THESIS

EFFECTS OF MONENSIN SODIUM, PLANT EXTRACTS AND INJECTABLE TRACE MINERALS ON FEEDLOT PERFORMANCE, FERTILITY AND MORBIDITY OF BEEF CATTLE

Submitted by

Mariah Fischer

Department of Animal Science

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2015

Master's Committee:

Advisor: Jason Ahola
Co-Advisor: Kraig Peel

George Seidel
Terry Engle

ABSTRACT

EFFECTS OF MONENSIN SODIUM, PLANT EXTRACTS AND INJECTABLE TRACE MINERALS ON FEEDLOT PERFORMANCE, FERTILITY AND MORBIDITY OF BEEF CATTLE

Two studies were conducted to evaluate the effects monensin sodium, plant extracts, and injectable trace minerals on heifer and bull fertility, and calf feedlot performance and morbidity.

In the first study, Angus heifers (n = 107; 259.3 ± 21.0 d of age), blocked by weaning BW (262.7 ± 29.9 kg; d -19), were randomly assigned to treatments in a 2 x 2 factorial design, where all heifers received the same basal ration consisting of a 30% CP liquid supplement containing 200 mg/0.45 kg monensin sodium. Treatments were applied daily to the basal ration as topdressed supplements and were fed at a rate of 0.32 $kg \cdot hd^{-1} \cdot d^{-1}$. Treatments were as follows: 1) high level of monensin sodium (**MON**), where monensin sodium was topdressed at 200 $mg \cdot hd^{-1} \cdot d^{-1}$, 2) low level of monensin sodium plus the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (**CCE**), where plant extracts were topdressed at 11,000 $mg \cdot hd^{-1} \cdot d^{-1}$, 3) control (**CON**), low level of monensin sodium without topdressed supplements, or 4) high levels of monensin sodium with plant extracts (**COMB**), where monensin sodium was topdressed at 200 $mg \cdot hd^{-1} \cdot d^{-1}$ and plant extracts were topdressed at 11,000 $mg \cdot hd^{-1} \cdot d^{-1}$.

In both studies, heifers were weighed and estrus detection patch status was recorded every 11 d. Age at puberty was determined by patch status and was recorded as the d the patch was first activated. A 14 d CIDR-PG-AI protocol was utilized to inseminate heifers, when heifers were 427.3 ± 21.0 d of age. In the 14 d CIDR-PG-AI, a controlled internal drug release device (**CIDR**) was inserted 33 d prior to AI and removed 14 d later. Prostaglandin was injected 16 d

after CIDR removal, and heifers were inseminated 3 d later. Heifers were placed with bulls for natural service 21 d post AI. Pregnancy was determined 56 d post AI via ultrasound and 178 d post AI via rectal palpation. Calving records were used to validate ultrasound results.

In the first study, there were no treatment main effects for initial or final BW ($P > 0.05$). There were no interactions between the main effects of monensin sodium fed at high concentrations and plant extracts for any feedlot or fertility performance variable ($P > 0.05$); however, there was a main effect of high levels of monensin sodium for heifer DMI from d 0 to 8 and d 8 to 15, where MON and COMB heifers had reduced DMI compared to CCE and CON heifers ($P = 0.05$). From d 11 to 22 and d 44 to 66, heifers that received plant extracts (CCE and COMB) had lower ADG than CON and MON heifers ($P = 0.05$). Feed efficiency tended ($P = 0.08$) to be improved in heifers fed high levels of monensin sodium (MON and COMB) compared to heifers fed low levels of monensin sodium (CCE and CON); however overall DMI, ADG, age at puberty and pregnancy rate were not affected by the main effects of high levels of monensin sodium or plant extracts ($P > 0.05$).

In the second study, Angus bulls (n = 31, yr 1; n = 35, yr 2), heifers (n = 107) and steers (n = 105) were randomly assigned a treatment at weaning (278.6 ± 35.0 kg; 241.0 ± 19.6 d): 1) control (**CON**), no injection, or 2) injectable trace minerals (**MIN**), which included Cu, Zn, Se and Mn. The MIN treatment was administered at weaning (d 0) and again on d 64 (yr 1) and d 110 (yr 2) to bulls, and at weaning (d -19) and on d 135 to heifers. The second injections were 80 (yr 1) or 38 d (yr 2) prior to bull breeding soundness exams and 33 d prior to heifer AI. Steers received only the initial injection at weaning. Injections were administered at 1 mL/45.4 kg of BW at weaning and 1 mL/68.0 kg of BW prior to breeding soundness exams and AI, as per the product label. Bulls in both years exhibited no differences in overall ADG based on treatment (*P*

> 0.05); however, MIN bulls had reduced ADG from d 64 to 113 (P = 0.05) versus CON. Steer and heifer ADG was not different across treatments (P > 0.05). Incidence of morbidity was not different among treatments for any class of cattle (P > 0.05).

A licensed veterinarian performed the breeding soundness exams and Computer-Assisted Sperm Analysis was performed to analyze sperm motility, velocity, straightness and linearity objectively. Motility and morphology from breeding soundness exams were not effected by MIN (P > 0.05); however, in yr 2 there was a tendency for sperm from MIN bulls to have a higher percentage of secondary defects (P = 0.08). Sperm beat cross frequency was greater in CON in yr 2 (P < 0.01), and tended to be greater in CON in yr 1 (P = 0.10). Progressive velocity of sperm was greater in CON in yr 2 (P < 0.05). Neither BCS nor age at puberty in heifers was affected by treatment (P > 0.05). These results suggest there is no benefit to newly weaned calf performance or morbidity, or yearling bull or heifer fertility, when supplementing cattle with injectable trace minerals at weaning.

Key Words: ADG, beef cattle, fertility, monensin sodium, plant extracts, trace minerals

ACKNOWLEDEGEMENTS

I would like to take this opportunity to thank the many people who have helped and supported me through this journey. It has been an amazing experience and I feel blessed to have worked with my committee members, Jason Ahola, Kraig Peel, George Seidel and Terry Engle. I appreciate each and every one of you, and I am truly grateful to each of you for the time and effort you have invested in me throughout my graduate career. Jason, I admire your passion for the beef industry and your desire to share your enthusiasm and knowledge with students. Thank you for taking the time to mentor me, pushing me to think critically and helping me to be more concise and consistent. Thank you for allowing me to travel and develop my leadership skills through my involvement with the Seedstock team. Kraig, thank you for investing in me not only as a graduate student but also as a person. I am thankful for the "life" conversations we have had, as well as your continued support, mentorship and friendship throughout Graduate School. George, thank you for your continued support and dedication to the Beef Management Systems program. I am grateful to all of the hands-on experience you have shared with this program and for the ways in which you have challenged me to think critically. Terry, working with you has been a phenomenal experience. You are truly an amazing person, and I appreciate your constant positive attitude and willingness to teach me. I am also grateful to Jack Whittier for all of his wisdom, input and continued support through this experience.

Graduate school would not have been the same without the friendship and hard work of Ashley Adams, Alex Ohlheiser, Bo Bigler, Matthew Markwood, Miranda Culbertson and Katie Linton. I appreciate each one of you for the various ways in which you have supported and helped me through Graduate School. Katie Linton, I could not have made it through the last two

years without your constant support and friendship. I am blessed to say that you are one of my closest friends and I will always cherish the memories we have made.

Jesse French, I have enjoyed working with you at ARDEC and on several research projects. You are an incredible mentor and I will always treasure your friendship. Thank you for taking the time to teach me, and for your patience and willingness to work with me through the balance of Graduate School and ARDEC. David Escobar, you are a great friend and I have truly enjoyed working with and learning from you. I appreciate your constant patience with me and your amazing sense of humor.

Last but not least, I am incredibly thankful for my amazing family. To Orville and Sherrie, my wonderful grandparents, I appreciate your constant thoughts and prayers and the faith that you have in me. I am blessed to have the two of you as such incredible role models in my life. To my parents, Rick and Chris, thank you for always believing in me and pushing me to the best of my abilities. I am blessed to have such wonderful, supportive parents. To my brother Austin, I am lucky to have such an awesome friend in you. Thank you for always being there for me, understanding me and challenging me to be the best person I can be.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

REVIEW OF LITERATURE

SECTION I: MONENSIN & PLANT EXTRACTS

Introduction

Proper nutrition and management are vital to the profitability and productivity of cow-calf operations. The greatest influence on the profitability of cow-calf operations is the percentage of cows in the herd that become pregnant and calve every 12 months (Bohnert and Cooke, 2012). Age at which beef heifers obtain puberty is a major determining factor of their potential lifetime productivity (Schillo et al., 1992). Current beef management practices in the United States aim for heifers to calve by two years of age (Funston and Deutscher, 2004). Feed resources account for one of the greatest costs of production while developing heifers (Clark et al., 2005). Heifers reaching a BW of 50 to 57% of their mature body weight, in comparison to the traditional target weight of 60 to 65% by the breeding season can reduce development costs without impairing reproductive performance (Funston and Deutscher, 2004). Increasing ADG and improving feed efficiency can aid in heifers reaching target breeding weight at a younger age.

Ionophores, such as monensin sodium, have been used in beef diets due to their ability to increase feed efficiency and nutrient utilization (Bergen and Bates, 1984). When monensin sodium is fed in the diet, feed conversion increases, propionate to acetate ratio increases, and protein degradation decreases (Raun, 1990). Monensin sodium has also been shown to decrease the age of puberty (Lalman et al., 1993; Moseley et al., 1982; Purvis and Whittier, 1996) and increase first service conception rate in heifers (Purvis and Whittier, 1996). However, there is

some controversy over feeding monensin sodium due to classification as an antibiotic (Edrington et al., 2003).

With this in mind, researchers have discovered that natural plant extracts have some antimicrobial properties that may allow manipulation of microbial activity in the rumen, making them a potential alternative to ionophores (Benchaar et al., 2008). Plant extracts, such as cinnamaldehyde, capsicum oleoresin and eugenol, have been studied both *in vivo* and *in vitro* (Cardozo et al, 2006; Yang et al, 2010a; Yang et al, 2010b). Preliminary research has shown increased ADG (Geraci et al., 2012), decreased dry matter and water intake (Cardozo et al., 2006), and reduced ruminal crude protein (CP) degradation with the supplementation of plant extracts (Yang et al., 2010a). There is little information known on how plant extracts may effect the age of puberty or pregnancy rates in beef heifers.

Overall, research has indicated that animal productivity may be improved through the use of metabolic enhancing supplements such as monensin sodium, and that plant extracts may potentially be utilized to improve animal performance in place of the ionophores currently used in production today.

Monensin Sodium

Monensin sodium ($C_{36}H_{61}O_{11}Na$) is a biologically active compound produced by *Streptomyces cinnamonensis* (Hanley and Hoehn, 1967). Monensin sodium was originally used as a coccidiostat for poultry, and in December of 1975 it was registered with the commercial name Rumensin® (Elanco Products Co., a division of Eli-Lily, Indianapolis, IN) for improving feed efficiency in feedlot cattle (Tedeschi, Fox and Tylutki, 2003).

Ionophores, such as monensin sodium, have been found to favorably alter ruminal functions and improve feed efficiency in beef cattle. Improved productive efficiency is achieved

by increasing energy metabolism, improving nitrogen metabolism and inhibiting feedlot disorders such as bloat and lactic acidosis (Bergen and Bates, 1984). These improvements may occur in the ruminant animal by shifting rumen fermentation to increase ruminal propionic acid yield, decrease methanogenesis, decrease rapid ruminal proteolysis and decrease deamination of dietary proteins.

Mode of Action

Monensin sodium, in its anionic form, binds with a metal cation, such as Na^+, creating a lipophilic, cation-ionophore complex allowing for increased diffusion across the cell membrane (Ochinnikov et al., 1979; Bergen and Bates, 1984). Absorption of monensin sodium only occurs when it is protonated, either by H^+ or a metal cation. Sandeaux et al. (1982) measured Na^+ transport by monensin sodium under two different experimental conditions: 1) constant pH and differing concentrations of Na^+ on either side of the membrane, and 2) constant Na^+ concentration and differing pH levels on either side of the membrane. Researchers determined that Na^+ and H^+ move across the membrane in opposite directions of each other, dependent on the chemical potentials of each molecule (Bergen and Bates, 1984). Monensin sodium can effect this ion flux because it is considered to be a cation–H^+ antiporter (Harold, 1972).

Mediation of Na^+-H^+ exchange by monensin sodium is primarily due to its high affinity (10 times greater) for Na^+, compared to K^+ (Pressman, 1976). The Na^+/K^+ ATPase enzyme is the primary transport system in eukaryotic cytoplasmic membranes (DeVoe, 1974) and establishes a Na^+ gradient by exchanging 2 K^+ molecules and 3 Na^+ molecules through the Na^+/K^+ pump (Bergen and Bates, 1984). Ionophores disrupt normal function of the Na^+/K^+ pump, which disrupts the cellular solute uptake of other enzymes dependent on ion gradients. This interruption of movement across the membrane results in increased energy expenditure by cells (Bergen and

Bates, 1984). However, some cells cannot meet this energy demand due to dependence on substrate level phosphorylation of ATP, resulting in impaired cell division (Bergen and Bates, 1984).

Gram positive bacteria depend on substrate level phosphorylation for ATP and are therefore inhibited by ionophores, such as monensin sodium. Gram negative bacteria, in comparison, are not as sensitive to the effects of ionophores and can flourish within the rumen (Tedeschi, Fox and Tylutki, 2003). Increasing numbers of gram negative bacteria in the rumen can change the fermentation process, leading to an improvement in energy and nitrogen utilization.

Effects on Volatile Fatty Acids

Acetate, butyrate and propionate are the three principal VFAs found in the ruminant. Volatile fatty acids are produced from the fermentation of carbohydrates by the bacteria and protozoa in the rumen under anaerobic conditions. Volatile fatty acids provide the energy (ATP) to cows and bacteria for synthesizing protein from ammonia (Endecott and VanSaun, 2012). Microbes in the rumen obtain ammonia from the fermentation of proteins or from non-protein sources of nitrogen, such as urea.

Gram negative bacteria, many of which can survive the influences of monensin sodium, have the distinctive property of containing fumarate reductase (Kroger, 1977). Fumarate reductase is important in ruminal fermentation because it accounts for the shift in molar proportions of volatile fatty acids (VFA) towards propionic acid (Scheifinger and Wolin, 1973). Fumarate reductase achieves this shift by producing succinate from fumarate, which is later metabolized to propionate.

Propionate is the only volatile fatty acid (VFA) that can be utilized in gluconeogenesis, and is the major source (70%) of glycogen and glucose in the ruminant animal (Reece, 2009). Propionate's unique 3-Carbon structure allows it to enter the Citric Acid Cycle as an intermediate (succinate). Reece (2009) further explained that acetate (2C) and butyrate (4C) may only enter the Citric Acid Cycle as acetyl-CoA. This does provide some additional energy, however, a sufficient amount of oxaloacetate(OAA) must be present to react with acetyl-CoA to form citrate and start the TCA cycle. Excessive accumulation of acetyl-CoA leads to the production of ketone bodies, and potentially ketosis. Supplementing monensin sodium can lead to a favorable decrease in butyrate as well as a decrease in the acetate to propionate ratio without effecting the total VFA production (Richardson et al., 1976; Moseley et al, 1977; Boling et al., 1977; McCartor et al., 1979).

Recognizing that propionate is a major precursor for glucose, a 1978 study examined how monensin sodium would effect the propionate, and in turn, blood glucose concentrations in ruminants (Van Maanen et al., 1978). Researchers in this study used 4 fistulated steers and two successive rations, one that was high roughage and the second that was high concentrate. Feeding 150 $mg \cdot d^{-1}$ of monensin sodium resulted in an increase of propionate for both ration formulations however did not alter blood glucose concentration. Van Maanen et al., (1978) observed a numerical increase in the blood glucose entry rates. Raun et al. (1976), also reported that blood glucose concentration was not changed. Glucose concentration does not serve as an accurate means of determining glucose turnover. Additionally, researchers predict that the remaining propionate not converted to glucose may be used for CO_2 production, synthesis of additional compounds or compensation for the reduced acetate production (Van Maanen, 1978).

Moseley et al. (1977) analyzed the effects of monensin sodium in heifers that were randomly assigned to feedlot confinement, full time pasture grazing or part time confinement and part time grazing. Heifers that were assigned to part time grazing and monensin sodium supplement exhibited a 11.2% decrease in ruminal acetic acid ($P < 0.05$), 23.5% decrease in butyric acid ($P < 0.05$) and a 30.9% increase in propionic acid ($P < 0.05$) when compared to the control heifers. Moreover, heifers assigned to dry lot confinement and monensin sodium had similar total VFA concentrations, decreased acetate and butyrate concentrations ($P < 0.05$), and increased propionate concentration compared to the control ($P < 0.005$) (Moseley et al.,1977). Similarly, McCartor et al. (1979), researched the effects of monensin sodium in an 80% roughage, 20% concentrate diet, against the same 80:20 ration without monensin sodium (control) and a 50:50 ration without monensin sodium. Total VFA concentration was not effected by treatment ($P > 0.10$), however the monensin sodium and 50:50 treatments increased propionate molar concentrations ($P < 0.01$) and decreased molar acetate concentrations ($P < 0.05$) compared to the control.

Steers on a finishing ration and fed 33 ppm monensin sodium showed that acetate decreased by 16%, butyrate decreased by 46% and propionate increased by 75% compared to a control group of steers that did not receive monensin sodium ($P < 0.001$) (Perry et al., 1976). Boling et al. (1977) examined the effects of dosage of monensin sodium (0, 100, 200 or 300 mg) on VFA production and found that molar percentages of acetate and butyrate decreased and propionate increased as the dose amount of monensin fed increased. Additionally, in an *in vitro* study, utilizing dual-flow continuous culture fermenters with rumen fluid from two cows on a 50% concentrate ration, researchers found that the addition of monensin sodium increased the

proportion of propionate ($P < 0.001$) and decreased the proportions of acetate and butyrate ($P < 0.001$) (Lourenco, et al., 2008).

The effects of monensin sodium on VFA production have been well documented and its mode of action within the rumen is fairly well understood, however altering VFA concentration is not the only means by which monensin sodium can achieve improved efficiency in beef cattle (Schelling, 1984).

Effects on Methane Production

With a growing concern for methane produced by livestock and the influence toward greenhouse gases (Tedeschi, Fox and Tylutki, 2003), there is a focus on the relationship of total methane production as a proportion of energy intake associated with production (Baker, 1999). Methanogens, organisms naturally existing in the rumen microbial ecosystem, use hydrogen and carbon dioxide to produce methane. These methanogens rely on other rumen populations to replenish the hydrogen used for methanogensis. It was proposed that the patterns of VFA and methane production could be altered when the rate of fermentation in the rumen was high because other rumen microbial populations would out-compete methanogens for the metabolic hydrogen they required for methanogenesis (Baker, 1997). Monensin sodium has been noted for its capability to manipulate the VFA and methanogensis production patterns by selecting against hydrogen-producing rumen bacteria and selecting for succinate-forming bacteria (Chen and Wolin, 1979). As previously stated, succinate can later be metabolized to propionate (Scheifinger and Wolin, 1973). Propionate has been found to reduce methane (CH_4) production by conserving hydrogen for its own formation, compared to its VFA counterparts, acetate and butyrate which tend to promote CH_4 production (Wolin, 1960). It is more beneficial to the animal to form propionate for further use as energy and gluconeogenesis, rather than create CH_4 to be expended.

It is also thought that the reduction in methanogensis, through the use of an ionophore such as monesin sodium, was not solely due to the increase in propionate production but also a decrease in feed intake (Johnson et al., 1994). Furthermore, while monensin sodium allows for a consistent decrease in methane production, it accounts for only a small response and slight improvement in the efficiency of animal production (Schelling, 1984).

Effects on Protein Bypass

Bypassing the rumen and allowing for different digestion and absorption sites within the ruminant animal can increase animal performance (Chalupa, 1974). Efficiency of feed utilization can be determined by examining the site of digestion in the gastrointestinal tract (Roger and Davis, 1982). Nutrients that can be enzymatically digested post-ruminally can improve animal performance by bypassing the normal fermentation energy losses that would typically occur when dietary protein is converted to microbial protein in the rumen (Black, 1971). Microbial proteins synthesized in the rumen and undegraded or protected proteins that bypass the rumen can supply amino acids to be absorbed by the small intestine (Chalupa, 1974). Absorption in the small intestine may be beneficial to growing animals (Yang et al., 2010a).

