IMPACTS OF GESTATIONAL AND EARLY-LIFE NUTRITION ON PRODUCTIVE AND HEALTH RESPONSES OF BEEF CATTLE

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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December 2020

Major Subject: Animal Science

ABSTRACT

Nutritional management during gestation as well as postnatally is critical to optimize efficiency and profitability of cow-calf systems. Two experiments were performed to evaluate the effects of trace mineral supplementation during gestation and supplementation with calcium salts of soybean oil (**CSSO**) to nursing beef steers on performance and physiological responses. In experiment one, the impact of inorganic or organic Co, Cu, Mn, and Zn supplementation to beef cows during gestation were evaluated on parameters associated with offspring performance and physiological responses. One hundred and ninety non lactating pregnant beef cows were assigned to the experiment at 117 ± 2.2 days of gestation (d 0) and received diets containing either 1) sulfate sources of Cu, Co, Mn, and Zn (**INR**), or 2) organic complexed source of Cu, Co, Mn, and Zn (**AAC**). No treatment differences were detected ($P \geq 0.19$) for calf body weight at birth or at weaning. Heifers born to INR cows had delayed puberty attainment (treatment ×day interaction; $P < 0.01$), whereas no treatment differences were detected ($P \geq 0.24$) for carcass traits when male offspring were reared as feeder cattle. In experiment two, the impacts of supplementing CSSO at 2 months of age via creep-feeding and/or postweaning via preconditioning were evaluated on parameters associated carcass quality and development. Steers receiving CSSO at 2 mo of age had greater ($P \leq 0.01$) mRNA expression of genes associated with lipid metabolism in the *longissimus muscle* later in life, although this did not translate into improved carcass characteristics. Outcomes of these experiments may be used to develop nutritional strategies to enhance reproductive efficiency in female offspring and upregulate genes associated with lipogenesis during the finishing period. Research is still warranted to examine the effects of such supplementation during these periods of developmental plasticity on cattle productivity.

DEDICATION

This dissertation is dedicated to my mother Beth Schubach, and grandparents Ted and Margaret Schubach.

AKNOWLEDGEMENTS

First, I would like to thank my family for all the support they provided to me, especially my grandmother and grandfather. Thank you for showing me I was capable of anything I dreamed of and for your love and patience, I know you are with me each day. A very special thank you goes to my mother Beth Schubach. I could not have accomplished my dreams without your unwavering love and support, thank you for your belief in me and for the excellent example you set.

I would also like to express my sincere appreciation to my advisor, Dr. Reinaldo Cooke, for providing me with the opportunity to be part of his research group, and to attend Texas A&M University. Thank you for believing in my work, and for holding me to high expectations. In this environment I was able to grow not only as a scientist, but as a professional and a person. For this I am grateful. I would also like to thank Dr. Ky Pohler for his guidance and support during my time at Texas A&M, for serving on my committee, and his willingness to always make time to share his knowledge. I am especially grateful to Dr. Rodolfo Cardoso, Dr. Stephen Smith, and Dr. Kevin Washburn for agreeing to serve on my committee as well, their contribution to my education was invaluable.

I would like to extend my thanks to Dr. David Bohnert and the staff at the Eastern Oregon Agricultural Research Center. This was the place I fell in love with research, learned the most, and made the best memories. Burns will always mean a great deal to me, and I will forever be grateful this is where my scientific journey began. I would also like to thank Alice Brandão for her patience, her friendship, and her support. To say I could not have completed this without you is an understatement, thank you for teaching me, for being the reasonable one when I needed it, and for the countless laughs and memories. To Dr. Rodrigo Marques, thank you for your guidance and

your support throughout this program, your passion for research is inspiring. Thank you to all of the interns, graduate students, and professors from the Cooke-Pohler lab group for being my home away from home, and for all of the amazing memories. I am also incredibly thankful for the friendships I made in College Station, and for the happy hours and barbecues we enjoyed together. More specifically, I would like to thank Genevieve D'Souza, Sydney Reese, and Nicky Oosthuizen for their friendship, support, and for all of the laughs. To my Minnesotan friend Samantha Girtz, thank you for keeping me laughing, and for being my rock, you were my constant throughout this journey, and I don't know where I would be without you.

Finally, thank you to my husband Lorin Harvey. Words cannot describe my appreciation for your patience, understanding, compassion, and support. You were an integral part to my time in College Station, and I could not imagine completing this work without your help. I am incredibly blessed to have such a special person like you in my life, thank you.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Dr. Reinaldo Cooke, Dr. Ky Pohler, Dr. Rodolfo Cardoso, and Dr. Stephen Smith of the Department of Animal Science, and Dr. Kevin Washburn of the Department of Veterinary Medicine and Biomedical Sciences.

Collaborators included Dr. David Bohnert of the Eastern Oregon Agricultural Research Station at Oregon State University; Dr. Rodrigo Marques at the Department of Animal and Range Sciences at Montana State University; Dr. Donald Jump at the School of Biological and Population Health Sciences at Oregon State University; and Drs. Connie Larson and Jason Russell of Zinpro Corporation. In addition, sincere appreciation is expressed to Flavia Cooke, as well as the graduate students and staff members of Texas A&M University and Oregon State University who contributed to these projects.

Funding Sources

A portion of this work was supported by Zinpro Corporation as well as by Church and Dwight Corporation. A portion of graduate study was supported by a fellowship from the Tom Slick Graduate Research Fellowship Program at Texas A&M University. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Tom Slick Graduate Research Fellowship Program.

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

TABLE Page

TABLE Page

1. INTRODUCTION

The success of each cow-calf operation depends on its ability to produce one healthy calf per cow annually in order to meet the projected increase in demand for animal protein. Additionally, producers are challenged to improve growth, efficiency, carcass, muscle, and quality characteristics of offspring (Robinson et al., 2013). Nutritional management during gestation as well as postnatally is critical to optimize efficiency and profitability of cow-calf systems. The embryonic, fetal, and neonatal periods are the stages of life in which most developmental processes occur, and research demonstrates nutrient supply during these periods exerts long-term consequences on health and performance of the offspring (Koletzo et al., 2009; Fall, 2011), leading to the concept of developmental programming (Reynolds et al., 2010). Developmental programming refers to perturbations during the fetal period that promote adaptive responses, which have long-term effects on the physiology and metabolism of the organism (Wu et al., 2006; Reynolds et al., 2010). For example, maternal nutrient restriction during gestation in beef cattle results in compromised placental angiogenesis, cotyledon growth (Vonnahme et al., 2007), and reduced fetal growth (Long et al., 2009). Hence, nutritional manipulation during critical periods of developmental plasticity represents an opportunity to alter offspring development and performance.

Effective prenatal nutritional management is a crucial component of livestock production systems, and supplementation or manipulation strategies during this period have been shown to enhance performance of subsequent offspring (Larson et al., 2009; Bohnert et al., 2013; Marques et al., 2016; Marques et al., 2017). Moreover, steers from protein-supplemented dams were born heavier, and maintained this advantage concurrent with a greater percentage of body fat until

slaughter (Larson et al., 2009). Calves born to cows supplemented with organic trace minerals during the last trimester of gestation had greater body weight upon weaning until slaughter and reduced incidence of bovine respiratory disease (Marques et al., 2016). Furthermore, Marques et al. (2017) demonstrated supplementing essential fatty acids to late-gestating beef cows resulted in greater hot carcass weights and marbling scores of offspring. These results are suggestive of fetal programming effects on postnatal offspring growth and health (Funston et al., 2010). Additionally, nutritional management of beef cows affects fetal development throughout the entirety of gestation (Wu et al., 2006), whereas the previous literature investigated nutritional manipulation during the last trimester of gestation.

Another period of epigenetic susceptibility is during early postnatal life, given that in most species organ development is not complete at birth and continues postnatally (Patel and Srinivasan, 2011). Nutritional intervention during this period has been shown to increase expression of growth related and adipogenic genes (Moriel et al., 2014a) and marbling in cattle (Cooke et al., 2011; Scheffler et al., 2014; Mangrum et al., 2016), and accelerate puberty attainment in heifers (Moriel et al., 2014b). This phenomenon is known as metabolic imprinting, and these biological responses to nutritional intervention can permanently alter physiological outcomes later in life (Du et al., 2010). Some of these studies utilized early-weaned cattle, which may not be a feasible management strategy for many cow-calf operations in the United States. Furthermore, Cooke et al. (2011) and Mangrum et al. (2016) evaluated steers older than 5 months of age, however, younger animals appear to be more responsive to metabolic imprinting events (Lucas, 1991). One alternative to investigate developmental programming effects to younger animals in traditional cow-calf systems, is to provide supplementation via creep feeding (Reis et al., 2015). Therefore, additional

research is warranted to determine if nutritional intervention provided via creep-feeding is an alternative to promote metabolic imprinting effects without the need for early-weaning.

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2. LITERATURE REVIEW

2.1. Developmental Programming

Developmental programming, also called fetal programming, proposes that alterations in fetal nutritional or endocrine status may result in adaptations that permanently alter the trajectory of growth, physiology, and metabolism of the offspring (Barker and Clark, 1997; Wu et al., 2006). Moreover, recent reviews focused on developmental programming have suggested that nutritional manipulation during gestation can have profound influences on occurrence of adulthood disease and long-term performance (Bell, 2006; Wu et al., 2006), given that development of fetus is sensitive to direct and indirect effects of maternal nutrition at all stages between oocyte maturation to parturition (Robinson et al., 1999; Funston et al., 2010). Du et al. (2010) suggested the fetal stage is the most efficient stage to increase carcass traits and marbling in cattle given that the fetal stage is critical for skeletal muscle and intramuscular adipocyte development (Zhu et al., 2004; Yan et al., 2013).

A reduction in the number of muscle fibers permanently reduces animal performance, whereas adipocyte development provide sites for marbling formation during finishing (Du et al., 2010). Maternal nutrient restriction results in a reduction in the total number of secondary muscle fibers (Zhu et al., 2004), however, maternal overnutrition results in elevated expression of adipogenic genes in fetal skeletal muscle (Tong et al., 2008) and increased number and size of adipocytes in the skeletal muscle postnatally (Yan et al., 2011). The process of myogenesis occurs in utero, therefore postnatal muscle growth is mainly dictated by an increase in muscle fiber size without formation of new muscle fibers (Stickland, 1978). During fetal development, progenitor cells generate cells in a satellite position around developing myofibers, these are identified by the

expression of *paired box gene 7* (**Pax7**; Seale et al., 2000; Kassar-Duchossoy et al., 2005). These cells are responsible for postnatal skeletal muscle growth and repair, and are mitotically quiescent postnatally (Seale and Rudnicki, 2000; Le Grand and Rudnicki, 2007). Upon activation, satellite cells begin to cycle and coexpress Pax7 and myogenic regulatory factor *myogenic differentiation 1* (**MyoD**; Le Grand and Rudnicki, 2007). These cells then downregulate Pax7, and begin to express another regulatory factor *myogenin*, and differentiate and fuse with existing muscle fibers, contributing to muscle hypertrophy (Perdiguero et al., 2009).

Adipogenesis refers to differentiation of preadipocyte cells into adipocytes that are capable of lipogenesis as well as secretion of hormones and cytokines (Houseknecht et al., 2002). This process is initiated around mid-gestation in the ruminant, and adipogenic potency gradually declines postnatally due to depletion of multipotent cells (Du et al., 2010). The program of adipocyte differentiation and associated gene expression is coupled to *peroxisome proliferator-activated receptor gamma* (**PPAR-γ**) and *CCAAT/enhancer binding proteins α* (**C/EBPα**), which play a role in the transcription of many genes and proteins regulating the process (Houseknecht et al., 2002). Differentiation of adipocytes during the early postnatal period suggests nutritional management during this time may enhance fat deposition (Wang et al., 2009). Hence, proper fetal development and nutritional management throughout gestation is crucial to maximize growth and performance potential of animals.

The effect of maternal nutrient status during gestation can also have profound impacts on future reproductive performance of female progeny. Follicle assembly into primordial follicles is believed to begin around day 80 of gestation in cattle, and conclude by day 143 (Nilsson and Skinner, 2009). Fertility is related to ovarian characteristics, and prediction of ovarian reserve, determined through measuring antral follicle counts (**AFC**) via ultrasonography, has been used to

predict the reproductive performance and lifetime productivity of females. Specifically, research has demonstrated number of antral follicles is positively correlated with pregnancy success in beef heifers and dairy cows (Ireland et al., 2008; Cushman et al., 2009; Mossa et al., 2012). Moreover, heifers born to energy restricted dams had a reduction in AFC compared to heifers born to control-fed cohorts (Mossa et al., 2009; Sullivan et al., 2009; Mossa et al., 2013). Maternal nutrient status during gestation also results in alterations within the neuroendocrine system of the female offspring, leading to delayed puberty attainment and altered reproductive function (O'Neil et al., 2019). These findings indicate that maternal nutrition during gestation impacts reproductive development of female progeny, although the impacts of specific maternal nutrition strategies on reproductive performance of replacement heifers deserves further investigation.

2.1.1. *Dietary Factors and Developmental Programming*

Within the beef production cycle, there is the potential for cows to undergo periods of undernutrition during pregnancy or lactation due to a variety of factors including forage quality or quantity, lactation demands, and management practices (Caton and Hess, 2010). Many extensive beef cattle production systems depend on forage to provide a vast majority of nutrients, which may be variable in quality and might not be nutritionally complete (Spears, 1994). As stated previously, maternal nutritional status during gestation significantly impacts offspring growth and development (Wu et al., 2006), leading to long-term effects on performance and quality of carcass traits (Wu et al., 2006; Larson et al., 2009). Protein supplementation to cows during late gestation has been shown to increase offspring birth and weaning weights (Bohnert et al., 2013), increase postweaning rate of body weight gain (Martin et al., 2007), and enhance carcass marbling (Larson et al., 2009). Increasing dietary energy during late gestation also results in increased progeny birth body weight (Wilson et al., 2016). Moreover, energy source during gestation also affects fetal

growth. Radunz et al. (2012) reported offspring from cows supplemented with corn or dried distillers' grains with soluble were heavier at birth and tended to be heavier at weaning compared with offspring from cows fed grass hay at isocaloric intake. Producers must design their supplementation strategies according to the nutritional requirements of the animal, quantity and quality of the forage, as well as the economic benefit of the supplement. Although, other nutrients including trace minerals (Hostetler et al., 2003) are known to impact fetal development, and offer an opportunity to enhance offspring productivity.

2.1.2. ***Minerals***

The majority of research conducted has primarily focused on energy and crude protein nutrition of gestating cows, and the subsequent impacts on health, productivity, and reproduction of offspring, however, other nutrients including trace minerals impact fetal development (Hostetler et al., 2003). Most extensive beef cattle operations depend on forage to meet nutritional demands, which provide a source of minerals, vital for nearly all processes within the body. Many factors affect mineral content of forage including soil, plant species, stage of maturity, yield, climate, and management practices (Spears, 1994), and only a fraction of the minerals in soils are actually taken up by the plants, as they can grow normally when they contain less Fe, Zn, Mn, or Cu than livestock require (McDowell, 1996). Hence, in order to maintain maximum long-term productivity, cow-calf producers must design mineral supplementation programs according to animal stage and level of production as well as forage quality and quantity (Greene, 2000). Minerals are divided into two classes; macro minerals which are required in amounts > 100 ppm of the diet, and trace minerals which are required in amounts < 100 ppm of the diet, and do not change with animal stage or level of production. Although these trace minerals, such as Cu, Zn, I, Mn, Se, Co, and Fe, are present in only minute amounts, they are essential for beef cattle (NASEM, 2016). If a deficiency occurs,

metabolic processes such as tissue growth and immune function are impaired (Underwood and Suttle, 1999), and development and postnatal performance of the offspring might be altered (Hostetler et al., 2003).

The form of mineral supplementation may also affect animal performance, given that organic trace minerals in ruminant diets may be of greater bioavailability compared to their inorganic counterparts (Brown and Zeringue, 1994). Organic trace minerals differ from inorganic forms due to their chemical association with an organic ligand, as trace minerals are usually found as inorganic salts (Spears, 1996). There are five different compounds in the United States that are commercially available as organically bound mineral and they are defined by the Association of American Feed Control Officials. These compounds are: 1) a metal amino acid complex, 2) a metal amino acid chelate, 3) a metal (specific amino acid) complex, 4) a metal proteinate, and 5) a metal polysaccharide complex (AAFCO, 2000). There has been considerable interest in the use of chelated, or organic trace minerals in ruminant diets due to indications that these may be of greater bioavailability compared to their inorganic counterparts (Brown and Zeringue, 1994). Metal chelates, or complexes are stable in the digestive tract, thus protecting the metal from forming complexes with other dietary components that would inhibit absorption and allowing the greater absorption of the trace mineral, as trace minerals within the body function almost entirely as organic complexes or chelates already. In order for an animal to utilize inorganic trace minerals, the animal must first convert them to organic biologically active forms (Spears, 1996). More specifically, Miles and Henry (2000) posited the following benefits of organic trace minerals: 1) the ring structure protects the trace mineral from unwanted reaction in the digestive tract; 2) chelates can pass intact through the intestine and enter the bloodstream; 3) decreased reactions between the mineral and other nutrients enhances passive absorption; 4) the mineral is circulated

in a form similar to that found in the body; 5) absorption of chelates occurs through a different route than inorganic minerals; 6) each mineral in a chelate participates in the enhances absorption of other minerals in the chelate; 7) the negative charge of chelates facilitates efficient absorption and metabolization; 8) chelation enhances solubility and movement through membranes; 9) chelation increases water and lipid solubility of the mineral thereby increasing passive absorption; 10) chelates are more stable at low pH; and 11) the amino acid transport system can facilitate absorption of chelates. Hence, the mode of action of organic trace minerals or chelates likely differs from that of inorganic trace minerals, thus providing benefits to the animal.

2.1.3. ***Metabolism and Absorption of Cu, Co, Mn, and Zn***

2.1.3.1. *Copper*

Absorption of Cu occurs in the small intestine, and is homeostatically regulated (Underwood and Suttle, 1999). Copper crosses the brush border via transporters and within the cytosol is bound by chaperones for use within the cell or to metallothionein for storage. Within these cells, Cu attaches to albumin and travels via portal circulation to the liver. In the liver, Cu may either be bound to metallothionein for storage, incorporated into Cu-containing enzyme, or bound to ceruloplasmin and secreted from hepatocytes (Cousins, 1985). Copper deficiency in ruminant animals can occur when copper intake is insufficient or as a secondary deficiency, whereby other factors within the diet can interfere with Cu absorption or metabolism (Suttle, 1991).

The NASEM (2016) lists the dietary requirement of beef cattle for Cu as 10 mg/kg of the diet. Altered Cu status can affect a wide range of biological functions such as fertility, mitochondrial activity, bone and joint strength, immune function, and erythropoiesis (Gooneratne et al., 1989). However, the ability of a particular feedstuff to meet the Cu requirements depends

on the absorbability rather than the actual concentration of Cu within the feedstuff (Suttle, 1983), variations of which are determined by events within the rumen such as interactions between Cu and its potential antagonists; Mo, S, and Fe (Underwood and Suttle, 1999). Within the rumen, Mo and S are involved in the formation of thiomolybdate complexes which bind Cu to form insoluble compounds that are poorly absorbed (Suttle, 1991). Those complexes that are absorbed affect systemic metabolism of Cu, resulting in Cu that is tightly bound to plasma albumin and therefore not functionally available (Spears, 1994). Dietary Fe may also inhibit Cu absorption through adsorption of Cu by insoluble Fe compounds, thereby adding to the effects of Mo and S (Underwood and Suttle, 1999), indicating that high Fe concentrations in forages may contribute to the development copper deficiency in the ruminant. Hence, Cu concentrations of available forage are of little value when attempting to assess Cu adequacy unless the concentrations of Cu antagonists are also assessed (Spears, 1994).

2.1.3.2. *Cobalt*

Cobalt is essential in the ruminant diet for the production of vitamin B_{12} by rumen microbes to meet requirements of both the host animal and ruminal bacteria (Stangl et al., 2000). Within the rumen, microbes partition cobalt between vitamin B_{12} (cobalamin) and cobalamin analogs (corrinoids), which are physiologically inactive (Underwood and Suttle, 1999). Once in the abomasum, cobalamins are bound by intrinsic factor and this complex flow to the intestines where it is readily absorbed by the epithelial cells (Seetharam, 1999). Some elemental Co may also be absorbed through the intestinal tract, which along with cobalamins is transported to various tissues throughout the body, with the liver containing the highest concentration and considered the main storage site (Underwood and Suttle, 1999). Cobalamin, with Co as its functional core, is required for two enzymatic processes that are critical for metabolism; propionate utilization and methyl

transfer processes (Mills, 1987). Clinical manifestations of Co deficiency in ruminants include infertility, immune suppression, fatty liver, loss of appetite, weight loss, and in extreme cases rapid muscular wasting (Underwood and Suttle, 1999). Performance and metabolism of cattle is decreased during the feedlot finishing phase when they are fed a moderately Co-deficient diet (0.04-0.05 mg/kg of DM; Tiffany et al., 2003).

The NASEM (2016) lists the dietary requirement of beef cattle for Co as 0.10 mg/kg of the diet. Research has shown that Co deficient diets result in unstable fermentation patterns within the rumen, possibly due to shifting microbial populations (McDonald and Suttle, 1986). Furthermore, relative production of cobalamins and corrinoids are affected by diet, as diets largely composed of roughages tend to favor production of cobalamin whereas those containing larger amounts of concentrates result in greater corrinoid production (Sutton and Elliot, 1972). Data also show diet neutral detergent fiber and sugar content are positively correlated with vitamin B_{12} production and non-fiber carbohydrates and starch content are negatively correlated with vitamin B_{12} production (Schwab et al., 2006). Level of intake is also positively related to vitamin B_{12} synthesis (Zinn et al., 1987).

2.1.3.3. *Manganese*

Manganese functions as a component of metalloenzymes in several different areas of metabolism including cartilage development, blood clotting, lipid and carbohydrate metabolism, and resistance to oxidants. Like Cu, the efficiency of absorption is controlled by homeostatic mechanisms within the organism which prevent accumulation of excessive amounts of metal within the tissues (Underwood and Suttle, 1999). Manganese is absorbed from the small intestine at very small amounts and transported by transferrin to the liver (Davidsson et al., 1989). Although Mn is one of the least abundant trace elements in all livestock tissues (Underwood and Suttle,

1999), it is generally higher in tissues rich in mitochondria such as: the liver, pancreas, liver, kidney, and pituitary gland (Hidiroglou, 1979).

According to the NASEM (2016), the dietary requirement of Mn for breeding cattle is 40 mg/kg of the diet. Symptoms of Mn deficiency include: small calf birth weights, spinal deformities in newborn calves, weakness, incoordination, abortions, altered estrous cycles, and seminal tubular degeneration (Graham, 1991). Unfortunately, determining the exact cause of a Mn deficiency can be difficult because it appears that other minerals and conditions in the diet can interfere with Mn utilization (Hidiroglou, 1979). Increased levels of Ca and P to the diet have been shown to lower the absorbability of dietary Mn (Underwood and Suttle, 1999). Moreover, excretion of Mn was increased in cows concurrently with increased Ca intake, although Mn absorption was unaffected (Vagg, 1971). Competition between Fe and Mn for common binding sites in the intestinal mucosa may also occur, as increased dietary Mn concentrations have been shown to reduced serum and tissue Fe concentrations (Matrone et al., 1959). However, the main antagonist of Mn absorption is phytate, which is broken down in the rumen and eliminates this source of concern (Underwood and Suttle, 1999).

