUCTION

Manifestations of developmental orthopedic disease (DOD), including osteochondrosis (OC) in young horses appears to be multifactorial; thus, understanding the etiology of disease onset has proven difficult (Yetrehus et al., 2007). Epidemiological studies provided key information to help explain the factors leading to the development of DOD including: genetics (Philipsson et al., 1993), breed (Lepeule et al., 2009), growth rate (Lepeule et al., 2009), nutritional imbalances (Hurtig et al., 1993) or excess (Glade and Belling, 1984; Savage et al., 1993) and exercise conditions or consistency (van Weeren and Barneveld, 1999; Lepeule et al., 2009). Nevertheless, the relative contribution of each factor remains unclear, and the disease is still considered idiopathic (Crenshaw, 2006; Yetrehus et al. 2007). Although causation of initial lesions is continuingly debated, modifications in type II collagen (Laverty et al., 2000; van de Lest et al., 2004) and proteoglycan (Laverty et al., 2000; de Grauw et al., 2011) metabolism have been suggested.

Maternal obesity (MO) resulting from a high-energy diet is harmful to fetal development. Multiple epidemiological and experimental studies demonstrate how modification of the nutritional environment throughout gestation affects offspring health into adulthood (Wu et al., 2006; Fowden et al., 2013). Hypertrophy of adipocytes caused by overnutrition leads to subacute inflammation that is characterized by an increased circulation of pro-inflammatory cytokines and acute phase proteins (Shoelson et al., 2007). This chronic low-grade systemic inflammation leads to activation of synoviocytes and macrophages, initiating production of cartilage-degrading proteases such as metatalloproteinases (MMPs) and pro-inflammatory cytokines and eicosanoids in the joint. Resulting localized inflammation impairs healing and causes tissue destruction (Sun et al., 2016).

Type II collagen is the primary component of the articular cartilage extracellular matrix (ECM) and the matrix is maintained through a balance of synthesis and degradation. Alterations in the ECM of articulating joints influence the prevalence of developmental orthopedic disease in horses (de Grauw et al., 2006). Development of cartilage and epiphysis of long bones occurs during the third trimester of pregnancy; therefore, overnutrition during this period could predispose foals to development of DOD with eventual progression to osteoarthritis (OA) through alterations in cartilage development. Although this has yet to be investigated in the horse, evidence in ovine have shown enhanced collagen accumulation and cross linkages in fetal muscle born to obese mothers (Huang et al., 2012), altering the integrity of the collagen.

Determining effects of maternal nutrition on ECM can be evaluated through usage of cartilage metabolism biomarkers measured in synovial fluid. Inflammatory conditions influence collagen metabolism and aggrecan synthesis by increasing catabolic collagenase cleavage neopeptide (C2C), anabolic carboxypropetide of type II collagen (CPII), and chondroitin sulfate 846 epitope (CS846; de Grauw et al., 2009; Lucia et al., 2013). Therefore, the objective of this study was to investigate effects of third trimester overnutrition on cartilage metabolism and aggrecan synthesis through direct ECM turnover products in neonatal foals.

MATERIALS AND METHODS

All care, handling, and procedures for experiment approved by the Texas A&M University Institutional Animal Care and Use Committee.

Horses and Dietary Treatments

Sixteen pregnant Quarter horse mares (initial BW 541 ± 17 kg; initial BCS 5.96 ± 0.91) were used in a completely randomized design to evaluate effects of third trimester overnutrition on cartilage metabolism in neonatal foals. All mares were bred to a single stallion to reduce

genetic variability. Prior to initiation of the study, mares were maintained on pasture at a BCS 6. Mares with confirmed breeding dates were stratified by BW, BCS, and expected foaling date, then randomly assigned to one of two dietary treatments: Control (**CON**) or overfed (**HIGH**). Treatments began on d 235 gestation and continued until parturition. Control mares (CON; n=8) were fed to meet 100% nutrient requirements during late gestation. Mares in the HIGH treatment group (n=8) received an additional 40% above CON receiving the same diet as control plus an additional 0.70% BW in commercial concentrate (SafeChoice Mare and Foal, Cargill Animal Nutrition, Elk River, MN) daily until parturition. All mares received *ad libitum* access to coastal bermudagrass hay (*Cynodon dactylon*) and water for the duration of the project. All mares also received 0.10% BW/d of a forage balancer (Empower Balance, Cargill Animal Nutrition, Elk River, MN). Composited hay and grain samples were collected and analyzed for nutrient composition (Table 1). Mares were fed individually twice per day, and adjustments were made according to changes in BW every 14 d.