Protein bypass and improved nitrogen utilization has been observed when monensin sodium is incorporated in the diet. A protein-sparing effect has also been reported by numerous studies (Hanson and Klopfenstein, 1979; Mies et al., 1979;). Poos et al., (1979) and Rogers and Davis (1982) described this effect as a result of decreased bacteria nitrogen passing to the lower gastrointestinal tract, in combination with an increase in dietary nitrogen passing through to the abomasum. If the abomasum contains a decreased amount of microbial nitrogen, this reflects a decrease in ruminal microbial protein synthesis (Whetstone et al., 1981).

Crude protein digestion was improved by feeding monensin sodium to cattle on a 50% concentrate-50% roughage ration and may in part be attributed to the reduction in feed intake (Rogers and Davis, 1982). However, when fed to cattle on a 90% concentrate diet, crude protein digestion was not improved, although it was numerically increased (Muntifering et al., 1980). Faulkner et al. (1985) also noted that monensin sodium had differing effects on animal performance when fed a high-fiber diet. Similarly, Poos et al. (1979) suggested that monensin sodium fed at 200 $mg \cdot hd^{-1} \cdot d^{-1}$ would reduce microbial protein synthesis and increase dietary protein ruminal bypass. However, Faulkner et al. (1985) saw no differences in protein bypass and concluded that cattle should instead be fed 100 $mg \cdot hd^{-1} \cdot d^{-1}$ when receiving a high fiber diet to improve nutrient utilization. Dose and diet may be influential factors for the effects of monensin sodium on nutrient efficiency.

Steers fed monensin sodium on a distiller grain diet exhibited increased concentration of essential and nonessential amino acids in the abomasum ($P < 0.05$; Poos et al., 1979). In contrast however, Whetstone et al. (1981) and Faulkner et al. (1985) found that amino acids were not effected by monensin sodium and that amino acid catabolism was in fact inhibited and the rate of catabolism was exceeded by the proteolysis rate.

While the mechanism for monensin sodium to impact protein bypass is still unclear and results are not in agreement, but it does appear that overall monensin sodium has the potential to decrease proteolysis and deamination of protein in the rumen (Whetstone et al., 1981; Rogers and Davis, 1982). A decrease in ruminal degradation would allow for more protein to be absorbed in the lower digestive tract and would in turn improve nutrient utilization and animal performance (Whetstone et al., 1981).

Effects on Feedlot Performance

In a dosage (0, 25, 50 or 100 mg) response study, steers on a finishing ration that were supplemented with 50 or 100 mg monensin sodium were reported to have higher ($P < 0.01$) average daily gain (ADG) than the control group or steers fed 25 mg·d^{-1} monensin sodium (Boling et al., 1977). Additionally, Potter et al. (1974) reported that cattle fed 100 or 200 mg·d^{-1} monensin sodium per head daily had higher ADG than cattle fed a control ($P < 0.01$). Oliver (1975) also found that all cattle fed monensin sodium (0, 25, 50, 100 or 200 mg·d^{-1}) exhibited greater gains than controls ($P < 0.05$) when cattle were on pasture. Dosage response to monensin sodium also reflected a decrease in feed intake as monensin sodium level increased, and as a result improved feed efficiency ($P < 0.01$) compared to control cattle (Boling et al., 1977).

In addition to a dosage response, a period response was also noted. For the first 59 d of the experiment, heifers that were fed monensin sodium while grazing pasture demonstrated greater gains than the controls ($P < 0.01$) (Moseley et al., 1977). However from d 60 to 158, the authors reported that all heifers gained at nearly identical rates. This disagrees with the increased gains exhibited by cattle fed monensin sodium grazing Coastal bermudagrass pastures (Oliver, 1975). Nevertheless, Moseley et al. (1977) pointed out that heifers were on a part time grazing diet which consisted of low quality forage, indicating that heifers fed monensin sodium were better able to utilize the available forage, making them more efficient.

In a feedlot setting, monensin sodium reduced feed intake by 10.9% compared to controls (Moseley et al., 1977). Nevertheless, the improved dry matter intake did not compensate for the similar ADG reported over the course of the experiment and as a result feed efficiency was not different between treatments due to monensin sodium supplementation (Moseley et al., 1977). Likewise, a high roughage feedlot study reported no differences in ADG, feed intake or feed

efficiency for heifers fed monensin sodium ($P > 0.05$) (McCartor et al., 1979). Yang et al. (2010b) fed 330 $mg \cdot d^{-1}$ to steers also found that monensin sodium supplementation had no differences in feed intake or efficiency compared to control steers.

When monensin sodium was fed in block form to finishing beef steers there were no effects on ADG, feed intake or feed efficiency (Berger et al., 1981). However, monensin sodium fed as part of the ration, instead of as a supplement block, resulted in a decreased feed intake and 4% increased feed efficiency compared to control steers (Berger et al., 1981). Similarly, a study analyzing the effects of gradual versus total introduction of monensin sodium into a finishing ration, found that monensin sodium severely decreased feed intake during the second 28 d period of the trial in which control cattle consumed 60% more feed than monensin fed cattle (Perry et al., 1976). Nearly identical gains, with a lesser feed intake than control cattle demonstrated a 10% less dry matter intake per kilogram of gain compared to the control group, implying that monensin sodium fed steers may have been more efficient in nutrient utilization (Perry et al., 1976). Similarly, heifers in a feedlot experiment were found to have reduced feed intake and improved nutrient utilization ($P < 0.05$), resulting in a greater overall feed efficiency ($P < 0.05$) if they were fed an ionophone (Purvis and Whittier, 1996).

Effects on Pubertal Onset and Pregnancy

Heifers that conceive earlier at first breeding, calve earlier in the season, and therefore wean heavier calves and potentially more calves throughout their productive lifetime (Lesmeister et al., 1973). Monensin sodium has been shown to reduce age of puberty in beef females, demonstrating that the percentage of heifers that can be bred to calve by 24 months of age can be increased (McCartor et al., 1979). Hereford, Brahman and F-1 crossed heifers revealed that pubertal onset could be decreased by 14 d (Moseley et al., 1977). In this experiment, 92% of

heifers that received monensin sodium supplementation reached puberty compared to only 58% of heifers fed the control. Conception rates were numerically higher from monensin sodium heifers compared to controls, in which 55% of monensin sodium heifers successfully conceived compared to only 47% of control heifers. McCartor et al. (1979) also observed that while heifer conception rates were not statistically different ($P > 0.05$), monensin sodium heifers achieved puberty 29 d earlier and 17 kg lighter than control heifers ($P < 0.009$). Likewise Purvis and Whittier (1996) reported that heifers fed an ionophore attained puberty 8 d earlier and 10 kg lighter ($P < 0.05$) and tended to have higher first service conception rates compared to control heifers ($P = 0.12$). Without effecting body weight and composition, Meinert et al. (1992) found that Holstein heifers fed monensin sodium exhibited reduced puberty by 15 d for heavy weight heifers and 24 d for light weight heifers. In contrast to all the previously mentioned data, English crossbred heifers fed a grass hay and barley straw ration demonstrated similar results for age of puberty and conception rates regardless of monensin sodium supplementation or no supplementation (Lalman et al., 1993). The reduced response to monensin sodium may be accounted for by the modest rate of gain utilized to develop these heifers. Nevertheless, monensin sodium has been shown to decrease age of puberty and possibly increase conception rates in beef females fed higher concentrate diets. This is beneficial to producers who can now decrease their input costs, while breeding and calving out their heifers earlier, allowing for more productivity from their females.

An Issue with Monensin Sodium

Considering previous research and the understanding the mechanism by which monensin sodium performs within the ruminant animal, it can be concluded that monensin sodium does not have deleterious effects on animal performance or reproduction (Moseley et al., 1977) and may

in fact be beneficial to achieving greater animal efficiency. However, monensin sodium is still considered to be an antibiotic (Edrington et al., 2003). Therefore, more research is being conducted to see if there are potential substitutes to feeding ionophores to cattle. Natural plant extracts have demonstrated some similar effects on animal performance, compared to ionophores, and are being considered as potential substitute supplements.

Plant Extracts: Cinnamaldehyde, Eugenol and Capsicum Oleoresin

Essential from medicinal, edible and herbal plants can be extracted from the leaves, flowers, seeds, roots, stem and bark (Benchaar et al., 2008) through steam distillation or solvent extractions (Greathead, 2003). These compounds, dependent on metabolic pathway of synthesis, are classified as either terpenoids or phenylpropanoids (Calsamiglia et al., 2007). Terpenoids have a basic 5 carbon structure and phenylpropanoids have a chain of 3 carbons attached to an aromatic ring. Terpenoids and phyenylpropanoids interact with the cell membrane and work against bacteria (Griffin et al., 1999; Dorman and Deans, 2000). This interaction results in a loss of membrane stability, leakage of ions across the membrane and a decrease in the transmembrane ion gradient. Large amounts of energy are diverted to this function and bacteria growth is slowed (Griffin et al., 1999). Changing the bacterial populations within a ruminant animal subsequently changes the fermentation process, which may be beneficial to improving nutrient utilization in ruminants (Calsamiglia et al., 2007).

RumeNext® (ADM Alliance Nutrition Inc., Quincy, IL) is a newer product being tested to evaluate the effects of plant extracts on beef cattle performance. The primary ingredients in RumeNext® are cinnamaldehyde, eugenol and capsicum oleoresin. Cinnamaldehyde, (C_9H_8O), is a phenylpropanoid with antimicrobial activities and is the main active component (75%) of

cinnamon oil, *Cinnmomum cassia* (Calsamiglia et al., 2007). Eugenol, ($C_{10}H_{12}O_2$) is a phenolic compound that appears to have antimicrobial properties against bacteria. It is the main active component in clove buds (85%). Capsicum oleoresin, *capsicum annun ssp.*, is the main active component in chili peppers and belongs to the tetraterpenoid family. Although research has reported that plant extracts may be favorable to animal performance, most of the data reported differs dependent on specific extract or combination of extracts used, dosage of extracts fed, species utilized and dietary treatment (Meyer et al., 2009).

Effects on Volatile Fatty Acids

A reduction in the production of VFA would be unfavorable to the animal (Busquet et al., 2005) because VFA represent the main supply of metabolizable energy as they are the end products of microbial fermentation in the rumen (Van Soest, 1982). Total VFA concentration was not effected in an *in vitro* experiment using eugenol and cinnamaldehyde at low doses (75 $mg \cdot kg^{-1}$), suggesting that the plant extract additives used did not modify diet fermentation and energy availability (Busquet et al., 2005). In contrast, Busquet et al. (2006) found that cinnamaldehyde and eugenol applied *in* vitro at high doses (3,000 mg/L) detrimentally decreased total VFA production, while capsicum oil had very small and non-significant effects on total VFA production. Similarly, Oh et al. (1967) observed inhibition of microbial fermentation at high dose supplementation of plant extracts.

Plant extracts have also been observed as being pH dependent (Cardozo et al., 2005). Decreasing pH from 7.0 to 5.5 resulted in lower total VFA concentrations ($P < 0.05$). At a pH of 5.5 and 7.0 high doses of cinnamaldehyde, eugenol and capsicum (300 mg/L) all resulted in a strong inhibition of total VFA concentration ($P < 0.05$). At a pH of 7.0, cinnamaldehyde and capsicum used at low doses (0.3, 3 and 30 mg/L) decreased total VFA production compared to

the control ($P < 0.05$). In contrast, at low doses (0.3, 3 and 30 mg/L), eugenol had no effect on total VFA concentration. Cardozo et al. (2005) also observed that at the lower pH of 5.5 and low doses (0.3, 3 and 30 mg/L), cinnamaldehyde, eugeol and capsicum, increased total VFA concentration ($P < 0.05$).

While *in vitro* studies are helpful in screening the effects of plant extracts on ruminal fermentation, there are some limitations to these methods and *in vivo* studies need to be taken into account (Calsamiglia et al., 2007). In fistulated heifers on a 90% concentrate diet, cinnamaldehyde and eugenol fed in combination had no effects on total VFA production (Cardozo et al., 2006). Similarly, heifers on an 80% concentrate diet supplemented with low, medium or high doses of cinnamaldhyde (400, 800 or 1600 $mg \cdot d^{-1}$, respectively) exhibited no changes in total VFA concentration compared to a control group of heifers not supplemented with cinnamaldhyde (Yang et al., 2010a). Moreover, steers on a finishing ration supplemented with a combination of cinnamaldehyde, capsicum and eugenol did not have different total VFA concentrations compared to steers that were supplemented with the ionophore, monensin sodium (Geraci et al., 2012).

Proportions of VFA, such as acetate, butyrate and propionate, were not effected due to the supplementation of plant extracts fed *in vivo* (Cardozo et al., 2006; Yang et al., 2010a; Geraci et al., 2012). In contrast, an *in vitro* experiment with eight dual flow culture fermenters observed that clove bud oil increased the molar proportion of butyrate, lowered the molar proportion of acetate and increased the proportion of propionate, resulting in a reduced acetate to propionate ratio (Busquet et al., 2005). This study also reported that cinnamaldehyde numerically decreased the molar proportion of acetate and increased the butyrate proportion, however it had no effect on the proportion of propionate. Busquet et al. (2006) found that the effects cinnamaldehyde and

eugenol had on the proportions of acetate, butyrate and propionate produced were dosage dependent. The effects of cinnamaldehyde, eugenol and capsicum on individual VFA proportions are additionally found to be pH dependent (Cardozo et al., 2005).

Effects on Ammonia Nitrogen Concentration

A decrease in ammonia N concentration would suggest that deamination activity has been reduced, whereas an increase in ammonia N concentration would imply deamination activity has been stimulated (Cardozo et al., 2005). Cinnamaldehyde and capsicum reduced ammonia N concentration and eugenol increased ammonia N concentration despite dosage supplied or pH environment when utilized *in vitro* (Cardozo et al., 2005). In contrast, cinnamaldehyde, eugenol and capsicum decreased ammonia N concentration only at higher dosages (300 and 3,000 mg/L) (Busquet et al., 2006). *In vivo* studies have observed that a combination of cinnamaldehyde and eugenol decreased the ammonia N concentration compared to the control (Cardozo et al., 2006) or compared to monensin sodium (Geraci et al., 2012), suggesting that deamination activity was inhibited.

Effects on Feedlot Performance

Supplementation of cinnamaldehyde to cattle on high grain diets effected feed intake in a dose dependent manner (Yang et al., 2010a). Cattle fed cinnamaldehyde at 400 $mg \cdot d^{-1}$ exhibited a slight increase ($P < 0.09$) in dry matter intake compared to cattle supplemented at 1600 $mg \cdot d^{-1}$ which showed a slight decrease in dry matter intake. In agreement with these results, Cardozo et al. (2006) reported that heifers fed a combination of cinnamaldehyde and eugenol at 270 $mg \cdot d^{-1}$ ($P < 0.03$) or capsicum at 1000 $mg \cdot d^{-1}$ ($P < 0.05$) exhibited decreased dry matter and water intake compared to a control. Another feedlot study reported that dry matter intake was only favorably effected by cinnamaldehyde for the first 4 weeks of the experiment, at which point

intake was then relatively stable and there were no differences between cinnamaldehyde supplemented steers or control steers throughout the end of the trial (Yang et al., 2010b).

When comparing monensin sodium to plant extracts, steers that received a combination of cinnamaldehyde, eugenol and capsicum had similar dry matter intakes to monensin sodium fed steers (Geraci et al., 2012). Researchers in this study were surprised by this outcome, yet acknowledged that there is limited data directly comparing plant extracts to monensin sodium therefore it is unknown if these results were expected. Geraci et al. (2012) observed that steers fed a combination of the plant extracts increased ADG by 16% during the later part of the study compared to monensin sodium steers. The increased ADG during the last half of the experiment, and the similar dry matter intake between plant extract and monensin sodium fed steers resulted in a treatment by period interaction, however there were no treatment effects on overall feed to gain efficiency. Similarly, finishing steers fed increasing levels of cinnamaldehyde exhibited no overall improvement in ADG except during the first time period in which ADG was quadratically effected by cinnamaldehyde dose level (Yang et al., 2010b). Feed efficiency in those first 28 d was also improved due to increasing levels of cinnamaldehyde in the diet. Feed efficiency was only then quadratically effected by dosage level from d 29-56 and d 85-112, however overall cinnamaldhyde supplementation did not effect feed efficiency.

Effects Pubertal Onset and Pregnancy

Bigler (2014) utilized beef heifers in 2 consecutive years to examine the effects of plant extracts on age of puberty and conception rates. In the first year, there were no differences between treatment groups, however in the second year, heifers that received plant extracts had reduced age of puberty compared to control heifers. Additionally, Bigler (2014) reported that neither pregnancy rates nor the percentage of heifers to calve within the first 21 d of the calving

season were different between heifers that received plant extracts and those that were fed monensin sodium.

Rationale for Current Experiment

Unfortunately there is limited literature on the effects of plant extracts on feedlot performance and in the literature published there appears to be conflicting results. The influence on feedlot performance seems to be effected by plant extract source, combination of extracts, dosage of extracts, time period and dietary treatment (Cardozo et al., 2006; Yang et al., 2010a; Yang et al., 2010b; Geraci et al., 2012). It is also difficult to understand the exact mode of action a combination of plant extracts perform within the rumen environment; as well as to explain how a combination of extracts may lose their efficacy at some point in time due to the adaptive functions of the microbial ecosystem (Calsamiglia et al., 2007).

Currently, there is little published literature regarding the effects of plant extracts, specifically cinnamaldehyde, eugenol and capsicum, on pubertal onset or conception rates in beef heifers. Utilizing a natural form of an ionophore to decrease age and weight of puberty in heifers could be beneficial to producers for achieving greater herd productivity and efficiency, all the while improving public perception of cattle production.

Furthermore, additional research is warranted to observe the mode of action of plant extracts in the rumen, analyze plant extracts effects on feedlot performance, pubertal onset and conception rates and evaluate the potential utilization of plant extracts as a substitute for ionophores such as monensin sodium.

SECTION II: TRACE MINERALS

COPPER, ZINC, SELENIUM AND MANGANESE

Introduction

Minerals are essential to animal health, growth and reproductive performance. Structural, physiological, catalytic and regulatory functions that occur within the animal body rely on minerals and their existence in body tissues and cells (Underwood and Suttle, 1999). Because these functions are dependent on minerals for proper activity, such as tissue growth, enzyme and immune function, homeostasis and hormone regulation, it is vital that mineral concentrations are managed in narrow margins (Underwood and Suttle, 1999). Unbalanced or improper mineral intake can lead to metabolic disease or toxicity, as well as reduced animal performance or health (Paterson and Engle, 2005).

Mineral requirements vary by stage of production for the animal (ie: maintenance, growth or reproduction) (Underwood and Suttle, 1999). When minerals are required at greater than 100 mg/kg diet, they are classified as macro minerals; whereas minerals required at less than 100 mg/kg diet are micro, or trace, minerals (McDowell, 1992). There are at least 17 minerals that are considered to be essential for beef cattle according to the NRC (1996). Phosphorus, potassium, sulfur, magnesium, sodium, chlorine and calcium are macro minerals which must be sufficiently supplied in beef cattle diets (NRC, 1996). The trace minerals essential for beef cattle are chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium and zinc (NRC, 1996). Unfortunately, cattle diets do not always supply sufficient amounts of minerals to adequately meet the body's demand (Pogge et al., 2012). Minerals can be supplemented to cattle

by mixing them in a ration, providing them to animals free choice or administering them via bolus or injectable sources.

Dietary mineral sources are often subjected to antagonists (ie: Mo, Fe, or S) present in the diet and can therefore result in decreased mineral absorption (Spears, 1996). Research indicates that the ability to avoid these dietary antagonists and the detrimental effects of decreased mineral absorption in the ruminant could help to improve animal health and productivity (Richeson and Kegley, 2011). Use of injectable minerals may be advantageous to bypass the negative interactions occurring in the gastrointestinal tract because upon injection the minerals would be directly circulated throughout the bloodstream and incorporated into cells as needed (Berry et al., 2000). The remaining minerals that are not immediately utilized by cells would be filtered through the liver for storage or excretion from the body (Suttle, 1987).

A current commercially available source of injectable trace minerals is the product commonly known as MultiMin® (MultiMin® USA, Fort Collins, CO). MultiMin® provides 60 mg/mL zinc (Zn), 15 mg/ mL copper (Cu), 10 mg/ml manganese (Mn) and 5 mg/mL selenium (Se).

Acknowledging that proper mineral supplementation is a necessity for successful animal production, research has indicated that utilizing injectable trace minerals in beef cattle may have advantageous effects on animal health, growth and performance. Although all macro and micro minerals are equally important to animal productivity, this review will focus solely on the 4 trace minerals Cu, Zn, Se and Mn, as they are the minerals that make up the mineral supplement MultiMin®.