2.1.3.4. *Zinc*

Zinc plays a critical role in well over 200 metalloenzymes which are necessary for metabolism of many nutrients including proteins, nucleic acids, and carbohydrates (Spears, 1994). Zinc also plays a role in stabilization of protein and membrane structure, control of gene transcription and cell signaling, as well as DNA and RNA polymerases (Underwood and Suttle, 1999). Absorption of Zn occurs in the small intestine via transporters and within the cytosol is bound to metallothionein similar to Cu (Cousins, 1985). Zinc is absorbed according to need in ruminants (Suttle et al., 1982), and mucosal absorption is limited at high rates of Zn intake. Once

transported to the portal bloodstream, Zn is primarily bound to plasma albumin (Underwood and Suttle, 1999). Turnover rate of Zn within the body is high as the capacity to store Zn is poorly developed. Although, significant amounts of Zn may be redistributed from pools within the body during a deficient period, delaying the onset of clinical deficiency (Underwood and Suttle, 1999).

The NASEM (2016) lists the dietary requirement of beef cattle as 30 mg/kg of Zn in the diet. However, when ruminants are fed a Zn deficient diet there a rapid reduction in Zn content in many tissues, and prolonged feeding of such a diet causes a clinical deficiency (Miller, 1969). Symptoms of such a deficiency include anorexia, skin lesions, decreased growth, skeletal disorders, reproductive disturbances, as well as increased susceptibility to infection (Underwood and Suttle, 1999). When ruminants are fed a diet deficient in Zn, there is also a rapid increase in percentage of Zn absorbed from the diet and a large decrease in endogenous fecal losses. A reduction in animal growth rate as well as increase in age will decrease percentage of dietary Zn absorbed (Miller, 1969).

2.1.4. *Inorganic vs. Organic Trace Mineral Sources*

Due to the hypothesized increased bioavailability of organic trace mineral sources, ruminants may respond to supplementation in the form of enhanced growth, milk production, reproduction, and immune responses. Supporting this rationale, liver mineral status of cows has been shown to be affected by trace mineral supplementation, with those receiving organic forms of trace minerals having greater liver trace mineral concentrations compared to their unsupplemented counterparts (Ahola et al., 2004; Marques et al., 2016). Moreover, Nocek et al. (2006), found cows can be supplemented with organic trace minerals at 75% of NRC (2001) requirements with no reduction in reproductive or productive performance compared with supplementing at 100% of NRC (2001) requirements using only sulfate sources. Organic trace

mineral supplementation to dairy cows significantly increased milk production, milk fat yield, and milk protein yield compared to inorganic trace mineral supplemented cows (Kellogg et al., 2003). Additionally, improved reproductive performance has been reported in dairy cows receiving organic trace mineral supplements both pre and post-calving (Rabiee et al., 2010), possibly due to improved repair of uterine tissues. Stanton et al. (2000) also reported greater pregnancy rates to AI in cows receiving organic trace minerals than those receiving inorganic trace mineral supplements.

During gestation, the fetus is completely dependent on the dam for its supply of trace minerals (Hidiroglou and Knipfel, 1981). Inadequate maternal intake or transfer to the fetus can result in impaired fetal development and postnatal performance (Weiss et al., 1983). Given the necessity of trace minerals for processes such as protein synthesis, bone formation, lipid metabolism, and DNA synthesis, trace mineral supplementation during gestation may directly affect embryonic and fetal development (Hostetler et al., 2003). However, research examining the effects of source of trace mineral supplementation during gestation on beef cattle offspring performance is limited. No differences in puberty attainment or pregnancy status were reported for heifers born to organic supplemented dams compared to inorganic supplemented cohorts (Price et al., 2016). Although, the previous authors began supplementation approximately 82 prior to calving, well after ovarian development of the female progeny was complete (Nilsson and Skinner, 2009). Recent research from our group demonstrated that supplementing late-gestating beef cows with organic Cu, Co, Mn, and Zn enhanced offspring productivity (Marques et al., 2016). More specifically, calves born from cows supplemented with these organic trace minerals were > 20 kg heavier at weaning until slaughter and had reduced bovine respiratory disease incidence compared with calves from non-supplemented cohorts. These results were suggestive of developmental

programming effects on postnatal offspring growth and health (Funston et al., 2010). The physiological mechanism underlying these outcomes deserves further investigation, specifically when cows are provided organic trace minerals throughout a greater duration of gestation.

2.2. Metabolic Imprinting

As stated previously, developmental programming refers to the concept that perturbations during critical prenatal stages may have lasting impacts on adult growth and physiology (Barker and Clark, 1997; Wu et al., 2006). Although, organ development and tissue differentiation are not complete at birth in most mammals and continue into the early postnatal period. During this time, organisms have the ability to respond to environmental stimuli that are foreign to normal development through adaptations at the cellular, biochemical, and molecular level (Patel and Srinivasan, 2002). The term metabolic imprinting defines these biological responses to a nutritional intervention early life that permanently alters physiological outcomes later in life (Du et al., 2010). Specifically, Waterland and Garza (1999) describe metabolic imprinting as a phenomenon characterized by: 1) a susceptibility limited to a critical window in early development; 2) an effect persisting into adulthood; 3) a specific and measurable outcome; and 4) exhibiting a dose-response relation between the specific exposure and the outcome. Given the different rates of tissue differentiation and accretion, it is possible that multiple critical windows exist for metabolic imprinting.

2.2.1. *Dietary Factors and Metabolic Imprinting*

In beef cattle research, early weaning has been used to study metabolic imprinting effects, given that it allows for nutritional manipulation of animals at a young age. For example, Gasser et al. (2006) demonstrated heifers weaned at 4 months of age and receiving a high-concentrate diet

for 70 days post-weaning had accelerated puberty attainment. Additionally, Scheffler et al. (2014) reported calves weaned between 3 to 4 months of age and consuming a high concentrate diet produced heavier carcasses with enhanced marbling scores compared with forage fed, normally weaned cohorts. Similarly, Moriel et al. (2014) reported enhanced muscle expression of growth-related and adipogenic genes in steers receiving a high-concentrate diet immediately after weaning at 3 months of age, which translated into enhanced growth performance of these steers. The aforementioned studies utilized early-weaned animals as a model to study metabolic imprinting, however, early weaning may not be a feasible management alterative for many commercial cow-calf operations in the United States, where calves are typically weaned at 7 months of age (Whittier, 1995). Additionally, it may be difficult, depending on the study design, to separate the effects of supplementation and weaning.

An alternative to early weaning may be to provide supplements to calves via creep feed. Creep feeding is a tool used by cattle producers to provide additional nutrients to calves prior to weaning that may not be obtained from the nursing or forage. Most often, this results in greater calf weights upon weaning (Sexten et al., 2004; Moriel and Arthington, 2013a; Reis et al., 2015). Further, creep-fed calves exhibit enhanced dry matter intake (Moriel and Arthington, 2013b) and body weight gain during the receiving period (Arthington et al., 2008). Reis et al. (2015) reported transient and long-term increases in genes regulating adipocyte development in creep-fed heifers. Hence, creep feeding may be a strategy to provide supplements during a critical window of development, with the intent of stimulating metabolic imprinting events.

2.2.2. ***Essential Fatty Acids***

Supplemental fat has been traditionally added to ruminant diets for three reasons: 1) to add energy density to the diet, 2) to manipulate digestion and absorption of different nutrients, or 3) to

alter the fatty acid (**FA**) profile of meat or milk (Chilliard, 1993). Moreover, specific FA may impact tissue metabolism, gene expression, and synthesis of other FA and steroids (Sumida et al., 1993; Houseknecht et al., 2002). In humans and livestock, ω-3 and ω-6 FA are considered essential given they cannot be synthesized in the body (Hess et al., 2008). Specifically, ω-3 and ω-6 FA are polyunsaturated FA (**PUFA**), indicating they contain more than one double bond. The family to which a PUFA belongs depends on the parent FA from which it is synthesized. The ω-3 series are derived from linolenic acid, whereas the ω-6 series are derivatives of linoleic acid (Das, 2006). Humans and livestock lack the delta 12 and 15 desaturase enzymes required to insert a double bond at the n-6 or n-3 position of a fatty acid, hence the essentiality of ω-3 and ω-6 PUFA (Lee et al., 2016).

Dietary fats undergo extensive modification performed by the microbial population, resulting in differences between FA profile of the diet and FA entering the bloodstream from the small intestine (Jenkins et al., 2008). Upon entry to the rumen, microbial lipases hydrolyze the ester linkage in the lipids, separating the FA from their glycerol backbone, a process termed lipolysis (Funston, 2004). Following lipolysis, microbes isomerize the unsaturated FA to trans FA intermediates, followed by hydrogenation of the double bonds, resulting in saturated FA (Jenkins et al., 2008). This process of biohydrogenation results in several intermediate partially hydrogenated FA, available to flow out of the rumen to be absorbed in the small intestine. Hence, essential FA may be consumed by the ruminant, however stearic acid (a saturated FA) comprises most of the FA leaving the rumen and reaching tissues. As such, methods to protect PUFA from microbial activity within the rumen have been developed to circumvent the impacts of biohydrogenation on dietary PUFA, and to increase flow of these FA to the small intestine for absorption. Treatment processes that can achieve rumen protection of FA include formaldehyde

treatment, fatty amides, and calcium salts, with the latter being more commercially available and considerably more researched (Jenkins and Bridges, 2007). Calcium salts of FA achieve rumen protection through blocking of the carboxyl group of the FA by calcium, a bond which is stable at rumen pH. Once in the abomasum, FA are released from calcium salts through acidic dissociation, and are available for absorption in the small intestine (Jenkins and Bridges, 2007). Although, degree of protection and profile of FA reaching circulation has varied among feeding trials examining calcium salts of FA.

2.2.2.1. *Linoleic Acid.*

Dietary FA can have profound effects on gene expression in metabolic tissues, and often effects are observed in a tissue-specific manner. Further, research indicates that specific dietary FA may differentially regulate gene expression. Omega-3 PUFA prevents adipose tissue accumulation through suppression of adipocyte differentiation (Okuno et al., 1997; Raclot et al., 1997). On the other hand, ω-6 PUFA have been described as pro-adipogenic (Cleary et al., 1999; Massiera et al., 2003). Linoleic acid, an ω-6 PUFA, is converted to arachidonic acid through elongation and desaturation (Nakamura and Nara, 2004). As mentioned previously, during adipogenesis, up regulation of C/EBPα leads to increased expression of PPAR-γ, a pivotal transcription factor in adipose tissue development (Houseknecht et al., 2002). Activation of C/EBPα requires two redundant cell surface receptor-ligand systems in functional preadipocytes; the prostacyclin receptor and leukemia inhibitor factor receptor (Ailhaud, 2006). In the preadipocyte, arachidonic acid leads to synthesis of prostacyclin, which is secreted and acts externally in a paracrine/autocrine fashion (Belmonte et al., 2001). Moreover, arachidonic acid increases intracellular cyclic AMP (**cAMP**) production (Gaillard et al., 1989). This coupled with prostacyclin binding to its receptor activates the protein kinase A pathway, in part regulating the

expression of C/EBPα (Vassaux et al., 1992), and ultimately leading to increased PPAR-γ expression and adipogenesis.

Both ω-3 and ω-6 PUFA also regulate gene expression through interaction with specific transcription factors, acting as hydrophobic hormones to regulate gene expression by binding directly to receptors (Heuvel, 1999; Price et al., 2000; Houseknecht et al., 2002). Endogenous dietary fatty acids and their metabolites are similar in structure to peroxisome proliferators, the fatty-acid like chemicals responsible for activating PPAR-γ (Heuvel, 1999). That is, they contain a carboxylic acid functional group and a hydrophobic tail and are therefore able to convert PPAR-γ into a transcriptionally active complex. Research has demonstrated that certain FA, including linoleic and arachidonic acid and their metabolites bind directly to PPAR-γ at physiologic concentrations (Kliewer et al., 1995; Kliewer et al., 1997). Hence, an increase in circulating linoleic acid could lead to an increase in expression of PPAR-γ, and ultimately adipogenesis.

2.3. Potential Mechanisms

According to Waterland and Garza (1999), nutrition can permanently affect an organisms' structure or function through a variety of routes, although alterations in cell number and epigenetics appear to be the most prominent mechanisms. Rate of cellular proliferation and growth is tissue specific, indicating that different tissues may have different windows of hyperplastic growth (Waterland and Garza, 1999). Nutrient deprivation or excess might affect rates of cell division, leading to permanent changes in cell number.

Additionally, the process of developmental programming and metabolic imprinting can occur through epigenetics (Wu et al., 2006), which refers to alterations in gene expression resulting from changes in chromatin structure, leading to gene silencing or activation occurring independent

of changes in DNA sequence (Funston and Summers, 2013). This can result in heritable variations in gene expression (Delcuve et al., 2009). More specifically, epigenetic mechanisms elicited by nutritional factors include DNA methylation, histone modifications, and non-coding microRNAs (**miRNA**; Canani et al., 2011). These changes can alter the postnatal growth and performance trajectory of the offspring (Wu et al., 2006).

Changes in DNA methylation, are an essential part of normal development, and most mammalian DNA is methylated (Canani et al., 2011). Specifically, methylation refers to the addition of a methyl group by DNA methyltransferase at sites located at cytosine bases followed by a guanosine (**CpG**; Holliday and Grigg, 1993). Most of these sites are methylated, however specific CpG-rich areas within the genome, known as CpG islands are not methylated and are often associated with the promotor region of a gene (Simmons, 2011). Methylation of DNA is negatively associated with gene expression such that hypermethylation of DNA results in highly condensed heterochromatin, unavailable to transcriptional machinery and therefore transcriptionally silent, whereas the opposite results in transcriptionally active euchromatin (Waterland and Garza, 1999; Simmons, 2011).

Histones are also key players in epigenetics, given that histone tails protruding from the globular core of packaged DNA are subject to posttranslational modification (McKay and Mathers, 2011). These modifications include methylation, acetylation, phosphorylation, and ubiquitination of amino-terminal histone tails (Portela and Esteller, 2010). Generally speaking, acetylation induces transcription activation, whereas the opposite leads to transcription repression (Waterland and Garza, 1999). Methylation, phosphorylation, and ubiquitination, however, have been associated with both transcription activation and repression (Sun and Allis, 2002; Portela and Esteller, 2010). Furthermore, the existence of multiple modifications within a short stretch of the

same histone tail suggests precise combinations of modifications leads to specific activation or repression effects (Peterson and Laniel, 2004).

Another factor regulating epigenetic changes are miRNA, which are usually 22 nucleotides in length and bind to target mRNA impacting stability and translation of target mRNA (Canani et al., 2011). Therefore, these molecules are involved in many cellular processes including development, differentiation, and metabolism. Each mRNA may be regulated by more than one miRNA, and each miRNA is predicted to have numerous targets (Lewis et al., 2003). Moreover, miRNA have effects on DNA methylation and histone modification, and vice versa, creating a highly controlled feedback mechanism (Chuang and Jones, 2007).

2.4. Nutritional Strategies to Enhance Beef Cattle Productivity

Nutritional manipulation during periods of developmental plasticity such as the embryonic, fetal, and neonatal periods exerts long term effects on skeletal muscle and adipose tissue development, health, and overall performance of offspring (Wu et al., 2006; Patel and Srinivasan, 2011). Identifying specific nutritional strategies that are targeted at these critical periods of development provide a unique opportunity to optimize efficiency and profitability of beef cattle systems. Improved skeletal muscle development and adipogenesis in skeletal muscle enhances growth performance and carcass marbling upon slaughter (Du et al., 2010), traits which benefit carcass prices (USDA, 1997), and beef palatability. Moreover, development of replacement beef heifers is a critical component of cow-calf systems, and production efficiency increases with improved female longevity (Roberts et al., 2015).

Research from our group has demonstrated that supplementation of dietary organic Co, Cu, Mn, and Zn during the last trimester of gestation resulted in heavier offspring at weaning, and this

advantage was maintained until slaughter (Marques et al., 2016). Furthermore, calves from cows supplemented with organic Co, Cu, Mn, and Zn had reduced incidence of bovine respiratory disease compared with inorganic or unsupplemented cohorts. These results were suggestive of a developmental programming effect on postnatal offspring health and productivity, although the biological mechanisms underlying these results were not explored. Furthermore, supplementation with organic trace minerals throughout the entire duration of gestation may have consequences on female progeny reproductive development.

Nutritional intervention during the early postnatal period also has the potential to permanently alter physiological outcomes later in life, via metabolic imprinting (Du et al., 2010). Cooke et al. (2011) and Mangrum et al. (2016) demonstrated that CSSO supplementation to beef steers during a 28-d preconditioning period or 110 d after weaning, respectively, increased carcass marbling upon slaughter. These studies suggest CSSO supplementation may stimulate metabolic imprinting events related to carcass marbling in cattle. However, younger animals are more responsive to metabolic imprinting events (Lucas, 1991), whereas the aforementioned authors utilized steers older than 5 months of age. Therefore, research is warranted to investigate the effects of CSSO supplementation to young beef steers, during this period of developmental plasticity on growth and carcass characteristics.

To address these gaps in knowledge, two experiments were conducted evaluating 1) source of trace mineral supplementation to beef cows during gestation on performance and physiological responses of the offspring; and 2) supplementation with CSSO to nursing beef steers on carcass development and quality. Results from these experiments are reported and discussed in the following chapters.

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3. EFFECTS OF ORGANIC OR INORGANIC COBALT, COPPER, MANGANESE, AND ZINC SUPPLEMENTATION TO GESTATING BEEF COWS: I, PHYSIOLOGICAL AND PRODUCTIVE RESPONSES OF THE COW AND OFFSPRING

3.1. Introduction

Maternal nutrition is a major extrinsic factor programming nutrient partitioning and development of fetal organ systems associated with health, production, and reproduction (Long et al., 2009; Long et al., 2010), leading to long-term effects on offspring performance. The majority of research to date has focused primarily on energy and CP intake of gestating beef cows (Bohnert et al., 2013; Wilson et al., 2016); however, little is known about the effects of trace mineral status of gestating cows on offspring productivity. The fetus is completely dependent on the dam for its supply of trace minerals (Hidiroglou and Knipfel, 1981), which are essential for fetal developmental processes such as protein synthesis, bone formation, lipid metabolism, and DNA synthesis (Hostetler et al., 2003). Furthermore, inadequate maternal intake or transfer of trace minerals to the fetus can result in impaired fetal development and postnatal performance (Weiss et al., 1983).

One strategy to enhance trace mineral status in cattle is to feed organic complexed sources (Spears, 1996). There has been considerable interest in the use of organic trace minerals in ruminant diets due to indications that these may be of greater bioavailability compared to their inorganic counterparts (Brown and Zeringue, 1994). For example, Hostetler et al. (2003) reported that Cu, Mn, and Zn concentrations in fetal tissues collected from sows supplemented with organic sources of these elements were greater compared with fetal tissues from sows supplemented with inorganic sources, resulting in reduced fetal loss by d 30 of gestation. Recent research from our

group demonstrated that supplementing late-gestating beef cows with organic Co, Cu, Mn, and Zn enhanced offspring productivity (Marques et al., 2016). More specifically, supplementing beef cows with organic or sulfate sources of Co, Cu, Mn, and Zn increased cow liver concentrations of Co, Cu, and Zn compared with non-supplemented cows. However, liver Cu and Zn concentrations in the neonatal calf were only increased in organic vs. non-supplemented cows. Calves born from cows supplemented with organic trace minerals were also > 20 kg heavier at weaning compared to calves born from non-supplemented cows (Marques et al., 2016). Collectively, these results were suggestive of fetal programming effects of organic trace mineral supplementation on postnatal offspring growth and health (Funston et al., 2010). However, the physiological mechanisms underlying these outcomes still warrants investigation.

Nutritional management of beef cows impacts fetal development throughout the entirety of gestation (Wu et al., 2006), whereas Marques et al. (2016) investigated supplementation during the last trimester of gestation. Hence, organic trace mineral supplementation may be even more beneficial to offspring health and performance if offered to beef cows over a greater duration of gestation. Based on the results reported by Marques et al. (2016) and the rationale presented above, we hypothesized that organic trace mineral supplementation to gestating beef cows would improve cow production responses and offspring productivity via programming effects. To test this hypothesis, this experiment compared performance and physiological responses of offspring from beef cows supplemented with organic or sulfate sources of Co, Cu, Mn, and Zn.

3.2. Materials and Methods

This experiment was conducted at the Texas A&M – Beef Cattle Systems (College Station, TX, USA). All animals were cared for in accordance with acceptable practices and experimental

protocols reviewed and approved by the Texas A&M – Institute of Animal Care of Use Committee (#2018/0093). This paper is one of two companion papers addressing the impacts of supplementing organic or sulfate sources of Co, Cu, Mn, and Zn. The current paper addresses pre- and postpartum responses of the dam as well as responses of the progeny from birth until weaning, whereas the companion paper (Chapter 4) addresses the post-weaning responses of the male and female progeny managed as replacement heifers or feeder cattle.

3.2.1. *Cow Management and Dietary Treatments*

One hundred and ninety non-lactating, pregnant beef cows [average ¾ *Bos taurus* and ¼ *Bos indicus*; 138 multiparous, 52 primiparous, initial body weight (**BW**) = 509.3 ± 5.7 kg; age = 4.6 ± 0.2 yr; initial BCS = 5.5 ± 0.1 according to Wagner et al. (1988)] were assigned to this experiment at 117 ± 2.2 d of gestation (d 0 of the experiment). Cows were pregnant to AI using semen from a single Brangus sire (n = 83) or pregnant to 6 Brangus bulls via natural service (n = 107; cows were exposed to bulls for 40 d beginning 15 d after AI), according to the breeding management and pregnancy diagnosis described by Oosthuizen et al. (2020). At the beginning of the experiment (d 0), pregnancy length was expected to be 141 ± 1.8 d for cows pregnant to AI and 217 d or less for cows pregnant via natural service.

Prior to the beginning of the experiment (d – 30), cows were ranked by pregnancy type (AI or natural service), parity, BW, and BCS, and assigned to receive diets that contained 1) sulfate sources of Cu, Co, Mn, and Zn (**INR**; custom blend manufactured by Anipro Xtraformance Feeds, Pratt, KS; n = 95) or 2) organic complexed source of Cu, Co, Mn, and Zn (**AAC**; Availa 4; Zinpro Corporation, Eden Prairie, MN; n = 95). The AAC trace mineral source was based on a metal:AA complex ratio of 1:1 for Zn, Cu, and Mn in addition to cobalt glucoheptonate (Zinpro Corporation). Diets were isocaloric and isonitrogenous and formulated to meet requirements for macrominerals,

Se, I, and vitamins (Table 3.1) for pregnant cows (NRC, 2000). The INR and AAC sources were mixed with dried distillers grain and formulated to provide the same daily amount of Cu, Co, Mn, and Zn (based on 7 g/cow daily of Availa 4; Marques et al., 2016) as described in Table 3.1. Cows were maintained in a single pasture dominated by bermudagrass (*Cynodon spp*.) and were supplemented with 12 kg of sorghum-sudangrass hay and 0.76 kg of pasture supplement cubes daily from d 93 until calving. Upon calving, cow-calf pairs were moved to an adjacent pasture dominated by perennial ryegrass (*Lolium perenne L*.). Nutritional profile of feedstuffs is provided in Table 3.2.