Foal Sample Collection

When parturition was imminent, mares were moved into 3.7 x 3.7 m box stalls, were continuously monitored, and parturition was attended. Immediately following parturition, foals were removed from sight, smell and sound of the mare. At 5-hr postpartum, foals were euthanized with 15 mL pentobarbitol (Beuthanasia-D, Merck & Co. Inc., Madison, NJ), and the synovial fluid was immediately collected via carpal arthrocentesis. The carpal joint was aseptically aspirated using a location medial to the extensor *carpi radialis* tendon in the palpable depression between the radial carpal bone and the third carpal bone, to a depth of approximately 12.7 mm to avoid unnecessary contact with articular cartilage (McIlwraith and Trotter, 1996).

Pooled synovial fluid (1 to 4 mL) was collected and transferred into 1.5 mL micro-centrifuge tubes and immediately stored at -20^{o}C until later analysis of C2C, CPII, and CS846.

Table 6. 1 Nutrient composition of concentrates and hay (DM basis) fed to mares.

	Coastal bermudagrass hay	Forage balancer[a]	Mare and foal concentrate[b]
DE, Mcal/lb	1.07	1.56	1.45
DM, %	90.06	89.14	89.94
Nutrient, % DM			
CP	6.5	34.16	18.26
ADF	36.46	7.63	16.84
NDF	61.58	16.88	32.17
Ca	0.29	3.46	1.45
P	0.16	1.81	1.07
K	1.09	1.82	1.48
Na	0.02	0.5	0.49
Cl	0.3	0.98	0.98

[a]Nutrena Empower® Topline Balance™, (Cargill Animal Nutrition, Elk River, MN) fed at 0.1% BW daily (as-fed).
[b]Nutrena SafeChoice Mare and Foal (Cargill Animal Nutrition, Elk River, MN) fed at 0.7% BW

Sample Analysis

Concentration of synovial fluid CPII, C2C, and CS-846 were measured using commercially available ELISA kits (IBEX Pharmaceuticals Inc., Montreal, QC, Canada) previously validated for use in equine synovial fluid (de Grauw et al. 2009; Lucia et al., 2013). Standards were prepared according to manufacturer's protocol with samples prepared at a 1:2 dilution for both CPII and C2C. Sample dilutions for CS846 were 1:50. All samples were

analyzed in duplicate, with the mean detectable concentrations for CPII, C2C, and CS846 being 50 ng/mL, 10 ng/mL, and 20 ng/mL, respectively. Shifts in cartilage metabolism were evaluated by the ratio of CPII to C2C. Individual ratios were calculated for each animal. Final concentrations of all markers were read using a microplate reader with optical density set at 450 nm. The CPII, C2C, and CS846 intra-assay precision within plates was <10 %.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS. The model contained an effect of treatment. Significance was declared at $P \leq 0.05$ and trends were identified at $P \leq 0.10$.

RESULTS AND DISCUSSION

The ability to understand and decrease prevalence of developmental orthopedic diseases, is a substantial challenge facing the equine industry today. Nutritional factors have previously been connected with the manifestation of the disease in growing horses. Excessive energy intake, especially in the form of non-structural carbohydrates, can lead to dysregulation of glucose through development of insulin resistance; thus, increasing risk of developing OC lesions (Ralston 1996; Ott et al., 2005). However, limited data exists on the evaluating the overfeeding of mares in all nutrients, rather than carbohydrates specifically. Although, feeding management of young horses are more widely understood, the role of maternal nutrition or alterations in nutritional environment during gestation and their impact on cartilage development has not been fully elucidated in the horse. Although often negatively implicated, effects of high concentrate diets on foal skeletal development has produced inconsistent results. Heyden et al. (2013) reported the percentage of OC incidence was higher in foals born to mares whose diets included concentrate both with and without roughage (33.7 and 38.9%, respectively) during gestation

compared to foals born to mares supplied forage only (4.2%) diets in an epidemiological study. Furthermore, authors also reported an increased risk factor of 26% for OC lesions development when mares are feed concentrate only compared to roughage only in the gestational period (Heyden et al., 2013). In contrast, recent studies reported no changes to the osteoarticular status in foals at 6 and 24 mo between foals born to overfed mares receiving diets containing rich in soluble carbohydrates (Peugnet et al., 2016; Robles et al., 2017).