Trace Minerals: Cu, Zn, Se and Mn

The four main functions of minerals include: 1) structural components of body organs, tissues, molecules and membranes; 2) maintenance of physiological processes such as osmotic pressure, acid-base balance, membrane permeability and tissue irritability; 3) catalyst for enzyme and hormone systems and component of metalloenzymes; and 4) regulation of cell replication and differentiation (Underwood and Suttle, 1999). Copper, zinc, selenium and manganese, the four trace minerals focused on in this review, are all considered to be important metalloenzymes in livestock (Underwood and Suttle, 1999).

Trace mineral deficiencies have a significant impact on the functions occurring within the animal body and may occur in one of two ways: 1) a primary deficiency which is due to inadequate amount, availability or intake from the environment, or 2) a secondary deficiency which is caused by the presence of one or more antagonists that reduce the availability of the trace mineral (Ahola, 2004). Trace mineral deficiencies, toxicities or imbalances require the animal to metabolically compensate for the nutrient deviation, leading to the manifestation of metabolic diseases that are capable of decreasing energy metabolism, immune function and overall animal performance (McDowell, 1989; Underwood and Suttle, 1999). Each mineral, Cu, Zn, Se and Mn, is uniquely utilized within the animal body and thus its deficiencies or toxicities result in varying diseases or illnesses.

Copper

Copper is essential for reproduction, bone development, growth, connective-tissue development and pigmentation of hair, fleece and feathers (Underwood and Suttle, 1999). While it is still under investigation, it appears that Cu is important to erythropoieses function due to a mechanism in which it can impair the transport of iron (Suttle, 1987). Copper is important for

development of the central nervous system as well as a normal functioning immune system (Underwood and Suttle, 1999). Copper is surpassed only by Zn in the number of enzymes it is responsible for activating (Underwood and Suttle, 1999). Among the enzymes requiring Cu for activation are tyrosinase (pigmentation), lysyl oxidase (connective-tissue development), ceruloplasmin (erythropoiesis), Cu-Zn superoxide dismutase (oxidant protection), cytochrome *c* oxidase (electron transport chain), and peptidylglycine-α-amidating monooxygenase (brain function) (Underwood and Suttle, 1999).

The primary organ for Cu storage is the liver and thus the first biochemical change to be seen when an animal is deprived of Cu would be a decline in liver Cu concentrations (Underwood and Suttle, 1999). Molybdenum, a common antagonist, when in the presence of sulfate, can also decrease the deposition of Cu into organs (Paterson and Engle, 2005). Sulfur, iron and calcium will also influence absorption of Cu in the ruminant animal (Underwood and Suttle, 1999).

Clinical manifestations of Cu deficiencies can result in anaemia, bone disorders, connective-tissue disorders, cardiac lesions, neonatal ataxia, depigmentation, scours and diarrhea, infertility, susceptibility to infections or mortality (Underwood and Suttle, 1999). Conversely, it is pertinent to remember that Cu at high enough levels can be toxic to cattle, causing nausea, vomiting, abdominal pain, convulsions, paralysis and death (Paterson and Engle, 2005).

Zinc

While Zn is widely distributed throughout the animal body, the highest concentrations of Zn were found in the following locations, listed from highest to lowest concentrations: pancreas, liver, pituitary gland, kidney and adrenal gland (McDowell, 1992). Additionally, research has

shown that the testicles and accessory sex glands contain high concentrations of zinc as well (McDowell, 1992). Unfortunately, the ability to store Zn in a form that can later be mobilized is poorly developed in the animal body (Paterson and Engle, 2005).

Zinc metalloenzymes, retinene reductase and alcohol dehydrogenase, are involved in the process of converting vitamin A alcohol (retinol) to vitamin A aldehyde (retinene), a process that is vital to normal vision (Underwood and Suttle, 1999). Likewise, Zn plays vital roles in digestion, glycolysis, DNA synthesis, nucleic acid metabolism and protein metabolism, roles derived from its effects on gene expression (Chesters, 1992). Zinc is involved in regulation of appetite, and thus a reduced appetite is one of the initial clinical signs of a Zn deficiency (Paterson and Engle, 2005). Inflammation of the nose and mouth with submucous hemorrhages, unthrify appearance, rough hair coats, stiffness of joints with swelling of feet, cracks in the coronary bands and dry scaly skin on the ears are additional clinical signs of Zn deprivation (McDowell, 1992). Zinc deficiency can additionally result in retardation, subnormal growth, reduced fertility, and impaired immune function (Underwood and Suttle, 1999).

Selenium

Interestingly, in the 1930s Se was only of interest because it was thought to be a toxic element that made animals lose their hair, nails and hooves (McDowell, 1992). Selenium wasn't considered to be beneficial to livestock until some 20 years later when research showed that Se helped to prevent liver necrosis in swine and muscular dystrophy in calves (McDowell, 1992). Additionally, Se is vital for growth, fertility, disease prevention, immune function, cardiovascular disease, gastrointestinal function and thyroid function (Underwood and Suttle, 1999; Kumar and Priyadarsini, 2014). Selenium and Vitamin E are often utilized together and are

considered to be closely linked because they both protect biological membranes from degeneration and without them tissues would breakdown (Paterson and Engle, 2005).

In ruminants, a Se deficiency often leads to white muscle disease, or muscular dystrophy, which is a degeneration of the striated muscles (Paterson and Engle, 2005). Deprivation of Se may also lead to anemia, lowered fertility, embryonic loss and retained placenta (Underwood and Suttle, 1999). Animals that are suffering from Se toxicity will have loss of appetite, lack of thriftiness, cirrhosis of the liver, loss of hair, lameness and elongated hooves (McDowell, 1992).

Manganese

Although Mn is implicated in many of the same processes as Cu and Zn, it is diverse in that it is widely distributed in cells and tissues throughout the body at very low concentrations (Corah and Arthington, 1993). Manganese metalloenzymes are involved in many functions of the body such as bone and cartilage development (glycosyl transferase), blood clotting (prothrombin), lipid and carbohydrate metabolism (pyruvate carboxylase) and oxidant resistance (superoxide dismutases) (Underwood and Suttle, 1999). Reproductively, it has been suggested that Mn may be essential for normal ovarian function (Hidiroglou, 1975), and that Mn deprivation may be associated with the anestrus condition in cattle (Maas, 1987). Deficiencies in magnesium can lead to ataxia, impaired growth, defective ovulation and testicular degeneration (Underwood and Suttle, 1999).

The Immune System

Health is a term that describes a subjective observation of an animals' general appearance along with a few clinical measurements such as rectal temperature or serum profiles (Galyean et al., 1999). *Immunity* refers to the manner in which an animals' body reacts to foreign substances (Abbas et al., 1991). The primary goal of the immune system is to recognize and defend itself

against bacteria, viruses and other foreign substances, also known as antigens, that may be harmful to the body (Ahola and Engle, 2012).

Calves initially receive immunity through the immunoglobulins passed to them in utero through the dam's placental barrier and then once they are born from the immunoglobulins provided in the dam's colostrum (Reece, 2009). Cattle producers typically take advantage of a calf's ability to recognize and respond to antigens by vaccinating them at branding or weaning (Ahola and Engle, 2012). Vaccinations are comprised of only a small dose of the antigenic substance so that the calf will not get mortally ill but will be able to hastily and efficiently stimulate an immune response the next time the antigen is present (Ahola and Engle, 2012).

A primary immune response would occur when it is the first time the immune system is responding to the antigen; whereas a secondary response is a response to an antigen that has previously been responded to, such as at vaccination (Ahola and Engle, 2012). Typically secondary responses are quicker, larger and more efficient when responding to the antigen.

Immunity is classified as either innate (natural) or acquired (specific) (Galyean et al., 1999). Innate immunity is a non-specific response in which phagocytes such as macrophages, neutrophils and natural killer cells attack the foreign antigen (Abbas et al., 1991). Aquired immunity is either: 1) humoral, in which B-lymphocytes respond to antigens by becoming antibody producing cells or memory cells to provide defense against the foreign substance, or 2) cell mediated, where defense against the extracellular pathogen is brought upon by T-lymphocytes (Galyean et al., 1999). Furthermore, to efficiently produce the antibodies and lymphocytes necessary to stimulate an immune response proper plane of nutrition is vital (Ahola and Engle, 2012). Minerals such as Cu, Zn, Se and Mn are imperative to physiological activities

including antioxidant systems, energy production, protein synthesis and membrane integrity (Nockels and Whittier, 1995).

Effects on Animal Morbidity and Mortality

Morbidity and mortality resulting from bovine respiratory disease (BRD) in newly weaned and received cattle continues to be the most significant health and economic problem facing the U.S. beef industry (Duff and Galyean, 2007). Newly weaned calves transported to the feedlot are exposed to numerous, possibly new, infectious substances. Additionally, the weaning and transportation processes increase the stress of the animal resulting in depressed immunological function to the antigens they encounter (Blecha et al., 1984; Paterson and Engle, 2005).

Injection of the required trace minerals Cu, Zn, Se and Mn at initial processing may be advantageous to animal performance by ensuring that newly received, stressed cattle have necessary trace minerals readily available to them (Berry et al., 2000). It has also been suggested that administering trace minerals concurrently with vaccinations enhances the production of neutralizing antibodies to that vaccine antigen (Arthington and Havenga, 2012). Research has found that the injectable trace minerals increased humoral immune response to antigens and increased acute phase protein concentrations in weaned and transport stressed calves (Arthington et al., 2014b). Richeson et al. (2011) reported that utilizing the injectable trace mineral during processing of stressed calves improved BRD morbidity upon arrival in the feedlot and throughout the feeding period. The reduced treatment and retreatment incidences of cattle while in the feedlot resulted in decreased antibiotic costs over the entire feeding period (Richeson et al., 2011).

In contrast to these results, research has indicated that a lifetime supplementation of Cu, Zn and Mn, administered in combination and maintained at levels above deficiency, have little impact on performance and health of feedlot cattle (Ahola et al., 2005). This study analyzed the effects of mineral supplementation starting as a neonate and ending through the finishing phase of production. Mineral supplementation had no impact on humoral or cell mediated responses, morbidity or number of times cattle were treated. Additionally, administering Cu, Zn, Mn and Co at the NRC (1996) recommended level, 2 times above, or 3 times above the NRC recommendation had little impact on overall morbidity, incidence of respiratory disease, foot rot, bloat or percentage of times feedlot steers required retreatment (Rhoads et al., 2003).

Utilizing an injectable trace mineral with Cu, Zn, Se and Mn 28 d prior to weaning in calves also exposed to a free choice mineral had no influence on calf health (Kegley et al., 2011). Researchers implicated that the free choice mineral may have played a role in their results. However, when calves did not receive free choice mineral and were injected with trace minerals 90 d pre-weaning and again at weaning, there were still no differences found between calves that received treatment and those that did not (Gadberry and Simon, 2012).

The effects of minerals on morbidity and mortality in cattle are contradicting and may be due to the differences in initial mineral status, vaccination protocols, class of cattle or diet provided. Future research should attempt to use similar backgrounded cattle and experimental environments, units and design, in order to allow for more consistency in results and a better understanding of trace minerals within beef cattle.

Effects on Feedlot Performance

Proper growth in beef cattle is vital to the animal's feedlot performance and in turn, the producer's profitability. Trace minerals are important for the growth of livestock because of their

involvement in biochemical processes such as bone, muscle and skeletal development (Underwood and Suttle, 1999). The trace minerals Cu, Zn, Se and Mn have been evaluated to observe their effects on calf feedlot performance when administered prior to weaning, at weaning, upon arrival to the feedlot and during shipping.

Calves that received the injectable trace mineral 150 d and again 50 d prior to weaning did not show improved ADG compared to control calves (Arthington et al., 2014b). Likewise, injecting Cu deficient calves 28 d prior to weaning with Cu, Zn, Se and Mn did not influence ADG (Kegley et al., 2011). Calves in both studies were additionally supplied with unlimited access to minerals, which may have provided for adequate mineral intake for both supplemented and control treatment groups.

Administration of injectable trace minerals Cu, Zn, Se and Mn upon entering the feedlot has been found to improve overall ADG and feed efficiency (Richeson and Kegley, 2011). Beef heifer calves served as a control or received one of two levels of trace minerals. Heifers in both the high and low mineral level treatment groups gained 0.18 to 0.19 kg per kilogram of feed consumed compared to control heifers which gained 0.16 kg per kilogram of feed consumed. In contradiction to this, bull calves that received trace mineral injections upon arrival to the feedlot showed no improvement in feed conversion when compared to their control contrasts (Berry et al., 2000). Bulls did have a tendency to exhibit improved ADG if they received trace minerals, however there was also a period of increased DMI which may account for the lack of differences seen in overall feed efficiency between treatments. Differences between studies could be due to class of cattle or prior mineral status.

Heifers that received pre-weaning injectable trace minerals were shipped 1,600 km to a feedlot where they were then given another injection upon arrival (Arthington et al., 2014b).

Heifers were monitored for the first 14 d for improvement or decline in feedlot performance. Supplemented heifers had reduced ADG over the 14 d period when compared to non-supplemented heifers. In another experiment involving cattle shipping, researchers implemented an 84 d depletion period in which they utilized dietary antagonist to induce mild mineral deficiencies in growing and finishing steers (Genther ande Hansen, 2014). Throughout the depletion period, there were no treatment effects on ADG, DMI or G:F. Two d after shipment, an equal number of steers on the control and deficient diets were injected with Cu, Zn, Se and Mn. During the repletion period, steers that were deficient and received an injection of mineral had greater ADG compared to those steers which were deficient but did not receive the injection. Steers on the control diet exhibited no differences in ADG despite mineral injection or lack thereof. These results are consistent with Engle et al. (1995) who noted that Zn depletion decreased gain by an average of 45.6%, but upon Zn repletion there were no evident differences in gain.

Additionally, organic trace mineral supplementation improved growth performance of shipping stressed calves compared to inorganic supplemented calves (Kegley et al., 2012). During the receiving period calves were supplemented with inorganic or organic forms of Cu, Zn, Mn and Co in the diet. Organic supplemented calves had a 4.6 kg greater final weight and a 0.11 kg greater ADG than inorganic supplemented calves. These results agree with Dorton et al. (2006) who found that weaned calves supplemented with organic trace minerals for 30 d and then shipped to the feedlot had greater ADG than steers that were fed inorganic trace minerals. In contrast, for the first 56 d in the feedlot calves supplemented with organic or inorganic Cu, Zn and Mn exhibited no differences in BW, ADG or DMI (Ahola et al., 2005). Also for the finishing phase (140 to 181 d), no effect of mineral source on ADG was observed (Ahola et al., 2005).

However, researchers in this study did find that there was a tendency for supplemented cattle to have increased DMI compared to non-supplemented cattle. Non-supplemented cattle therefore had greater feed efficiency than organic supplemented cattle, and organic supplemented cattle were more efficient with feed conversion compared to inorganic supplemented cattle.

The research regarding utilization of Cu, Zn, Mn and Se to influence feedlot performance is variable and limited. Time of mineral injection, along with mineral source and combination of minerals used appear to influence the effects on cattle growth and performance, however more research is needed to better understand these impacts.

Effects on Bull Fertility

Bull fertility is often overlooked and the emphasis of calf production tends to be placed more on female reproductive performance (Cooke, 2012).This is unfortunate because bull infertility would result in multiple calf losses compared to one cow's infertility which would only result in the loss of a single calf (Cooke, 2012). Bull infertility could be detrimental to a producer's profitability, especially if the producer is not achieving the output of calves expected. Fertilization failure can be due to the lack of normal viable sperm reaching the ova, age, disease, injury, unsoundness or malnutrition (Burns et al., 2010; Cooke, 2012). Therefore, breeding soundness exams and analysis of sperm should be performed prior to the breeding season in order to ensure high pregnancy rates in females (Cooke, 2012). Additionally, adequate nutrition and mineral supplementation is essential to reproductive performance.

Spermatogenesis, or sperm production, requires approximately 60 d in bulls (Rowe et al., 2011). With this in mind, researchers supplemented bulls with inorganic or organic mineral treatments 60 d prior to beginning the experiment and then throughout the 9 week study on semen quality and production analysis (Rowe et al., 2011). The 9 week period was chosen to

represent another 60 d spermatogenesis phase in order to accurately identify the effects of minerals on sperm production. Sperm motility is one of the most important factors effecting bull fertility (Rowe et al., 2011). Bulls subjected to organic Zn, Cu, Mn and Co had 65.5% motile sperm compared to 56.1% motile sperm exhibited by bulls fed the inorganic minerals (Rowe et al., 2011). Organic mineral supplemented bulls also had higher percentages of progressive motile sperm (47.0%) and motile sperm with rapid motility (62.3%) in comparison to inorganic supplemented bulls (38.4%, 52.8%, respectively) (Rowe et al., 2011). Rowe et al. (2011) also reported that progressive and motile sperm percentages after thawing semen samples remained greater for bulls receiving organic minerals versus those receiving inorganic minerals. Similarly, crossbred bulls that received only organic Zn outperformed bulls supplemented with inorganic Zn by exhibiting increased ejaculate volume, sperm concentration, percent live sperm and percent motility (Kumar et al., 2006). Researchers inferred that this result may be due to the fact that organic Zn had more bioavailability than inorganic Zn, and therefore allowed for better absorption, distribution and uptake of Zn, improving its effects (Kumar et al., 2006).

Similarly, *in vitro* studies have observed that utilizing solely Mn improved semen quality (Bansal and Bilaspuri, 2008). Manganese is a chain-breaking antioxidant (Cavallini et al., 1984). An antioxidant that is capable of reducing oxidative stress and improving sperm motility could be beneficial to managing male infertility (Verma and Kanwar, 1999). Results have indicated that Mn (60, 100 or 200 µM) efficiently reduced the oxidative stress caused by ferrous ascorbate and improved sperm motility and viability in crossbred bull semen samples utilized *in vitro* (Bansal and Bilaspuri, 2008). In fact, motility, morphology and viability were all improved upon the addition of Mn in untreated samples and ferrous ascorbate treated samples (Bansal and Bilaspuri, 2008). Although Mn appears to improve sperm quality *in vitro*, research has also

indicated that Mn supplementation level in diets needs to be carefully managed (Reis et al., 2014). This is a challenging concept because many countries have naturally occurring concentrations of Mn that already exceed NRC (2000) requirements 30-fold, and then feed manufacturers add Mn to mineral supplements increasing the level of Mn in cattle diets even more. Moreover, Reis et al. (2014) found that Mn fed at greater than 540 mg/kg resulted in detrimental effects to the integrity of the plasma and acrosomal membranes of bull sperm.

The individual use, combination of minerals or method of mineral administration may have varying effects on bull semen characteristics. For example, ram lambs receiving soluble boluses of Zn, Co and Se exhibited increased motility, proportion of live sperm and proportion of intact membranes when compared to an non-supplemented group of rams (Kendall et al., 2000). In contradiction to these results, another experiment utilized an injectable source of Cu, Zn, Mn and Se and reported no difference in sperm quality between mineral treated and untreated bulls (Kirchhoff et al., 2015). Additionally, the injectable mineral had no influence on the semen trace mineral concentration (Kirchhoff et al., 2015).

Injecting Se alone into dairy bulls with marginal Se status had no effects on sperm quality (Bartle et al., 1980). However, researchers in this study found that Se levels in the blood were 4 times greater in bulls that received the injection compared to those that did not (Bartle et al., 1980). Likewise, the semen Se concentration was 2.5 times greater for treated bulls versus untreated bulls. The semen Se concentration was 10 times greater than the Se blood levels in both treated and untreated bulls. Although Bartle et al. (1980) could not determine what accounted for this significant increase in semen selenium, it appears that the male reproductive system does have a high affinity for Se and may be the preferred organ system for Se metabolism (Bartle et al., 1980). Segerson and Johnson (1981) found that the reproductive tract of the beef

bull is capable of concentrating high levels of selenium. Serum Se concentrations were highly correlated to Se concentrations in the seminal vesicle and testis tissue, extended semen, supernatant sample and seminal plasma portion of semen sample, as well as the kidney and liver (Segerson and Johnson, 1981). In rats, it has been reported that testicular and epididymal function were also extremely sensitive to Se deficiencies (Wu et al., 1969). Despite Se concentration improving in reproductive tissues, the sperm concentration in the testis and caput, corpus and caudal epididymis were not different between bulls injected with Se and bulls left non-supplemented (Segerson and Johnson, 1981). Likewise, Se treatment did not improve sperm cell viability or Se concentration (Segerson and Johnson, 1981).

While it appears that trace minerals play a role in bull fertility, results from various studies tend to disagree. Also, the current literature published on the effects of mineral supplementation, in particular the combination of Cu, Zn, Mn and Se, directly on bull fertility is limited. Therefore additional research is necessary to understanding the impact of these 4 trace minerals on bulls.