From d 0 of the experiment until calving, cows were gathered 3 times weekly (Tuesdays, Thursdays, and Saturdays) and individually sorted into 1 of 24 feeding pens (1 cow per pen; 6 × 9 m). Cows individually received treatments (0.5 kg of treatment/feeding, dry matter basis) and returned to pasture after their treatment was completely consumed. This process was repeated until all cows had been individually sorted into pens and had consumed their treatments. Immediately after calving, cow-calf pairs were removed from the pasture and were assigned to the general management of the research herd, which included free-choice inorganic trace mineral supplementation (Producers Special Pasture Mineral; Producers Cooperative Association, College Station, TX; containing 14% Ca, 7% P, 13% NaCl, 5% Mg, 9,900 mg/kg Zn, 2,500 mg/kg Cu, 100 mg/kg I, 4,000 mg/kg Mn, 26 mg/kg Se, 91 IU/g of vitamin A, 10 IU/g of vitamin D3, and 0.05 IU/g of vitamin E). Male calves were castrated using an elastic bander at 43 ± 0.5 d of age and all calves received vaccination against respiratory viruses (Triangle 5; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA) and Clostridium (Covexin 8; Merck Animal Health, Omaha, NE) on d 345 of the experiment. Cows were assigned to the same reproductive management (d 214 to d 287) and pregnancy diagnosis (d 345 of the experiment) described by Oosthuizen et al. (2020).

3.2.2. ***Sampling***

3.2.2.1. *Feedstuffs*

Samples of all ingredients fed to gestating cows were collected before the beginning of the experiment and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of crude protein (method 984.13; AOAC, 2006), acid detergent fiber (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY: AOAC, 2006), and neutral detergent fiber (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.), and macro- and trace minerals using inductively coupled plasma emission spectroscopy (Sirois et al., 1991), as well as Se according to method 996.16 of the AOAC (2006). Calculations for TDN used the equation proposed by Weiss et al. (1992), whereas calculations for NEm and NEg used the equations proposed by NRC (2000).

3.2.2.2. *Cows and Newborn Calves*

Prior to the beginning of the experiment (d – 30) and 2 wk before the beginning of the estimated calving season (d 97), individual BW and BCS (Wagner et al., 1988) were recorded from all cows and a liver biopsy was performed via needle biopsy (TruCut biopsy needle; CareFusion Corporation, San Diego, CA) as in Marques et al. (2016) from a subset of cows (n = 60 per subset; 30 cows/treatment each sampling). Upon calving, cow BW and BCS were recorded from all cows. Additionally, a liver sample was collected (Marques et al., 2016), while a colostrum sample was collected via hand milking (100 mL) from a subset of cows (n = 30 cows/treatment). Concurrently with cow post-calving sampling, calf birth BW and gender were recorded. Furthermore, a liver sample was collected (Marques et al., 2016), and biopsy of the *longissimus muscle* (**LM**) was performed as in Schubach et al. (2019) from calves born from cows preselected for sampling (n =

30 calves/treatment). Cows and calves were sampled immediately after calving was identified and completed, although cows that calved at night and their calves were sampled at first light the next morning, but within 8 h from calving. Another liver sample was collected from sampled calves 24 h after birth (Marques et al., 2016). When feasible, the expelled placenta was retrieved and immediately rinsed with nanopure water for 5 min, with a total of 32 placentas retrieved (n = 16/treatment). All placentas were expelled within 12 h after calving; therefore, were not considered retained fetal membranes (Takagi et al., 2002). The 5 largest cotyledons were dissected from each placenta using curved scissors, given that the largest cotyledons are expected to be the most active regarding nutrient transfer from the dam to the fetus (Senger, 2003). Cotyledons from each placenta were pooled and stored at -80°C.

Milk production was estimated via the weigh-suckle-weigh method (Aguiar et al., 2015) when cows were 42 ± 0.5 days postpartum. More specifically, calves were separated from their dams for 8 h, weighed, allowed to suckle for 30 min, and were then weighed again. Milk yield was calculated as the difference in pre- and post-suckling calf BW and was adjusted to 24 h by multiplying by 3 h. Fresh milk samples were manually collected from a subset of cows (n = 30 cows/treatment), and liver samples were collected from sampled cows and their calves (Marques et al., 2016) when cows were 43 ± 0.5 days postpartum.

3.2.2.3. *Weaning*

Cow BW and BCS (Wagner et al., 1988) were recorded at weaning (d 367). Calf BW was recorded over 2 consecutive days after weaning (d 367 and 368). A subset of cow-calf pairs were selected for tissue collection (n = 30 pairs/treatment), where liver samples were collected from both cows and their calves (Marques et al., 2016), and biopsy of the LM was performed in calves (Schubach et al., 2019).

3.2.3. ***Laboratorial Analyses***

Liver, cotyledon, colostrum, and milk samples were analyzed via inductively coupled plasma mass spectrometry for concentrations of Co, Cu, Mn, and Zn by the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI) according to Braselton et al. (1997). Additional liver and muscle samples were stored in 2-mL tubes containing 1 mL of RNA stabilization solution (RNAlater, Ambion, Inc., Austin, TX) and stored at -80°C until further processing. Total RNA was extracted from tissue samples using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop Lite; Thermo Fisher Scientific, Wilmington, DE) at 260 nm and 260/280 nm ratio; respectively (Fleige and Pfaffl, 2006). Reverse transcription of extracted RNA and real-time reverse-transcription polymerase chain reaction (PCR) using gene specific primers (20 pM each; Table 3.3) were completed as described by Rodrigues et al. (2015). Responses from the genes of interest were quantified based on the threshold cycle (CT), the number of PCR cycles required for target amplification to reach a predetermined threshold. The CT responses from liver genes of interest were normalized to the geometrical mean of CT values of *ribosomal protein L12* and *cyclophilin*, whereas the CT responses from muscle genes of interest were normalized to the geometrical mean of CT values of *ribosomal protein S9* and *β-actin* (Vandesompele et al., 2002). The CV for the geometrical mean of reference genes across all liver and LM samples were 3.0 and 2.6%, respectively. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

3.2.4. ***Statistical Analysis***

All cow and calf variables were analyzed with cow as the experimental unit, and cow(treatment × parity) as the random variable. Quantitative data were analyzed using the MIXED

procedure of SAS (SAS Inst. Inc., Cary, NC), whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). All data were analyzed using gestation days receiving treatment as an independent covariate, and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Model statements for cow-related responses included the effects of treatment, parity, and the treatment × parity interaction. Model statements for calf-related responses, and placental cotyledons analysis included the effects of treatment, calf sex, and the treatment × calf sex interaction. Calf liver samples collected at birth and 24 h after birth were analyzed as repeated measures, using day as fixed effect, and all resultant interactions with treatment and calf sex. Nonetheless, results for liver analyses are reported in tables according to sampling days to facilitate assessment and interpretation. The subject for the repeated statement was cow(treatment × parity) and the covariance structure utilized was autoregressive which provided the best fit according to the lowest Akaike information criterion. No treatment × parity interactions were significant therefore, results are reported across parities as least square means and separated using least square differences. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

3.3. Results and Discussion

Nutrient composition and profile of treatments offered to INR- and AAC-supplemented cows are described in Table 3.1. Both diets provided adequate amounts of macronutrients and trace minerals, based on the requirements of pregnant cows (NRC, 2000). Moreover, it is important to note that the minimum requirements for Cu, Mn, and Zn were exceeded by nearly 200% whereas the minimum requirements for Co were exceeded by over 2,000% for both INR and AAC treatments based on NRC requirements (NRC, 2000), as in Marques et al. (2016). Hence, results

from this experiment should not be associated with differences in trace mineral intake or a trace mineral deficiency, but rather the potential impacts of supplemental organic Co, Cu, Mn, and Zn intake by AAC cows.

3.3.1. ***Cow Parameters***

Cow age at the beginning of the experiment, as well as length of treatment administration did not differ ($P \geq 0.61$) between INR and AAC cows (Table 3.4). As per the experimental design, initial cow BW and BCS were also similar ($P \geq 0.35$) between treatments (Table 3.4). Cows receiving AAC had greater ($P = 0.04$; Table 3.4) pre-calving BW compared to INR cows; however, this difference was insufficient to impact pre-calving BCS, calving BW, or calving BCS, which did not differ ($P \geq 0.31$) between treatments. These outcomes were expected given that AAC and INR supplemented cows were managed as a single group from d 0 until calving. Others have also reported that Cu, Co, Mn, and Zn supplementation, either as organic or inorganic sources, failed to substantially impact BW and BCS during gestation in cows receiving diets with adequate contents of these elements (Stanton et al., 2000; Ahola et al., 2004; Marques et al., 2016).

No treatment differences were detected ($P \geq 0.18$) between INR and AAC for initial (d –30) liver Cu, Mn, and Zn concentrations (Table 3.5); however, initial liver Co concentrations were greater ($P < 0.01$) for INR vs. AAC cows. Nonetheless, both treatments had adequate Co, Cu, Mn, and Zn liver status before the beginning of the experiment (Kincaid, 2000). In pre-calving (d 97) samples, liver concentrations of Co were greater ($P < 0.01$) for AAC compared to INR cows, whereas liver concentrations of Cu were greater ($P < 0.01$) for INR compared to AAC cows. No treatment differences were detected ($P \geq 0.30$) for pre-calving liver Mn or Zn concentrations (Table 3.5). These results are similar to those reported by Marques et al. (2016) for inorganic and organic supplemented cow trace mineral liver concentrations prior to calving. At calving, INR

supplemented cows had greater ($P \leq 0.01$) liver Cu and Zn concentrations compared to AAC cows, whereas no treatment differences were detected ($P \geq 0.11$) for liver Mn or Co concentrations (Table 3.5). Liver concentrations are often used as the standard for assessing mineral status in cattle, however, research suggests that a significant portion of some trace minerals may be stored to a greater degree in other body tissues, such as Mn, and Zn in kidney and bone (Underwood and Suttle, 1999). Nevertheless, organic trace mineral forms are expected to have enhanced absorption, retention, and biological activity compared with sulfate minerals (Spears, 1996; Hostetler et al., 2003), only liver Co supported this rationale in the pre-calving analysis. The effect of supplementing organic Zn, Cu, and Co on liver mineral status in beef cows has been variable (Stanton et al., 2000; Ahola et al., 2004; Arthington and Swenson, 2004; Marques et al., 2016), supporting the inconsistency in treatment effects detected for Cu, Co, and Zn in samples collected both pre-calving (d 97) and at calving in INR and AAC supplemented cows. Nonetheless, both treatments had adequate Co, Cu, Mn, and Zn liver status before and at calving (Kincaid, 2000), corroborating that INR and AAC diets exceeded the recommended amount of these trace minerals for gestating beef cows (NRC, 2000).

No treatment differences were detected ($P \geq 0.51$; Table 3.6) for liver mRNA expression of genes associated with Cu and Zn metabolism in samples collected prior to treatment administration (d -30) or prior to calving (d 97). More specifically, *Cu-transporter protein* (**CUT**) is associated with Cu transport into hepatic cells and distribution of Cu into cellular organelles (Prohaska and Gybina, 2004; Han et al., 2009). *Metallothioneins* (**MT**) are a superfamily of intracellular metal binding proteins, most abundantly found in the liver, kidney, intestine, and pancreas (Coyle et al., 2002). Induction of hepatic MT synthesis is triggered by a variety of metals, although Zn is the primary physiological inducer, and it is proposed that regulation of MT

expression controls free Zn concentration as well as that of Zn transporter proteins (Coyle et al., 2002; López-Alonso et al., 2005). *Copper-Zinc-superoxide dismutase* (**SOD**) is an enzyme located in the cytoplasm, nucleus, or mitochondrial membrane that, using Cu or Zn as cofactors, functions to eliminate superoxide anion radicals thereby protecting cells from oxidative damage (Sturtz et al., 2001; Miao and St. Clair, 2009). At calving, MT expression was greater in AAC vs. INR cows ($P = 0.02$), whereas no treatment differences were detected for mRNA expression of CUT or SOD ($P \geq 0.19$; Table 3.6). The effect of liver Cu status on hepatic CUT mRNA expression has been variable (Bauerly et al., 2005; Fry et al., 2013), corroborating lack of treatment effects detected for CUT mRNA expression prior to calving and at calving, despite treatment differences in liver Cu concentrations. Furthermore, research has demonstrated that hepatic MT expression may be an indication of Zn bioavailability, as local Zn levels dictate expression (Wang et al., 2012); however, Zn concentration in the liver was greater for INR vs. AAC-supplemented cows. Moreover, previous research demonstrated hepatic SOD mRNA expression is reduced in Cu deficient cattle (Hansen et al., 2008), further indicating both treatments had adequate Cu liver status at the time of calving. Nevertheless, the impacts of supplemental AAC Co, Cu, Mn, and Zn on these liver genes remains unclear.

By design, all cows had similar ($P = 0.69$) days postpartum when assessed for milk production (Table 3.7). No treatment differences were detected ($P \geq 0.17$) for concentrations of Co, Cu, Mn, and Zn in the liver when cows were assessed for milk production, whereas no treatment differences were detected for milk yield and mineral composition (Table 3.5; $P \geq 0.19$). These results differ from those reported in dairy cows (Kellogg et al., 2003; Nocek et al., 2011), whereas milk production was increased with organic trace mineral supplementation. Although the

previous authors provided organic trace minerals throughout the entire lactation whereas cows in the present study were removed from treatments upon calving.

No treatment effects were detected for cow BW (P = 0.48) or liver Co, Mn, or Zn concentrations at weaning ($P \geq 0.72$; Table 3.5), whereas INR cows had greater BCS at weaning compared with AAC supplemented cows ($P < 0.01$; Table 3.4). However, a treatment difference was detected for liver Cu concentrations, where INR cows had greater ($P < 0.01$) liver Cu concentrations compared to AAC cows (Table 3.4). No treatment effects were detected ($P \geq 0.41$) for pregnancy rates to AI, natural service, or overall pregnancy rates (AI +natural service; Table 3.4). These results can be attributed to the similar nutritional management that both INR and AAC cows received from calving until weaning, and indicate that Co, Cu, Mn, and Zn supplementation during gestation as organic or inorganic sources, did not impact post calving cow reproductive performance (Stanton et al., 2000; Marques et al., 2016) despite treatment differences detected for BCS at weaning.

3.3.2. ***Calf Birth and Weaning Parameters***

In the placental cotyledons (Table 3.8), no treatment differences were detected ($P \geq 0.46$) for Co, Cu, Mn, or Zn concentrations. In addition, no treatment differences were detected ($P \geq 0.67$) for liver Co, Cu, Mn, or Zn liver concentrations in calves at birth or 24 h after birth, although day effects were detected ($P < 0.01$) for liver concentrations of Co, Cu, Mn, and Zn where these elements decreased in concentration from birth until 24 h after birth across treatments (Table 3.8). This was expected, given that the bovine neonate depends heavily on liver stores of trace minerals, specifically Cu, for postnatal utilization due to low concentrations of these elements in milk (Underwood and Suttle, 1999). Corroborating these results, no treatment differences were detected for concentrations of Cu, Co, Mn, and Zn in colostrum collected from cows ($P \geq 0.40$; Table 3.7).

Given that the fetus relies completely on the dam for proper supply of trace minerals (Hidiroglou and Knipfel, 1981), lack of treatment effects detected for cotyledon and calf liver trace mineral concentrations indicates transfer of these elements from maternal to fetal tissues was not enhanced when the AAC diet was provided to gestating beef cows compared to the INR diet (Hostetler et al., 2003).

No treatment effects were detected ($P \geq 0.15$) for mRNA expression of CUT, IGF-I, MT, or SOD in liver of calves at birth or 24 h after birth (Table 3.9). Day effects were observed ($P < 0.01$) for calf liver mRNA expression of CUT and MT, both of which increased 24 h after birth, reflecting the greater activity in hepatic tissue and utilization of trace minerals in the neonate (Underwood and Suttle, 1999; López-Alonso et al., 2005; Han et al., 2009). No treatment differences were detected ($P \geq 0.29$) for mRNA expression of genes associated with adipogenic or muscle development activities in the LM at birth or at weaning (Table 3.10). More specifically, *myogenin* is a regulatory factor in the LM that influences postnatal muscle growth through differentiation and fusion of satellite cells with existing fibers (Le Grand and Rudnicki, 2007; Du et al., 2010). These cells, both quiescent and activated, are marked by expression of paired box gene 7 (**PAX7**), which is necessary for satellite cell specification and survival (Seale et al., 2000; Li et al., 2011). Trace minerals, such as Zn, have been proposed to be required for muscle differentiation through activation and proliferation of satellite cells (Petrie et al., 1996; Ohashi et al., 2015), although trace mineral source provided during gestation did not impact genes involved in myogenesis herein. Peroxisome proliferator-activated receptor gamma (**PPAR-γ**), plays a pivotal role in adipocyte differentiation and associated gene expression (Houseknecht et al., 2002), the process of which is initiated around mid-gestation in the ruminant (Du et al., 2010). A target of PPAR-γ, adipocyte fatty acid-binding protein (**FABP4**), is also highly involved in

differentiation of adipocytes, acting as an intracellular fatty acid chaperone (Michal et al., 2006). Accordingly, several Zn-finger proteins, stabilized by one or more Zn ions, participate in adipocyte determination and differentiation (Wei et al., 2013), whereas Zn has been shown to enhance adipogenesis *in vitro* (Tanaka et al., 2001), demonstrating the importance of trace minerals in adipose tissue development. Nevertheless, supplementing gestating beef cows with AAC had no effect on genes involved with adipogenesis in the LM of calves at birth or at weaning compared to INR supplemented cohorts.

No treatment differences were detected ($P \geq 0.11$) for liver concentrations of Co, Cu, Mn, and Zn in the liver of calves when cows were assessed for milk production (Table 3.7). In addition, no treatment effects were detected ($P \geq 0.26$) for calving rate, calf birth BW (adjusted or not; BIF, 2010), or kilograms of calf born per cow assigned to the experiment (Table 3.11). Previous research has also failed to demonstrate impacts of organic trace mineral supplementation of gestating beef cows on calf birth BW (Stanton et al., 2000; Marques et al., 2016). Hence, the AAC diet did not impact fetal growth, despite treatment differences detected for cow liver trace mineral concentrations. At weaning, no treatment differences were detected ($P \geq 0.12$) for weaning rate, proportion of male calves weaned, weaning age, calf weaning BW (adjusted or not; BIF, 2010), or kilograms of calf weaned per cow (Table 3.11). Nonetheless, the effect of inorganic or organic trace mineral supplementation to gestating beef cows on offspring weaning BW has been inconsistent (Stanton et al., 2000; Ahola et al., 2004; Marques et al., 2016). Furthermore, no treatment differences were detected ($P \geq 0.17$) for liver concentrations of Co, Cu, Mn, or Zn in calves upon weaning (Table 3.8). No treatment differences were detected ($P \geq 0.26$) for mRNA expression of CUT, IGF-1, or MT in the liver of calves at weaning, although mRNA expression of SOD was greater ($P = 0.04$) in AAC calves compared with INR calves (Table 3.9). The impacts

of trace mineral status and hepatic gene expression on calf performance after weaning is discussed in the following chapter.

3.4. Overall Conclusions

In conclusion, supplementing gestating beef cows with organic sources of Co, Cu, Mn, and Zn increased cow liver concentrations of Co, and altered hepatic trace mineral metabolism and gene expression compared with INR cohorts. Liver trace mineral status was not altered in the neonatal calf when cows were supplemented with organic sources of Co, Cu, Mn, and Zn; however, the liver is not the absolute indicator of trace mineral status in livestock (Underwood and Suttle, 1999). Moreover, at weaning, calves born to AAC supplemented cows had greater hepatic mRNA expression of genes associated with antioxidant activity and mineral metabolism. The physiological mechanisms underlying these effects, as well as the specific role of each trace mineral supplemented herein on developmental programming, still warrants further investigation. Nonetheless, results from this experiment are novel, and suggest that supplementing gestating beef cows with an organic complexed source of Co, Cu, Zn, and Mn in place of sulfate sources may impact offspring hepatic metabolism and may be a viable alternative to inorganic supplementation.

3.5. References

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Table 3.1. Ingredient composition and nutrient profile of diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**) or organic complexed sources of Co, Cu, Mn, and Zn (**AAC**)

Item	INR	AAC
Ingredients, g/d (as-fed basis)		
Dried distillers grains	193	193
Macromineral mix[1]	60	60
Inorganic trace mix[2]	4.21	0
Organic trace mix[3]	0	7
DM intake, g/d	233.5	236.5
Nutrient profile[4] (DM basis)		
Net energy for maintenance[5], Mcal/kg	2.18	2.18
Crude protein, %	36.6	36.6
Ca, %	4.53	4.53
P, %	2.93	2.90
Mg, %	0.64	0.63
K, %	1.13	1.12
Na, %	1.90	1.93
S, %	0.77	0.73
Co, mg/kg	43.35	66.71
Cu, mg/kg	626.66	601.15
Fe, mg/kg	1870.97	1947.69
Mn, mg/kg	1001.72	965.14
Se, mg/kg	66.65	68.73
Zn, mg/kg	1745.88	1695.07

[1]Containing (DM basis) 339.7 g/kg $CaHPO_4$, 312.7 g/kg $CaCO_3$, 197.5 g/kg NaCl, 39.1 g/kg KCl, 18.9 g/kg MgO, 4.0 g/kg Fe_2O_3, 2.5 g/kg S, 0.9 g/kg Vit E, 0.8 g/kg Na_2O_3Se 3%, 0.7 g/kg Vit A, 0.1 g/kg $C_2H_{10}I_2N_2$, and 0.1 g/kg Vit D 400.

[2]Containing (DM basis) 420 g/kg ground corn, 213 g/kg $ZnSO_4$, 133 g/kg $MnSO_4$, 106 g/kg $CuSO_4$, and 8 g/kg $CoSO_4$.

[3]Availa 4 (Zinpro Corporation; Eden Prairie, MN), which contained (DM basis) 5.15% Zn from 1:1 Zn and AA complex, 2.86% Mn from 1:1 Mn and AA complex, 1.80% Cu from 1:1 Cu and AA complex, and 0.18% Co from cobalt glucoheptonate.

[4]Values obtained via wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

[5]Calculated with equations described by the NRC (2000).

Table 3.2. Nutritional profile of feedstuffs offered to cows[1,2]

Item	Pasture			
	A	B	Cubes	Sorghum-Sudan Hay
Total digestible nutrients, %	58	69	80	53
Net energy for maintenance, Mcal/kg	1.16	1.62	2.00	1.00
Net energy for growth, Mcal/kg	0.59	1.02	1.33	0.44
Crude protein, %	10.7	20.5	48.0	11.8
Neutral detergent fiber, %	57.7	19.6	24.0	61.5
Ca, %	0.98	2.07	0.32	0.76
P, %	0.27	0.32	1.26	0.25
Mg, %	0.29	0.41	0.67	0.31
K, %	1.62	2.72	1.86	4.11
Na, %	0.02	0.05	0.28	0.02
Co, mg/kg	1.61	3.05	1.10	0.69
Cu, mg/kg	8	12	15	11
Fe, mg/kg	3855	5120	303	1566
Mn, mg/kg	106	144	36	54
Se, mg/kg	0.40	0.20	0.19	0.12
Zn, mg/kg	42	35	66	57

[1]Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). Total Digestible nutrients were calculated according to equations described by Weiss et al. (1992). Net energy for maintenance and growth were calculated with equations described by the NRC (2000).