Inconsistent results bring about more questions in regard to nutritional management of mares during gestation. However, it is important to note, these studies did not evaluate cartilage alterations through direct biomarkers, but rather relied on radiographs exhibiting presence of developed lesions and evaluated foals at an older age (6-24 mo). In the current study authors evaluated markers of metabolism within the synovial fluid, which is in direct contact with relevant tissues and allows for increased understanding of cartilage metabolism before the production of lesions.

Cartilage biomarkers

Overfeeding mares in the third trimester did not affect (P = 0.51; Table 6.2) level of synovial CS846 epitope of cartilage aggrecan in their foals (58,492 ± 8,963 ng/mL), when compared to foals born to control mares (66,983 ± 8,963 ng/mL). The CS846 epitope is most concentrated in newborn articular cartilage and decreases progressively with age, to the point it is almost absent from adult cartilage (Antoniou et al., 1996). The CS846 epitope increases in synovial fluid post-injury and reappears in cartilage on the largest aggrecan molecules in OA (Rizzkalla et al., 1992). Onset of OC has shown to decrease the CS846 epitope, suggesting that aggrecan synthesis is impaired in horses 9-18 months (Laverty et al., 2005). Moreover, de Grauw et al. (2011) reported reduced concentrations of CS846 in tarsocrural synovial fluid of 18 wk old

foals with confirmed radiological OC compared to foals without OC. Conversely, localized inflammation within the joint results in increased CS846 synthesis (Millican et al., 2018), suggesting, synthesis of aggrecan molecules is regulated differently under pathological conditions and play a role in either the onset or progression of disease. Alterations at the neonatal age could potentially be an underlying cause of disease onset; however, changes may not be present at birth and based on the current study are not affected by overnutrition in the third trimester.

Under the conditions of the present study, collagen synthesis, as determined by CPII synovial concentration was not significantly affected ($P = 0.99$) foals from the overfed and control mares (1461.22± 241.24 and 1464.14± 241.24, respectively). As the primary component of articular cartilage, type II collagen and the rate of recent collagen synthesis within the joint can be measured using CPII (de Grauw et al., 2009). During fibril formation, this molecule is proteolytically cleaved from the procollagen strand and has a half-life of 16 h in synovial fluid. Frisbie et al. (2008) reported an increased CPII concentration was associated with arthritic joints and osteochondrosis in exercising 2 yr old horses. In contrast, de Grauw et al. (2011) reported no differences in synovial fluid CPII concentrations in normal versus foals affected with osteochondrosis. Although limited data exists on the affect of fetal programming on type II collagen, influence of MO on collagen synthesis and accumulation within fetal muscle demonstrated by Huang et al. (2012). They reported collagen content of the *longissimus dorsi* muscle increased in offspring born to obese ewes and attributed enhanced collagen accumulation, likely a result of increased synthesis (Huang et al., 2012; Huang et al., 2010b). In the present study maternal obesity was not induced (Bradbery et al., 2017); therefore, indicating

alterations in collagen synthesis are not impacted in horses that overfed, but have not yet reached a stage of obesity.

In addition to increased accumulation of collagen within the muscles, cross-linking was also increased, thought to be a result of inhibited remodeling from decreased MMP expression. Overactive collagen synthesis within the articular cartilage would alter the homeostatic balance and cause dysregulation of cartilage repair and remodeling. Thus, our hypothesis was foals born to overfed mares would exhibit decreased degradation of type II cartilage; however, our observations revealed no differences in cartilage degradation between the two treatments, as measured by C2C ($P = 0.99$). Concentration of synovial C2C in foals born to HIGH and CON mares were 183.26 ± 14.29 and 183.18 ± 14.29, respectively. Breakdown of type II collagen has been measured using the C2C biomarker (de Grauw et al., 2009). Collagenases cause unwinding or cleavage of collagen, exposing normally hidden epitopes that increase with joint inflammation (Matyas et al., 2004 and Lucia et al., 2013). Foals affected with OC showed no changes in levels of synovial C2C at 18, 22, and 52 weeks of age (de Grauw et al., 2011). Although, an additional biomarker measuring the breakdown of type II collagen (Coll2-1) was reported to be elevated in horses (median age: 3.14 yr) with OC (Verwilghen et al., 2011) with values increasing with increased articular degradation from 147.61 nM to 207.54 and 227.02 nM Coll2-1. Furthermore, the molecular expression of aggrecan or collagen type II did not differ between affected and clinically normal cartilage collected from equine stifle or shoulder joints (Semevolos et al., 2001). However, studies have shown a decrease in MMP expression in fetal muscle of offspring born to obese dams (Huang et al., 2012). The MMP family is responsible for the breakdown of collagen, including type II.