Effects on Cow and Heifer Fertility

The reproductive efficiency of a beef female can be determined by factors such as age of puberty and first conception, duration of post-partum anestrus, total lifetime productivity and total weight of calves weaned (Burns et al., 2010). Dietary Cu, Zn and Mn are important to reproduction, and dietary Cu and Zn are often found in levels below those recommended for breeding cattle and thus should be supplemented in the diet (NRC, 1996).

Research has indicated that supplementing crossbred heifers with dietary Cu, Zn and Mn had no impact on age of puberty (Grings et al., 1999). Age of puberty was instead influenced by sire breed, age of dam (Grings et al.,1999), and the use of a progestin implantation (Grings et al.,

1998). Although supplementation of Cu, Zn and Mn did not effect the percentage of heifers pregnant (Grings et al., 1999), the combination of Cu, Zn, Mn and Se supplemented to virgin beef heifers through an injectable source improved overall pregnancy rate (93.0%) compared to the control set of heifers (83.0%) (Brasche, 2014). Brasche (2014) also reported that while there was a mineral treatment by synchronization protocol interaction for heifers conceiving to AI, there were no differences between supplemented and control heifers within the 5 d Co-Synch plus CIDR protocol or within the 14 d CIDR-PG protocol. Additionally, lactating beef cows on Florida pastures administered injectable trace minerals exhibited increased pregnancy rates as BCS increased, however, in contrast to previous results, mineral supplementation did not effect this outcome (Arthington et al., 2014a).

Previous researchers suggested that administering an injectable trace mineral source 30 d prior to the breeding season may improve reproductive performance in heifers (Brasche, 2014). However, other researchers found that utilizing the same injectable trace minerals in beef cows, 30 d prior to breeding and again 30 d prior to calving, had no effect on reproductive performance for a two year period (Whitworth et al., 2014). Authors in this study noted that these cows had additional access to free choice minerals and that their initial mineral status was unknown. This indicates that it is possible for future results to differ if the initial mineral status was observed and mineral intake was controlled.

When observing cows that were not exposed to free choice mineral supplementation, and only received the injectable source of minerals, supplemented cows did not exhibit differences in pregnancy rate, post-partum interval or BCS at weaning, calving or rebreeding in comparison to non-injected cows (Gadberry and Simon, 2012). Although previous research has indicated that supplementation of Cu, Zn, Mn and Se in conjunction with Vitamin E can improve reproductive

performance (Ishak et al., 1983), injectable Cu, Zn, Mn and Se administered with Vitamin E to beef cows did not improve post-partum interval or conception rates (Daugherty et al., 2002),

Interestingly, one injection of trace minerals before breeding had no beneficial effects on first service conception rates in intensely managed dairy cows, however when dairy cows received 2 injections (one before calving and one before breeding) they exhibited lower conception at first service (Vanegas et al., 2004). Researchers originally thought that these unique results were due to retained placentas or mastitis in cows, however when the data was analyzed there were no differences among these illnesses prior to breeding. The physiological activity that caused these results is unknown and further research is warranted to explain what occurred.

Ahola et al. (2004) noted that mineral source (organic or inorganic) may influence pregnancy rate to AI in beef cattle. This was a 2 year study in which cows received no supplement (control) or received free choice mineral (organic or inorganic) at NRC recommended concentrations of Cu, Mn and Zn. In the first year organic supplemented cows tended to have higher pregnancy rates than inorganic supplemented cows. Only in the second year did both groups of supplemented cows have higher pregnancy rates than control cows. Combining both years, supplemented cows tended to have higher 60 d pregnancy rates than control cows. In contrast, feeding Cu alone, or Cu with Zn, did not improve 60 d pregnancy rates in supplemented cows compared to control cows (Muehlenbein et al., 2001). While there is a source effect on pregnancy in beef cows, it also appears there may be an interaction between the combination of minerals utilized that influences attainment of pregnancy. Additionally, source and level of minerals supplied to the animal may also impact pregnancy rates. For example, organic and inorganic Cu, Co, Mn and Zn supplied above nutrient requirements reduced

reproductive performance (Olson et al., 1999). Organic supplemented cows conceived later than inorganic supplemented or control cows. Incidence of organic and inorganic supplemented cows not being pregnant was greater than for control cows. Researchers believed that the cows utilized in this study had adequate mineral supplementation (as seen by the control cows), and that excess supplementation of trace minerals may cause imbalances or toxicities that result in reduced reproductive performance (Olson et al., 1999).

It appears female fertility is influenced differently depending on the source, level and combination of minerals supplemented to cattle. More research should be conducted to analyze how these characteristics may benefit or hinder female reproductive performance.

Rationale for Current Experiment

It is known that trace minerals are vital to animal productivity, however the effects of trace minerals on animal health and performance differ depending on the source (organic or inorganic), administration (dietary, bolus, injectable) or combination of minerals utilized. Research has indicated that the use of injectable minerals may be advantageous to bypass dietary antagonists within the gastrointestinal tract, because upon injection the minerals would be directly circulated throughout the bloodstream and incorporated into cells as needed (Berry et al., 2000). By increasing mineral absorption and utilization, animal health and productivity may also be improved (Richeson and Kegley, 2011). Unfortunately, the current literature regarding injectable trace minerals is limited and contradicting.

Moreover, further research is warranted to determine the effects of injectable trace minerals in cattle, in particular, on fertility in yearling bulls and heifers, as well as feedlot performance and morbidity in newly received beef calves.

LITERATURE CITED

Abbas, A.K., A.H. Lichtman, J.S. and Prober. 1991. Cellular and molecular immunology. W.B. Saunders Co., Philadelphia, PA.

Ahola, J.K., D.S. Baker, P.D. Burns, R.G. Mortimer, R.M. Enns, J.C. Whittier, T.W. Geary, and T.E. Engle. 2004. Effect of copper, zinc and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two year period. J. Anim. Sci. 82:2375-2383.

Ahola, J.K., L.R. Sharpe, K.L. Dorton, P.D. Burns, T.L. Stanton, and T.E. Engle. 2005. Effects of lifetime copper, zinc and manganese supplementation and source on performance, mineral status, immunity and carcass characteristics of feedlot cattle. Prof. Anim. Sci. 21:305-317.

Ahola, J.K., and T.E. Engle. 2012. Trace minerals and the immune system in cattle. Cattle Producers Handbook. (3rd Ed.) Nutrition Section. p. 315:1-5.

Arthington, J.D., and L.J. Havenga. 2012. Effect of injectable trace minerals on the humoral immune response to multivalent vaccine administration in beef calves. J. Anim. Sci. 90:1966-1971.

Arthington, J.D., P.G.M.A. Martins, P. Moriel, and L. Havenga. 2014a. Effects of injectable trace minerals at the start of the breeding season on attainment of pregnancy in commercial beef cows. ADSA-ASAS-CSAS Joint Annual Meeting. Kansas City Convention Center. Kansas City, MO. p. 1469 (Abstr.)

Arthington, J.D., P. Moriel, P.G.M.A. Martins, G.C. Lamb, and L.J. Havenga. 2014b. Effects of trace mineral injections on measures of performance and trace mineral status of pre- and postweaned beef calves. J. Anim. Sci. 92:2630-2640.

Baker, S.K. 1997. Gut microbiology and its consequences for the ruminant. Proc. Nutr. Soc., Australia. 21:6-13.

Baker, S.K. 1999. Rumen methanogens and inhibition of methanogenesis. Aust. J. Agric. Res. 50:1293-1298.

Bansal, A.K., and G.S. Bilaspuri. 2008. Effect of manganese on bovine sperm motility, viability and lipid peroxidation *in vitro.* Anim. Reprod. 5:90-96.

Bartle, J.L., P.L. Senger, and J.K. Hillers. 1980. Influence of injected selenium in dairy bulls on blood and semen selenium, glutathione peroxidase and seminal quality. Bio. Reprod. 23:1007-1013.

Benchaar, C., S. Calsamiglia, A.V. Chaves, G.R. Fraser, D. Colombatto, T.A. McAllister, and K.A. Beauchemin. 2008. A review of plant-derived essential oils in ruminant nutrition and production. Animal Feed Science and Technology. 145:209-228.

Bergen, W.G., and D.B. Bates. 1984. Ionophores: Their effect on production efficiency and mode of action. J.Anim. Sci. 58:1465-1483.

Berger, L.L., S.C. Ricke, and G.C. Jr. Fahey. 1981. Comparsion of two forms and two levels of lasolocid with monensin on feedlot cattle performance. J. Anim. Sci. 53(6):1440-1445.

Berry, B. A., W. T. Choat, D. R. Gill, C. R. Krehbiel, and R. Ball. 2000. Efficacy of Multimin™ in improving performance and health in receiving cattle. Anim. Sci. Rep. Oklahoma Agric. Exp. Stn. Accessed March 15, 2015. http://www.ansi.okstate.edu/ research/2000rr/12.htm.

Bigler, B.J. 2014. Effects of monensin sodium and plant extracts containing cinnamaldehyde, capsicum oleoresin and eugenol on d to puberty, gain, pregnancy rate and feed efficiency in developing beef heifers fed a high roughage diet. Master Thesis. Colorado State University, Fort Collins.

Black, J.L. 1971. A theoretical consideration of the effect of preventing rumen fermentation on the efficiency of utilization of dietary energy and protein in lambs. Brit. J. Nutr. 25:31-55.

Blecha, F., S.L. Boyles, and J.G. Riley. 1984. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman x Angus feeder calves. J. Anim. Sci. 59:576-583.

Bohnert, D.W., and R.F. Cooke. 2012. Nutritional management of the mature beef cow. Cattle Producers Handbook. (3rd Ed.) Nutrition Section. p. 330:1-4

Boling, J.A., N.W. Bradley, and L.D. Campbell. 1977. Monensin levels for growing and finishing steers. J. Anim. Sci. 44(5):867-871.

Brasche, C.J., J.B. Hall, and M.E. Drewnoski. 2014. Effect of an injectable trace mineral on pregnancy rate in virgin heifers when synchronized using 5 day Co-Synch plus CIDR or 14 day CIDR-PG protocol. ADSA-ASAS-CSAS Joint Annual Meeting. Kansas City Convention Center, Kansas City, MO. p. 926. (Abstr.)

Burns, B.M., G. Fordyce, and R.G. Holroyd. 2010. A review of factors that impact on the capacity of beef cattle females to conceive, maintain a pregnancy and wean a calf-Implications for reproductive efficiency in northern Australia. Anim. Reprod. Sci. 122:1-22.

Busquet, M., S. Calsamiglia, A. Ferret, and C. Kamel. 2005. Screening for the effects of plant extracts and active compounds of plants on dairy cattle rumen microbial fermentation in a continuous culture system. Anim. Feed Sci. and Tech. 123-124:597-613

Busquet, M., S. Calsamiglia, A. Ferret, and C. Kamel. 2006. Plant extracts effect in vitro rumen microbial fermentation. J. Dairy Sci. 89:761-771.

Calsamiglia, S., M. Busquet, P.W. Cardozo, L. Castillejos, and A. Ferret. 2007. Invited Review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90:2580-2595.

Cardozo, P.W., S. Calsamiglia, A. Ferret, and C. Kamel. 2005. Screening for the effects of natural plant extracts at different pH on in vitro rumen microbial fermentation of a high-concentrate diet for beef cattle. J. Anim. Sci. 83:2572-2579.

Cardozo, P.W., S. Calsamiglia, A. Ferret, and C. Kamel. 2006. Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. J. Anim. Sci. 84:2801-2808.

Cavallini, L., M. Valente, and A. Bindoli. 1984. On the mechanism of inhibition of lipid peroxidation by manganese. Inorganica Chim Acta. 91:117-120.

Chalupa, W. 1974. Rumen bypass and Protection of proteins and amino acids. J. Dairy Sci. 58:1198-1218.

Chen, M., and M.J. Wolin. 1979. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. Appl. Environ. Microbiol. 38:72-77.

Chesters, J.K. 1992. Trace element-gene interactions. Nutr. Rev. 50:217-223.

Clark, R.T., K.W. Creighton, H.H. Patterson, and T.N. Barrett. 2005. Symposium Paper: Economic and tax implications for managing beef replacement heifers. Prof. Anim. Sci. 21:164-173.

Cooke, R.F. 2012. Reproductive tract anatomy and physiology of the bull. Cattle Producers Handbook. (3rd Ed.) Reproduction Section. 424:1-3.

Corah, L.R., and J. Arthington. 1993. Mineral nutrition-identifying problems and solutions. Proc., Range Cow Beef Symposium XIII, Cheyenne, WY. p. 100-119.

Daugherty, S.R., G.E. Carstens, D.B. Herd, K.S. Barling, and R.D. Randel. 2002. Effects of prenatal and prebreeding trace mineral/vitamin E injections on calf health and reproductive performance of beef cows. Beef Cattle Research in Texas Report. Dep. Anim. Sci., Texas A&M Univ., College Station, TX.

DeVoe, R.D. 1974. Principles of cell homeostatis. In: Medical Physiology (13th Ed.) p. 3. C.V. Mosby Co., St. Louis, MO.

Dorman, H.J.D., and S.G. Deans. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile fatty oils. J. Appl. Microbiol. 88:308-316.

Dorton, K.L., T.E. Engle, and R.M. Enns. 2006. Effects of trace mineral supplementation and source, 30 days post-weaning and 28 days post receiving, on performance and health of feeder cattle. Asian-australas. J. Anim. Sci. 19:1450-1454.

Duff, G.C., and M.L. Galyean. 2007. Board invited review: recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85:823-840.

Edrington, T.S., T.R. Callaway, P.D. Varey, Y.S. Jung, K.M. Bischoff, R.O. Elder, R.C. Anderson, E. Kutter, A.D. Brabbin, and D.J. Nisbet. 2003. Effects of the antibiotic

ionophores monensin, lasalocid, laidlomycin propionate and bambermycin on salmonella and E. coli 0157:H7 in vitro. Journal of Applied Microbiology. 94:207-217.

Endecott, R.L., and R.J. VanSaun. 2012. Beef cattle nutrition: Feeding the cow and the rumen. Cattle Producers Handbook. (3rd Ed.) Nutrition Section. 313: 1-4.

Engle, T.E., C.F. Nockels, C.V. Kimberling, R.E. Toombs, R.S. Yemm, D.L. Weaber, and A.B. Johnson. 1995. The effect of feeding organic and inorganic zinc on biochemical parameters in zinc-deficient calves. Proc., West. Sect. Am. Soc. Anim. Sci. 46:471-474.

Faulkner, D.B., T.J. Klopfenstein, T.N. Trotter, and R.A. Britton. 1985. Monensin effects on digestibility, ruminal protein escape and microbial protein synthesis on high fiber diets. J. Anim. Sci. 61:654-660.

Funston, R.N., and G.H. Deutscher. 2004. Comparsion of target breeding weight and breeding date for replacement beef heifers and effects on subsequent reproduction and calf performance. J. Anim. Sci. 82:3094-3099.

Gadberry, M.S., and K. Simon. 2012. Response of beef cows, not exposed to mineral supplements, to an injectable trace mineral supplement. Univ. of Arkansas Anim. Sci. Rep. Fayetteville, AR.

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

Genther, O.N., and S.L. Hansen. 2014. Effects of dietary trace mineral supplementation and a multi-element trace mineral injection on shipping response and growth performance of beef cattle. J. Anim. Sci. 92:2522-2530.

Geraci, J.I., A.D. Garciarena, G.A. Gagliostro, K.A. Beauchemin, and D. Colombatto. 2012. Plant extracts containing cinnamaldehyde. Eugenol and capsicum oleoresin added to

feedlot cattle diets: Ruminal environment, short term intake pattern and animal performance. Anim. Feed Sci. and Tech. 176:123-130.

Greathead, H. 2003. Plant and plant extract for improving animal productivity. Proc. Nutr. Soc. 62:279-290.

Griffin, S.G., S.G. Wyllie, J.L. Markham, and D.N. Leach. 1999. The role of structure and molecular properties os terpenoids in determining their antimicrobial activity. Flavour Fragr. J. 14:322-332.

Grings, E.E., J.B. Hall, R.A. Bellows, R.E. Short, S.E., Bellows, and R.B. Staigmiller. 1998. Effect of nutritional management, trace mineral supplementation and norgestomet implant on attainment of puberty in beef heifers. J. Anim. Sci. 76:2177-2181.

Grings, E.E., R.B. Staigmiller, R.E. Short, R.A. Bellows, and M.D. MacNeil. 1999. Effects of stair-step nutrition and trace mineral supplementation on attainment of puberty in beef hifer of three sire breeds. J. Anim. Sci. 77:810-815.

Hanley, M.E., and M.M Hoehn. 1967. Monensin, a new biologically active compound. I. Discovery and isolation. Antimicrob. Agents Chemother. p.349-352.

Hanson, T.L., and T.J. Klopfenstein. 1979. Monensin, protein source and protein levels for growing steers. J. Anim. Sci. 48:474.

Harold, F.M. 1972. Conservation and transformation of energy. Bacteriol. Rev. 36:172-230.

Hidiroglou, M. 1975. Manganese uptake by the ovaries and reproductive tract of cycling and anestrous ewes. Can. J. of Physio. and Pharm. 53:969-972.

Ishak, M.A., L.L. Larson, F.G. Owens, S.R. Lawry, and E.D. Erickson. 1983. Effects of selenium, vitamins and ration fiber on placental retention and performance of dairy cattle. J.Dairy. Sci. 66:99-106.

Johnson, D.D., J.S. Abo-Omar, and B.R. Carmean. 1994. Persistence of methane suppression by propionate enhancers in cattle diets. In Energy Metabolism in Farm Animals. p. 339-342. CSIC Publishing Service: Madrid, Spain.

Kegley, E.B., K.P. Coffey, and J.T. Richeson. 2011. Effects of trace mineral injection 28 days before weaning on calf health, performance and carcass characteristics. Univ. of Arkansas Anim. Sci. Rep. Fayetteville, AR.

Kegley, E.B., M.R. Pass, J.C. Moore, and C.K. Larson. 2012. Supplemental trace minerals (zinc, copper, manganese and cobalt) as Availa-4 or inorganic sources for shipping-stressed beef cattle. Professional Animal Scientist. 28:313-318.

Kendall, N.R., S. McMullen, A. Green, and R.G. Rodway. 2000. The effect of a zinc, cobalt and selenium soluble glass bolus on trace element status and semen quality of ram lambs. J. Anim. Repro. Sci. 62:277-283.

Kirchhoff, A.A., K.E. Fike, and R. Breiner. 2015. An injectable trave mineral supplement in yearling bulls causes short term increase in circulating trace mineral levels but does not improve sperm quality. Kansas Ag. Exp. Stn. Rep. 1:1-5.

Kumar, N., R.P. Verma, L.P. Singh, V.P. Varshney, and R.S. Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* x *Bos taurus*) bulls. Reprod. Nutr. Dev. 46:663-675.

Kumar, B.S., and K.I. Priyadarsini. 2014. Selenium nutrition: How important is it? Biomed. & Prev. Nutr. 4:333-341.

Kroger, A. 1977. Phosphorylative electron transport with fumarate and nitrate as terminal hydrogen acceptors. B.A. Haddock and W.A. Hamilton (Ed.) Microbial Energetics. p. 61. Cambridge Univ. Press, Cambridge, U.K.

Lalman, D.L., M.K. Petersen, R.P. Ansotegui, M.W. Tess, C.K. Clark, and J.S. Wiley. 1993. The effects of ruminally undegradeable protein, propionic acid, and monensin on puberty and pregnancy in beef heifers. J. Anim. Sci. 71:2843-2852.

Lesmeister, J.L., P.J. Burfening, and R.L. Blackwell. 1973. Date of first calving in beef cows and subsequent calf production. J. Anim. Sci. 36:1-6.

Lourenco, M., P.W. Cardozo, S. Calsamiglia, and V. Fievez. 2008. Effects of saponins, quercetin, eugenol and cinnamaldehyde on fatty acid biohydrogenation of forage polyunsaturated fatty acids in dual-flow continuous fermenters. J. Anim. Sci. 86:3045-3053.

Maas, J. 1987. Relationship between nutrition and reproduction in beef cattle. Veterinary Clinics of North America. Food Animal Practice. 3:633-646.

McCartor, M.M., R.D. Randel, and L.H. Carroll. 1979. Dietary alteration of ruminal fermentation on efficiency of growth and onset of puberty in Brangus heifers. J. Anim. Sci. 48:488-494.

McDowell, L.R. 1989. Vitamins in Animal Nutrition. Academic Press Inc. Harcourt Brace Jovanovich Publishers, San Diego, CA.

McDowell, L.R. 1992. Minerals in Animal and Human Nutrition. Academic Press Inc., New York, NY.