[2]Cows were maintained in a single pasture (A) dominated by bermudagrass (*Cynodon spp.*) and were supplemented with 12 kg of sorghum-sudangrass hay and 0.76 kg of pasture supplement cubes daily from d 93 until calving. Upon calving, cow-calf pairs were moved to an adjacent pasture (B) dominated by perennial ryegrass (*Lolium perenne L.*)

Table 3.3 Primer sequences, accession number, and reference for all gene transcripts analyzed by real-time reverse transcription PCR

Target[1]	Primer sequence	Accession nº	Source
Liver			
CUT			
Forward	GGGTACCTCTGCATTGCTGT	NM_001100381	Han et al. (2009)
Reverse	ATGGCAATGCTCTGTGATGT		
MT			
Forward	ATCCGACCAGTGGATCTGCTTTGCC	NM_001040492.2	Gessner et al. (2013)
Reverse	AGACACAGCCCTGGGCACACT		
SOD			
Forward	TGTTGCCATCGTGGATATTG	NM_174615	Gessner et al. (2013)
Reverse	CAGCGTTGCCAGTCTTTGTA		
IGF-1			
Forward	ATGCCCAAGGCTCAGAAG	NM_001077828	Moriel et al. (2014)
Reverse	GGTGGCATGTCATTCTTCACT		
Ribosomal protein L12			
Forward	CACCAGCCGCCTCCACCATG	NM_205797.1	Gessner et al. (2013)
Reverse	CGACTTCCCCACCGGTGCAC		
Cyclophilin			
Forward	GGTACTGGTGGCAAGTCCAT	NM_178320.2	Moriel et al. (2014)
Reverse	GCCATCCAACCACTCAGTCT		

Table 3.3. Continued

Target[1]	Primer sequence	Accession nº	Source
Longissimus muscle			
FABP4			
Forward	AAACTTAGATGAAGGTGCTCTGG	AJ4160220	Li et al. (2018)
Reverse	CATAAACTCTGGTGGCAGTGA		
Myogenin			
Forward	GAGAAGCGCAGACTCAAGAAGGTGAATGA	AF09174	Muroya et al. (2002)
Reverse	TCTGTAGGGTCCGCTGGGAGCAGATGATC		
PAX7			
Forward	GGGCTCAGATGTGGAGTCAG	XM_616352.6	Moriel et al. (2014)
Reverse	GCTCCTCTCGGGTGTAGATG		
PPAR-γ			
Forward	GCATTTCCACTCCGCACTAT	AY137204	Li et al. (2018)
Reverse	GGGATACAGGCTCCACTTTG		
B-actin			
Forward	AGCAAGCAGGAGTACGATGAGT	NM_173979	Bong et al. (2012)
Reverse	ATCCAACCGACTGCTGTCA		
Ribosomal protein S9			
Forward	CCTCGACCAAGAGCTGAAG	AF479289	Jeong et al. (2012)
Reverse	CCTCCAGACCTCACGTTTGTTC		

[1]CUT = Cu-transporter protein; MT = metallothionein 1A; SOD = superoxide dismutase 1; IGF1 = insulin-like growth factor I; FAPB4 = adipocyte fatty acid-binding protein; PAX7 = paired box gene 7; PPAR-γ = peroxisome proliferator-activated receptor-γ.

Table 3.4. Performance of beef cows receiving diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1,2]

Item	INR	AAC	SEM	*P*-value
Average cow age, yr	3.8	3.9	0.2	0.70
Days on trial, d	167	166	3	0.91
BW, kg				
Initial (d -30)	485	496	8	0.35
Intermediate (d 97)	428	443	5	0.04
Calving	419	437	12	0.31
Weaning (d 367)	463	469	6	0.48
BCS				
Initial (d -30)	5.6	5.5	0.08	0.55
Pre-calving (d 97)	4.1	4.2	0.07	0.93
Calving	4.5	4.5	0.06	0.73
Weaning (d 367)	5.3	5.0	0.06	< 0.01
Pregnancy rates[3], %				
To AI	46.2 (30/65)	40.3 (29/72)	6.0	0.49
To bull	51.8 (29/56)	49.2 (32/65)	6.5	0.78
Overall	68.6 (59/86)	62.8 (59/94)	5.0	0.41

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 days of gestation (d 0 of the experiment).

[2]BW and BCS (Wagner et al., 1988) were recorded before the beginning of the experiment (d -30), 2 wk before the beginning of the calving season (intermediate BW; d 97), upon calving, and at weaning (d 367).

[3]Cows that were ≥ 45 d postpartum at the beginning of the breeding season (d 214) were assigned to an estrus synchronization + fixed-time AI protocol (Oosthuizen et al., 2020). All cows were exposed to mature Brangus bulls for 60 d following AI. Cow pregnancy status to AI was verified by detecting a fetus via transrectal ultrasonography 27 d after AI. Final pregnancy status was determined on d 345. Values within parenthesis report number of pregnant cows divided by total cows exposed to AI, number of cows non pregnant to AI and those that were only exposed to natural service, and number of pregnant cows divided by total cows exposed to breeding (AI + natural service), respectively.

Table 3.5. Liver concentrations of Co, Cu, Mn, and Zn of beef cows receiving diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1,2]

Item	INR	AAC	SEM	*P*-value
Co, mg/kg				
Initial (d -30)	0.18	0.15	0.01	< 0.01
Pre-calving (d 97)	0.59	0.68	0.02	0.01
Calving	0.58	0.55	0.11	0.83
Early lactation	0.14	0.15	0.01	0.28
Weaning (d 367)	0.21	0.21	0.01	0.72
Cu, mg/kg				
Initial (d -30)	71.5	64.6	9.4	0.60
Pre-calving (d 97)	133.2	74.9	12.9	< 0.01
Calving	118.5	42.9	9.9	< 0.01
Early lactation	133.5	152.2	15.0	0.39
Weaning (d 367)	117.4	63.6	12.5	< 0.01
Mn, mg/kg				
Initial (d -30)	9.1	8.6	0.3	0.18
Pre-calving (d 97)	10.5	10.0	0.3	0.30
Calving	12.0	10.6	0.6	0.11
Early lactation	8.6	7.8	0.4	0.17
Weaning (d 367)	9.4	9.4	0.2	0.79
Zn, mg/kg				
Initial (d -30)	155.4	142.2	7.4	0.21
Pre-calving (d 97)	314.1	341.5	22.3	0.39
Calving	173.2	129.5	11.5	0.01
Early lactation	134.2	142.0	8.0	0.52
Weaning (d 367)	151.0	153.0	6.5	0.83

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 days of gestation (d 0 of the experiment).

[2]Liver samples were collected before the beginning of the experiment (d -30), 2 wk before the beginning of the calving season (d 97), upon calving, when cows were 43 ± 0.5 days postpartum

(early lactation), and at weaning (d 367) via needle biopsy (Arthington and Corah, 1995). Concentrations of Co, Cu, Mn, and Zn were determined by the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI; Braselton et al., 1997)

Table 3.6. Expression of liver genes in beef cows receiving diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1,2]

Item[3]	INR	AAC	SEM	*P*-value
CUT				
Initial (d -30)	1.75	1.44	0.12	0.11
Pre-calving (d 97)	1.67	1.70	0.08	0.82
Calving	1.95	1.75	0.11	0.19
MT				
Initial (d -30)	4.0	3.6	0.7	0.71
Pre-calving (d 97)	26.1	22.8	3.8	0.54
Calving	36.4	65.6	11.6	0.04
SOD				
Initial (d -30)	2.65	2.75	0.23	0.78
Pre-calving (d 97)	1.99	2.11	0.13	0.51
Calving	2.02	2.04	0.13	0.90

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0 of the experiment).

[2]Liver samples were collected via needle biopsy (Arthington and Corah, 1995) before the beginning of the experiment (d -30), 2 wk before the beginning of the calving season (d 97), and upon calving. Values are expressed as relative fold change compared within threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

[3]CUT = Cu-transporter protein; MT = metallothionein 1A; SOD = superoxide dismutase.

Table 3.7. Lactation responses of beef cows receiving diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1]

Item	INR	AAC	SEM	*P*-value
Milk production				
Days in milk	42.6	42.2	0.7	0.69
Milk yield, kg/d	7.1	7.4	0.6	0.70
Mineral analysis[2]				
Co, ppm				
Colostrum	0.005	0.005	0.001	0.50
Milk	0.0003	0.0003	0.001	0.40
Cu, ppm				
Colostrum	0.99	1.00	0.01	0.58
Milk	0.05	0.06	0.01	0.19
Mn, ppm				
Colostrum	0.06	0.06	0.01	0.53
Milk	0.01	0.02	0.002	0.42
Zn, ppm				
Colostrum	15.35	13.71	1.62	0.48
Milk	4.21	4.26	0.30	0.89

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0 of the experiment). Upon calving colostrum samples were collected manually from a subset of cows (n =30 cows/treatment). Milk production was estimated via the weigh-suckle-weigh method (Aguiar et al., 2015) and milk samples were manually collected when cows were 42 ± 0.5 days postpartum.

[2]Concentrations of Co, Cu, Mn, and Zn were determined by the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI; Braselton et al., 1997).

Table 3.8. Concentrations of Co, Cu, Mn, and Zn in cotyledons and liver from calves born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1,2]

Item	INR	AAC	SEM	*P*-value
Co, mg/kg				
Cotyledon	0.46	0.53	0.08	0.55
Calf birth	0.19	0.20	0.01	0.79
Calf 24 h after birth	0.15	0.15	0.01	0.96
Calf WSW	0.23	0.23	0.01	0.94
Calf weaning (d 367)	0.16	0.16	0.01	0.88
Cu, mg/kg				
Cotyledon	8.2	9.2	0.9	0.46
Calf birth	394.5	399.1	19.1	0.87
Calf 24 h after birth	303.1	291.3	19.5	0.67
Calf WSW	83.4	94.0	11.1	0.50
Calf weaning (d 367)	67.9	73.0	8.6	0.68
Mn, mg/kg				
Cotyledon	16.8	19.0	4.9	0.75
Calf birth	6.2	6.4	0.3	0.67
Calf 24 h after birth	4.7	4.7	0.3	0.91
Calf WSW	11.6	12.0	0.3	0.33
Calf weaning (d 367)	9.2	8.8	0.2	0.17
Zn, mg/kg				
Cotyledon	93.0	93.6	3.3	0.89
Calf birth	823.4	868.6	79.2	0.69
Calf 24 h after birth	675.8	637.0	79.3	0.73
Calf WSW	168.0	149.3	8.1	0.11
Calf weaning (d 367)	139.2	151.5	7.0	0.22

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0 of the experiment).

[2] Cotyledon and calf liver samples were collected via needle biopsy (Arthington and Corah, 1995) within 8 h after calving, 24 h after birth, when calves were 43 ± 0.5 days of age (WSW), and at

weaning (d 367). Concentrations of Co, Cu, Mn, and Zn were determined by the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI; Braselton et al., 1997).

Table 3.9. Expression of liver genes in calves born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1,2]

Item[3]	**INR**	**AAC**	**SEM**	***P*-value**
CUT				
Birth	2.19	2.23	0.11	0.78
24 h after birth	2.51	2.53	0.11	0.91
Weaning (d 367)	2.13	2.01	0.10	0.40
IGF-I				
Birth	4.79	6.07	0.65	0.17
24 h after birth	5.79	4.42	0.65	0.14
Weaning (d 367)	9.22	9.88	1.06	0.66
MT				
Birth	33.7	32.9	7.2	0.94
24 h after birth	59.4	68.4	7.2	0.38
Weaning (d 367)	39.9	54.1	8.8	0.26
SOD				
Birth	2.92	2.96	0.20	0.88
24 h after birth	2.77	2.70	0.20	0.79
Weaning (d 367)	1.78	1.98	0.07	0.04

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0 of the experiment).
[2]Liver samples were collected via needle biopsy (Arthington and Corah, 1995) at birth, 24 h after birth, and at weaning (d 367). Values are expressed as relative fold change compared within threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).
[3]CUT = Cu-transporter protein; IGF-I = insulin-like growth factor-I; MT = metallothionein 1A; SOD = superoxide dismutase 1.

Table 3.10. Expression of *longissimus* muscle genes in calves born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1,2]

Item[3]	INR	AAC	SEM	*P*-value
FABP4				
Birth	155.7	108.0	31.4	0.29
Weaning	45.9	38.4	9.0	0.56
Myogenin				
Birth	5.05	4.54	0.58	0.53
Weaning	4.20	4.42	0.51	0.77
PAX7				
Birth	3.42	3.42	0.25	0.99
Weaning	3.23	3.22	0.16	0.98
PPAR-γ				
Birth	4.28	4.24	0.76	0.97
Weaning	2.14	2.03	0.21	0.71

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0 of the experiment).
[2]Muscle samples were collected via needle biopsy (Schubach et al., 2019) at birth and at weaning (d 367). Values are expressed as relative fold change compared within threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).
[3]FABP4 = adipocyte fatty acid binding protein; PAX7 = paired box gene 7; PPAR-γ = peroxisome proliferator-activated receptor-γ.

Table 3.11. Calving and weaning outcomes from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC;** n = 95) during gestation[1]

Item	INR	AAC	SEM	*P*-value
Calving results				
Calving rate, %	97.8	99.0	1.3	0.51
Percent of male calves born	60.6	49.0	5.1	0.11
Percent of AI – sired calves born	42.7	44.9	5.1	0.76
Calf birth BW, kg	30.7	31.4	0.4	0.26
Kilograms of calf born per cow[2]	30.7	31.4	0.4	0.26
Adjusted calf birth BW[3], kg	32.5	32.9	0.5	0.61
Adjusted kilograms of calf born per cow[2]	31.9	32.6	0.4	0.31
Weaning results				
Weaning rate, %	93.4	91.9	2.7	0.70
Percent of AI – sired calves weaned	41.2	44.0	5.3	0.71
Percent of male calves weaned	60.0	48.4	5.3	0.12
Calf weaning age, d	199	200	3	0.79
Calf weaning BW, kg	183	178	3	0.19
Kilograms of calf weaned per cow[4]	173	164	5	0.21
205-d adjusted weaning BW[3], kg	192	187	3	0.18
Adjusted kilograms of calf weaned per cow[4]	184	174	5	0.22
Birth to weaning ADG, kg/day	0.76	0.73	0.01	0.12

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0 of the experiment).
[2]Calculated based on calving rate and calving rate and calf birth BW.
[3]Calculated according to the Beef Improvement Federation (2010).
[4]Calculated based on weaning rate and calf weaning BW.

4. EFFECTS OF ORGANIC OR INORGANIC COBALT, COPPER, MANGANESE, AND ZINC SUPPLEMENTATION TO GESTATING BEEF COWS: II, IMPACTS ON OFFSPRING REPRODUCTIVE DEVELOPMENT, PRODUCTIVE RESPONSES, AND CARCASS QUALITY

4.1. Introduction

Maternal nutrition is a major extrinsic factor programming nutrient partitioning and development of fetal organ systems associated with health, production, and reproduction (Long et al., 2009; Long et al., 2010), leading to long-term effects on offspring performance. The majority of research to date has focused primarily on energy and CP intake of gestating beef cows (Bohnert et al., 2013; Wilson et al., 2016), however little is known about the effects of trace mineral status of gestating cows on offspring productivity. The fetus is completely dependent on the dam for its supply of trace minerals (Hidiroglou and Knipfel, 1981), which are essential for fetal developmental processes such as protein synthesis, bone formation, lipid metabolism, and DNA synthesis (Hostetler et al., 2003). One strategy to enhance trace mineral status in cattle is to feed organic complexed sources (Spears, 1996), due to indications that these may be of greater bioavailability compared to their inorganic counterparts (Brown and Zeringue, 1994).

Recent research from our group demonstrated that supplementing late gestating beef cows with organic complexed sources of Co, Cu, Mn, and Zn enhanced offspring productivity (Marques et al., 2016). More specifically, calves born to cows supplemented with organic complexed sources of the aforementioned trace minerals were heavier at weaning and had reduced bovine respiratory (**BRD**) incidence during the receiving period compared with calves from non-supplemented cohorts. However, the fetus is sensitive to the effects of maternal nutrition from all stages between

oocyte maturation through parturition, whereas the previous authors only investigated the effects of trace mineral supplementation to beef cows during the last trimester of gestation. Additionally, Marques et al. (2016) focused specifically on calves reared as feeder cattle destined for slaughter. Given that maternal nutrient status during gestation has profound impacts on future reproductive performance of female progeny, research is warranted to examine the impacts of organic trace mineral supplementation to gestating beef cows on female offspring productivity. Therefore, we hypothesized that organic trace mineral supplementation to gestating beef cows would improve offspring health and productivity via programming effects. To test this hypothesis this experiment compared performance and physiological responses of male offspring raised as feeder cattle and female offspring raised as replacement heifers from beef cows supplemented with organic or sulfate sources of Co, Cu, Mn, and Zn.

4.2. Materials and Methods

This experiment was conducted at the Texas A&M – Beef Cattle Systems (College Station, TX, USA). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Texas A&M – Institute of Animal Care of Use Committee (#2018/0093).

4.2.1. *Cow Management and Dietary Treatments*

This paper is one of two companion papers addressing the impacts of supplementing organic or sulfate sources of Co, Cu, Mn, and Zn. The companion paper (Chapter 3) addresses the pre- and postpartum responses of the dam as well as responses of the progeny from birth until weaning. Detailed materials and methods have been presented in Chapter 3. Briefly, 190 non-lactating, pregnant beef cows were assigned at 117 ± 2.2 d of gestation (d 0 of the experiment).

Cows were pregnant to AI using semen from a single Brangus sire (n = 83) or pregnant to 1 of 6 Brangus bulls via natural service (n = 107; cows were exposed to bulls for 40 d beginning 15 d after AI), according to the breeding management and pregnancy diagnosis described by Oosthuizen et al. (2020). At the beginning of the experiment (d 0), pregnancy length was expected to be 141 ± 1.8 d for cows pregnant to AI and 217 d or less for cows pregnant via natural service.

Prior to the beginning of the experiment (d – 30), cows were ranked by pregnancy type (AI or natural service), parity, BW, and BCS, and assigned to receive diets that contained 1) sulfate sources of Cu, Co, Mn, and Zn (**INR**; custom blend manufactured by Anipro Xtraformance Feeds, Pratt, KS; n = 95) or 2) organic complexed source of Cu, Co, Mn, and Zn (**AAC**; Availa 4; Zinpro Corporation, Eden Prairie, MN; n = 95). The AAC trace mineral source was based on a metal:AA complex ratio of 1:1 for Zn, Cu, and Mn in addition to cobalt glucoheptonate (Zinpro Corporation). Diets were isocaloric and isonitrogenous and formulated to meet requirements for macrominerals, Se, I, and vitamins (Table 3.1, Chapter 3) for pregnant cows (NRC, 2000). The INR and AAC sources were mixed with dried distillers' grain and formulated to provide the same daily amount of Cu, Co, Mn, and Zn (based on 7 g/cow daily of Availa 4; Marques et al., 2016). Nutritional profile of treatments is described in Chapter 3.

From d 0 of the experiment until calving, cows were gathered 3 times weekly (Tuesdays, Thursdays, and Saturdays) and individually sorted into 1 of 24 feeding pens (1 cow per pen; 6 × 9 m). Cows individually received treatments (0.5 kg of treatment/feeding, dry matter basis) and were returned to pasture after their treatment was completely consumed. This process was repeated until all cows had been individually sorted into pens and had consumed their treatments. Immediately after calving, cow-calf pairs were removed from the pasture and assigned to the general management of the research herd, which included free-choice inorganic trace mineral

supplementation (Producers Special Pasture Mineral; Producers Cooperative Association, College Station, TX; containing 14% Ca, 7% P, 13% NaCl, 5% Mg, 9,900 mg/kg Zn, 2,500 mg/kg Cu, 100 mg/kg I, 4,000 mg/kg Mn, 26 mg/kg Se, 91 IU/g of vitamin A, 10 IU/g of vitamin D3, and 0.05 IU/g of vitamin E). Male calves were castrated using an elastic bander at 43 ± 0.5 d of age and all calves received vaccination against respiratory viruses (Triangle 5; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA) and Clostridium (Covexin 8; Merck Animal Health, Omaha, NE) on d 345 of the experiment. Cows were assigned to the same reproductive management (d 214 to d 287) and pregnancy diagnosis (d 345 of the experiment) described by Oosthuizen et al. (2020).

4.2.2. ***Calf Management***

Calves were weaned on d 367 of the experiment and transferred to a 5.3 ha pasture for a 45-d preconditioning period as a single group. Calves were revaccinated against respiratory viruses (Titanium 5; Elanco Animal Health, Greenfield, IN), and Clostridium (Covexin 8; Merck Animal Health) and received a pour-on anthelmintic (Dectomax; Zoetis, Floram Park, NJ) upon weaning. During preconditioning, calves received a mixed bermuda-rye grass hay, a total mixed ration (**TMR**; Table 4.1), and water for *ad libitum* consumption. On d 412, all calves were transferred to a single 52.6 ha oat (*Avena sativa L.*) pasture for a 25-d backgrounding phase as a single group.

4.2.2.1. *Heifer Development*

On d 437, heifers (*n* = 81; 34 INR, 47 AAC) were moved to an adjacent 44.5 ha oat pasture where they remained throughout the remainder of the experiment (until d 620). Heifers had access to water and the same commercial mineral and vitamin mix previously described (Producers Special Pasture Mineral) for *ad libitum* consumption.

4.2.2.2. *Feeder Cattle*

Following separation from the heifers, steers (*n* = 95; 51 INR, 44 AAC) remained in a single oat pasture for an additional 56 days with access to water and the same commercial mineral and vitamin mix previously described (Producers Special Pasture Mineral) for *ad libitum* consumption. On d 493 steers were loaded into a double-deck commercial livestock trailer (Legend 50' cattle liner; Barrett LLC., Purcell, OK) and transported 202 km to a commercial feedlot (Graham Land and Cattle Company; Gonzales, TX). Upon arrival, steers were administered Bovi-Shield Gold 5 (Zoetis, Inc.), UltraChoice 8 (Zoetis, Inc.), Dectomax pour-on (Zoetis, Inc.), and were implanted with Synovex Choice S (Zoetis, Inc.). Steers received the same diets (Table 4.2) provided *ad libitum* and were managed as a single group until slaughter at a commercial packing facility (d 724). Steers were observed daily for BRD signs during the feedlot period based on the DART system (Zoetis), and received medication according to the management criteria of the commercial feedlot.

4.2.3. ***Sampling***

4.2.3.1. *Feedstuffs*

Samples of all ingredients fed to calves were collected weekly, pooled across weeks, and analyzed for nutrient content (Dairy One Forage Laboratory). All samples were analyzed by wet chemistry procedures for concentrations of crude protein (method 984.13; AOAC, 2006), acid detergent fiber (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY: AOAC, 2006), and neutral detergent fiber (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.), and macro- and trace minerals using inductively coupled plasma emission spectroscopy (Sirois et al., 1991), as well as Se according to method 996.16 of the AOAC (2006). Calculations for TDN used the

equation proposed by Weiss et al. (1992), whereas calculations for NEm and NEg used the equations proposed by NRC (2000).

4.2.3.2. *Preconditioning*

Blood samples were collected on d 345 and 367 prior to vaccination from a subset of calves (*n* = 30 calves/treatment) via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing no additive for serum collection. Calf BW was recorded over 2 consecutive days after weaning (d 367 and 368) and at the end of preconditioning (d 411 and 412), which were averaged to calculate preconditioning average daily gain (**ADG**). Additional blood samples were collected from the same subset of calves (*n* = 30 calves/treatment) on d 367, 368, 370, 373, 377, 382, and 397 via jugular venipuncture into commercial blood collection tubes (Vacutainer) containing either no additive or freeze-dried sodium heparin for serum and plasma collection, respectively. Calves were observed daily for BRD signs during the 45-d preconditioning period according to the subjective criteria described by Berry et al. (2004).