After review of the individual markers, no difference in CPII/C2C between treatments was observed ($P = 0.85$). Foals maintained an average CPII:C2C ratio of 8.16 and 7.83 ± 1.15 for CON and HIGH treatments, respectively. Potential dilution effects of biomarkers could be a confounding factor in synovial fluid biomarker analysis, thus, evaluation of ratios looking at the anabolic to catabolic processes in the joint may prevent biases (te Moller and van Weeren, 2017). Evaluating the ratios allows for observation of any metabolic shifts. Significant correlation between radiographic appearance of OC at 5.5 months and CPII and the CPII/C2C ratio, reflecting higher collagen turnover due to remodeling and repair at lesion sites (Donabedian et al., 2008).

Table 6. 2 Effect of maternal overnutrtion on neonatal foal cartilage biomarkers within carpal joint synovial fluid

	Dietary Treatments[1]			
	CON	HIGH	SEM	*P*-Value
Synovial Fluid Biomarkers				
CS846[5], ng/mL	58429	66983	8963	0.51
CPII[3], ng/mL	1461.22	1464.14	241.24	0.99
C2C[4], ng/mL	183.26	183.18	14.29	0.99
CPII:C2C	8.16	7.84	1.1493	0.85

[1]All mares received ad libitum access to coastal bermudagrass hay (Cynodon dactylon) and 0.1% BW forage balancer daily (Nutrena Empower® Topline Balance™, Cargill Animal Nutrition, Elk River, MN). Control mares (CON; n=8) were fed to meet nutrient requirements during late gestation. The overfed treatment (HIGH; n=8) received an additional 40% above CON receiving the same diet plus 0.7% BW commercial concentrate (SafeChoice Mare and Foal, Cargill Animal Nutrition, Elk River, MN) daily until parturition.
[3]Carboxypropetide of type II collagen
[4]Collagenase cleavage neopeptide
[5]Chondroitin sulfate 846 epitope

Conclusion

According to the data, maternal overnutrition in the third trimester did not affect cartilage metabolism based on type II collagen and aggrecan synthesis within the carpal joint of neonatal foals. Nevertheless, in the present study true obesity was not induced, thus could explain why no effects were observed. Influence of maternal nutrition on the ECM development and homeostasis, has not been extensively studied. This data supplies foundational data for further investigation into the implications of late gestation nutrition and maternal obesity's influence on articular cartilage development, to better understand and or eliminate factors that are potentially predisposing foals to OC, or development joint diseases later in life.

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CHAPTER VII

CONCLUSION

Trace minerals are essential in metabolic processes such as growth, development, immunity, and reproduction. Current dietary requirements for cattle and horses are based on preventing deficiency rather than optimal performance. Relying on mineral composition of forage and rations alone are often unable to prevent deficiencies due to poor bioavailability, mineral-mineral interactions, and ruminal interactions. Thus, supplementation of TMs has become industry standard and utilization of organic sources is increasingly more common. The research presented and described allowed for further evaluation the impact of supplemental organic TM sources in finishing beef steers and growing horses.

CATTLE

Summary

In beef cattle, inorganic TM, most often in the sulfate form, are typically fed to meet the needs of beef cattle; however, other sources of TM, like amino acid complex, are thought to reduce the potential of ruminal interactions that ultimately reduce the bioavailability of minerals through the formations of insoluble complexes.

The objective of studies presented in Chapter III and IV were to further evaluate how Zn amino acid complex supplementation influences live performance and carcass characteristics in finishing beef cattle and its impact on liver abscess prevalence. Our hypothesis was supplementing ZnAA in conjunction with ractopamine hydrochloride would result in the highest

performing cattle and ZnAA supplementation would result in a lower percentage of cattle plagued with liver abscesses.

The initial pooled analysis revealed cattle receiving RAC and ZnAA complex had the highest live performance, exhibited through increased ADG and final BW. Alone and in combination with ZnAA, RAC fed cattle had increased feed efficiency over steers not supplemented with RAC. Furthermore, under the conditions of the two large-scale studies included in the analysis, addition of ZnAA to RAC supplemented finishing diets resulted in improved HCW and carcass-based gain with minimal impact on carcass characteristics. Ultimately, these data suggested that the inclusion of ZnAA and RAC in finishing diets provide incremental improvements in steer performance over RAC alone, conveying a potential opportunity for cattle feeders to optimize performance and increase economic returns.