Meinert, R.A., C.M.J. Yang, A.J. Heinrichs, and G.A. Varga. 1992. Effect of monensin on growth, reproductive performance and estimated body composition in Holstein heifers. J. Dairy Sci. 75:257-261.

Meyer, N.F., G.E. Erickson, T.J. Klopfenstein, M.A. Greenquist, M.K. Luebbe, P. Williams, and M.A. Engstrom. 2009. Effect of essential oils, tylosin and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation and digestibility. J. Anim. Sci. 87:2346-2354.

Mies, W.L., L.B. Sherrod, C.B. Summers, and N.G. Elliston. 1979. Effect of rumensin upon protein efficiency of finishing steers. J. Anim. Sci. 47:339.

Moseley, W.M., T.G. Dunn, C.C. Kaltenbach, R.E. Short, and R.B. Staigmiller. 1982. Relationship of growth and puberty in beef heifers fed monensin. J. Anim. Sci. 55:357-362.

Moseley, W.M., M.M. McCartor, and R.D. Randel. 1977. Effects of monensin on growth and reproductive performance of beef heifers. J. Anim. Sci. 45:961-968.

Muehlenbein, E.L., D.R. Brink, G.H. Deutscher, M.P. Carlson, and A.B. Johnson. 2001. Effects of inorganic and organic copper supplementated to first calf cows on cow reproduction and calf health and performance. J. Anim. Sci. 79:1650-1659.

Muntifering, R.B., B. Theurer, R.S. Swingle, and W.H. Hale. 1980. Effect of monensin on nitrogen utilization and digestibility of concentrate diets by steers. J. Anim. Sci. 50:930-936.

National Research Council (NRC). 1996. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington D.C. p. 54-74.

National Research Council (NRC). 2000. Nutrient requirements of beef cattle. 7th rev. ed. Update 2000. Natl. Acad. Press, Washington D.C. p. 54-74.

Nockels, C.F., and J.C. Whittier. 1995. Micronutrients and the immune system of cattle. Western Beef Resource Committee Cattle Producer's Library 315:1-4.

Oh, H.K., T. Sakai, M.B. Jones, and W.M. Longhurst. 1967. Effect of various essential oils isolated from Douglas fir needles upon sheep and deer rumen microbial activity. Appl. Microbiol. 15:777-784.

Oliver, W.M. 1975. Effect of monensin on gains of steers grazed on coastal bermudagrass. J. Anim. Sci. 41:999-1001.

Olson, P.A., D.R. Brink, D.T. Hickok, M.P. Carlson, N.R. Schneider, G.H. Deutscher, D.C. Adams, D.J. Colburn, and A.B. Johnson. 1999. Effects of supplementation on organic and inorganic combinations of copper, cobalt, manganese and zinc above nutrient requirement levels on postpartum two year old cows. J. Anim. Sci. 77:522-532.

Ovchinnikov, J.A. 1979. Physico chemical basis of ion transport through biological membranes: Ionophores and ion channels. Eur. J. Biochem. 94:321-336.

Paterson, J.A., and T.E. Engle. 2005. Trace Mineral Nutrition in Beef Cattle. Nutrition Conference. Dept. Anim. Sci., UT.

Perry, T.W., W.M. Beeson, and M.T. Mohler. 1976. Effect of monensin on beef cattle performance. J. Anim. Sci. 42:761-765.

Pogge, D.J., M.E. Richter, M.E. Drewnoski, and S.L. Hansen. 2012. Mineral concentrations of plasma and liver after injection with a trace ineral complex differ among Angus and Simmental cattle. J. Anim. Sci. 90:2692-2698.

Poos, M.I., T.L. Hanson, and T.J. Klopfenstein. 1979. Monensin effects on diet digestibility, ruminal protein bypass and microbial protein synthesis. J. Anim. Sci. 48:1516-1524.

Potter, E.L., C.O. Cooley, A.P. Raun, L.F. Richardson, and R.P. Rathmacher. 1974. Effect of monensin on daily gain of cattle on pasture. J. Anim. Sci. 38:1344 (Abstr).

Pressman, B.C. 1976. Biological application of ionophores. Ann. Rev. Biochem. 45:501-530.

Purvis, H.T., and J.C. Whittier. 1996. Effects of ionophore feeding and anthelmentic administration on age and weight at puberty in spring born beef heifers. J. Anim. Sci. 74:736-744.

Raun, A.P., C.O. Cooley, E.L. Potter, R.P. Rathmacher, and L.F. Richardson. 1976. Effect of monensin on feed efficiency of feedlot cattle. J. Anim. Sci. 43:670-677.

Raun, A.P. 1990. Rumensin "then and now." In Rumensin. Elanco Animal Health, Denver, Colorado. p. A1-A20.

Reece, W.O. 2009. Functional anatomy and physiology of domestic animals. (4th Ed.) Blackwell Publishing. Ames, Iowa. p. 406-410.

Reis, L.S., L.S. Reis, A.A. Ramos, A.S. Camargos, and E. Oba. 2014. Effect of manganese supplementation on the membrane integrity and the mitochondrial potential of the sperm of grazing Nelore bulls. J. Anim. Repro. Sci. 150:1-6.

Rhoads, A.R., T.L. Stanton, T.E. Engle, and C.V. Kimberling. 2003. Effects of concentration and source of trace minerals on performance, immunity, mineral and lipid metabolism, and carcass characteristics of beef steers. Prof. Anim. Sci. 19:150-158.

Richardson, L.F., A.P. Raun, E.L. Potter, C.O. Cooley, and R.P. Rathmacher. 1976. Effect of monensin on rumen fermentation in vitro and in vivo. J. Anim Sci. 43:657-664.

Richeson, J. T., and E. B. Kegley. 2011. Effect of supplemental trace minerals from injection on health and performance of highly stressed, newly received beef heifers. Prof. Anim. Sci. 27:461–466.

Rogers, J.A., and C.L. Davis. 1982. Rumen volatile fatty acid production and nutrient utilization in steers fed a diet supplemented with sodium bicarbonate and monensin. J. Dairy Sci. 65:944-952.

Rowe, M.P., J.G. Powell, E.B. Kegley, T.D. Lester and R.W. Rorie. 2014. Effect of supplemental trace mineral source on bull semen quality. Prof. Anim. Sci. 30(1):68-73.

Sandeaux, R., J. Sandeaux, C. Gavach, and B. Brun. 1982. Transport of Na^+ by monensin across bimolecular lipid membranes. Biochim. Biophys. Acta. 684:127-132.

Scheifinger, C.C., and M.J. Wolin. 1973. Propionate formation from cellulose and soluble sugars by combined cultures of *bacteriodes succinogenes* and *selenomonas ruminantium.* Appl. Microbiol. 26:789-795.

Schelling, G.T. 1984. Monensin mode of action in the rumen. J. Anim. Sci. 58(6):1518-1527.

Schillo, K.K., J.B. Hall, and S.M. Hileman. 1992. Effects of nutrition and season on the onset of puberty in the beef heifer. J. Anim. Sci. 70:3994-4005.

Segerson, E.C., and B.H. Johnson. 1980. Selenium and reproductive function in yearling bulls. J. Anim. Sci. 51:395-401.

Spears, J.W. 1996. Organic trace minerals in ruminant nutrition. Anim. Sci. Feed Technol. 58:151–163.

Suttle, N.F. 1987. The nutritional requirements for sopper in animals and man. In: Howell, J.McC. and Gawthorne, J.M. *Copper in Animal and Man.* Vol. 1. CRC Press, Boca Raton, FL. p.21-44.

Tedeschi, L.O., D.G. Fox, and T.P. Tylutki. 2003. Potential environment benefits of ionophores in ruminant diets. J. Environ. Quality. 32:1591-1602.

Underwood, E.J., and N.F. Suttle. 1999. The Mineral Nutrition of Livestock (3rd ed.). CABI Publishing, Wallingfor, UK.

Van Maanen, R.W., J.H. Herbein, A.D. McGilliard, and J.W. Young. 1978. Effects of monensin on in vivo rumen propionate production and blood glucose kinetics in cattle. J. Nutr. 108:1002-1007.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. Comstock, Cornell Univ. Press, New York, NY.

Vanegas, J.A., J. Reynolds, and E.R. Atwill. 2004. Effects of an injectable trace mineral supplement on first-service conception rate of dairy cows. Dairy Sci. 87:3665–3671.

Verma, A., and K.C. Kanwat. 1999. Effect of vitamin E on human sperm motility and lipid peroxidation *in vitro*. Asian J. Androl. 1:151-154.

Whetstone, H.D., C.L. Davis, and M.P. Bryant. 1981. Effect of monensin on breakdown of protein by ruminal microorganisms in vitro. J. Anim. Sci. 53:803-809.

Whitworth, W.A., T.G. Montgomery, A.J. Hood, and M.S. Gadberry. 2014. Effects of injectable trace mineral supplementation on reproductive rates in *Bos indicus* cattle. Univ. of Arkansas Anim. Sci. Rep. Fayetteville, AR. p. 42-43.

Wolin, M.J. 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43:1452-1459.

Wu, S.H., J.E. Oldfield, O.H. Muth, P.D. Whanger, and P.H. Weswig. 1969. Effect of selenium on reproduction. Proc., West. Sec. ASAS 20:85.

Yang, W.Z., B.N. Ametaj, C. Benchaar, and K.A. Beauchemin. 2010a. Dose response to cinnamaldehyde supplementation in growing beef heifers: Ruminal and intestinal digestion. J. Anim. Sci. 88:680-688.

Yang, W.Z., B.N. Ametaj, C. Benchaar, M.L. He, and K.A. Beauchemin. 2010b. Cinnamaldehyde in feedlot cattle diets: Intake, growth performance, carcass characteristics and blood metabolites. J. Anim. Sci. 88:1082-1092.

CHAPTER II

EFFECTS OF HIGH LEVELS OF MONENSIN SODIUM AND PLANT EXTRACTS CINNAMALDEHYDE, CAPSICUM OLEORESIN AND EUGENOL ON BEEF HEIFER FEEDLOT AND REPRODUCTIVE PERFORMANCE

SUMMARY

The objectives of this study were to determine the effects of high levels of monensin sodium fed with or without the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol on ADG, feed efficiency, age at puberty and pregnancy rate in developing beef heifers. In mid-October, Angus heifers (n = 107; 259.3 ± 20.98 d of age), blocked by weaning BW (262.7 ± 29.85 kg; d -19), were randomly assigned to treatments in a 2 x 2 factorial design, where all heifers received the same basal ration consisting of a 30% CP liquid supplement containing 200 mg/0.45 kg monensin sodium. Treatments were applied daily to the basal ration as topdressed supplements and were fed at a rate of 0.32 kg·hd^{-1}·d^{-1}. Treatments were as follows: 1) high level of monensin sodium (**MON**), where monensin sodium was topdressed at 200 mg·hd^{-1}·d^{-1}, 2) low level of monensin sodium plus the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (**CCE**), where plant extracts were topdressed at 11,000 mg·hd^{-1}·d^{-1}, 3) control (**CON**), low level of monensin sodium without topdressed supplements, or 4) high levels of monensin sodium with plant extracts (**COMB**), where monensin sodium was topdressed at 200 mg·hd^{-1}·d^{-1} and plant extracts were topdressed at 11,000 mg·hd^{-1}·d^{-1}. Heifers were weighed and estrus detection patch status was recorded every 11 d. Age at puberty was determined by patch status

and recorded as the d the patch was first activated. A 14 d CIDR-PG-AI protocol was utilized to inseminate heifers, when heifers were 427.3 ± 20.98 d of age. In the 14 d CIDR-PG-AI, a controlled internal drug release device (**CIDR**) was inserted 33 d prior to AI and removed 14 d later. Prostaglandin-F-2_{α} was injected 16 d after CIDR removal and heifers were inseminated 3 d later. Heifers were placed with bulls for natural service 21 d post AI. Pregnancy was determined 56 d post AI via ultrasound and 178 d post AI via rectal palpation. Calving records were used to validate ultrasound results. There were no differences between treatments for initial or final BW ($P > 0.05$). There were no interactions between the main effects of monensin sodium fed at high concentrations and plant extracts for any feedlot or fertility performance variable ($P > 0.05$); however, there was a main effect of high levels of monensin sodium for heifer DMI from d 0 to 8 and d 8 to 15, where heifers fed high levels of monensin sodium had reduced DMI compared to heifers fed low levels of monensin sodium ($P = 0.05$). From d 11 to 22 and d 44 to 66, heifers that received plant extracts (CCE and COMB) had lower ADG than heifers that did not receive plant extracts ($P = 0.05$). Feed efficiency tended ($P = 0.08$) to be improved in heifers fed high levels of monensin sodium (MON and COMB) compared to heifers fed low levels of monensin sodium (CCE and CON); however overall DMI, ADG, age at puberty and pregnancy rate were not affected by the main effects of high levels of monensin sodium or plant extracts ($P > 0.05$).

Key Words: beef heifer development, feed efficiency, monensin sodium, plant extracts, puberty

INTRODUCTION

Current beef management practices in the United States include heifers calving by 2 yr of age (Funston and Deutscher, 2004). One of the greatest costs of developing heifers is feed

resources (Clark et al., 2005). Heifers reaching a BW of 50 to 57% of their mature BW, in comparison to the traditional target weight of 60 to 65%, by breeding season can reduce development costs without impairing reproductive performance (Funston and Deutscher, 2004). Increasing ADG and improving feed efficiency can aid in heifers reaching target breeding weight at a younger age while also decreasing feed input costs.

Ionophores, such as monensin sodium, have been used in beef cattle rations to improve feed efficiency and nutrient utilization (Bergen and Bates, 1984). Monensin sodium has been found to improve feed conversion (Raun, 1990), decrease age at puberty (Moseley et al., 1982; Lalman et al., 1993) and increase first service pregnancy rate (Purvis and Whittier, 1996). Plant extracts have also demonstrated antimicrobial properties that may allow manipulation of microbial activity in the rumen (Benchaar et al., 2008). Plant extracts have been found to improve ADG by 16% (Geraci et al., 2012) and reduce DMI when fed to cattle on high concentrate diets (Cardozo et al., 2006). Unfortunately, there is limited published literature regarding the effects of plant extracts on animal performance and fertility. The objectives of this study were to evaluate the effects of high levels of monensin sodium, fed with or without the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol, on ADG, feed efficiency, age at puberty and pregnancy in beef heifers.

MATERIALS AND METHODS

Experimental procedures conducted with animals were approved by the Institutional Animal Care and Use Committee at Colorado State University prior to initiation of the study.

Experimental Design

Angus heifers (n = 107; 259.3 ± 20.98 d of age), blocked by weaning BW (262.7 ± 29.85 kg; d -19), were randomly assigned to treatments using a random number generator. Pens were 488 m^2 with 6 or 7 heifers and were also randomly assigned a treatment. All heifers received the same basal ration (Table 2.1) consisting of a 30% CP liquid supplement containing 200 mg/0.45 kg monensin sodium. Treatments were applied daily to the basal ration as topdressed supplements and were fed at a rate of 0.32 $kg \cdot hd^{-1} \cdot d^{-1}$. There were 4 replicates per treatment. In a 2 x 2 factorial design, the treatments were: 1) high level of monensin sodium (**MON**), where monensin sodium was topdressed at 200 $mg \cdot hd^{-1} \cdot d^{-1}$, 2) low level of monensin sodium plus the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (**CCE**), where plant extracts were topdressed at 11,000 $mg \cdot hd^{-1} \cdot d^{-1}$ (RumeNext®, ADM Alliance Nutrition Inc., Quincy, IL), 3) control (**CON**), low level of monensin sodium without topdressed supplements, or 4) high levels of monensin sodium with plant extracts (**COMB**), where monensin sodium was topdressed at 200 $mg \cdot hd^{-1} \cdot d^{-1}$ and plant extracts were topdressed at 11,000 $mg \cdot hd^{-1} \cdot d^{-1}$. These dosage levels were recommended by ADM Alliance Nutrition (Archer Daniels Midland Inc.). Researchers were unaware of the monensin supplied in the basal ration and it was unintentionally used. It is important to note that results from Fischer et al. (2014) are therefore inaccurate.

Data Collection

Feed refusals were estimated and recorded and feed offered was adjusted daily based on evaluation by a trained bunk reader to minimize feed refusals. Feed samples and orts were collected weekly and intake was calculated on a DM basis by pen. The DM and nutrient analysis was performed by a private laboratory (SDK Laboratories, Hutchinson, KS).

Heifers were weighed every 11 d to measure ADG. Estrus detection patches (Estrotect™ Heat Detector, Rockway Inc., Spring Valley, WI) were applied on d 0. Patch status was recorded every 11 d as activated, not activated or missing. Missing patches were not considered activated, and if a patch was missing, it was replaced with a new patch. Age at puberty was estimated based on patch status and recorded as the d that the patch was first activated. A 14 d CIDR-PG-AI protocol was utilized to inseminate heifers on d 168, when heifers were 427.3 ± 20.98 d of age. In the 14 d CIDR-PG-AI, a controlled internal drug release device (**CIDR**) was inserted 33 d prior to AI and removed 14 d later. Prostaglandin-F-2_{α} was injected 16 d after CIDR removal and heifers were inseminated 3 d later. Heifers were placed with bulls for natural service 21 d post AI. Pregnancy was determined via ultrasound at 56 d post AI and again at 178 d post AI via rectal palpation. Calving records were used to validate ultrasound results.

Chute exit velocity was observed every 11 d using an infrared sensor timing system (FarmTek Inc., North Wylie, TX) in order to evaluate the effects of plant extracts on heifer behavior. The first sensor was placed 1.83 m from the front of the chute, and the second sensor was 3.66 m from the front of the chute. Velocity was measured in m/s as the time it took heifers to move from the first to second sensor after exiting the chute.

Data Analysis

Average daily gain, DMI, G:F, chute exit velocity, age at puberty, BCS, pregnancy rate and d of calving season were analyzed using a mixed model and the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Pen was the experimental unit. Feedlot performance data were analyzed by periods of time since previous research had found differences in the effects of monensin sodium (Moseley et al., 1977) and plant extracts over time (Yang et al., 2010b; Geraci et al., 2012). The model included treatment as a fixed effect. Predictor variables included BW,

BCS, age, AI tech and sire, depending on the variable analyzed. If predictor variables were considered to have an effect ($P \leq 0.10$), they were left in the model statement.

RESULTS AND DISCUSSION

Feedlot Performance

There were no time x treatment interactions or interactions between the main effects of high levels of monensin sodium and plant extracts for any feedlot performance variable ($P > 0.05$; Table 2.2). Heifers fed high levels of monensin sodium had reduced DMI from d 0 to 8 and d 8 to 15 compared to heifers that were fed low levels of monensin sodium ($P = 0.05$). From d 15 to the end of the experiment, there were no additional period differences between treatments for DMI ($P > 0.05$). There was a plant extracts main effect on ADG from d 11 to 22 and d 44 to 66, in which heifers fed plant extracts had lower ADG compared to heifers that did not receive plant extracts ($P = 0.05$). Overall DMI and ADG was not affected by the main effects of monensin sodium fed at high levels or plant extracts ($P > 0.05$). Heifers that were supplemented with high levels of monensin sodium tended to have improved G:F compared to low level monensin sodium supplemented heifers ($P = 0.08$).

In agreement with the current study, Yang et al. (2010b) supplemented steers on a finishing ration with 330 $mg \cdot hd^{-1} \cdot d^{-1}$ monensin sodium and found that steers had a 17% decrease in DMI for the first wk of the experiment compared to the wk prior to starting the experiment. The authors reported that after wk 1, and for the duration of the experiment, monensin sodium fed steers did not have different feed intake compared to control cattle. While a reduction in DMI can benefit animals starting a new ration, by aiding in the reduction of feedlot disorders such as

lactic acidosis or bloat (Yang et al., 2010b), this effect is more valuable for animals on high concentrate diets. In the current study, heifers were fed a receiving ration and maximum intake was desired. Regardless, DMI results from the current study were not surprising, since several studies have observed that cattle fed monensin sodium had reduced DMI compared to control cattle (Moesely et al., 1977; Benchaar et al., 2006). Additionally, researchers fed steers 0, 25, 50 or 100 mg monensin sodium and found that feed intake tended to be decreased as monensin sodium dosage increased (Boling et al., 1977). In contrast to the previously mentioned studies, a feedlot efficiency study utilizing 0, 100, 500 or 750 $mg \cdot hd^{-1} \cdot d^{-1}$ monensin sodium found that there were no differences in feed intake between supplementing cattle with 0, 100 or 500 $mg \cdot hd^{-1} \cdot d^{-1}$ (Raun et al., 1976). However, the authors reported that cattle feed intake was reduced by the 750 $mg \cdot hd^{-1} \cdot d^{-1}$ dosage level. Raun et al. (1976) concluded that the optimal dosage of monensin sodium was between 0 and 500 mg.