4.2.3.3. *Heifer Development*

Heifer full BW was recorded over 2 consecutive days at the end of the growing phase (d 619 and 620) and used with the two BW obtained at the end of preconditioning (d 411 and 412) to calculate heifer ADG. Each week beginning on d 437, heifer full BW was recorded, and blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer) containing freeze-dried sodium heparin for plasma collection. All plasma samples were analyzed for progesterone concentrations to estimate onset of puberty. Heifers were considered pubertal once plasma progesterone concentrations were ≥ 1.0 ng/mL followed by a cyclic pattern of plasma progesterone < and ≥ 1.0 ng/mL, suggestive of normal estrous cycles

(Schubach et al., 2017). Puberty attainment was declared at the first sampling that resulted in plasma progesterone ≥ 1.0 ng/mL. Heifer age and BW at puberty was calculated based on weekly full BW measurements and heifer age at the week of puberty attainment. On d 584, biopsies of the liver (Marques et al., 2016) and *longissimus muscle* (**LM**; Schubach et al., 2019) were performed via needle biopsy (TruCut biopsy needle; CareFusion Corporation, San Diego, CA) from a subset of heifers (*n* = 30; 15 heifers/treatment), and stored separately in 2-mL tubes containing 1 mL of RNA stabilization solution RNAlater, Ambion, Inc., Austin, TX) and stored at -80°C until further processing. Additionally, on d 620, the ovaries of each heifer were examined via transrectal ultrasonography equipped with a 7.5 mHz linear array-transducer. Pairs of ovaries were classified by antral follicle count (AFC) according to modified methods of Ireland et al. (2008). Ovarian pairs with < 10 antral follicles were classified as low AFC; ovarian pairs with 11 to 20 antral follicles were classified as intermediate AFC; and ovarian pairs with > 20 antral follicles were classified as high AFC.

4.2.3.4. *Feeder Cattle*

Steer BW was recorded over two consecutive days prior to transport to the feedlot (d 492 and 493) and values were averaged and used for calculation of ADG. On d 586, biopsies of the liver (Marques et al., 2016) and LM (Schubach et al., 2019) were performed via needle biopsy (TruCut biopsy needle; CareFusion Corporation, San Diego, CA) in a subset of steers (*n* = 30; 15 steers/treatment), were stored separately in 2-mL tubes containing 1 mL of RNA stabilization solution (RNAlater, Ambion, Inc., Austin, TX) and were stored at -80°C until further processing. At the commercial packing plant, hot carcass weight (**HCW**) was collected upon slaughter (d 724). Final BW was estimated based on HCW adjusted to a 63% dressing percentage to minimize variation associated with gut fill (Loza et al., 2010), and was used to estimate feedlot ADG. After

a 24-h chill, trained personnel assessed carcass backfat thickness at the 12th rib and LM area, and all other carcass measures were recorded by a USDA grader.

4.2.4. ***Laboratorial Analyses***

After collection, all blood samples were immediately placed on ice, centrifuged (2,500 × g for 30 min; 4 °C) for plasma or serum harvest and stored at -80°C on the same day of collection. Plasma samples collected on d 367, 368, 370, 373, 377, 382, and 397 were analyzed for cortisol (radioimmunoassay kit #07221106, MP Biomedicals, Santa Ana, CA; Burdick et al., 2009) and haptoglobin concentrations (Cooke and Arthington, 2013). Serum samples collected on d 345, 367, 377, and 382 were analyzed for antibodies against BRD viruses (Gonda et al., 2012), bovine herpesvirus-1 (**BHV-1**; BHV-1 Ab ELISA number 99-41459; IDEXX) and bovine viral diarrhea viruses type I and II (**BVDV**; BVDV Ab Elisa number 99-44000; IDEXX Switzerland AG, Liebefeld-Bern, Switzerland). The intra- and inter-assay CV were, respectively 4.0 and 4.6% for cortisol, 4.7 and 7.4% for haptoglobin, 6.1 and 5.1% for BHV-1, and 1.1 and 4.9% for BVDV. Plasma samples collected from heifers each week from d 437 to 619 were analyzed for progesterone using a radioimmunoassay as previously described (Pohler et al., 2016). The intra- and inter-assay CV were 6.7 and 11.6%, respectively, whereas the minimal detectable concentration was 0.05 ng/mL for progesterone.

Total RNA was extracted from tissue samples using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop Lite; Thermo Fisher Scientific, Wilmington, DE) at 260 nm and 260/280 nm ratio, respectively (Fleige and Pfaffl, 2006). Reverse transcription of extracted RNA and real-time reverse-transcription polymerase chain reaction (**PCR**) using gene specific primers (20 pM each; Table 3.3, Chapter 3) were completed as described by Rodrigues et al. (2015). Responses

from the genes of interest were quantified based on the threshold cycle (**CT**), the number of PCR cycles required for target amplification to reach a predetermined threshold. The CT responses from liver genes of interest were normalized to the geometrical mean of CT values of *ribosomal protein L12* and *cyclophilin*, whereas the CT responses from muscle genes of interest were normalized to the geometrical mean of CT values of *ribosomal protein S9* and *β-actin* (Vandesompele et al., 2002). The CV for the geometrical mean of reference genes across all liver and LM samples were 2.3 and 1.6%, respectively. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

4.2.5. ***Statistical Analysis***

All data were analyzed with cow as the experimental unit, and cow(treatment × parity) as the random variable. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). All data were analyzed using gestation days receiving treatment as an independent covariate, and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Model statements for calf responses during preconditioning contained the effects of treatment, calf sex, and the treatment × calf sex interaction. Model statements for all other heifer and feeder cattle responses contained the effects of treatment. Blood variables were analyzed as repeated measures, using day as fixed effect and all result interactions with treatment and calf sex when appropriate. The model statement for puberty attainment contained the effects of treatment, day, and the treatment × day interaction. The specified term used in the repeated statement was day, the subject was cow(treatment × parity), and the covariance structure utilized was autoregressive which provided the best fit according to the lowest Akaike information

criterion. Results are reported as least square means and separated using least square differences. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

4.3. Results and Discussion

4.3.1. *Preconditioning*

Upon weaning, mean plasma haptoglobin concentrations were greater ($P = 0.03$) in calves born to INR-supplemented cows compared with AAC-supplemented cohorts, and increased in all calves after weaning (day effect, $P < 0.01$; Table 4.3). Circulating cortisol concentrations have been positively associated with plasma haptoglobin concentrations (Cooke et al., 2012), whereas in the present experiment a treatment × day interaction was detected ($P = 0.03$; Figure 4.1) for plasma cortisol given that AAC calves had greater ($P = 0.05$) plasma cortisol concentrations on d 367 compared to INR calves, and tended to have lower ($P = 0.10$) plasma cortisol concentrations on d 370. The day effects reported herein for plasma cortisol and haptoglobin concentrations were expected based on the neuroendocrine stress response and acute-phase protein reaction elicited by weaning and vaccination against BRD pathogens (Rodrigues et al., 2015; Marques et al., 2016).

Moreover, *Cu, Zn-superoxide dismutase* (**SOD**) has been accredited with essential antioxidant activity, removing potentially deleterious reactive oxygen species (**ROS**; Tsang et al., 2014). These compounds are produced under normal circumstances; however, they are efficiently eliminated by powerful antioxidant systems within the cell. It is when the balance between ROS production and antioxidant capacity is lost that oxidative stress occurs, and cellular dysfunction and tissue damage ensue (Bandyopadhyay et al., 1999). Furthermore, glucocorticoids, such as cortisol, participate in elimination of ROS generated by immune cells (Dandona et al., 1999). Overproduction of ROS, leading to oxidative stress, is influenced by a variety of environmental

factors, including psychological and physiological stressors (Møller et al., 1996). Hence, treatment differences detected herein for hepatic SOD mRNA expression upon weaning (Chapter 3) and plasma cortisol concentrations, suggest that AAC supplementation to gestating beef cows impacted offspring hepatic metabolism and steroidogenesis required to cope with the stress of weaning procedures.

No treatment differences were detected for mean serum concentrations of antibodies against BVDV or BHV-1 ($P \geq 0.22$; Table 4.3) during preconditioning. Day effects were detected ($P < 0.01$) for both of these serum variables (Table 4.4), denoting that calves effectively acquired humoral immunity against these pathogens upon vaccination (Richeson et al., 2008). Haptoglobin and oxidative stress status are positively correlated (Royer et al., 2016; Sauerwein et al., 2013), with both being closely related to the immune system and inflammatory status (Lykkesfeldt and Svendsen, 2007). Lack of treatment differences for serum concentrations of antibodies against these pathogens do not corroborate with results reported herein for hepatic mRNA expression of SOD upon weaning (Chapter 3), as well as mean plasma haptoglobin concentrations.

During the 45-d preconditioning period no treatment effects were detected ($P \geq 0.40$) for incidence of calves that required treatment for BRD or calf ADG (Table 4.3), indicating that treatments did not influence calf preconditioning performance and health parameters despite treatment differences detected for plasma haptoglobin and cortisol. Although BRD incidence was not as prevalent as typically observed in recently weaned cattle (Marques et al., 2016; Silva et al., 2017), and may have hindered proper assessment of this variable and contributed to lack of treatment differences. Furthermore, at the end of preconditioning, calf BW was similar ($P = 0.23$) for AAC and INR calves, as well as kg of preconditioned calf produced/cow ($P = 0.25$; Table 4.3). These outcomes indicate that supplementing an organic source of Co, Cu, Mn, and Zn to gestating

beef cows did not impact postnatal offspring preconditioning performance compared with inorganic supplemented cohorts.

4.3.2. ***Heifer Development***

To our knowledge, no other study has compared the effects of supplementing gestating beef cows with sulfate or organic complexed Co, Cu, Mn, and Zn on heifer progeny reproductive performance. No treatment differences were detected ($P \geq 0.48$) for mRNA expression of genes associated with Cu and Zn in liver samples collected on d 584 (Table 4.5). More specifically, *Cu-transporter protein* (**CUT**) is associated with Cu transport into hepatic cells, and distribution of Cu into cellular organelles (Prohaska and Gybina, 2004; Han et al., 2009). *Metallothioneins* (**MT**) are a superfamily of intracellular metal binding proteins, most abundantly found in the liver, kidney, intestine, and pancreas (Coyle et al., 2002). Induction of hepatic MT synthesis is triggered by a variety of metals, although Zn is the primary physiological inducer, and it is proposed that regulation of MT expression controls free Zn concentration as well as that of Zn transporter proteins (Coyle et al., 2002; López-Alonso et al., 2005). Additionally, SOD is an enzyme located in the cytoplasm, nucleus, or mitochondrial membrane that, using Cu or Zn as cofactors, functions to eliminate superoxide anion radicals thereby protecting cells from oxidative damage (Sturtz et al., 2001; Miao and St. Clair, 2009). No treatment differences were detected ($P = 0.81$; Table 4.5) for liver mRNA expression of *Insulin-like growth factor-I* (**IGF-I**). Collectively, AAC supplementation to beef cows during gestation did not modulate mRNA expression of genes involved in growth or trace mineral metabolism in the liver of female offspring.

No treatment differences were detected ($P \geq 0.77$) for mRNA expression of genes involved in adipogenic activities in the LM of heifers on d 584 (Table 4.6). More specifically, *peroxisome proliferator-activated receptor gamma* (**PPAR-γ**), plays a pivotal role in adipocyte differentiation

and associated gene expression (Houseknecht et al., 2002), whereas a target of PPAR-γ, *adipocyte fatty acid-binding protein* (**FABP4**), is also highly involved in differentiation of adipocytes, acting as an intracellular fatty acid chaperone (Michal et al., 2006). However, the intramuscular region is typically the last depot for adipose tissue to be deposited in the growing animal (Oliveira et al., 2011); therefore, mRNA expression of the aforementioned genes in subcutaneous adipose tissue samples may have been more appropriate when assessing heifer fat accretion and body composition during the growing phase, both of which influence puberty attainment in beef heifers (Yelich et al., 1995).

A treatment difference was detected ($P = 0.05$) for mRNA expression of *myogenin* (Table 4.6), a regulatory factor in the LM that influences postnatal muscle growth through differentiation and fusion of satellite cells with existing fibers (Le Grand and Rudnicki, 2007; Du et al., 2010) which was greater for INR vs. AAC heifers. Corroborating this result, INR heifers tended to have greater ($P = 0.09$) mRNA expression of *paired box gene 7* (**PAX7**) compared with AAC cohorts, expression of which is necessary for satellite cell specification and survival (Seale et al., 2000; Li et al., 2011). Therefore, it is plausible that heifers born to INR supplemented dams had a greater population of satellite cells undergoing differentiation on d 584. Although, no treatment differences were detected ($P = 0.39$) for heifer ADG throughout the growing phase (d 412 to 620; Table 4.7), corroborating similar hepatic IGF-I mRNA expression (Table 4.5).

In beef females, the ovarian reserve is determined during gestation, with follicle assembly into primordial follicles beginning around d 80 of gestation and concluding by d 143 (Nilsson and Skinner, 2009). Prediction of reproductive performance and lifetime productivity of females has been accomplished through estimation of the ovarian reserve through AFC via ultrasonography (Ireland et al., 2008). In the present experiment, maternal supplementation with AAC did not

influence ($P \geq 0.49$) the proportion of heifers classified as high, or intermediate AFC (Table 4.7), although a greater ($P = 0.05$) proportion of AAC heifers were classified as low AFC compared with INR heifers. These results are in contrast to other studies that have reported dietary manipulation during gestation severely altered the development of the ovarian reserve in female offspring (Sullivan et al., 2009; Ireland et al., 2011). However, the previous authors imposed severe nutrient deprivation during the first and second trimesters of gestation in beef heifers, whereas treatments in the present experiment were designed to only differ in form of supplemental Co, Cu, Mn, and Zn, and utilized mature cows. Moreover, treatment differences detected for low AFC could be due to a low number of heifers assigned this classification ($n = 9$), thereby inflating statistical differences beyond biological relevance.

A treatment × day interaction was detected ($P < 0.01$) for puberty attainment, as AAC heifers exhibited accelerated puberty attainment compared with INR heifers (Figure 4.3). Heifers bred on their pubertal estrus have reduced pregnancy rates compared with cohorts bred on their second or third estrus (Byerly et al., 1987). Thus, it can be speculated that AAC heifers would have a greater first service conception rate compared with INR heifers, and likely calve earlier in the breeding season, contributing to a more biologically and economically efficient cowherd (Lesmeister et al., 1973). Nevertheless, these variables were not measured in the present study to validate this rationale. Heifer age and BW at puberty attainment did not differ between treatments ($P \geq 0.17$), nor BW evaluated on a weekly basis (Table 4.7; Figure 4.2). Puberty in cattle is highly influenced by nutritional status and body development, including growth rate and body composition (Schillo et al., 1992), although no treatment differences were detected in the LM for genes associated with adipogenic activities. Conversely, myogenic factors are downregulated as cattle mature and muscle fibers are fully developed (Picard et al., 2002; Du et al., 2010); therefore,

treatment differences noted in the LM for mRNA expression of *myogenin* and PAX7 corroborate the accelerated physiological maturity of AAC heifers compared with INR cohorts.

The mechanisms by which supplementing gestating beef cows with organic complexed supplemental Co, Cu, Mn, and Zn accelerates reproductive development remains unknown. Although, previous research has demonstrated supplemental organic complexed trace minerals improved reproductive performance in cattle, such as decreased interval to first estrus and increased pregnancy rates (Griffiths et al., 2007; Uchida et al., 2001), as well as improved *in vitro* embryo production efficiency (Dantas et al., 2019). More specifically, lactating beef cows supplemented with organic trace minerals yielded a greater number of culturable oocytes and transferable embryos compared to cohorts receiving sulfate forms of the same elements (Dantas et al., 2019). Primordial germ cells present in the developing ovary actively utilize machinery and enzymes against ROS to maintain cell integrity (Hayeshi et al., 2017), and research conducted in both humans and animals reported increased antioxidant enzyme activity and decreased production of ROS during germ cell development when supplemented with trace minerals (Özkaya,. and. Nazıroğlu. 2010; Shi et al., 2010). Therefore, trace mineral availability may contribute to the increased internal protection by the developing ovary from ROS produced via its own metabolism, leading to formation of a more favorable follicular environment and oocyte, and enhanced reproductive development of the female.

The role of trace mineral source on reproductive performance is complex, especially given the plethora of processes each element in the present study participates in, and the essentiality of each in all aspects of development (Hostetler et al., 2003). For example, Zn is an integral component of over 200 metalloenzymes, and participates in developmental processes from DNA and RNA synthesis to membrane structure and stability (Underwood and Suttle, 1999). More

specifically, marginal Zn deficiency leads to abnormal estrous cycles (Swenerton and Hurley, 1980) and impaired ovarian development during the fetal period (Hurley and Keen, 1988). In turn, supplementing gestating beef cows with organic complexed Co, Cu, Mn, and Zn accelerated reproductive development of heifer progeny, though the exact mechanism contributing to such results warrants further investigation.

4.3.3. *Feeder Cattle*

All steers were shipped to the packing plant on d 723 and slaughtered on d 724 of the experiment; hence, days on feed for both treatments was 230 d. No treatment differences were detected ($P = 0.56$) for proportion of steers treated for BRD symptoms (Table 4.8), and no steer mortality was observed during this phase. It should be noted, however, that BRD incidence observed herein was not as elevated as in research conducted at commercial receiving yards (Snowder et al., 2006; Marques et al., 2016). and may have hindered proper assessment of this variable and contributed to lack of treatment differences. In the present experiment, steers underwent a 45-d preconditioning program and were backgrounded at the same facility for an additional 82 d according to the management of the research station. This management scheme likely contributed to the decreased morbidity observed herein, given that such programs are known to prepare cattle for the feedlot in the form of vaccination against respiratory pathogens and adaptation to dry feed (Duff and Galyean, 2007).

No treatment differences were detected ($P \geq 0.15$) for mRNA expression of CUT, IGF-I, or SOD in the liver on d 586. However, mRNA expression of MT was greater ($P = 0.02$) for INR vs. AAC steers (Table 4.5). As previously mentioned, hepatic Cu and Zn concentrations are correlated with MT expression (Coyle et al., 2002), and previous research has demonstrated that increased liver Zn concentrations are associated with increased feed intake (Littledike et al., 1995).

Therefore, it can be speculated that the increased MT expression in the liver of INR steers could be due to a potential increase in DMI while in the feedlot, although feedlot ADG, final BW, and HCW did not differ ($P \geq 49$) between treatments. No treatment differences were detected ($P \geq 0.30$) for mRNA expression of genes associated with adipogenic activities or muscle development in the LM during the feedlot phase (Table 4.6). In the present experiment, similar mRNA expression was supported by equivalent phenotypic responses, given that no treatment differences were noted ($P \geq 0.24$) for the remaining carcass traits evaluated, including LM area, marbling, and proportion of carcasses graded as Choice (Table 4.8). Marques et al. (2016) also failed to demonstrate impacts of AAC supplementation to gestating beef cows on carcass merit traits, despite differences in finishing BW compared to non-supplemented cohorts. Moreover, no treatment effects were detected for percentage of steers slaughtered per cow assigned to the experiment ($P = 0.44$; Table 4.8), indicating that mortality rate of male offspring was similar throughout the entire offspring productive life. Similar to weaning outcomes, the proportion of AI-sired steers that were slaughtered did not differ ($P = 0.44$) among treatments (Table 4.8). Collectively, these outcomes suggest supplementing gestating beef cows with AAC did not impact performance and carcass characteristics of male offspring raised as feeder cattle.

4.4. Overall Conclusions

Supplementing gestating beef cows with organic sources of Co, Cu, Zn, and Mn resulted in a reduced acute phase response in the offspring upon weaning, although this was not translated into greater offspring performance during a 45-d preconditioning period. Supplementing gestating beef cows with organic sources of Co, Cu, Zn, and Mn did not impact calf performance when steers were managed as feeder cattle, although genes involved in trace mineral metabolism were

altered in INR offspring. Moreover, heifers born to AAC supplemented cows had accelerated puberty attainment compared to their INR cohorts, independent of growth rate. Nonetheless, results from this experiment are novel, and suggest that supplementing gestating beef cows with an organic complexed source of Co, Cu, Zn, and Mn compared to sulfate sources may be a viable alternative to enhance reproductive efficiency of female offspring raised as replacement heifers.

4.5. References

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Table 4.1. Nutritional profile of feedstuffs offered to offspring[1,2]

Item	Bermuda-Rye Grass Hay	TMR	Pasture A	Pasture B
Total digestible nutrients, %	54	72	63	59
Net energy for maintenance, Mcal/kg	1.00	1.76	1.36	1.22
Net energy for growth, Mcal/kg	0.44	1.13	0.78	0.62
Crude protein, %	8.7	16.4	22.3	13.6
Neutral detergent fiber, %	72.4	30.0	41.0	54.2
Ca, %	0.61	0.80	0.37	0.48
P, %	0.17	0.61	0.42	0.26
Mg, %	0.13	0.30	0.18	0.11
K, %	1.87	1.64	4.41	1.85
Na, %	0.06	0.23	0.23	0.06
Co, mg/kg	0.36	0.73	0.36	0.27
Cu, mg/kg	8	51	10	8
Fe, mg/kg	221	305	784	272
Mn, mg/kg	72	127	68	37
Se, mg/kg	0.07	0.68	0.25	0.18
Zn, mg/kg	30	165	30	36

[1]Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). Total Digestible nutrients were calculated according to equations described by Weiss et al. (1992). Net energy for maintenance and growth were calculated with equations described by the NRC (2000).

[2]During preconditioning (d 367 to 412), calves received a mixed bermuda-rye grass hay and a total mixed ration for *ad-libitum* consumption. Total mixed ration consisted of (as-fed basis) 31.8% cracked corn, 30.0% dried distillers grains, 28.8% alfalfa hay, 7.0%

liquid molasses, and 2.1% inorganic mineral mix containing 14% Ca, 7% P, 13% NaCl, 0.27% K, 0.4% Mg, 0.25% Cu, 0.003% Se, 0.99% Zn, 90.91 IU/kg of vitamin A, 9.09 IU/kg of vitamin D3, and 0.045 IU/kg of vitamin E. On d 412, all calves were transferred to a single oat pasture (*Avena sativa L.*; Pasture A), where steers were maintained until d 493, whereas heifers were transferred to an adjacent oat pasture (Pasture B) on d 437 and remained there until d 620.

Table 4.2. Ingredient composition (as-fed basis) of diets offered to steers in the feedlot[1]

Ingredients, % as-fed basis	**A**	**B**	**C**	**D**
Brewers grain	35.0	28.0	21.0	21.0
Cotton seed hulls	16.0	9.5	2.5	2.5
Dried corn	33.5	47.0	64.0	69.0
Liquid molasses	6.0	3.0	0.0	0.0
Mineral and vitamin mix[2]	2.5	2.5	2.5	2.5
Rice bran	7.0	10.0	10.0	5.0

[1]Steers were transported to the feedlot on d 493 where they remained until slaughter. Diet A = offered for 15 d on receiving; B = offered for 20 d after diet A; C = offered for 32 d after diet B; D = offered until slaughter.

[2]Diets included a customized blend of minerals, vitamins, and feed additives (Purina Animal Nutrition, Arden Hills, MN), which contained one-third of Zn, Mn, and Cu as metal:AA complex ratio (Zinpro Corporation, Eden Prairie, MN) and two-thirds as sulfate sources.