The initial study established opportunity for ZnAA to augment RAC response when supplemented at 360 mg ZnAA·hd^{-1}·d^{-1} (38 mg ZnAA/kg DM), but did not evaluate benefits of ZnAA alone. Therefore, establishing ZnAA's effect on cattle not receiving RAC and determining the most effective concentration of ZnAA supplementation led to our second analysis.

Chapter IV included reports of our second pooled analysis, including data from 9 independent feedlot trials. The primary analysis evaluated the effects of ZnAA supplemented throughout the entire finishing period at a range of 30 -120 mg/kg DM and RAC incorporated at 200 to 320 mg·hd^{-1}·d^{-1}. Results revealed the inclusion of ZnAA and RAC independently improved cattle performance. The addition of ZnAA in finishing rations resulted in increased BW, ADG, and HCW without altering intakes. Furthermore, carcass-adjusted measures were improved with the addition of ZnAA.

The secondary analysis evaluated varying ZnAA concentrations: 1) No supplemental ZnAA 2) LOW, 30-54 mg/kg DM and 3) HIGH, ≥ 60 mg/kg DM. Results conveyed that HIGH supplementation rates had the greatest response in performance, although, liver abscess presence was lowest in the low supplementation group. Due to the variability in range of supplementation, optimal concentrations are difficult to predict; however, adding dietary ZnAA at a concentration of ≥ 60 mg/kg DM in the finishing program may have potential gains for producers economically. Ultimately, the added value of supplementing of ZnAA throughout the finishing period in cattle with or without RAC is an increased opportunity to produce more beef from a smaller cattle inventory.

Information collected from this work, identifies benefits of utilizing supplemental ZnAA throughout the finishing period; although, it is important to recognize limitations of pooled data sets. Further work evaluating varying total Zn levels would likely benefit cattle producers, in addition to evaluating the impact of utilizing multiple sources.

EQUINE

Summary

Similar to beef cattle, traditional programs utilize inorganic sources of TM to meet the requirements of their horses; however, the incorporation of organic TM into equine diets has become increasingly more popular. Limited data on TM supplementation in horses is available; therefore, this studies objective was to evaluate the impact of source (AA complexed vs. inorganic) on growth and body composition of young horses. An additional objective was to measure mineral source impact on cartilage metabolism, synovial inflammation, and synovial fluid mineral concentration. The study completed and described revealed potential roles for complexed TM to positively impact performance in finishing beef cattle and influenced equine

metabolism. In addition to gaining novel information about the impact of TM source and LPS on synovial fluid trace mineral concentrations.

Although no impact on growth or growth composition was observed, compared to inorganic mineral sources, our data suggest that sufficient intake of a CTM source may support a quick robust pro-inflammatory immune response to an LPS challenge that was evidenced by an increase in type II collagen degradation and a more rapid rise in aggrecan synthesis. Additionally, the intra-articular LPS challenge was sufficient in inducing inflammation, cartilage turnover, and aggrecan synthesis, thus allowing for the determination of dietary impact on these synovial fluid biomarkers.

Individual TM responses to the inflammatory challenge provided novel information of equine synovial fluid TM concentrations. Overall data indicated acute joint inflammation altered synovial fluid TM concentrations and dietary source (Co, Cu, Zn, Mn) impacted degree of response to LPS and repeated arthrocentesis of Co, Cu and Se. Compared to traditional inorganic Co, Co glucoheptonate appears to be more readily incorporated into synovial fluid. Data reported in Chapter V are a preliminary exploration and do not fully explain the potential role of complex changes of trace minerals in joint inflammation. Understanding specific mechanisms regarding interaction networks of these minerals requires additional in-depth studies; however, this data provides a foundation for future work in TM role in joint health.

Lastly, according to the data, maternal overnutrition (all nutrients fed at 160% of requirements) in the third trimester did not alter cartilage metabolism based on type II collagen and aggrecan synthesis within the carpal joint of neonatal foals. True obesity was not induced in the study described in Chapter VI, which could explain why no alterations in cartilage biomarkers were observed. The influence of maternal nutrition on the ECM development and

homeostasis has not been extensively studied. Thus, this data supplies foundational data of biomarker concentrations in the neonatal foal for further investigation into the implications of maternal nutrition's influence on articular cartilage development to better understand and/or eliminate factors that are potentially predisposing foals to OC, or OA development.