Yang et al. (2010b) observed the effects of 400, 800 or 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ cinnamaldehyde on cattle intake. Contrary to the current study, the authors reported that steers supplemented with 800 or 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ cinnamaldehyde had reduced DMI for the first wk of the study compared to the week prior to starting the experiment. Additionally, the authors reported that DMI for steers supplemented with 400 $mg \cdot hd^{-1} \cdot d^{-1}$ cinnamaldehyde was not effected for the first wk of the experiment; however, after wk 1, DMI continuously increased until reaching a plateau at wk 4. Yang et al. (2010b) reported that after d 29, through the end of the experiment, there were no differences between cinnamaldehyde supplemented cattle and control cattle. These results are inconsistent with the current experiment, in which CCE had no effects on overall DMI or period DMI. Perhaps plant extracts have different effects on cattle fed finishing versus receiving rations. Additionally, Yang et al. (2010b) observed a tendency for DMI to be effected

by level of cinnamaldehyde over the entire experiment. The authors used a much lower dosage of plant extract compared to the current experiment and observed fluctuations in DMI. Dosage may influence the effects of plant extracts on cattle intake. This statement appears to have merit based on another study by Yang et al. (2010), which also found that a lower dose of cinnamaldehyde (400 mg·hd^{-1}·d^{-1}) tended to increase DMI, whereas higher doses, such as 800 mg·hd^{-1}·d^{-1} and 1,600 mg·hd^{-1}·d^{-1} cinnamaldehyde, tended to decrease DMI (Yang et al., 2010). While dosage could be another explanation for the different effects of plant extracts on DMI found by Yang et al. (2010b) and those of the current study, it is unclear how the 11,000 mg·hd^{-1}·d^{-1} level of CCE had no effect when the increase in dosage appeared to decrease intake for Yang et al. (2010; 2010b). It is also possible that Yang et al. (2010b) saw different effects on DMI because of the author's sole use of cinnamaldehyde compared to the combination of cinnamaldehyde, capsicum oleoresin and eugenol used in the current study. In support of this, Cardozo et al. (2006) observed the effects of capsicum oil by itself and a mixture of cinnamaldehyde and eugenol on DMI in Holstein heifers fed a high concentrate diet and reported that intake was changed depending on the extracts used. The authors found that cinnamaldehyde and eugenol maintained or reduced DMI, whereas capsicum oil increased DMI. The effects observed by Cardozo et al. (2006) could help explain the results found in the current study. Perhaps the 3 extracts, when used in combination, balanced each other and resulted in the impartial effects on DMI. It is probable that the monensin sodium provided in the basal ration to heifers in the current experiment also counteracted any effect the plant extracts may have had on heifer DMI. Both the high and low dosages of monensin sodium used in the current study are lower than 500 mg·hd^{-1}·d^{-1} utilized by Raun et al. (1976). Raun et al., (1976) found that supplementing cattle with monensin sodium between 0 and 500 mg·hd^{-1}·d^{-1} had no effect on DMI.

Consistent with the current experiment, Lalman et al. (1993) observed the effects of monensin sodium on crossbred beef heifers' ADG. Over a 120 d experiment, the authors found that heifers supplemented with 200 mg·hd^{-1}·d^{-1} monensin sodium did not have improved ADG compared to control heifers. Similarly, the current study did not find an effect of monensin sodium on ADG, regardless of dosage. In contrast, Boling et al. (1977) grazed steers for a 140 d trial and found that steers fed 50 or 100 mg·hd^{-1}·d^{-1} monensin sodium had greater ADG compared to those fed 0 or 25 mg·hd^{-1}·d^{-1} monensin sodium. The current study utilized dosages greater than Boling et al., (1977) indicating that monensin sodium may lose efficacy for improving ADG at higher dosages. However, Moseley et al. (1977) supplemented beef heifers with higher dosages than Boling et al. (1977; 200 mg·hd^{-1}·d^{-1}) and reported that heifers fed monensin sodium had higher ADG for the first 59 d of the experiment compared to control heifers. The CON treatment of the current study utilized 200 mg·hd^{-1}·d^{-1} monensin sodium, however there was no effect found. Previous research, along with the current study, indicate that monensin sodium may lose efficacy for improving ADG somewhere between 200 and 400 mg·hd^{-1}·d^{-1}.

The current study found that there was a main effect of plant extracts on ADG. Geraci et al. (2012) compared the effects of monensin sodium (46.7 mg/kg) and a mixture of plant extracts (266 mg·hd^{-1}·d^{-1} cinnamaldehyde and eugenol, and 133 mg·hd^{-1}·d^{-1} capsicum oleoresin) on ADG of feedlot steers. The authors reported that animals receiving plant extracts had 16% higher ADG compared to those receiving monensin sodium. The authors also noted that ADG was only different among treatments at the end of the study from d 45 to 84. These results are not consistent with the current study, which observed that heifers supplemented with plant extracts had reduced ADG from d 11 to 22 and d 44 to 66 compared to heifers not supplemented with

plant extracts, regardless of monensin sodium supplementation. The variation in these results could be due to the lower dosage of plant extracts used by Geraci et al. (2012), or the fact that monensin sodium was included in all treatments in the current study.

In a plant extract dosage response study, Yang et al. (2010b) found that cattle supplemented with 400 $mg \cdot hd^{-1} \cdot d^{-1}$ cinnamaldehyde tended to have higher ADG than control cattle or cattle supplemented with high doses of cinnamaldehyde (800 or 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$). However, unlike Geraci et al. (2012) who observed plant extract effects from d 45 to 84, Yang et al. (2010b) observed the plant extract effects from d 0 to 28. Both of these experiments fed cattle high concentrate diets; however, steers in Yang et al. (2010b) had an initial BW of 390 ± 25.2 kg, whereas steers in Geraci et al. (2012) had an initial BW of 141 ± 6.6 kg. This information suggests there may be a dosage effect on the administration of plant extracts based on kg of BW instead of kg per animal. Additionally, different treatment effects may be observed between previous research, and the current experiment, because of the combination of plant extracts cinnmaldehyde, capsicum oleoresin and eugenol (Geraci et al., 2012), versus the sole use of cinnamaldehyde (Yang et al., 2010b).

Monensin sodium has not been found to improve feed efficiency in cattle when fed at rates greater than 360 $mg \cdot hd^{-1} \cdot d^{-1}$ (Elanco Animal Health, Greenfield, IN); however the current study administered monensin sodium at a rate greater than 360 $mg \cdot hd^{-1} \cdot d^{-1}$ and found that heifers tended to have an improved feed efficiency. In the current experiment, heifers supplemented with high levels of monensin sodium (MON and COMB) tended to have better feed efficiency than heifers supplemented with low levels of monensin sodium (CCE and CON; $P = 0.08$). This result could be due to the decreased DMI that MON and COMB heifers demonstrated during specific time periods throughout the study. Consistent with the current experiment, Moseley et

al. (1977) also reported that monensin sodium supplemented heifers had a period of increased ADG and decreased DMI; however, those heifers were not more efficient than control heifers. Moseley et al. (1977) used 200 $mg \cdot hd^{-1} \cdot d^{-1}$ monensin sodium and a high roughage diet, whereas the current experiment used 200 $mg \cdot hd^{-1} \cdot d^{-1}$ as the low level of monensin and 400 $mg \cdot hd^{-1} \cdot d^{-1}$ as the high level monensin sodium.

Contrary to the current experiment, Yang et al. (2010b) did not observe improved feed efficiency in finishing steers supplemented with 330 $mg \cdot hd^{-1} \cdot d^{-1}$ monensin sodium compared to control steers. This brings into question if there may be a dosage effect of monensin sodium on feed efficiency. However, Raun et al. (1976) observed the effects of monensin sodium supplemented at 0, 100, 500 or 750 $mg \cdot hd^{-1} \cdot d^{-1}$ and reported that there were no differences in efficiency between cattle supplemented with any dosage of monensin sodium. Additionally, the authors made it clear that steers supplemented with monensin sodium were more efficient than their control cohorts. Similarly, Boling et al. (1977) supplemented finishing steers with 0, 25, 50 or 100 $mg \cdot hd^{-1} \cdot d^{-1}$ monensin sodium and found that while feed intake tended to decrease as level of monensin sodium increased, feed efficiency was improved in cattle by all levels of monensin sodium compared to controls.

Geraci et al. (2012) also studied the effects of monensin sodium and the plant extracts cinnamaldehyde, eugenol and capsicum on efficiency of beef cattle. However, unlike the current study, the authors did not find that monensin sodium improved feed efficiency compared to plant extracts. Because steers had similar DMI throughout the entire 84 d study and plant extract supplemented steers had increased ADG for the second half of the experiment (Geraci et al., 2012), it would not have been surprising for plant extract supplemented steers to be more efficient than monensin sodium supplemented steers. However, the authors reported that the

overall feed efficiency between plant extract and monensin sodium supplemented steers was not different. The current experiment may have observed an effect of monensin sodium on feed efficiency, where Geraci et al. (2012) did not, due to the use of a much lower dosage of monensin sodium (400 $mg \cdot hd^{-1} \cdot d^{-1}$ versus 46.7 mg/kg).

Consistent with the current experiment, Yang et al. (2010b) found that supplementing cattle with a plant extract had no effect on overall feed efficiency. However, the authors did report that from d 0 to 28, increasing dose of cinnamaldehyde (400, 800 or 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$) decreased feed efficiency compared to controls. This effect was then changed from d 29 to 56, in which the high dose of cinnamaldehyde had greater G:F than the low or medium doses. Benchaar et al. (2006) also observed that higher dosages (2000 versus 4000 $mg \cdot hd^{-1} \cdot d^{-1}$) of eugenol, thymol, vanillin and limonene depressed feed efficiency of beef cattle fed a total mixed ration. The authors reported that overall feed efficiency was not different between cattle that were supplemented with eugenol, thymol, vanillin and limonene versus control cattle, which is consistent with the current experiment.

Animal Behavior

There was no interaction between high levels of monensin sodium and the plant extract treatments, nor were there main effects of high level monensin sodium or plant extracts on exit velocity by d or overall exit velocity ($P > 0.05$; Table 2.4). There was a time x treatment interation for d 44 ($P = 0.01$); however, the reason for this interaction is unclear.

Chute exit velocity was measured to evaluate heifer's escape inclination to assess whether or not treatment effected animal behavior. Bigler (2014), similar to the current study, observed the effects of monensin sodium and plant extract cinnamaldehyde, capsicum oleoresin and eugenol on chute exit velocity in Angus heifers. The author reported that while initial and

mid-point exit velocities were not different among treatments, monensin sodium supplemented heifers had a reduced final exit velocity when compared to plant extract, plant extract and monensin sodium, and control supplemented heifers. (Bigler, 2014). To date there is very little literature about the effects of monensin sodium and plant extracts on animal behavior, therefore, more research should be conducted to better understand these effects.

Reproductive Performance

There were no interactions between the main effects of high levels of monensin sodium (MON) or plant extracts (CCE) for any reproductive variables ($P > 0.05$; Table 2.3). There were no treatment effects for age at puberty, BCS, pregnancy to AI, season long pregnancy rate or day of calving season ($P > 0.05$).

Consistent with the current experiment, Bigler (2014) reported that there were no differences in heifer age at puberty when supplemented with 200 mg·hd^{-1}·d^{-1} monensin sodium, 1,100 mg·hd^{-1}·d^{-1} plant extracts (cinnmaldehyde, capsicum oleoresin and eugenol), a combination of monensin sodium (200 mg·hd^{-1}·d^{-1}) and plant extracts (1,100 mg·hd^{-1}·d^{-1}) or a control. However, in yr 2 of an experiment by Bigler (2014), using the same treatments with a different dose, the author reported that heifers supplemented with monensin sodium (200 mg·hd^{-1}·d^{-1}), plant extracts (1,400 mg·hd^{-1}·d^{-1}) or a combination of monensin sodium (200 mg·hd^{-1}·d^{-1}) and plant extracts (1,100 mg·hd^{-1}·d^{-1}), had decreased age at puberty when compared to control heifers. The difference in results between years could have been due to a dosage effect of plant extracts (Yang et al., 2010).

Similar to the second experiment by Bigler (2014), several other experiments have reported a decrease in age at puberty when heifers were supplemented with monensin sodium (Moseley et al.; 1977; McCartor et al., 1979; Moseley et al.1982; Lalman et al., 1993; and Purvis

and Whittier, 1996). One possible explanation for not observing a difference in age at puberty in the current study may be that as a result of genetic selection for younger age at puberty in beef heifers by selection pressure on bull scrotal circumference, puberty in heifers is now occurring at an age younger than the threshold that was previously overcome nutritionally by feeding an ionophore. This argument appears to have merit based on reported age of puberty of 377 d in 1982 (Moseley et al., 1982) and 369 d as reported by Lalman et al (1993) in heifers fed monensin sodium, while the age at puberty in heifers in the current study was an average of 315 d, an approximate 60 d decrease. A second explanation for no observed difference in age of puberty in the current study may be that the method used to detect age at puberty (activated estrus detection patches monitored at 11 d intervals) was not efficient to identify differences between heifers fed monensin, plant extracts or a combination of the 2, compared to control heifers.

Contrary to the current experiment, Purvis and Whittier (1996) reported that heifers supplemented with an ionophore tended to have higher first service conception rates compared to control heifers. The authors noted that this improvement was likely due to an increased number of ionophore supplemented heifers being pubertal prior to the breeding season. Similarly, Moseley et al., (1977) observed that 92% of monensin sodium supplemented heifers reached puberty compared to only 58% of control heifers; however the authors also noted that there was not a difference in pregnancy to AI, despite the higher percentage of pubertal heifers.

There was no significant difference in the number of d into the calving season in which heifers calved ($P > 0.05$) in the current experiment. Similarly, Bigler (2014) found that the supplementation of plant extracts or monensin sodium did not effect the number of females to calve within the first 21 d of the calving season. Regardless, it would be beneficial for heifers to

calve earlier in the calving season so that they can have a longer post partum interval and have more time to regain condition for rebreeding.

Unfortunately, there is limited published literature on the effects of plants extracts on beef female reproductive performance; therefore, more research should be conducted to better understand these effects.

IMPLICATIONS

Results observed in this study indicate that there are no differences in the level of monensin sodium supplemented, with or without the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol, for beef heifer feedlot or reproductive performance. Research has indicated that plant extracts utilized on their own may improve cattle performance; however, results vary dependent on plant extract or combination of plant extracts, dosage and class of cattle used. Further research should be conducted on plant extract's mode of actions and pathways within the ruminant animal, to better understand their effects on feedlot performance, age at puberty and pregnancy in heifers. Gathering this information could be valuable to animal scientists, as well as producers, looking to decrease input costs for heifer development. Previous research has clearly demonstrated improvements in ADG, feed to gain ratio and age at puberty in heifers fed monensin sodium, yet this effect was not observed in heifers fed monensin sodium in this study. Therefore, caution should be used in making conclusive changes in nutritional management or adoption of plant extracts in heifer development programs based solely on this study.

Table 2.1. Ingredient composition and proximate analysis of the basal diet, and monensin sodium and plant extract supplements, fed to developing beef heifers (DM basis)

Item	(%)
Ingredient[1]	
Wheat straw, ground	18.26
Alfalfa hay, ground	16.92
Corn silage	33.48
Whole corn	17.83
Dried distillers grain	10.00
Liquid supplement[2]	3.50
Proximate analysis[3]	
DM, %	67.97
CP, %	32.03
$NE_{G,}$ Mcal/kg	0.97
$NE_{M,}$ Mcal/kg	1.68
TDN, %	67.98
ADF, %	29.21
Supplement[4]	
CP, %	26.00
Crude fat, %	9.50
Crude fiber, %	12.50
Ca, %	2.75
P, %	0.75
Salt, %	6.00

[1] Basal diet composition

[2] 30% CP liquid supplement containing 200 mg/0.45kg monensin, 0.60% P, 4.00 ppm Co, 220.0 ppm Cu, 6.00 ppm I, 699.0 ppm Mn, 998.0 Zn, 3.30 ppm Se, 110,231 IU/kg vitamin A, 27,557 IU/kg vitamin D and 110 IU/lb vitamin E.

[3] DM basis

[4] Supplement composition: monensin sodium at 200 $mg \cdot hd^{-1} \cdot d^{-1}$, plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (RumeNext®, ADM Alliance Nutrition Inc., Quincy, IL) at 11,000 $mg \cdot hd^{-1} \cdot d^{-1}$, combination of monensin sodium and plant extracts delivered at 200 $mg \cdot hd^{-1} \cdot d^{-1}$ and 11,000 $mg \cdot hd^{-1} \cdot d^{-1}$, respectively, or control, which included neither monensin sodium nor plant extracts. Treatments fed at a rate of 0.32 $kg \cdot hd^{-1} \cdot d^{-1}$. Supplement minerals included: K, Mg, Mn, Zn, Co, Cu, I, Se, vitamin A and vitamin E, in very discrete amounts.

Table 2.2. Least squares means for DMI and ADG in developing heifers fed a high roughage diet supplemented with high or low levels monensin sodium, with or without the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol, from d 0 to 84

	Treatment[1]					*P*=		
Item	MON	CCE	CON	COMB	SEM	INT[2]	MON[3]	CCE[4]
n	27	27	26	27				
Initial BW, kg	280.79	278.14	280.05	285.97	6.415	0.59	0.52	0.80
Final BW, kg	363.66	350.84	361.29	361.45	8.206	0.63	0.45	0.46
DMI[5], kg								
d 0 to 8	4.98	5.78	5.56	4.88	0.335	0.64	0.05	0.86
d 8 to 15	4.87	5.76	6.03	4.67	0.529	0.95	0.05	0.67
d 15 to 22	5.55	6.17	7.84	5.61	0.878	0.34	0.13	0.38
d 22 to 32	7.90	8.17	8.62	8.49	2.021	0.80	0.92	0.97
d 32 to 38	7.83	8.23	8.59	8.73	1.986	0.76	0.95	0.89
d 38 to 43	6.69	6.53	7.20	6.71	0.414	0.42	0.71	0.45
d 43 to 50	7.49	7.52	8.02	7.71	0.540	0.52	0.76	0.80
d 50 to 57	8.44	8.32	8.99	8.71	0.586	0.43	0.90	0.74
d 57 to 64	9.12	9.55	9.71	9.71	0.646	0.57	0.74	0.74
d 64 to 71	9.38	10.11	10.18	9.79	0.732	0.75	0.46	0.82
d 71 to d 84	9.83	10.11	10.17	10.35	0.766	0.71	0.95	0.77
Overall DMI, kg	7.46	7.84	8.26	7.76	0.640	0.58	0.50	0.92
ADG[6], kg								
d 0 to 11	1.75	1.97	2.02	1.84	0.203	0.75	0.35	0.92
d 11 to 22	0.44	-0.04	0.48	0.24	0.165	0.36	0.47	0.05
d 22 to 33	0.98	1.15	0.93	1.06	0.252	0.79	0.93	0.58
d 33 to 44	0.69	0.62	0.54	0.91	0.366	0.85	0.56	0.69
d 44 to 66[7]	1.21	0.78	1.04	0.87	0.141	0.78	0.39	0.05
d 66 to 77	1.12	0.90	1.06	0.94	0.240	0.96	0.85	0.50
d 77 to 84	0.19	0.69	0.19	0.41	0.401	0.73	0.72	0.39
Overall ADG, kg	1.04	1.02	1.04	1.08	0.098	0.77	0.76	0.88
G:F[8]	0.10	0.09	0.08	0.10	0.002	0.68	0.08	0.77

[1] All heifers received a basal ration with a 30% CP liquid supplement containing 200 mg/0.45 kg monensin sodium. Treatments were applied daily as topdressed supplements and fed at a rate of 0.32 kg·hd^{-1}·d^{-1}. Treatments: 1) high level of monensin sodium (MON), where monensin sodium was topdressed at 200 mg·hd^{-1}·d^{-1}, 2) low level of monensin sodium plus the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (CCE), where plant extracts were topdressed at 11,000 mg·hd^{-1}·d^{-1}, 3) control (CON), low level of monensin sodium without topdressed supplements, or 4) high levels of monensin sodium with plant extracts (COMB), where monensin sodium was topdressed at 200 mg·hd^{-1}·d^{-1} and plant extracts were topdressed at 11,000 mg·hd^{-1}·d^{-1}.
[2] Interaction between the main effects of MON and the main effects of CCE
[3] Main effects of MON
[4] Main effects of CCE
[5] Treatment x time interaction for DMI (P = 0.99)
[6] Treatment x time interaction for ADG (P = 0.98)
[7] ADG was calculated from d 44 to d 66, omitting d 55 data due to malfunctions with the scale.
[8] Calculated from heifers overall ADG (d 0 to 84) and average DMI.