Table 4.3. Preconditioning performance of calves born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1]

Item	INR	AAC	SEM	*P*-value
Calf performance				
Treated for BRD symptoms[2], %	1.0	0.0	0.8	0.40
End of preconditioning BW, kg	206	201	3	0.23
Preconditioning ADG[3], kg/day	0.56	0.55	0.02	0.76
Kilograms of preconditioned calf per cow	198	187	6	0.20
Overall calf loss[4], %	6.6	8.1	2.7	0.70
Plasma hormones and metabolites[5],				
Cortisol, ng/mL	10.66	10.54	0.82	0.92
Haptoglobin, mg/dL	0.42	0.32	0.03	0.03
Serum antibodies against respiratory viruses[6]				
Bovine viral diarrhea viruses type I and II	0.74	0.65	0.05	0.22
Bovine herpesvirus-I	175.6	168.5	6.0	0.40

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0). Calves were weaned on d 367 and assigned to a 45-d preconditioning period as a single group.

[2]BRD = bovine respiratory disease. Calves were classified as positive for BRD symptoms according to the subjective criteria described by Berry et al. (2004) and received 1 mL/10 kg of BW of Baytril 100 (Bayer Animal Health, Shawnee Mission, KS).

[3]Calculated based on the average of two BW recorded upon weaning (d 367 and 368) and at the end of preconditioning (d 411 and 412).

[4]Calculated based on number of calves lost during gestation and until the end of preconditioning divided by the number of pregnant cows assigned to the experiment.

[5]Blood samples were collected on d 367, 368, 370, 373, 377, 382, and 397 for plasma extraction.

[6]Calves received vaccination against respiratory viruses on d 345 (Triangle 5; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA) and d 367 (Titanium 5; Elanco Animal Health, Greenfield, IN). Blood samples were collected on d 345, 367, 377, and 382 for serum extraction. Serum samples were analyzed and results expressed as sample:positive control ratio as in Gonda et al. (2012).

Table 4.4. Serum concentrations of antibodies against *bovine viral diarrhea viruses type I and II* (BVDV) and *bovine herpesvirus-I* (BHV), and plasma concentration of cortisol (ng/mL) and haptoglobin (mg/dL) in beef calves during a 45-d preconditioning program[1,2]

	Serum antibodies against respiratory viruses		Plasma hormones and metabolites	
Day	**BVDV**	**BHV**	**Cortisol**	**Haptoglobin**
-21	0.254^{b}	110.49^{c}	-	-
0	0.337^{b}	152.96^{b}	14.68^{a}	0.400^{c}
1	-	-	14.89^{a}	0.571^{b}
3	-	-	11.58^{b}	0.774^{a}
6	-	-	7.46^{d}	0.335^{cd}
10	-	-	4.63^{e}	0.204^{e}
15	1.143^{a}	217.34^{a}	12.35^{b}	0.220^{de}
30	1.087^{a}	213.17^{a}	9.21^{c}	0.113^{e}
SEM			0.84	0.048
P – value	< 0.01	< 0.01	< 0.01	< 0.01

[1] Within columns, values with different superscripts differ ($P \leq 0.05$). Serum antibodies reported as in Gonda et al. (2012).
[2]Blood samples were collected on d -21, 0, 1, 3, 6, 10, 15, and 30 relative to weaning (d 0). Calves received vaccination against respiratory viruses on d -21 (Triangle 5; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA) and d 0 (Titanium 5; Elanco Animal Health, Greenfield, IN)

Table 4.5. Expression of liver genes in calves born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1,2]

Item[2]	**INR**	**AAC**	**SEM**	***P*-value**
CUT				
Heifer	2.01	1.89	0.12	0.48
Steer	1.91	2.01	0.11	0.50
IGF-I				
Heifer	1.99	1.93	0.17	0.81
Steer	1.62	1.87	0.12	0.15
MT				
Heifer	126.4	161.2	63.0	0.71
Steer	5.35	3.08	0.63	0.02
SOD				
Heifer	2.17	2.15	0.16	0.95
Steer	1.92	2.01	0.11	0.58

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0). All calves were weaned on d 367 of the experiment at 200 ± 2.2 d of age. Heifers were managed as a single group receiving the same nutritional and overall management, whereas steers were transported on d 493 to a feedlot and managed a single group receiving the same nutritional and overall management until slaughter.

[2]Liver samples were collected via needle biopsy (Arthington and Corah, 1995) from heifers on d 584 and from steers on d 586. Values are expressed as relative fold change compared within threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

[3]CUT = Cu-transporter protein; IGF-I = insulin-like growth factor-I; MT = metallothionein 1A; SOD = superoxide dismutase 1.

Table 4.6. Expression of *longissimus* muscle genes in calves born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 95) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 95) during gestation[1,2]

Item[3]	**INR**	**AAC**	**SEM**	***P*-value**
FABP4				
Heifer	4.68	5.10	1.5	0.85
Steer	7.56	7.12	1.3	0.82
Myogenin				
Heifer	4.59	2.87	0.58	0.05
Steer	2.98	2.56	0.28	0.30
PAX7				
Heifer	1.91	1.70	0.08	0.09
Steer	1.67	1.56	0.11	0.51
PPAR-γ				
Heifer	1.62	1.53	0.21	0.77
Steer	2.22	2.52	0.30	0.49

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0). All calves were weaned on d 367 of the experiment at 200 ± 2.2 d of age. Heifers were managed as a single group receiving the same nutritional and overall management, whereas steers were transported on d 493 to a feedlot and managed a single group receiving the same nutritional and overall management until slaughter.
[2]Muscle samples were collected via needle biopsy (Schubach et al., 2019) from heifers on d 584 and from steers on d 586. Values are expressed as relative fold change compared within threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).
[3]FABP4 = adipocyte fatty acid binding protein; PAX7 = paired box gene 7; PPAR-γ = peroxisome proliferator-activated receptor-γ.

Table 4.7. Growth and reproductive responses of replacement beef heifers born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 34) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 47) during gestation[1]

Item	**INR**	**AAC**	**SEM**	***P*-value**
Final BW[2], kg	332	326	5	0.37
Average daily gain, kg/d	0.62	0.60	0.01	0.39
Pubertal by breeding[3], %	83.5	86.4	5.1	0.49
Age at puberty, d	413	399	7	0.17
Body weight at puberty, kg	314	306	6	0.36
Antral follicle count classification[4]				
High AFC, %	70.7	58.3	8.0	0.32
Intermediate AFC, %	27.0	23.7	7.5	0.77
Low AFC, %	2.3	18.0	5	0.05

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0). Heifers from both treatments were weaned on d 367 of the experiment at 202 ± 3 d of age and managed as a single group receiving the same nutritional and overall management.

[2]Heifer initial and final body weight (BW) were calculated, respectively according to the average of 2 BW recorded at the end of preconditioning (d 411 and 412) and average of BW recorded on d 619 and 620. Average daily gain was calculated using initial and final BW.

[3]Evaluated according to plasma progesterone concentrations in samples collected weekly from days 437 to 619 (Schubach et al., 2017).

[4]AFC = antral follicle count; on d 619 the ovaries of each heifer were examined via transrectal ultrasonography. Pairs of ovaries were AFC according to modified methods of Ireland et al. (2008). Ovarian pairs with < 10 antral follicles were classified as low AFC; ovarian pairs with 11 to 20 antral follicles were classified as intermediate AFC; and ovarian pairs with > 20 antral follicles were classified as high AFC.

Table 4.8 Feedlot performance of feeder steers born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 51) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 44) during gestation[1]

Item	INR	AAC	SEM	*P*-value
Feedlot performance				
Treated for BRD symptoms[2], %	2.2	4.3	2.5	0.56
BW at the end of feedlot period, kg	590	582	8	0.49
Average daily gain[3], kg/d	1.29	1.29	0.03	0.98
Percent of calves slaughtered	93.3	88.8	4.0	0.44
Percent of AI – sired calves slaughtered	38.7	41.0	4.5	0.72
Carcass characteristics[4]				
HCW, kg	372	367	5	0.49
Back fat, cm	1.63	1.68	0.08	0.72
LM area, cm	80.2	80.8	1.0	0.70
Marbling score	407	402	9	0.70
Yield grade	3.74	3.71	0.11	0.85
Choice, %	49.8	37.5	7.3	0.24
Kilograms of carcass produced per cow,[5] kg	346	326	16	0.38

[1]INR and AAC cows received the same amount of supplemental Co, Cu, Mn, and Zn from sulfate sources or Availa 4 (Zinpro Corporation, Eden Prairie, MN). Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0). Steers from both treatments were weaned on d 367 of the experiment at 197 ± 3 d of age and shipped to the feedlot on d 493 where they were managed as a single group receiving the same nutritional and overall management until slaughter (d 724).
[2]BRD = bovine respiratory disease. Calves were classified as positive for BRD symptoms according to the DART system (Zoetis Inc., Florham Park, NJ) and received medication according to feedlot management criteria.
[3]Calculated based on HCW (assuming 63% dressing; Loza et al., 2010).
[4]Back fat thickness measured at the 12th rib. Marbling score: 400 = Small^{00}, 500 = Modest^{00}, 600^{00}; yield grade calculated as reported by Lawrence et al. (2010).
[5]Calculated based on total kilograms of carcass harvested divided by number of pregnant cows assigned to the experiment that sired a male calf.

Figure 4.1. Plasma cortisol concentration from weaned calves (d 367 of the experiment) born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**) during gestation. Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0). A treatment × day interaction was detected ($P = 0.03$). Within days: † $0.05 \leq P \leq 0.10$; * $P \leq 0.05$.

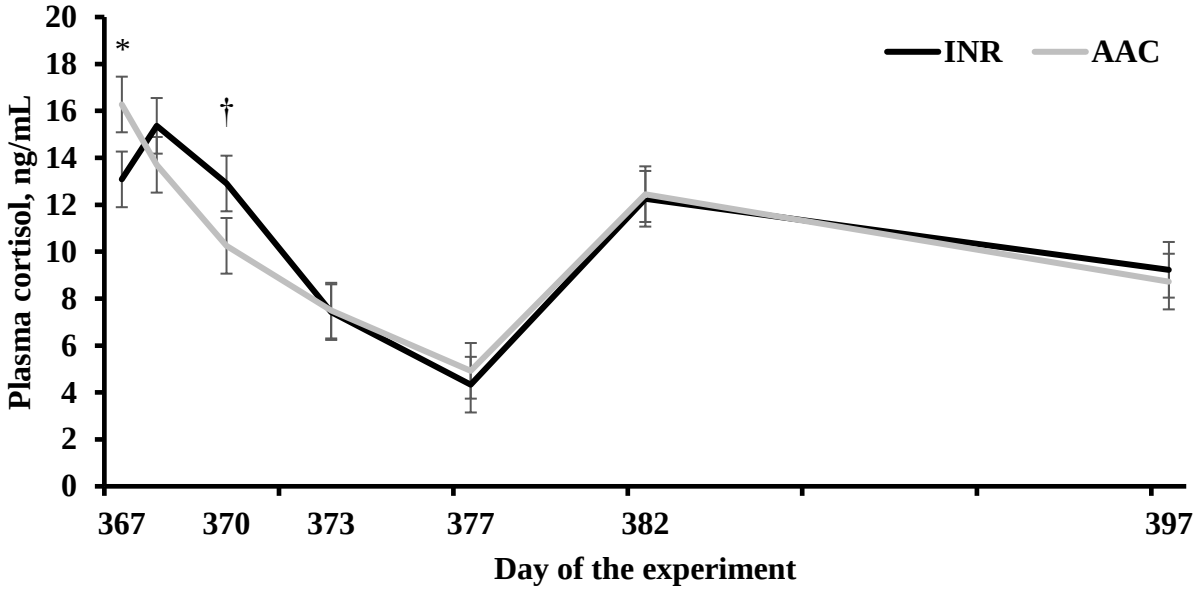


Figure 4.2. Weekly body weight of replacement beef heifers born from cows born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 34) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 47) during gestation. Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0). Heifers from both treatments were weaned on d 367 of the experiment at 202 ± 3 d of age and managed as a single group receiving the same nutritional and overall management. Growth rate of each heifer was modeled by linear regression of body weight against sampling days, and each regression coefficient was used as individual response. No treatment differences were noted for body weight ($P \geq 0.32$) or growth rate from d 437 to 619 (0.68 vs. 0.67 kg/day for INR and AAC heifers, respectively; SEM = 0.01).

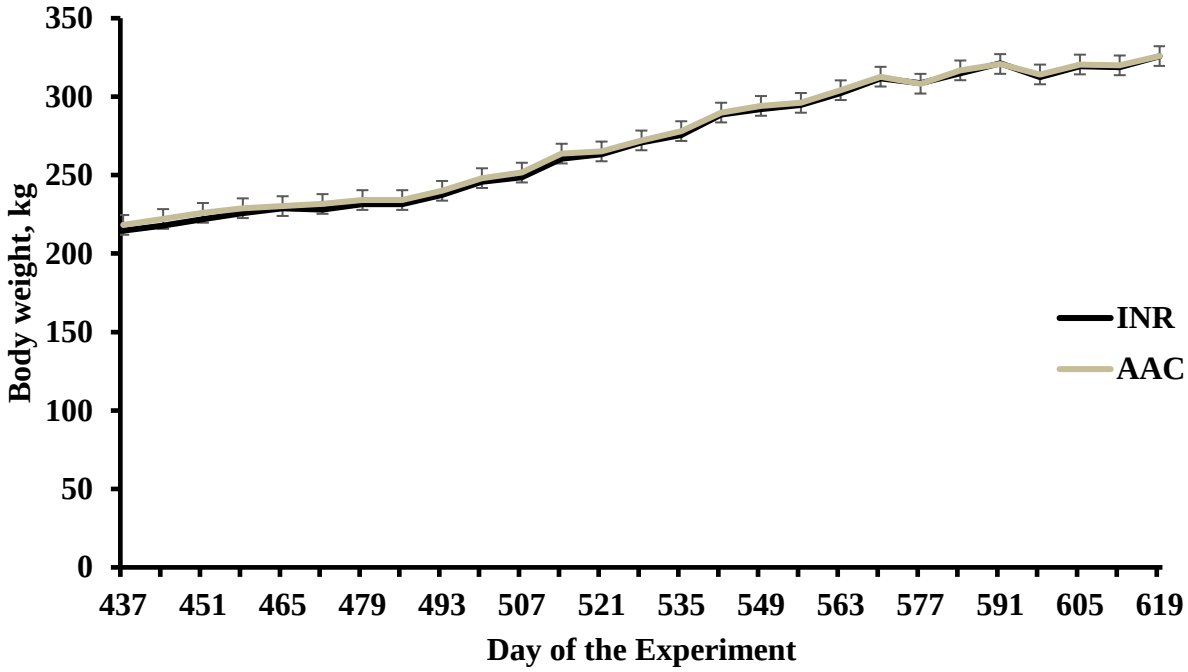
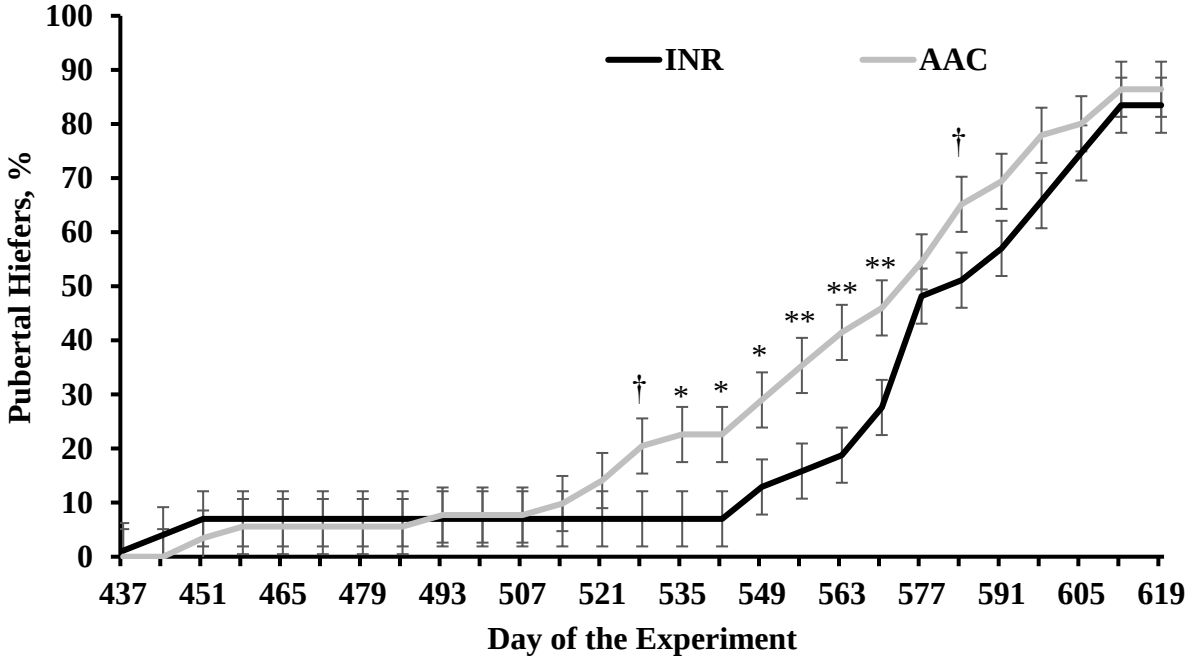


Figure 4.3. Puberty attainment of replacement beef heifers born from cows born from beef cows that received diets containing supplemental sulfate sources of Co, Cu, Mn, and Zn (**INR**; n = 34) or organic complexed source of Co, Cu, Mn, and Zn (**AAC**; n = 47) during gestation. Cows were assigned to the experiment at 117 ± 2.2 d of gestation (d 0). Heifers from both treatments were weaned on d 367 of the experiment at 202 ± 3 d of age and managed as a single group receiving the same nutritional and overall management. Puberty was estimated based on blood samples collected every 7 days from d 437 to 619. Heifers were considered pubertal once plasma progesterone concentrations were ≥ 1.0 ng/mL followed by a cyclic pattern of plasma progesterone < and ≥ 1.0 ng/mL indicative of normal estrous cycles. Puberty attainment was declared at the first sampling that resulted in plasma progesterone ≥ 1.0 ng/mL (Schubach et al., 2017). A treatment × day interaction was detected ($P < 0.01$). Within days: † $0.05 \leq P \leq 0.10$; * $P \leq 0.05$; ** $P < 0.01$.



5. SUPPLEMENTING CALCIUM SALTS OF SOYBEAN OIL TO BEEF STEERS EARLY IN LIFE TO ENHANCE CARCASS DEVELOPMENT AND QUALITY[1]

5.1. Introduction

Metabolic imprinting, defined as biological responses to a nutritional intervention during early life that permanently alters physiological outcomes later in life (Du et al., 2010), has been shown to enhance carcass characteristics in cattle (Graugnard et al., 2009; Moriel et al., 2014). As an example, Scheffler et al. (2014) reported feeding a high-concentrate diet to early-weaned beef steers from 100 to 205 d of age increased marbling compared with forage fed-steers weaned at 205 d of age. Improved carcass quality and increased marbling benefits carcass prices (USDA, 1997) and beef palatability through greater tenderness, juiciness, and flavor (Jost et al., 1983). Therefore, nutritional management to stimulate metabolic imprinting appears to be an effective strategy to improve marbling and quality of beef carcasses.

Our research group reported that supplementation with Ca salts of soybean oil (**CSSO**) to beef steers weaned at 6 mo of age during a 28-d preconditioning period, at 0.07% of weaning body weight (**BW**), increased marbling and percent of choice carcasses upon slaughter compared with cohorts not receiving supplemental fat (Cooke et al., 2011). Similarly, Mangrum et al. (2016) reported beef steers weaned at 5 mo of age and supplemented with CSSO for 110 d after weaning, at 0.09% of weaning BW, had greater marbling upon slaughter compared with nonsupplemented cohorts. These studies suggested that CSSO supplementation stimulates metabolic imprinting events related to carcass marbling in cattle, likely due to its essential fatty acid (**FA**) content. More

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. *Journal of Animal Science*; 97, 4182-4192, Copyright 2019 by Journal of Animal Science.

specifically, ω-6 FA have been shown to modify genes associated with adipose development (Azain, 2004; Benatti et al., 2004). However, Cooke et al. (2011) and Mangrum et al. (2016) evaluated steers older than 5 mo of age, whereas younger animals appear to be more responsive to metabolic imprinting events (Lucas, 1998).

One alternative to provide CSSO supplementation to younger steers in traditional cow-calf systems and stimulate metabolic imprinting events is via creep-feeding (Reis et al., 2015). Therefore, we hypothesized that inclusion of CSSO into a creep-feeding supplement provided to suckled steers, beginning at 2-mo of age would promote carcass marbling via metabolic imprinting effects beyond the outcomes reported by Cooke et al. (2011) and Mangrum et al. (2016). Moreover, we also theorized that inclusion of CSSO into both creep-feeding and preconditioning supplements would result on additive benefits to carcass marbling. To test these hypotheses, this experiment evaluated growth, physiological parameters, and carcass characteristics of beef steers receiving CSSO at 2 mo of age via creep-feeding, and/or postweaning via preconditioning supplementation.

5.2. Materials and Methods

This experiment was conducted over 2 consecutive years (2016 and 2017) at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns station) and was divided into 4 periods: creep-feeding (**CF**: day 0 to 60), preweaning period (day 61 to weaning), preconditioning (**PC**; weaning to feedlot shipping), and a feedlot period (feedlot arrival to slaughter). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (#4863).

5.2.1. *Animals and Treatments*

A total of 64 Angus × Hereford steers were enrolled in this study (32 steers per year). Steers were maintained on a 6,500-ha semiarid range pasture (Ganskopp and Bohnert, 2009) with their respective dams from birth until the beginning of the study (day −1). On day 0, steers were ranked by BW, age (initial BW = 114 ± 4 kg; initial age = 66.1 ± 0.9 d), as well as dam age and body condition score (**BCS**; Wagner et al., 1988) and allocated to 1 of 16 drylot pens (20 × 7 m; 2 steers per pen) in a manner that mean calf age, BW, dam age, and dam BCS were equivalent across all pens. Pens were randomly assigned to receive CSSO supplementation or not (**CON**) during the CF and/or PC periods, in a 2 × 2 factorial arrangement of treatments. As a result, 4 treatment combinations were generated (4 pens per treatment each year): 1) CSSO during CF and PC, 2) CSSO during CF and CON during PC, 3) CON during CF and CSSO during PC, and 4) CON during CF and PC.

During the CF period (day 0 to 60), steers were housed with their respective dams in the aforementioned drylot pens containing a creep-feeder that allowed both steers in the pen to have simultaneous access to the supplement. Pens received (as-fed basis) 500 g per steer daily of a pelletized corn-based creep supplement + 100 g soybean meal in addition to 80 g per steer daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ; n = 8 pens per year), or 67 g per steer daily of prilled saturated fat (EnergyBooster, Milk Specialties, Eden Prairie, MN) + 13 g per steer daily of limestone (CON, n = 8 pens per year). Supplemental CSSO was provided at a rate of 0.07% of steer initial BW, which was the CSSO supplementation rate used by Cooke et al. (2011). Treatments were formulated to be isocaloric, isonitrogenous, and isolipidic, but differing in FA composition (Table 5.1). Limestone was added to CON to compensate for the Ca included in the CSSO source (Table 5.1). Pens received 25% of their supplement treatments beginning on

day 0, and were offered the remaining portion in a step-up manner so that 100% of supplement was received by day 10 of the CF period (25% from day 0 to 2, 50% from day 3 to 6, and 75% from day 7 to 9). Supplement was readily consumed by steers within 24 h of being offered. Cows from both treatments received and readily consumed 20 kg per cow daily (DM basis) of alfalfa-grass hay during the CF phase. Hay consumption by steer calves was negligible given that steer height was insufficient to reach feed bunks containing hay, and milk is still the major dietary component of calves at this age (Ansotegui et al., 1991).

During the preweaning period (day 61 to weaning), cows and steers were returned to the same 6,500-ha semiarid range pasture (Ganskopp and Bohnert, 2009) and managed as a single group with no FA supplementation. Upon weaning (day 124 of year 1 and day 127 of year 2), steers were managed as a single group for 8 d in a semiarid range pasture, with ad libitum access to grass-alfalfa hay and no concentrate supplementation. This interval served as a transition period between weaning and experimental procedures to alleviate behavioral stress caused by maternal separation (Weary et al., 2008). Steers were then returned to the same drylot pens used in the CF period (16 pens; 2 steers per pen each year), for a 40-d PC period. During the PC period (day 132 to 172 of year 1 and day 135 to day 175 of year 2), steers had access to free-choice mixed alfalfa-grass hay and received a corn-based concentrate (Table 5.1) in addition to 150 g per steer daily of CSSO (Essentiom; Church and Dwight Co., Inc.; n = 8 pens per year), or 125 g per steer daily of prilled saturated fat (EnergyBooster, Milk Specialties) + 25 g per steer daily of limestone (CON, n = 8 pens per year). As in the CF phase, CSSO supplementation was provided at a rate of 0.07% of steer weaning BW, and limestone added to CON to compensate for the Ca included in the CSSO source (Table 5.1). Treatments were isocaloric, isonitrogenous, isolipidic, differed in FA composition (Table 5.1). Steers were observed daily for bovine respiratory disease (**BRD**) signs

according to the DART system (Zoetis, Florham Park, NJ), as detailed by Sousa et al. (2018). Throughout the CF, preweaning, and PC periods, water and a commercial mineral and vitamin mix (Cattleman's Choice; Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 6,000 ppm Zn, 3,200 ppm Cu, 65 ppm I, 900 ppm Mn, 140 ppm Se, 136 IU/g of vitamin A, 13 IU/g of vitamin D3, and 0.05 IU/g of vitamin E were available for ad libitum consumption.

At the end of the PC period (day 173 of year 1 and day 176 of year 2), steers were loaded onto a commercial livestock trailer, and transported for 218 km to a commercial feedlot (Lightning Feeders, Nyssa, OR), where they were managed as a single group (feedlot period). Upon feedlot arrival, steers received a hormonal implant (Component TE 200; Elanco Animal Health, Greensfield, IN) and received the same diets (Table 5.2) until slaughter at a commercial packing facility (day 378 of year 1 and day 385 of year 2; Tyson Fresh Meats, Inc., Pasco, WA). Steers were observed daily for BRD signs during the feedlot period based on the DART system (Zoetis), and received medication according to the management criteria of the commercial feedlot.

5.2.2. ***Sampling***

Samples of hay and supplement ingredients utilized during CF and PC periods were collected before the beginning of the experiment and analyzed for nutrient concentration by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of crude protein (method 984.13; AOAC, 2006), acid detergent fiber (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY: AOAC, 2006), neutral detergent fiber (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.), and FA concentrations using gas chromatography (Autosystem XL Gas Chromatograph, Perkin Elmer,

Inc., Waltham, MA) according to Sukhija and Palmquist (1988). Only FA that were individually identified in the analysis are reported herein. Calculations for total digestible nutrients used the equations proposed by Weiss et al. (1992), whereas net energy for maintenance and gain were calculated with the equations proposed by the NRC (2000). Nutritional and FA concentrations of all feedstuffs utilized are described in Table 5.3.

Steer BW was recorded on 2 consecutive days to determine BW prior to (day −1 and 0) and at the end of the CF period (day 60 and 61) and used to calculated average daily gain (**ADG**). Steer BW was also recorded on 2 consecutive days at weaning (day 124 and 125 of year 1 and day 127 and 128 of year 2) and used to calculate ADG during the preweaning period. Individual shrunk BW was recorded prior to the beginning of the PC period (day 131 of year 1 and day 134 of year 2) and prior to feedlot shipping (day 171 of year 1 and day 174 of year 2) after 16 h of feed and water withdrawal, and values were used to calculate ADG during PC. At the commercial packing plant, hot carcass weight (**HCW**) was collected upon slaughter (day 378 of year 1 and day 385 of year 2). Final BW was estimated based on HCW adjusted to a 63% dressing percentage to minimize variation associated with gut fill (Loza et al., 2010), and was used to estimate ADG during the feedlot period. After a 24-h chill, trained personnel assessed carcass backfat thickness at the 12th rib and LM area, and all other carcass measures were recorded by a USDA grader. Cow BW and BCS were also recorded on day - 1, 61, and at weaning.

Supplement, hay, and total DM intake were evaluated daily during the PC period by collecting and weighing offered and nonconsumed feed. All samples were dried to 96 h at 50 °C in forced-air ovens for DM calculation. Hay, supplement, and total daily DM intake (**DMI**) of each pen were divided by the number of steers within each pen and expressed as kg per steer per day. Total BW gain and DMI of each pen during the PC period were used for feed efficiency (**G:F**)

calculation. Blood samples were collected from all steers on day 0, 60, weaning (day 124 of year 1 and day 127 of year 2), prior to shipping (day 172 of year 1 and day 175 of year 2), and during the feedlot period (day 328 of year 1 and day 337 of year 2). Blood was collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing freeze-dried sodium heparin. Immediately after blood sampling, biopsies of the *longissimus muscle* (**LM**) between the 11th and 12th rib were performed in all calves via needle biopsy (Tru-Cut biopsy needle; CareFusion Corporation, San Diego, CA) according to Meijer et al. (1995). Muscle biopsy samples were stored in 2-mL tubes containing 1 mL of RNA stabilization solution (RNAlater, Ambion, Inc., Austin, TX), and stored at −80 °C until further processing.

5.2.3. ***Laboratorial Analyses***

After collection, all blood samples were immediately placed on ice, centrifuged (2,500 × g for 30 min; 4 °C) for plasma harvest, and stored at −80 °C on the same day of collection. Plasma samples were analyzed for FA concentration using gas chromatography (Agilent 7890, Agilent Technologies, Inc.) using the same procedures described by Tripathy et al. (2010). Only FA that were individually identified in the analysis are reported herein. Total RNA was extracted from muscle samples using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop Lite; Thermo Fisher Scientific, Wilmington, DE) at 260 nm and 260/280 nm ratio, respectively (Fleige and Pfaffl, 2006). Reverse transcription of extracted RNA and real-time reverse transcription-PCR using gene specific primers (20 pM each; Table 5.4) were completed as described by Rodrigues et al. (2015). A portion of the amplified products were purified with the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced at the Oregon State University – Center for Genome Research

and Biocomputing to verify the specificity of amplification. All amplified products represented only the genes of interest. Responses from the genes of interest were quantified based on the threshold cycle (**CT**), the number of PCR cycles required for target amplification to reach a predetermined threshold. The CT responses from muscle genes of interest were normalized to the geometrical mean of CT values of *ribosomal protein S9* and *β-actin* (Vandesompele et al., 2002). The CV for the geometrical mean of reference genes across all samples was 3.6%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

5.2.4. ***Statistical Analysis***

All data were analyzed using pen as experimental unit and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), whereas binary data were analyzed using the GLIMMIX procedure of SAS. During the CF and preweaning periods, the model statements used for steer and cow BW, cow BCS, and steer ADG contained the effects of CF treatment. Model statements used for mRNA expression of LM genes and plasma FA concentrations contained the effects of CF treatment, day, and the CF treatment × day interaction. Data from CF and preweaning periods were analyzed using pen(CF treatment × year), steer(pen), and year as random variables. During the PC and feedlot periods, model statements for steer BW, ADG, incidence of BRD, feed efficiency, and carcass variables contained the effects of CF treatment, PC treatment, and the resultant interaction. Model statements used for mRNA expression of LM genes, plasma FA concentrations, and precondition DMI contained the effects of CF treatment, PC treatment, day, and all resultant interactions. Data from the PC and feedlot periods were analyzed using pen(CF treatment × PC treatment × year), steer(pen), and year as random variables, but for DMI and feed efficiency that used pen(CF treatment × PC treatment ×

year) and year as random variables. For all analysis using repeated measures, the specified term was day, whereas the subject was steer(pen) for mRNA expression of LM genes and plasma FA, or pen(CF treatment × PC treatment × year) for preconditioning DMI. The covariance structure utilized was autoregressive, which provided the best fit for these analyses according to the lowest Akaike information criterion. Values obtained on day 0 were not used as independent covariate for each respective analysis given the experimental length and sampling schedule. All results are reported as least square means, and least square differences or PDIFF were used for simple or multiple mean separation, respectively. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. In the text, results are reported according to main treatment effects if higher-order interactions containing treatments were nonsignificant, or according to highest-order interaction detected. Nonetheless, results are reported in tables according to sampling days to facilitate assessment and interpretation

5.3. Results and Discussion

5.3.1. *Creep-Feeding and Preweaning Periods*

As designed, steers receiving CON and CSSO during CF were of similar age ($P = 0.63$) at the beginning of the experimental period (Table 5.5). No CF treatment differences were detected ($P \geq 0.69$) for BW or ADG prior to weaning (Table 5.5), which was expected given CF treatments were isocaloric, isonitrogenous, and isolipidic. Cow milk yield was not evaluated in the present experiment to estimate milk consumption and its contribution to steer daily nutrient intake during the CF and preweaning periods. Nevertheless, cows nursing CSSO and CON steers received the same nutritional management during lactation, and had similar age, days in milk (based on calf age), BW, and BCS at the beginning of the CF period, which are known to impact milk production

in cattle (NRC, 2000). Moreover, cow BW and BCS remained similar between treatment groups throughout the CF and preweaning periods, which further mitigates potential differences in steer milk intake during the experiment (Reis et al., 2015).

Interactions between CF treatment × day were detected ($P \leq 0.05$) for plasma concentrations of palmitoleic, linoleic, linolenic, ω-6, PUFA, and ω-6: ω-3 ratio (Table 5.6). Concentrations of linoleic, ω-6, PUFA, and ω-6: ω-3 ratio in plasma were greater ($P < 0.01$) in CSSO vs. CON steers on day 60, but similar ($P \geq 0.68$) between CF treatment groups on day 0 and at weaning (Table 5.6). In turn, plasma concentrations of palmitoleic and linolenic were greater ($P \leq 0.01$) in CON vs. CSSO steers on day 60, but similar ($P \geq 0.40$) between CF treatment groups on day 0 and at weaning (Table 5.6). No CF treatment differences were detected ($P \geq 0.22$) for plasma concentrations of palmitic, stearic, oleic, dihomo-gamma-linolenic, docosadienoic, arachidonic, docosapentaenoic, SFA, MUFA, ω-3, and total FA (data not shown). As in Brandão et al. (2018), these results corroborate the FA content and profile of treatments (Table 5.1), given that plasma FA concentrations reflect intake and duodenal flow of FA (Lake et al., 2007; Scholljegerdes et al., 2007; Hess et al., 2008). Previous research also reported that CSSO supplementation increased plasma concentrations of linoleic acid, ω-6 FA, and total PUFA while reducing plasma concentrations of linolenic acid and ω-3 FA in growing steers, without immediate impacts on their growth rates (Cooke et al., 2011). Hence, supplementing 80 g of CSSO to suckled beef steers via creep-feeding effectively increased intake and circulating concentrations of linoleic and ω-6 FA.

No CF treatment differences ($P \geq 0.45$) were detected for mRNA expression of genes associated with adipogenic activities in the LM prior to and at the time of weaning (Table 5.7). More specifically, *peroxisome proliferator-activated receptor gamma* (**PPAR-γ**) plays a pivotal role in the regulation of adipogenesis and lipid metabolism, through induction of genes mediating the

process (Houseknecht et al., 2002), and has been identified as a candidate gene related to adipogenesis of bovine intramuscular adipose tissue (Lim et al., 2011). *Adipocyte fatty acid-binding protein* (**FABP4**) is a target gene of PPAR-γ (Taniguchi et al., 2008), and is highly involved in adipocyte differentiation, lipid hydrolysis, and acts as an intracellular FA chaperone (Michal et al., 2006). *Stearoyl-CoA desaturase* (**SCD**) is a key regulatory enzyme in the lipogenic pathway (Ntambi, 1999), and increased expression is associated with adipocyte hypertrophy (Martin et al., 1999). Increased expression of *fatty acid synthase* (**FASN**), an enzyme that modulates de novo synthesis of FA, is also a marker of adipogenesis (Graugnard et al., 2009). Similar to PPAR-γ, *sterol regulatory element-binding protein-1* (**SREBP1**) is a regulator of lipid metabolism, induced during the early stages of adipogenesis binding to the response element of PPAR-γ (Du et al., 2010). Increased expression of SREBP1 indicates enhanced capacity for de novo FA synthesis, and therefore increased lipid accumulation in the muscle tissue (Zhao et al., 2010). No CF treatment differences were also detected ($P \geq 0.52$) for mRNA expression of *myogenin* or *myogenic differentiation 1* (**MyoD**), which are myogenic regulatory factors in the LM that regulate postnatal muscle growth through differentiation and fusion of satellite cells with existing muscle fibers (Perdiguero et al., 2009; Du et al., 2010). Nevertheless, the impacts of dietary FA on these LM genes are still unclear (Price et al., 2000). Collectively, CSSO supplementation during a 60-d creep-feeding did not modulate mRNA expression of genes involved with lipid accumulation or myogenesis analyzed herein in the LM of suckled beef calves.

5.3.2. ***Preconditioning and Feedlot Periods***

During the PC period, a CF × PC treatment interaction was detected for hay and total DMI. These variables were greater ($P \leq 0.03$) in steers receiving CON during both CF and PC compared with steers receiving CON during CF and CSSO during PC (Table 5.8). No other CF or PC

treatment differences were detected ($P \geq 0.12$) for preconditioning hay, supplement, and total DMI (Table 5.8). Addition of CSSO to preconditioning diets has been shown to reduce forage and total DMI in cattle naïve to CSSO supplementation (Araujo et al., 2010; Cooke et al., 2011). In turn, lack of similar results in steers that received CSSO during CF suggest that these animals may have been previously adapted to this FA source, preventing substantial hay and total DMI depression when supplemented during the PC period. Nonetheless, these outcomes were not sufficient to impact ($P \geq 0.53$) BW, ADG, or feed efficiency during preconditioning (Tables 5.8 and 5.9). These results support previous studies indicating CSSO supplementation to preconditioning cattle did not affect ADG or feed efficiency measures, compared to cohorts offered isocaloric, isonitrogenous, and isolipidic control diets (Araujo et al., 2010; Cooke et al., 2011). It also should be noted that BRD signs were not observed during the PC period, despite this experiment not being specifically designed to assess this response.

Interactions between PC treatment × day were detected ($P \leq 0.05$) for plasma concentrations of palmitic, palmitoleic, linoleic, linolenic, arachidonic, MUFA, PUFA, ω-6, ω-3, total FA, and ω-6: ω-3 ratio (Table 5.10). Plasma concentrations of palmitic, linoleic, arachidonic, ω-6, PUFA, total FA, and ω-6: ω-3 ratio were greater ($P \leq 0.04$) in steers receiving CSSO compared with CON during PC prior to feedlot shipping, but similar ($P \geq 0.24$) between PC treatment groups during the feedlot period (Table 5.10). In turn, plasma concentrations of palmitoleic, linolenic, ω-3, and MUFA were greater ($P \leq 0.05$) in steers receiving CON compared with CSSO during PC prior to feedlot shipping, and similar ($P \geq 0.12$) between PC treatment groups during the feedlot period (Table 10). No PC treatment differences were noted ($P \geq 0.12$) for plasma concentrations of stearic, oleic, dihomo-gamma-linolenic, docosadienoic, docosapentaenoic, and SFA (data not shown). Moreover, no CF treatment effects were detected

($P \geq 0.12$) for plasma FA profile prior to feedlot shipping and during the feedlot period (Table 5.6). Similar to treatment differences noted during the CF period, these outcomes corroborate the FA content and profile of PC treatments (Table 5.1; Hess et al., 2008), and also implicate that there are no long-term carryover effects of CSSO supplementation during the CF period on plasma FA profile. Hence, supplementing 150 g of CSSO to beef steers during preconditioning had immediate effects on circulating concentration of linoleic and ω-6 FA, as previously reported by others (Araujo et al., 2010; Cooke et al., 2011; Mangrum et al., 2016).

A PC treatment × day interaction was detected ($P = 0.05$) for PPAR-γ mRNA expression, which was greater ($P = 0.04$) in steers receiving CSSO vs. CON during PC prior to shipping but similar ($P = 0.42$) during the feedlot period (Table 5.11). Dietary fat may regulate PPAR-γ mRNA expression in a ligand-dependent manner (Houseknecht et al., 2002). Polyunsaturated FA, specifically ω-6 such as linoleic acid, are known to stimulate PPAR-γ function (Kliewer et al., 1997; Xu et al., 1999; Thoennes et al., 2000), and PPAR-γ mRNA expression is upregulated with dietary supplementation of such FA (Spurlock et al., 2000). No additional effects of PC treatment were noted for mRNA expression of genes associated with lipogenesis or myogenesis in the LM after weaning ($P \geq 0.27$; Table 5.11). Interactions between CF treatment × day were detected ($P \leq 0.01$) for mRNA expression of FABP4, FASN, PPAR-γ, and SCD in the LM (Table 5.7). Expression of these genes did not differ ($P \geq 0.13$) between CF treatment groups prior to feedlot shipping, but were greater ($P \leq 0.02$) in samples collected during the feedlot period in steers receiving CSSO during CF. No CF treatment effects ($P \geq 0.28$) were noted for mRNA expression of MyoD, myogenin, and SREBP1 in the LM after weaning (Table 5.7). Collectively, CSSO supplementation during CF elicited alterations in mRNA expression of adipogenic genes, which were noted during the feedlot period when lipogenesis is substantial (Pethick et al., 2004). These

outcomes are suggestive of a metabolic imprinting effect (Du et al., 2010), given CF treatments were offered to steers during a period of elevated epigenetic susceptibility (Lucas, 1998). One could also attribute upregulated mRNA expression of adipogenic genes in calves that received CSSO during CF due to a potential increased DMI during the finishing period, as dietary fat may also epigenetically modulate appetite (Gupta et al., 2009). Conversely, PC treatments had no major impacts on the LM genes after weaning besides an immediate increase in PPAR-γ mRNA expression, whereas providing CSSO during both CF and PC also failed to yield additive benefits on these variables.

No CF or PC treatment differences were noted ($P \geq 0.18$) for performance responses during the feedlot period, not carcass traits upon slaughter (Table 5.9). No CF or PC treatment effects were also detected ($P \geq 0.30$) for BRD incidence during the feedlot period (Table 5.9), which was not a major variable of interest herein despite the potential immune benefits of CSSO (Cooke et al., 2011). Lack of LM area and HCW differences corroborate similar MyoD and myogenin mRNA expression (te Pas et al., 1999), as well as similar performance between CF and PC treatment groups throughout the experimental period. However, differential mRNA expression of FABP4, FASN, PPAR-γ, and SCD during the feedlot period did not translate into greater marbling in steers receiving CSSO during CF. Increased mRNA expression may not be supported by equivalent final phenotypic differences (Clancy and Brown, 2008). Graugnard et al. (2010) also reported altered PPAR-γ and FASN mRNA expression in the longissimus lumborum, but similar carcass marbling scores, in beef steers receiving high- or low-starch diets for 112 d after weaning at 5 mo of age. Reis et al. (2015) reported increased FASN and PPAR-γ mRNA expression, but similar backfat thickness in heifers provided concentrate ad libitum via creep-feeding for 50 d or not. Urrutia et al. (2015) also reported differential mRNA expression of SCD in the longissimus thoracis muscle

of lambs fed a 10% linseed compared to a control diet for 5 wk but found no differences in longissimus thoracis fat content. Nonetheless, the lack of PC treatment effects on marbling score do not corroborate with Cooke et al. (2011) or Mangrum et al. (2016), who reported increased marbling score in steers supplemented with CSSO after weaning for 28 and 110 d, respectively. However, treatments offered in these previous experiments (Cooke et al., 2011; Mangrum et al., 2016) were not isonitrogenous or isolipidic as utilized herein; hence, their results may also be related to the energy contribution of CSSO supplementation rather than the specific treatment FA profile.

In summary, supplementing CSSO to beef steers at 2 mo of age via creep-feeding stimulated mRNA expression of some genes involved in lipid metabolism later in life compared with cohorts receiving an isocaloric, isonitrogenous, and isolipidic supplement based on prilled saturated fat. However, increased mRNA expression of these genes, which included FABP4, FASN, PPAR-γ, and SCD, did not translate into improved carcass characteristics. Research is still warranted to investigate the effects of CSSO supplementation to nursing beef cattle during the period of epigenetic susceptibility on lipogenic gene expression, growth, and carcass characteristics. Nonetheless, CSSO supplementation to young cattle appears to be a nutritional alternative to upregulate mRNA expression of LM genes associated with lipogenesis during the finishing period.

5.4. References

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Table 5.1. Composition and nutritional profile of treatments during the 60-d creep-feeding (**CF**) and 40-d preconditioning (**PC**) periods[1]

	CF[1]		PC[2]	
Item	**CSSO**	**CON**	**CSSO**	**CON**
Ingredients, g/day (as-fed)				
Pellet	500	500	0	0
Ground corn	0	0	1135	1135
Soybean meal	100	100	200	200
Essentiom[2]	80	0	150	0
EnergyBooster[3]	0	67	0	125
Limestone	0	13	0	25
Nutrient Profile, DM basis				
DM, %	90.39	90.66	90.22	90.45
Total digestible nutrients, %[4]	96.93	96.24	98.88	98.17
Net energy for maintenance, Mcal/kg[5]	2.67	2.74	2.69	2.75
Net energy for growth, Mcal/kg[5]	1.89	1.97	1.91	1.97
Crude protein, %	20.36	20.26	13.75	13.68
Neutral detergent fiber, %	9.93	9.98	7.78	7.83
Ca, %	1.23	0.78	1.06	0.68
Fatty acids, %	12.77	12.70	12.15	12.05
Palmitic (16:0), %	3.77	3.76	3.31	3.39
Stearic (18:0), %	0.49	5.03	0.42	4.30
Oleic (18:1), %	3.40	1.28	3.28	1.46
Linoleic (18:2), %	4.29	1.16	4.53	1.83
Linolenic (18:3), %	0.43	0.10	0.36	0.09

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[2] Church and Dwight Co., Inc. (Princeton, NJ).
[3]Milk Specialties (Eden Prairie, MN).
[4]Calculated according to the equations described by Weiss et al. (1992).
[5]Calculated with equations described by the NRC (2000).

Table 5.2. Ingredient composition (as-fed basis) of diets offered to steers during the feedlot period[1]

	Diets[2]				
Ingredients, % as fed	**A**	**B**	**C**	**D**	**E**
Alfalfa hay	53.0	39.0	19.0	12.0	4.0
Distillers grains	0.0	0.0	2.0	2.0	4.0
Dried corn	0.0	0.0	11.0	10.0	18.0
High-moisture corn	42.0	55.0	46	53.3	54.5
Corn Silage	0.0	0.0	18.0	17.0	11.0
Mineral and vitamin mix[3]	5.0	6.0	4.0	4.5	6.5
Tallow	0.0	0.0	0.0	1.2	2.0

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. *Journal of Animal Science*; 97, 4182-4192, Copyright 2019 by Journal of Animal Science. Feedlot period was d 174 to slaughter (d 378) of year 1, and d 177 to slaughter (d 385) of year 2.
[2]A = offered for 5 d after arrival receiving; B = offered for 5 d after diet A; C = offered for 7 d after diet B; D = offered for 10 d after diet C; E = offered until slaughter.
[3]Customized blend of minerals, vitamins, and feed additives (Performix Nutrition Systems, Nampa, ID).