Table 2.3. Least squares means for age at puberty and pregnancy rate in developing heifers fed a high roughage diet supplemented with high or low levels of monensin sodium, with or without the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol, from d 0 to 84

	Treatment[1]					*P* =		
Item	MON	CCE	CON	COMB	SEM	INT[2]	MON[3]	CCE[4]
n	27	27	26	27				
Age at puberty[5], d	322.9	313.9	314.2	310.2	5.49	0.29	0.67	0.26
BCS[6] at breeding	6.04	5.94	5.92	5.91	0.122	0.56	0.70	0.67
Pregnant to AI[7], %	63.85	71.61	61.13	75.87	0.083	0.93	0.68	0.18
Season long pregnancy rate[8], %	95.83	95.65	90.91	100	0.043	0.95	0.28	0.30
Day of calving season[9], d	19.3	16.6	19.0	18.6	47.93	0.99	0.99	0.99

[1] All heifers received a basal ration with a 30% CP liquid supplement containing 200 mg/0.45 kg monensin sodium. Treatments were applied daily as topdressed supplements and fed at a rate of 0.32 kg·hd^{-1}·d^{-1}. Treatments: 1) high level of monensin sodium (MON), where monensin sodium was topdressed at 200 mg·hd^{-1}·d^{-1}, 2) low level of monensin sodium plus the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (CCE), where plant extracts were topdressed at 11,000 mg·hd^{-1}·d^{-1}, 3) control (CON), low level of monensin sodium without topdressed supplements, or 4) high levels of monensin sodium with plant extracts (COMB), where monensin sodium was topdressed at 200 mg·hd^{-1}·d^{-1} and plant extracts were topdressed at 11,000 mg·hd^{-1}·d^{-1}

[2] Interaction between the main effects of MON and the main effects of CCE

[3] Main effects of MON

[4] Main effects of CCE

[5] Age at puberty determined by estrus detection patches (Estrotect™ Heat Detector, Rockway Inc., Spring Valley, WI) applied on day 0 and recorded every 11 d as activated, not activated or missing.

[6] Analyzed using the 9-point scale, where 1=thin and 9=obese (Richards et al., 1986).

[7] Heifers were estrus synchronized and inseminated using a 14 d CIDR-PG-AI protocol, in which a CIDR was inserted 33 d prior to artificial insemination and removed 14 d later. Prostaglandin was injected 16 d after CIDR removal and heifers were inseminated 3 d later (d 168). Pregnancy to AI was determined via ultrasound at 56 d post AI.

[8] Season long pregnancy rate was determined via rectal palpation 178 d post AI.

[9] d 0 = date first calf was born among heifers.

Table 2.4. Least squares means for chute exit velocity of developing heifers fed a high roughage diet supplemented with high or low levels of monensin sodium, with or without the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol, from d 0 to 84

	Treatment[1]					*P* =		
Item	MON	CCE	CON	COMB	SEM	INT[2]	MON[3]	CCE[4]
n	27	27	26	27				
Exit velocity[5,6], m/s								
d 11	0.89	0.99	0.94	0.86	0.104	0.70	0.39	0.89
d 22	0.65	0.65	0.60	0.55	0.071	0.33	0.79	0.75
d 33	0.73	0.75	0.84	0.68	0.117	0.88	0.47	0.58
d 44	1.20	1.62	0.83	1.23	0.393	0.36	0.97	0.32
d 55	0.79	0.82	0.97	0.75	0.113	0.64	0.28	0.42
d 66	0.89	0.92	0.95	0.94	0.120	0.74	0.89	0.90
d 77	0.84	0.84	1.04	0.83	0.102	0.36	0.31	0.29
d 84	0.67	0.84	0.73	0.72	0.136	0.83	0.53	0.54
Avg. velocity, m/s	0.83	0.92	0.86	0.82	0.083	0.66	0.43	0.76

[1] All heifers received a basal ration with a 30% CP liquid supplement containing 200 mg/0.45 kg monensin sodium. Treatments were applied daily as topdressed supplements and fed at a rate of 0.32 kg·hd^{-1}·d^{-1}. Treatments: 1) high level of monensin sodium (MON), where monensin sodium was topdressed at 200 mg·hd^{-1}·d^{-1}, 2) low level of monensin sodium plus the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (CCE), where plant extracts were topdressed at 11,000 mg·hd^{-1}·d^{-1}, 3) control (CON), low level of monensin sodium without topdressed supplements, or 4) high levels of monensin sodium with plant extracts (COMB), where monensin sodium was topdressed at 200 mg·hd^{-1}·d^{-1} and plant extracts were topdressed at 11,000 mg·hd^{-1}·d^{-1}.

[2] Interaction between the main effects of MON and the main effects of CCE

[3] Main effects of MON

[4] Main effects of CCE

[5] Measured over a distance from 1.83 m in front of chute to a point 3.66 m from front of chute, using an infrared sensor timing system (FarmTek Inc., North Wylie, TX).

[6] Treatment x time interaction for exit velocity ($P = 0.60$)

LITERATURE CITED

Benchaar, C., J.L. Duynisveld, and E. Charmley. 2006. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. Can. J. Anim. Sci. 86:91-96.

Benchaar, C., S. Calsamiglia, A.V. Chaves, G.R. Fraser, D. Colombatto, T.A. McAllister, and K.A. Beauchemin. 2008. A review of plant-derived essential oils in ruminant nutrition and production. Animal Feed Science and Technology. 145:209-228.

Bergen, W.G., and D.B. Bates. 1984. Ionophores: Their effect on production efficiency and mode of action. J.Anim. Sci. 58:1465-1483.

Bigler, B.J.. 2014. Effects of monensin sodium and plant extracts containing cinnamaldehyde, capsicum oleoresin and eugenol on days to puberty, gain, pregnancy rate and feed efficiency in developing beef heifers fed a high roughage diet. Master Thesis. Colorado State University, Fort Collins.

Boling, J.A., N.W. Bradley, and L.D. Campbell. 1977. Monensin levels for growing and finishing steers. J. Anim. Sci. 44 (5):867-871.

Cardozo, P.W., S. Calsamiglia, A. Ferret, and C. Kamel. 2006. Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. J. Anim. Sci. 84:2801-2808.

Clark, R.T., K.W. Creighton, H.H Patterson, and T.N. Barrett. 2005. Symposium Paper: Economic and tax implications for managing beef replacement heifers. Prof. Anim. Sci. 21:164-173.

Edrington, T.S., T.R. Callaway, P.D. Varey, Y.S. Jung, K.M. Bischoff, R.O. Elder, R.C. Anderson, E. Kutter, A.D. Brabbin, and D.J. Nisbet. 2003. Effects of the antibiotic ionophores monensin, lasalocid, laidlomycin propionate and bambermycin on salmonella and E. coli 0157:H7 in vitro. Journal of Applied Microbiology. 2003. 94:207-217.

Fischer, M.F., J.K. Ahola, B.J. Bigler, J.C. Whittier and R.K. Peel. 2014. Effects of monensin sodium and plant extracts containing cinnmaldehyde, eugenol, and capsicum oleoresin on days to puberty, average daily gain and feed efficiency in developing beef heifers receiving a high roughage diet. Proc. Western Sect. Anim. Sci. Mtgs. 65: 166-170.

Funston, R.N. and G.H. Deutscher. 2004. Comparsion of target breeding weight and breeding date for replacement beef heifers and effects on subsequent reproduction and calf performance. J. Anim. Sci. 82:3094-3099.

Geraci, J.I., A.D. Garciarena, G.A. Gagliostro, K.A. Beauchemin, and D. Colombatto. 2012. Plant extracts containing cinnamaldehyde. Eugenol and capsicum oleoresin added to feedlot cattle diets: Ruminal environment, short term intake pattern and animal performance. Animal Feed Science and Technology. 176:123-130.

Lalman, D.L., M.K. Petersen, R.P. Ansotegui, M.W. Tess, C.K. Clark, C.K. and J.S. Wiley. 1993. The effects of ruminally undegradeable protein, propionic acid, and monensin on puberty and pregnancy in beef heifers. J. Anim. Sci. 71:2843-2852.

McCartor, M.M., R. D. Randel, and L. H. Carroll. 1979. Dietary alteration of ruminal fermentation on efficiency of growth and onset of puberty in Brangus heifers. J. Anim. Sci. 48:488.

Moseley, W.M., T.G. Dunn, C.C. Kaltenbach, R.E. Short, and R.B. Staigmiller. 1982. Relationship of growth and puberty in beef heifers fed monensin. J. Anim. Sci. 55:357.

Moseley, W.M., M.M. McCartor, and R.D. Randel. 1977. Effects of monensin on growth and reproductive performance of beef heifers. J. Anim. Sci. 45:961.

Purvis, H.T. and J.C. Whittier. 1996. Effects of ionophore feeding and anthelmentic administration on age and weight at puberty in spring born beef heifers. J. Anim. Sci. 74:736-744.

Raun, A.P., C.O. Cooley, E.L. Potter, R.P. Rathmacher, and L.F. Richardson. 1976. Effect of monensin on feed efficiency of feedlot cattle. J. Anim. Sci. 43:670-677.

Raun, A.P.. 1990. Rumensin "then and now." In Rumensin. Elanco Animal Health, Denver, Colorado. p. A1-A20.

Richards, M.W., J.C. Spitzer, and M.B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300-306.

Yang, W.Z., B.N. Ametaj, C. Benchaar, and K.A. Beauchemin. 2010. Dose response to cinnamaldehyde supplementation in growing beef heifers: Ruminal and intestinal digestion. J. Anim. Sci. 88:680-688.

Yang, W.Z., B.N. Ametaj, C. Benchaar, M.L. He, and K.A. Beauchemin. 2010b. Cinnamaldehyde in feedlot cattle diets: Intake, growth performance, carcass characteristics and blood metabolites. J. Anim. Sci. 88:1082-1092.

CHAPTER III

EFFECTS OF INJECTABLE TRACE MINERALS ON WEANED CALF PERFORMANCE AND MORBIDITY, AND YEARLING BULL AND HEIFER FERTILITY

SUMMARY

The objectives of this experiment were to determine the effects of an injectable trace mineral on newly weaned calf performance and morbidity and yearling bull and heifer fertility. Over 2 yr, Angus bulls (n = 31, yr 1; n = 35, yr 2), heifers (n = 107, yr 1) and steers (n = 105, yr 1) were utilized. Cattle were randomly assigned a treatment at weaning (278.6 ± 35.0 kg; 241.0 ± 19.6 d): 1) control (**CON**), no injection, or 2) injectable trace minerals (**MIN**), which included Cu, Zn, Se and Mn. The MIN treatment was administered at weaning (d 0) and again on d 64 (yr 1) and d 110 (yr 2) to bulls, and at weaning (d -19) and on d 135 to heifers. Treatment administration corresponded to 80 (yr 1) or 38 d (yr 2) prior to the breeding soundness exams in bulls and 33 d prior to heifer AI. Bulls in both yr exhibited no differences in overall ADG based on treatment ($P > 0.05$); however, MIN bulls had reduced ADG from d 64 to 113 ($P = 0.05$) vs. CON bulls. Steer and heifer ADG was not different across treatments ($P > 0.05$). Incidence of morbidity was not different among treatments for any class of cattle ($P > 0.05$). A licensed veterinarian performed breeding soundness exams, and Computer-Assisted Sperm Analysis was used to analyze sperm motility, velocity, straightness and linearity objectively. Motility and morphology from breeding soundness exams were not affected by MIN ($P > 0.05$); however, in

yr 2 there was a tendency ($P = 0.08$) for sperm from MIN bulls to have a higher percentage of secondary defects. Sperm beat cross frequency was greater in CON bulls in yr 2 ($P < 0.01$), and tended to be greater in CON bulls in yr 1 ($P = 0.10$) than MIN bulls. Progressive velocity of sperm was greater in CON bulls in yr 2 ($P < 0.05$) than MIN bulls. Neither BCS nor age at puberty in heifers were affected by treatment ($P > 0.05$). Results suggest there was no benefit to newly weaned calf performance, morbidity, or yearling bull or heifer fertility, when supplementing well nourished and managed cattle with injectable trace minerals at weaning.
Keywords: ADG, age at puberty, beef cattle, fertility, morbidity, trace minerals

INTRODUCTION

Trace minerals are essential to animal health, growth and reproductive performance (Underwood and Suttle, 1999). Cattle can be administered supplemental trace minerals through dietary, oral bolus, or injectable methods. Unfortunately, providing minerals in the diet can be challenging because dietary minerals are subject to antagonists present in the diet and can therefore result in decreased mineral absorption (Spears, 1996). Additionally, stressed cattle often have reduced feed intake, causing reduced mineral intake (Galyean et al., 1999). Consequently, animal performance may be impaired (Spears and Kegley, 2002).

Administration of injectable trace minerals may avoid the detrimental interaction between minerals and antagonists in the gastrointestinal tract, since injected minerals circulate through the bloodstream (Berry et al., 2000). This process may benefit animal health and productivity (Richeson and Kegley, 2011). The authors reported that administering injectable trace minerals (Cu, Zn, Mn and Se) to stressed calves improved morbidity and decreased antibiotic costs

compared to control cattle. Beef heifers injected with trace minerals upon entering the feedlot had improved overall ADG and feed efficiency in comparison to control heifers (Richeson and Kegley, 2011). Relative to reproduction, supplementing mature bulls with trace minerals (Cu, Zn, Mn and Co) can improve sperm motility (Rowe et al., 2011); and injecting heifers with Cu, Zn, Mn and Se can improve pregnancy rates (Brasche, 2014).

The objectives of the current study were to evaluate the effects of the injectable trace minerals Cu, Zn, Se and Mn on newly weaned calf performance and morbidity, and yearling bull and heifer fertility.

MATERIALS AND METHODS

Experimental procedures conducted with animals were approved by the Institutional Animal Care and Use Committee at Colorado State University prior to initiation of the study.

Experimental Design

Angus bulls (n = 31, yr 1; n = 35, yr 2), heifers (n = 107) and steers (n = 105) from the same source and of genetic similarity were utilized over a 2-yr period. In mid-October, bulls, heifers and steers were weaned and randomly assigned to a treatment: 1) control (**CON**), no injection, or 2) trace mineral injection of 60 mg/mL Zn, 15 mg/mL Cu, 10 mg/mL Mn and 5 mg/mL Se (**MIN;** MultiMin®, MultiMin USA, Fort Collins, CO). The MIN treatment was administered at weaning (d 0) and again on d 64 (yr 1) and d 110 (yr 2) to bulls, and at weaning (d -19) and on d 135 to heifers. The second injections corresponded to 80 (yr 1) or 38 d (yr 2) prior to bull breeding soundness exams (**BSE**) and 33 d prior to heifer AI. Steers received only the initial injection at weaning. Injections were administered at 1 mL/45.4 kg of BW at weaning

and 1 mL/68.0 kg of BW prior to breeding soundness exams and AI, as per the product label. Immediately after weaning, all cattle were shipped 57 km to Colorado State University Agriculture Research, Development and Education Center (**ARDEC**) in Fort Collins.

Feedlot Performance

In both years, bulls were housed together in a single 804 m^2 pen. In yr 1 bulls (312.0 ± 19.45 kg) were fed an approximate 70% roughage, 30 % concentrate diet (Table 3.1). Bulls in yr 1 were transitioned from a receiving ration to a developing bull ration 80 d after weaning. In yr 2, bulls (306.8 ± 14.41 kg) received an approximate 50% roughage, 50% concentrate diet (Table 3.1). Bulls in yr 2 were transitioned to a developing bull ration 46 d after weaning. Bulls in both yr were weighed on d 0, 64, 113 and 144 (yr 1 only) or 148 (yr 2 only).

In yr 1, steers (275.2 ± 36.32 kg) were housed in the same feedlot pen as bulls until d 28, at which point steers were shipped 195 km to Colorado State University's Eastern Colorado Research Center (**ECRC**) located in Akron, CO. Steers were then housed in 3 feedlot pens with 35 or 36 steers per pen. Steers were castrated prior to shipment to ECRC. All steers were transitioned to a finishing ration by d 62. At the beginning of the finishing phase, initial (d 62), mid (d 123) and final (d 195 or 216) BW were recorded. Steers were harvested based on a visual, subjective evaluation by trained professionals after 146 or 170 d on the finishing ration. Total BW gain and d on feed were also calculated for each steer.

From d 0 to 84, heifers (259.3 ± 20.98 kg) were housed in 488 m^2 pens with 6 or 7 heifers per pen. Shortly after d 84, heifers were transported back to the ranch of origin and maintained on pasture until AI. In the feedlot, all heifers received a high roughage diet (Table 3.1). Feed refusals were estimated and recorded and feed offered was adjusted daily based on evaluation by a trained bunk reader to minimize feed refusals. Feed samples and orts were collected weekly

and intake was calculated on a DM basis. The DM and nutrient analysis were performed by a private laboratory (SDK Laboratories, Hutchinson, KS). Additionally, heifers were weighed every 11 d.

Morbidity

Morbidity was evaluated daily by trained health professionals. If clinical signs existed, including droopy or dull appearance, abnormal nasal discharge, coughing, hard breathing, presence of abscesses, lameness or a rectal temperature exceeding 40ºC, cattle were treated according to a preplanned antibiotic regimen created by the Livestock Medicine Department of the Colorado State University Veterinary Teaching Hospital. All illnesses or lameness were recorded and monitored.

Fertility

Breeding soundness exams were performed in early March when bulls were 390.3 ± 10.63 (yr 1) or 394.8 ± 14.72 d (yr 2) of age. Breeding soundness exams occurred at the ARDEC working facility and were performed by a licensed veterinarian to analyze bull soundness, scrotal circumference (**SC**), percent motile sperm (**MOT**), percent normal sperm (**NORM)**, percent of sperm with primary defects (**PRI**), and percent of sperm with secondary defects (**SEC**). Semen samples were collected from bulls into small plastic bags via electroejaculation. Samples were placed in a 37ºC water bath until visually analyzed under a microscope by the veterinarian. Semen samples were replaced in the warm water bath and remained there until they were transported to the laboratory for further analysis. A Styrofoam box that contained 2 freezer gel packs warmed to 37ºC was used to transport samples from the working facility to the laboratory. Samples were transported to the laboratory approximately every h. In the laboratory, ends were cut off the plastic sample bags and drained into pre-weighed 15 mL conical tubes at room

temperature. Sample volume (**VOL**) was determined by weighing the filled tubes and subtracting the tube weight, and by using 1 mL of semen = 1 g of liquid weight. Concentration (**CONC**) of samples was determined using a spectrophotometer. If sample concentration was higher than 30 to 50 million sperm/mL, Tyrode's albumen lactate pyruvate was added to the sample to dilute it. Samples had to have concentrations 25 to 30 million cells/mL; otherwise the concentration was too high for accurate evaluation of sperm motility with Computer Assisted Sperm Analysis (**CASA**). The CASA was performed to objectively analyze percent motile sperm (**CMOT**), percent progressive sperm (**PROG**), sperm path velocity (**VAP**), sperm progressive velocity (**VSL**), sperm track speed (**VCL**), sperm lateral amplitude (**ALH**), sperm beat cross frequency (**BCF**), sperm straightness (**STR**) and sperm linearity (**LIN**).

Estrus detection patches (Estrotect™ Heat Detector, Rockway Inc., Spring Valley, WI) were applied to all heifers on d 0. Patch status was recorded every 11 d as activated, not activated or missing. If a patch was missing at the time of BW was collected every 11 d, it was replaced with a new patch. Age at puberty was determined to be the date that a heifer's patch was first observed as activated. A 14 d CIDR-PG-AI protocol was utilized to inseminate heifers on d 168, when heifers were 427.3 ± 20.98 d of age. In the 14 d CIDR-PG-AI, a controlled internal drug release device (**CIDR**) was inserted 33 d prior to AI and removed 14 d later. Prostaglandin F-2_{α} (**PG**) was injected 16 d after CIDR removal, and heifers were mass inseminated 3 d later. Pregnancy was determined via ultrasound at 56 d post AI and again at 178 d post AI via rectal palpation. Heifers were placed with bulls for natural service 21 d post AI. Calving records were used to validate ultrasound results.