Table 5.3. Nutritional and fatty acid profile (dry matter basis) of feedstuffs[1]

Item	Pellet	Corn	Soybean meal	Essentiom[2]	EnergyBooster[3]	Grass-alfalfa hay
Total digestible nutrients, %	84	89	81	190	219	61
Net energy for maintenance, Mcal/kg	2.09	2.22	1.96	6.82	8.73	1.28
Net energy for growth, Mcal/kg	1.43	1.54	1.32	5.19	6.86	0.70
Crude protein, %	17.5	8.9	50.9	0.7	0.4	17.8
Neutral detergent fiber, %	10.5	7.51	14.8	0.91	1.75	44.5
Fatty acids, %	2.69	3.74	2.71	82.51	96.11	1.86
Palmitic (16:0), %	0.48	0.49	0.44	26.54	31.08	0.40
Stearic (18:0), %	0.07	0.06	0.10	3.33	46.05	0.06
Oleic (18:1), %	0.68	1.03	0.37	22.60	6.84	0.04
Linoleic (18:2), %	1.19	2.07	1.47	25.44	0.80	0.32
Linolenic (18:3), %	0.09	0.07	0.24	2.57	0.01	0.61

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. Journal of Animal Science; 97, 4182-4192, Copyright 2019 by Journal of Animal Science. Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). Total digestible nutrients were calculated according to the equations described by Weiss et al. (1992). Net energy for maintenance and growth was calculated with equations described by the NRC (2000).
[2]Church and Dwight Co., Inc. (Princeton, NJ).
[3]Milk Specialties (Eden Prairie, MN)

Table 5.4. Primer sequences, accession number and reference for all gene transcripts analyzed by real-time reverse transcription PCR[1]

Target[2]	Primer sequence	Accession nº	Source
FABP4			
Forward	AAACTTAGATGAAGGTGCTCTGG	AJ4160220	Li et al. (2018)
Reverse	CATAAACTCTGGTGGCAGTGA		
FASN			
Forward	ATCGAGTGCATCAGGCAAGT	AF479289	Jeong et al. (2012)
Reverse	TGTGAGCACATCTCGAAAGCCA		
MyoD			
Forward	ATCCTGCGCAACGCCATCCGCTATATCGA	AF093675	Muroya et al. (2002)
Reverse	CTCGCTGTAGTAAGTGCGGTCGTAGCAGT		
Myogenin			
Forward	GAGAAGCGCAGACTCAAGAAGGTGAATGA	AF091714	Muroya et al. (2002)
Reverse	TCTGTAGGGTCCGCTGGGAGCAGATGATC		
PPAR-γ			
Forward	GCATTTCCACTCCGCACTAT	AY137204	Li et al. (2018)
Reverse	GGGATACAGGCTCCACTTTG		
SCD			
Forward	GCCAACAACTCTGCCTTTATG	GU947654	Li et al. (2018)
Reverse	CACCAATGACTGACCACCTG		

Table 5.4. Continued

Target[1]	Primer sequence	Accession nº	Source
SREBP1			
Forward	ACTACCACGCCAAGTTCCTG	JN790254	Li et al. (2018)
Reverse	CGATGCCAATCTCCTCCTT		
B-actin			
Forward	AGCAAGCAGGAGTACGATGAGT	NM_173979	Bong et al. (2012)
Reverse	ATCCAACCGACTGCTGTCA		
Ribosomal protein S9			
Forward	CCTCGACCAAGAGCTGAAG	AF479289	Jeong et al. (2012)
Reverse	CCTCCAGACCTCACGTTTGTTC		

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. Journal of Animal Science; 97, 4182-4192, Copyright 2019 by Journal of Animal Science.
[2]FABP4 = adipocyte fatty acid-binding protein; FASN = fatty acid synthase; MyoD = myogenic differentiation 1; PPAR-γ = peroxisome proliferator-activated receptor-γ; SCD = stearoyl-CoA desaturase; SREBP1 = sterol regulatory element-binding protein-1.

Table 5.5. Performance responses of cows and their steer calves, which were supplemented with Ca salts of soybean oil (**CSSO**; n= 16 pens) or prilled saturated fat (**CON**; n = 16 pens) via creep-feeding (d 0 to 60)[1,2]

Item	CON	CSSO	SEM	*P*-value
Steers				
Initial age, d	65.8	66.4	0.9	0.63
Body weight, kg				
d 0, kg	115	113	4	0.74
d 60, kg	189	193	4	0.50
Weaning, kg	229	233	4	0.47
Average daily gain, kg/d				
d 0 to 60	1.23	1.32	0.06	0.30
d 60 to weaning[2]	0.67	0.67	0.04	0.97
Cows				
Initial age, yr	6.09	6.31	0.45	0.73
Body weight, kg				
d 0, kg	536	526	12	0.57
d 60, kg	564	556	12	0.61
Weaning, kg	530	526	12	0.75
Body condition score[3]				
d 0	5.03	5.03	0.07	0.99
d 60	5.06	5.15	0.07	0.35
Weaning	4.75	4.60	0.07	0.14

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. Journal of Animal Science; 97, 4182-4192, Copyright 2019 by Journal of Animal Science. CSSO = daily supplementation (per steer) with 80 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per steer) with 67 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone.
[2]Weaning occurred on day 124 of year 1 and on day 127 of year 2.
[3]According to Wagner et al. (1988).

Table 5.6. Plasma fatty acid concentrations (μg/mL of plasma) in beef steers supplemented with Ca salts of soybean oil (**CSSO**; n = 16 pens) or prilled saturated fat (**CON**; n = 16 pens) via creep-feeding (day 0 to 60)[1,2]

Item	CON	CSSO	SEM	*P*-value
Palmitoleic (16:1)				
day 0	26.9	25.3	1.4	0.40
day 60	26.8	17.8	1.4	< 0.01
Weaning	11.7	10.9	1.4	0.70
Shipping	4.42	4.86	0.43	0.47
Finishing	7.32	7.68	0.43	0.55
Linoleic (18:2, ω-6)				
day 0	156	152	18	0.86
day 60	218	380	18	< 0.01
Weaning	162	155	18	0.68
Shipping	255	222	15	0.13
Finishing	361	366	15	0.80
Linolenic (18:3, ω-3)				
day 0	81.6	79.8	5.6	0.82
day 60	104	84.2	5.6	0.01
Weaning	57.8	53.8	5.6	0.62
Shipping	50.5	49.6	2.7	0.82
Finishing	12.1	12.3	2.7	0.94
Total ω-6				
day 0	215	221	20	0.83
day 60	291	453	20	< 0.01
Weaning	207	199	20	0.77
Shipping	298	263	16	0.12
Finishing	398	403	16	0.82
ω-6:ω-3 ratio				
day 0	1.91	1.92	0.20	0.99
day 60	2.10	4.56	0.20	< 0.01

Table 5.6. Continued

Item	CON	CSSO	SEM	*P*-value
Weaning	2.97	2.93	0.20	0.89
Shipping	6.46	5.33	0.55	0.15
Finishing	24.8	24.4	0.55	0.56
Total PUFA[3]				
day 0	317	321	25	0.91
day 60	417	560	25	< 0.01
Weaning	279	266	25	0.71
Shipping	360	324	17	0.15
Finishing	413	419	17	0.81

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. Journal of Animal Science; 97, 4182-4192, Copyright 2019 by Journal of Animal Science. CSSO = daily supplementation (per steer) with 80 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per steer) with 67 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone.

[2]Weaning occurred on day 124 of year 1 and on day 127 of year 2. Calves were preconditioning from day 132 to 172 and day 135 to day 175 of year 1 and 2, respectively. Steers were shipped to the feedlot and remained there from day 173 to slaughter (day 378) and day 176 to slaughter (day 385) of year 1 and 2, respectively. Blood samples were collected on day 0, 60, weaning (day 124 of year 1; day 127 of year 2), prior to shipping to the feedlot (shipping; day 172, year 1; day 175, year 2), and once during the feedlot period (finishing; day 328, year 1; day 337, year 2).

[3]PUFA = linoleic, linolenic, dihomo-gamma-linolenic, arachidonic, docosadienoic, and docosapentaenoic acids.

Table 5.7. Expression of *longissimus muscle* genes in beef steers supplemented with Ca salts of soybean oil (**CSSO**; n = 16 pens) or prilled saturated fat (**CON**; n = 16 pens) via creep-feeding (day 0 to 60)[1,2]

Item[3]	**CON**	**CSSO**	**SEM**	***P*-value**
FABP4				
day 0	21.4	17.4	7.1	0.69
day 60	29.8	24.9	7.1	0.63
Weaning	31.2	30.1	7.1	0.91
Shipping	66.7	54.3	8.3	0.16
Finishing	38.5	72.5	8.3	0.02
FASN				
day 0	29.8	24.4	4.3	0.37
day 60	37.5	32.4	4.3	0.43
Weaning	14.6	21.9	4.3	0.25
Shipping	91.0	80.2	18.3	0.39
Finishing	124	210	18.6	0.02
MyoD				
day 0	35.5	31.9	4.0	0.52
day 60	14.6	15.5	4.0	0.87
Weaning	38.9	33.8	4.0	0.37
Shipping	12.9	13.8	1.0	0.55
Finishing	8.02	9.00	1.05	0.39
Myogenin				
day 0	43.5	43.8	4.7	0.95
day 60	29.3	22.7	4.7	0.32
Weaning	61.2	49.0	4.7	0.08
Shipping	39.9	42.2	4.4	0.80
Finishing	7.42	8.58	4.5	0.77
PPAR-γ				
day 0	4.39	3.91	0.46	0.45
day 60	4.40	3.97	0.46	0.49

Table 5.7. Continued

Item[3]	CON	CSSO	SEM	*P*-value
Weaning	3.67	3.71	0.46	0.95
Shipping	7.18	6.11	0.73	0.13
Finishing	5.01	8.20	0.74	0.01
SCD				
day 0	26.6	22.7	3.9	0.48
day 60	28.6	22.6	3.9	0.28
Weaning	10.6	17.9	3.9	0.19
Shipping	65.8	61.8	17.1	0.68
Finishing	96.5	202	17.4	< 0.01
SREBP1				
day 0	2.76	2.84	0.14	0.68
day 60	2.67	2.54	0.14	0.51
Weaning	1.93	1.93	0.14	0.98
Shipping	3.25	3.59	0.19	0.31
Finishing	3.07	3.22	0.20	0.59

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. Journal of Animal Science; 97, 4182-4192, Copyright 2019 by Journal of Animal Science. CSSO = daily supplementation (per steer) with 80 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per steer) with 67 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone.

[2]Weaning occurred on day 124 of year 1 and on day 127 of year 2. Calves were preconditioning from day 132 to 172 and day 135 to day 175 of year 1 and 2, respectively. Steers were shipped to the feedlot and remained there from day 173 to slaughter (day 378) and day 176 to slaughter (day 385) of year 1 and 2, respectively. Samples of the longissimus muscle were taken via needle biopsy on day 0, 60, weaning (day 124 of year 1; day 127 of year 2), prior to shipping to the feedlot (shipping; day 172, year 1; day 175, year 2), and once during the feedlot period (finishing; day 328, year 1; day 337, year 2). Values are expressed as relative fold change compared with threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

[3]FABP4 = adipocyte fatty acid-binding protein; FASN = fatty acid synthase; MyoD = myogenic differentiation 1; PPAR-γ = peroxisome proliferator-activated receptor-γ; SCD = stearoyl-CoA desaturase; SREBP1 = sterol regulatory element-binding protein-1.

Table 5.8. Preconditioning feed intake and efficiency in beef steers supplemented with Ca salts of soybean oil (CSSO) or prilled saturated fat (**CON**) during the creep-feeding period (**CF**; day 0 to 60, top row; n = 16 pens) and/or a 40-d preconditioning period (PC; bottom row; n = 16 pens)[1,2,3]

	CON	CSSO				*P*-values		
Item	**CON**	**CSSO**	**CON**	**CSSO**	**SEM**	**CF**	**PC**	**CF × PC**
Hay, kg/d	6.11[a]	5.40[b]	5.70[ab]	5.87[ab]	0.22	0.89	0.23	0.05
Supplement, kg/d	1.15	1.08	1.03	1.05	0.04	0.11	0.52	0.82
Total, kg/d	7.07[a]	6.29[b]	6.54[a]	6.73[a]	0.22	0.84	0.19	0.03
Feed efficiency, kg/kg	0.138	0.137	0.135	0.143	0.011	0.88	0.74	0.68

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. Journal of Animal Science; 97, 4182-4192, Copyright 2019 by Journal of Animal Science. CSSO = daily supplementation (per steer) with 80 g (CF) and/or 150 g (PC) of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per steer) with 67 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone (CF) and/or 125 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 25 g of limestone (PC). Within rows, means with different superscripts (a, b) differ ($P \leq 0.05$).

[2]Weaning occurred on day 124 of year 1 and on day 127 of year 2. Calves were preconditioning from day 132 to 172 and day 135 to day 175 of year 1 and 2, respectively. Steers were shipped to the feedlot and remained there from day 173 to slaughter (day 378) and day 176 to slaughter (day 385) of year 1 and 2, respectively.

[3]Feed intake was recorded daily during preconditioning by measuring offer and refusals from each pen, divided by the number of calves within each pen, and expressed as kg per calf per day. Feed efficiency was calculated using total feed intake and body weight gain of each pen during preconditioning.

Table 5.9. Postweaning performance and carcass traits in beef steers supplemented with Ca salts of soybean oil (**CSSO**) or prilled saturated fat (**CON**) during the creep-feeding period (**CF**; day 0 to 60, n = 8 pens per year) or a 40-d preconditioning period (PC; n = 8 pens per year)[1,2,3]

	CF main effect				PC main effect			
Item	**CON**	**CSSO**	**SEM**	***P*-value**	**CON**	**CSSO**	**SEM**	***P*-value**
Body weight, kg								
Preconditioning	224	227	3	0.53	226	226	3	0.97
Shipping	262	265	4	0.61	264	263	4	0.87
Slaughter[4]	570	578	9	0.54	580	567	9	0.31
Average daily gain, kg/d								
Preconditioning to shipping	0.926	0.925	0.063	0.99	0.935	0.916	0.063	0.84
Shipping to slaughter[4]	1.48	1.51	0.04	0.62	1.52	1.46	0.04	0.26
Incidence of respiratory disease[5], %	57.6	49.1	10.2	0.56	60.9	45.8	10.2	0.30
Carcass[6]								
Hot carcass weight, kg	359	364	6	0.54	366	357	6	0.31
Longissimus muscle area, cm^2	80.0	79.3	1.4	0.72	80.4	78.9	1.4	0.45
Yield grade	3.48	3.55	0.09	0.62	3.56	3.48	0.09	0.57
Marbling	493	470	16	0.32	479	484	16	0.80
Backfat, cm	1.50	1.52	0.05	0.69	1.56	1.46	0.05	0.18

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. Journal of Animal Science; 97, 4182-4192, Copyright 2019 by Journal of Animal Science. CSSO = daily supplementation (per steer) with 80 g (CF) and/or 150 g (PC) of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per steer) with 67 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone (CF) and/or 125 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 25 g of limestone (PC).

[2]Weaning occurred on day 124 of year 1 and on day 127 of year 2. Calves were preconditioning from day 132 to 172 and day 135 to day 175 of year 1 and 2, respectively. Steers were shipped to the feedlot and remained there from day 173 to slaughter (day 378) and day 176 to slaughter (day 385) of year 1 and 2, respectively.
[3]Shrunk body weight was recorded after 16 h of feed and water withdrawal on day 131 (year 1) and 134 (year 2), and prior to shipping (day 171, year 1; day 174, year 2). Average daily gain during preconditioning was calculated using initial and final shrunk body weights. Feed intake was recorded daily during preconditioning by measuring offer and refusals from each pen, divided by the number of calves within each pen, and expressed as kg per calf per day. Feed efficiency was calculated using total feed intake and body weight gain of each pen during preconditioning.
[4]Calculated based on HCW (assuming 63% dressing; Loza et al., 2010).
[5]Observed at the commercial feedlot, according to the DART system (Zoetis, Parsippany, NJ).
[6]Backfat thickness measured at the 12th rib. Marbling score: 400 = Small[00]; 500 = Modest[00]; 600 = Medium[00].

Table 5.10. Postweaning plasma fatty acid concentrations (μg/mL of plasma) in beef steers supplemented with Ca salts of soybean oil (**CSSO**; n = 16 pens) or prilled saturated fat (**CON**; n = 16 pens) during a 40-d preconditioning period[1,2,3]

Item	CON	CSSO	SEM	*P*-value
Palmitic (16:0)				
Shipping	85.4	102	4.1	0.04
Finishing	90.7	89.6	4.1	0.79
Palmitoleic (16:1)				
Shipping	5.58	3.70	0.43	< 0.01
Finishing	8.07	7.02	0.43	0.12
Linoleic (18:2, ω-6)				
Shipping	177	301	15	< 0.01
Finishing	364	364	15	0.94
Linolenic (18:3, ω-3)				
Shipping	63.9	36.2	2.7	< 0.01
Finishing	12.5	11.9	2.7	0.47
Arachidonic (20:4, ω-6)				
Shipping	12.8	16.6	0.7	< 0.01
Finishing	14.9	15.8	0.7	0.24
Total ω-6				
Shipping	216	344	15	< 0.01
Finishing	401	399	15	0.97
Total ω-3				
Shipping	74.8	47.8	3.0	< 0.01
Finishing	16.5	16.5	2.9	0.98
ω-6:ω-3 ratio				
Shipping	2.90	8.90	0.55	< 0.01
Finishing	24.6	24.6	0.55	0.98
Total MUFA				
Shipping	68.7	58.3	3.8	0.05
Finishing	107.3	99.9	3.8	0.13

Table 5.10. Continued

Item	CON	CSSO	SEM	*P*-value
Total PUFA[3]				
Shipping	291	392	17	< 0.01
Finishing	417	416	17	0.97
Total FA				
Shipping	593	713	29	0.01
Finishing	831	811	29	0.58

[1]Reprinted with permission from "Supplementing calcium salts of soybean oil to beef steers early in life to enhance carcass development and quality" by Schubach et al., 2019. Journal of Animal Science; 97, 4182-4192, Copyright 2019 by Journal of Animal Science. CSSO = daily supplementation (per steer) with 80 g (CF) and/or 150 g (PC) of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per steer) with 67 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone (CF) and/or 125 g of prilled saturated fat (EnergyBooster 100, Milk Specialties, Eden Prairie, MN) + 25 g of limestone (PC).

[2]Weaning occurred on day 124 of year 1 and on day 127 of year 2. Calves were preconditioning from day 132 to 172 and day 135 to day 175 of year 1 and 2, respectively. Steers were shipped to the feedlot and remained there from day 173 to slaughter (day 378) and day 176 to slaughter (day 385) of year 1 and 2, respectively. Blood samples were collected prior to shipping to the feedlot (shipping; day 172, year 1; day 175, year 2) and once during the feedlot period (finishing; day 328, year 1; day 337, year 2).

[3]MUFA = palmitoleic and oleic acids; PUFA = linoleic, linolenic, dihomo-gamma-linolenic, arachidonic, docosadienoic, and docosapentaenoic acids.

Table 5.11. Postweaning mRNA expression of *longissimus muscle* genes in beef steers supplemented with Ca salts of soybean oil (**CSSO**; n = 16 pens) or prilled saturated fat (**CON**; n = 16 pens) during a 40-d preconditioning period[1,2]

Item[3]	**CON**	**CSSO**	**SEM**	***P*-value**
FABP4				
Shipping	65.3	68.1	45.7	0.25
Finishing	41.2	35.9	77.2	0.58
FASN				
Shipping	87.8	94.3	75	0.53
Finishing	134	113	243	0.23
MyoD				
Shipping	12.5	13.2	13.1	0.53
Finishing	9.46	6.58	9.37	0.22
Myogenin				
Shipping	46.3	33.4	38.4	0.79
Finishing	6.83	8.02	8.60	0.66
PPAR-γ				
Shipping	6.37	8.00	5.48	0.04
Finishing	4.71	5.31	9.54	0.42
SCD				
Shipping	64.6	67.0	54.5	0.42
Finishing	101	97.7	222	0.44
SREBP1				
Shipping	3.63	2.87	3.56	0.28
Finishing	3.15	2.99	3.29	0.37

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[2]Weaning occurred on day 124 of year 1 and on day 127 of year 2. Calves were preconditioning from day 132 to 172 and day 135 to day 175 of year 1 and 2, respectively. Steers were shipped to

the feedlot and remained there from day 173 to slaughter (day 378) and day 176 to slaughter (day 385) of year 1 and 2, respectively. Samples of the longissimus muscle were taken via needle biopsy prior to shipping to the feedlot (shipping; day 172, year 1; day 175, year 2) and once during the feedlot period (finishing; day 328, year 1; day 337, year 2). Values are expressed as relative fold change compared with threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

[3]FABP4 = adipocyte fatty acid-binding protein; FASN = fatty acid synthase; MyoD = myogenic differentiation 1; PPAR-γ = peroxisome proliferator-activated receptor-γ; SCD = stearoyl-CoA desaturase; SREBP1 = sterol regulatory element-binding protein-1.

6. CONCLUSIONS

In experiment one, supplementing gestating beef cows with organic sources of Co, Cu, Mn, and Zn increased cow liver concentrations of Co, and altered hepatic trace mineral metabolism and gene expression compared to INR cohorts. Liver trace mineral status was not altered in the neonatal calf when cows were supplemented with organic sources of Co, Cu, Mn, and Zn, however the liver is not the absolute indicator of trace mineral status in livestock. Moreover, calves born to AAC supplemented cows had greater hepatic mRNA expression of genes associated with antioxidant activity and mineral metabolism at weaning. Supplementing gestating beef cows with organic sources of Co, Cu, Zn, and Mn resulted in a reduced acute phase response in the offspring upon weaning, although this was not translated into greater offspring performance during a 45-d preconditioning period. Steers born to INR supplemented cows had greater hepatic mRNA expression of genes associated with trace mineral metabolism later in life, whereas steer performance and carcass merit was not impact. Heifers born to AAC supplemented cows had accelerated puberty attainment compared to their INR cohorts, independent of growth rate. The physiological mechanisms underlying these effects, as well as the specific role of each trace mineral supplemented herein on developmental programming still warrants further investigation. Nonetheless, results from this experiment are novel, and suggest that supplementing gestating beef cows with an organic complexed source of Co, Cu, Zn, and Mn compared to sulfate sources may be a viable alternative to enhance reproductive efficiency of female offspring reared as replacement heifers.

In experiment two, supplementing CSSO to beef steers at 2 mo of age via creep-feeding stimulated mRNA expression of some genes involved in lipid metabolism later in life compared

with cohorts receiving an isocaloric, isonitrogenous, and isolipidic supplement based on prilled saturated fat. However, increased mRNA expression of these genes, which included FABP4, FASN, PPAR-γ, and SCD, did not translate into improved carcass characteristics. Research is still warranted to investigate the effects of CSSO supplementation to nursing beef cattle during the period of epigenetic susceptibility on lipogenic gene expression, growth, and carcass characteristics. Nonetheless, CSSO supplementation to young cattle appears to be a nutritional alternative to upregulate mRNA expression of LM genes associated with lipogenesis during the finishing period.

Collectively, the results from these experiments may be utilized to develop nutritional strategies during periods of developmental plasticity such as fetal or neonatal periods, and in doing so may improve offspring performance parameters such as reproductive efficiency and carcass quality.