Data Analysis

Breeding soundness exam and CASA traits, heifer fertility, feedlot performance and morbidity were analyzed using a mixed model and the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Each model included treatment as a fixed effect. Animal was the experimental unit. Bull fertility data were analyzed separately by yr since the second MIN injection was given at a different d prior to BSE in each yr. There was a yr x treatment interaction for bull BW and ADG, thus performance data were analyzed separately. Morbidity data for bulls were pooled and analyzed across yr since there was no time x treatment interaction. Predictor variables included final BW, ADG, SC, DMI, G:F, BCS, sire, age, or AI technician depending on the response variable being analyzed. Initial BW was used as a covariate for bull ADG and BW gain in yr 1 to account for the unexplained difference in initial BW ($P < 0.05$) between treatments. A repeated measures statement was used to account for any time x treatment interactions for BW data. For all data, if predictor variables were considered to have an effect ($P \leq 0.10$), they remained in the model statement. Spearman and Pearson correlations were calculated in SAS for sperm motility from the BSE and CASA analyses.

RESULTS AND DISCUSSION

Bull, Heifer and Steer Feedlot Performance

Because there was a yr x treatment interaction ($P > 0.05$) for bull BW and ADG, data from yr 1 and yr 2 were analyzed separately. Initial BW between CON and MIN bulls were different in yr 1 ($P < 0.01$), but not yr 2 ($P > 0.05$; Table 3.2). Ideally, bulls would have been blocked by BW and then randomly assigned CON or MIN treatment to account for variation in

initial BW; unfortunately, this was not possible due to facilities used at weaning. To account for treatment effects over time, a repeated measures statement was used (Table 3.2). With repeated measures, there were time effects on d 144 BW and overall ADG in both yr (P <0.01); however, in both yr there were no treatment effects or time x treatment interactions (P > 0.05).

Steers in the current study exhibited no differences between treatments for initial BW (d 0), d 123 BW, final BW (d 195 or 216), overall ADG (from d 62 to 195 or 216) or total BW gain (P > 0.05; Table 3.3). However, MIN steers had lower d 62 BW at the start of the finishing phase when compared to CON cohorts (P = 0.04). Also, there was a trend (P = 0.07) for MIN steers to have fewer d on feed than CON steers. Because there was no difference between treatments for ADG or final BW, it is unclear why MIN steers had lighter initial BW yet similar final BW and reduced d on feed in the current study. There were no treatment effects for initial BW, final BW, overall ADG, average DMI or G:F for beef heifers (P > 0.05; Table 3.4).

In agreement with the current study, Brangus-crossbred calves that received three 1 mL subcutaneous trace mineral injection formulated at 15 mg/mL Cu, 60 mg/mL Zn, 5 mg/mL Se and 10 mg/mL Mn did not have improved overall ADG compared to control calves (Arthington et al., 2014b). The authors administered injections within 24 h of birth and then again approximately 150 and 50 d prior to weaning (d 250), when calves were 100 and 200 d of age. Average daily gain was calculated from BW recorded at birth and on d 100, 150, 200 and 250. Likewise, administering injectable trace minerals Cu, Zn, Se and Mn at 1 mL/45.36 kg of BW, with the same formulation as Arthington et al. (2014b), only 28 d prior to weaning had no effect on BW or overall ADG in crossbred steer and heifer calves (Kegley et al., 2011). Crossbred calves were fence-line weaned in a bermudagrass and fescue pasture adjacent their mothers until 42 d post weaning at which point they were moved to wheat pasture for a grazing phase (Kegley

et al., 2011). The authors calculated ADG from initiation of the study (d 0) to weaning, from weaning to 28 and 42 d post weaning, for the 154-d period on wheat pasture, and for overall ADG from d 0 to 224. Both Kegley et al. (2011) and Arthington et al. (2014b) reported that calves were also provided ad libitum access to loose minerals, which may have provided adequate mineral intake for both injected and control calves. Cattle in the current study were not provided ad libitum access to trace minerals; however, there were trace minerals present in their diet, which may have allowed adequate intake (Table 3.1).

Inconsistent with the current study, crossbred bull calves that received a 3 mL injection of a solution containing 10 mg/mL Cu, 20 mg/mL Zn, 5mg/mL Se, and 20 mg/mL Mn upon arrival to the feedlot showed a tendency for improvement in overall ADG from d 0-42 (Berry et al., 2000). The authors found no other differences between mineral injected and control bulls for period ADG or BW recorded on d 0, 14, 28 and 42. Although the current study also administered trace minerals the same day cattle were transported to the feedlot, results from Berry et al. (2000) through a 42-d period are inconsistent with the current study's first 64-d period. Similar to Berry et al. (2000), a second experiment by Arthington et al. (2014b) also observed a difference between control and mineral injected heifers for ADG in the first 14 d after arrival to the feedlot. However, while Berry et al. (2000) found that mineral supplementation improved ADG, Arthington et al. (2014b) found that mineral injection reduced ADG compared to control cattle. This second experiment by Arthington et al. (2014b) used a subset of heifer calves mentioned in the previous paragraph. The authors reported that at weaning, heifers were held in a drylot pen overnight and then transported 1,600 km the following d. In addition to the 3 previous injections administered prior to weaning, heifers receiving minerals were given another 5 mL injection of Cu, Zn, Se and Mn upon arrival to the feedlot (Arthington et al., 2014b). Although the current

study administered injections and then transported calves 57 km, we also observed a negative effect of mineral injection on period ADG, much like Arthington et al., (2014b). However, in the current study, there were no differences between treatments for ADG immediately following transportation. In fact, the only difference in ADG in the current study was in yr 2 from d 64 to 113, where MIN bulls had reduced ADG compared to CON bulls ($P = 0.05$). The reduced ADG immediately following arrival to a feedlot may be explained by shipping stress; however, the reason for a reduction in ADG during the middle portion of the current study, 64 d post shipment to the feedlot, remains unclear. The reduced ADG from d 64 to 113 in the current study was also difficult to explain because there were no differences between CON and MIN bulls in yr 2 for BW at d 64 or 113 ($P > 0.05$).

In contrast to Arthington et al. (2014b), Kegley et al. (2011) and the current study, Richeson and Kegley (2011) reported that heifers receiving injectable Cu, Zn, Se and Mn after shipment to the feedlot had increased overall ADG and feed efficiency. Richeson and Kegley (2011) blocked heifers by initial BW and then randomly allocated them to a treatment: 1) injectable mineral solution containing 20 mg/mL Zn, 20 mg/mL Mn, 10 mg/mL Cu and 5 mg/mL Se given at 1 mL/45 kg of BW; 2) injectable mineral solution containing 48 mg/mL Zn, 10 mg/mL Mn, 16 mg/mL Cu and 5 mg/mL Se given at 1 mL/45 kg of BW; or 3) no injection. In contrast to Arthington et al. (2014b), Richeson and Kegley (2011) mineral and control heifers did not have different ADG from d 0 to 14. However, similar to Berry et al. (2000), heifers that received either trace mineral treatment in the Richeson and Kegley (2011) experiment had improved ADG from d 0 to 55 in comparison to controls. Richeson and Kegley (2011) also reported that both mineral injected heifer treatment groups had increased overall DMI from d 0 to 55 compared to control heifers. This contradicts results from the current study in which overall

DMI from d 0 to 84 was not affected by mineral treatment. Additionally, heifers injected with trace mineral in the Richeson and Kegley (2011) gained 0.18 kg or 0.19 kg BW/kg feed fed, and control heifers gained 0.16 kg BW/kg feed fed. The G:F values reported by Richeson and Kegley (2011) were higher than the G:F values observed in the current study for MIN and CON treatments. The differences between Richeson and Kegley (2011) and the current study could be due to variation in cattle management. For example, the ration used by Richeson and Kegley (2011) was 93.2% DM, consisting mainly of 54.5% cracked corn, 28.0% cottonseed hulls and 11.5% soybean meal, whereas the diet in the current study was 67.9% DM consisting of 33.5% corn silage, 17.8% corn, 18.3% wheat straw and 16.9% alfalfa (Table 3.1). Moreover, while Richeson and Kegley (2011) injected heifers with the same dosage used for the first injection in the current study, they only injected heifers once, whereas heifers were injected twice in the current study. Additionally, the concentration of Cu, Zn, and Mn in the injectable trace mineral solution for the current study was different than the concentrations utilized by Richeson and Kegley (2011). The authors used a solution containing 10 mg/mL Cu, 20 mg/mL Zn, 20 mg/mL Mn and 5 mg/mL Se or 16 mg/mL Cu, 48 mg/mL Zn, 10 mg/mL Mn and 5 mg/mL Se.

Genther and Hansen (2014) evaluated the effect of an injectable trace mineral solution containing 15 mg/mL Cu, 60 mg/mL Zn, 5 mg/mL Se and 10 mg/mL Mn upon shipment of steers with an induced mild mineral deficiency. The authors indicated that steers were weighed every 28 d for an 84-d depletion period, in which half the steers received a ration supplemented with Fe and Mo as dietary antagonists to induce a mineral deficiency. Steers were shipped for 20 h on d 88, weighed on d 90 and 91 and injected with trace minerals on d 91 for the initiation of the repletion period (Genther and Hansen, 2014). According to the authors, during the mineral depletion stage, there were no treatment effects for ADG, DMI or G:F. However, steers that were

previously considered mineral deficient and then received a 1 mL/68.0 kg of BW trace mineral injection 2 d after shipping, exhibited greater ADG compared to deficient steers that were not injected (Genther and Hansen, 2014). The authors also stated that control steers (with no induced depletion) were not affected regardless of injectable mineral supplementation. Mineral depletion can be detrimental to performance, as noted by Engle et al. (1995), who found that Zn depletion decreased gain by 45.6%; however, upon Zn repletion there were no differences in gain between cattle. Results from Genther and Hansen (2014) and Engle et al. (1995) suggest that trace mineral supplementation may only be beneficial in mineral deficient cattle. Unfortunately, the initial and final mineral status of cattle in the current study were not evaluated. Prior to arrival at the feedlot, cattle in the current study were exposed to an ad libitum standard mineral block fortified with extra Cu. Additionally, the minerals supplied in the diet were provided at adequate levels for proper cattle health (NRC, 1996). It is probable that there were no observed effects on cattle feedlot performance in the current study because cattle already had sufficient mineral status.

Bull, Heifer and Steer Morbidity

There were no differences ($P > 0.05$) between MIN and CON cattle for the number of times bulls, steers or heifers were treated, or the illnesses in which they were treated for (Tables 3.5, 3.6 and 3.7, respectively), except for the percentage of heifers that received no treatment for any illness over the course of the study ($P = 0.03$). In the current study a larger proportion of CON heifers never received treatment when compared to MIN heifers. Contrary to the current study, Richeson and Kegley (2011) observed that administering injectable trace minerals Cu, Zn, Se and Mn at initial processing of calves into the feedlot improved morbidity and sequentially reduced antibiotic costs compared to control calves. The authors may have observed different

results for morbidity compared to the current study because they used injections containing either 20 mg/mL Zn, 20 mg/mL Mn, 10 mg/mL Cu and 5 mg/mL Se or 48 mg/mL Zn, 10 mg/mL Mn, 16 mg/mL Cu and 5 mg/mL Se, neither of which are the same mineral concentration used in the current study.

Injecting cattle prior to weaning and feedlot arrival may not provide any benefit to calf health. Kegley et al. (2011) injected calves with Cu, Zn, Se and Mn, given at 1 mL/45.4 kg of BW, 28 d prior to weaning and found that calf health status was not affected by mineral supplementation. The authors suggested that calf health was not affected by mineral injections because calves were also provided ad libitum access to minerals, possibly allowing for sufficient mineral levels in the body. Similar to Kegley et al. (2011), cattle in the current study may have had sufficient mineral levels in the body because they were provided dietary trace minerals in the daily feedlot ration, as well as ad libitum access to a mineral block prior to weaning.

Gadberry and Simon (2012) conducted an experiment to observe the effects on immune response when beef calves were not exposed to free choice mineral supplementation. The authors reported that calves were injected with the trace minerals Cu, Zn, Se and Mn 90 d pre-weaning and again at weaning. Calves whose dams either did not receive, or received, a trace mineral injection pre-breeding were randomly assigned to receive an injection or no injection within their dam treatment (Gadberry and Simon, 2012). The authors reported that mineral injections did not affect immune response in calves not exposed to ad libitum mineral supplementation. Thus, it appears that injectable minerals may not affect morbidity regardless of ad libitum mineral supplementation (Kegley et al., 2011; Gadberry and Simon, 2012). It is also plausible that the Gadberry and Simon (2012) calves received adequate mineral supplementation

from range forages they were grazing. Unfortunately, initial and final mineral status of calves were not evaluated in either study.

While pre-weaning and weaning injections do not appear to influence morbidity, continuous and lifelong mineral supplementation also does not appear to influence calf health (Ahola et al., 2005; Gadberry and Simon, 2012). Ahola et al. (2005) used cattle with adequate mineral status, according to liver and blood evaluations, to study the effects of dietary Cu, Zn and Mn supplemented in combination and maintained at levels above deficiency from neonatal through finishing phase of production. The authors observed that lifetime mineral supplementation at levels above deficiency did not influence morbidity or the number of times cattle required treatment. In addition, increasing the level of mineral supplementation to 2 or 3 times above the recommended NRC (1996) level did not affect overall morbidity, incidence of respiratory disease, foot rot, bloat or the number of times feedlot cattle require treatment (Rhoads et al., 2003).

Bull Fertility

In yr 1 and 2, BSE traits were not different between treatments ($P > 0.05$; Table 3.8), although there was a tendency ($P = 0.08$) for MIN bulls to have increased SEC in yr 2. Results from CASA indicated no differences between treatments for CMOT, PROG, VAP, VCL, ALH, STR, LIN, VOL or CONC ($P > 0.05$; Table 3.9). However, in yr 2 BCF was decreased in MIN ($P < 0.01$) vs. CON bulls, and there was a tendency ($P = 0.10$) for BCF to be decreased in MIN bulls in yr 1 vs. CON bulls. Additionally, VSL in yr 2 MIN bulls was reduced ($P = 0.04$) vs. CON bulls. Breeding soundness exam MOT was not correlated with CASA CMOT in yr 2 ($r^2 = 0.18$; $P = 0.34$); however, they were positively correlated in yr 1 ($r^2 = 0.60$; $P < 0.001$).

Results from the current study agree with Kirchhoff et al. (2015) who used Hereford, Angus and Simmental yearling bulls to determine the effects of a 1 mL/45.4 kg of BW injectable solution containing 60 mg/mL Zn, 15 mg/mL Cu, 10 mg/mL Mn and 5 mg/mL Se on semen quality. The authors fed bulls a similar ration to the bull ration utilized in the current study and injected bulls with the same dosage and mineral concentration used in the current study. The researchers reported that the injectable trace minerals did not affect percent progressive sperm, percent normal sperm, scrotal circumference or overall percentage of bulls passing their BSE when compared to control bulls. Kirchoff et al. (2015) noticed that injected bulls tended to have greater sperm concentrations on d 42, however that effect was not observed in the current study. Although the current study observed a tendency for treated bulls to have increased SEC, this was not reported by Kirchoff et al. (2015). However, the authors reported that the overall number of bulls passing their breeding soundness exam, despite treatment, was relatively low, and that proximal and distal droplets, along with poor sperm morphology were the primary causes of failure (Kirchoff et al., 2015). This indicates that poor morphology across treatments was more a reflection on bull and sperm quality as opposed to treatment effects. Kirchoff et al. (2015) concluded that a trace mineral injection administered to bulls with adequate dietary trace mineral levels did not improve yearling bull BSE traits, which was consistent with the current study.

The combination of Cu, Zn, Se and Mn may have different effects compared to each mineral being used by itself. For example, injecting only Se in yearling Angus bulls did not influence sperm quality or viability when compared to a control (Segerson and Johnson, 1981). The authors also reported that various tissues such as the testes, epididymides and seminal vesicles had the capability to concentrate Se; however, the uptake of Se by spermatozoa was not affected. Additionally, Bartle et al. (1980) injected Holstein bulls with marginal Se status with 5

mg/90 kg of BW when they were approximately 19 mo of age. Injections were given again at 10, 20 and 40 mg/90 kg of BW at wk 10, 16 and 22 to maintain high levels of Se. Semen was collected 3 times a wk for the first 15 wk, 4 times for the next 15 wk and 6 times the final wk to determine the effects of Se on semen production and quality in bulls subjected to increased sexual activity. Bartle et al. (1980) reported that semen Se concentrations were higher than blood Se concentrations in treated bulls as well as semen and blood Se concentrations of control bulls. The authors believed that the bovine male reproductive system must have a high affinity for Se and may be the preferred organ system for Se metabolism. However, the authors also reported that Holstein bull sperm quality was not affected by administration of Se; thus researchers suggested that perhaps the optimum Se status had not been reached for bulls to reach their peak reproductive performance. This suggestion could also be applied to the current study because Se was administered less frequently and in lower concentration and dosage compared to Bartle et al. (1980).

Knazicka et al. (2013) utilized semen samples from mature breeding bulls to observe the effects of Cu concentration on seminal plasma and spermatozoa motility. The authors analyzed sperm using CASA and it was determined that excess Cu in seminal plasma was detrimental to male reproductive capacity by reducing spermatozoa count, motility, viability and morphology. Although sperm quality in the current study was not detrimentally impacted, it was also not improved by 2 trace minerals injections. Perhaps the negative effects Cu had on sperm quality in previous experiments were balanced by the addition of Zn, Se and Mn in the injectable mineral used in the current study.

Bansal and Bilaspuri (2008) used Mn because of its molecular chain-breaking antioxidant capabilities. The authors evaluated the effects of Mn on crossbred bull sperm samples subjected

to oxidative stress inducers, such as ferrous ascorbate or ascorbic. The researchers found that Mn administered *in vitro* to semen samples reduced oxidative stress and improved sperm motility and viability. These results suggest that Mn would have a beneficial effect on sperm quality; however, that result was not observed in the current study. It is possible Mn reacted better when used *in vitro* and directly injected into semen samples vs. Mn injected into the animal body. Also, Mn may have different effects in the body when used in combination with other minerals, such as the Cu, Zn, and Se utilized in the current study.

Additionally, using dietary Zn by itself showed benefit to sperm quality in healthy *Bos Taurus* x *Bos indicus* crossbred 2 yr old bulls fed a 50:50 roughage to concentrate diet (Kumar et al., 2006). The authors observed that bulls supplemented with 35 or 70 ppm Zn sulfate or 35 ppm Zn propionate had higher ejaculate volume, sperm concentration, percent live sperm and percent motile sperm after a 6-mo period compared to control bulls. Results from Kumar et al. (2006) are not consistent with results observed in the current study. These differences could be due to variation in experiment period, method of administration of Zn, age and weight of bulls or combination of minerals used. For example, Kumar et al. (2006) used dietary Zn in 2 yr old bulls initially weighing 316 ± 0.77 kg for a 6-mo period, whereas the current study used injectable Cu, Zn, Mn and Se in yearling bulls weighing 312.0 ± 19.45 kg (yr 1) or 306.8 ± 14.41 (yr 2) for less than a 5-mo period. The current study collected semen samples from bulls 1 time and Kumar et al. (2006) collected semen samples at the start of the experiment and then 6 more times throughout the experiment. Kumar et al. (2006) chose a longer experiment since spermatogenesis in bulls was 60 d; thus allowing for the authors to observe the effects of dietary Zn for 3 sperm "cycles."

While supplementation of minerals may affect sperm quality, it appears that mineral source (organic vs. inorganic) also affects sperm quality (Rowe et al., 2011). Since spermatogenesis in bulls takes 60 d for a complete cycle, the authors supplemented dietary organic trace minerals Zn, Cu, Mn and Co to mature bulls for 60 d prior to initial semen collection. The dietary organic minerals were continually supplemented through an additional 9 wk semen collection period, in which semen was collected once a wk and then analyzed using CASA (Rowe et al., 2011). The authors reported that bulls supplemented with organic trace minerals had 65.5% vs. 56.1% CMOT among bulls supplemented with inorganic trace minerals. The authors also found that bulls supplemented with organic trace minerals had a higher percent of progressive and rapid sperm compared to their inorganic supplemented cohorts.

Rowe et al. (2011) described motile sperm as those having VAP greater than 30 µm/sec and VSL greater than 15 µm/sec. Progressive sperm had a VAP greater than 50 µm/sec and STR greater than 70% (Rowe et al., 2011). Rapid sperm had a VSL greater than 50 µm/sec (Rowe et al., 2011). If requirements for motile, progressive or rapid sperm proposed by Rowe et al. (2011) were applied to the current study, CON and MIN bulls in yr 1 would have had motile sperm; however, it would not have been considered progressive or rapid (Table 3.9). Conversely, both CON and MIN bulls in yr 2 would have been considered to have motile, progressive and rapid sperm. Additionally, CON bulls in the current study had increased VSL compared to MIN bulls, indicating those bulls may have had more motile and rapid sperm, if using the guidelines of Rowe et al. (2011). In contradiction to Rowe et al. (2011), the current study did not observe treatment effects for most of the CASA components. This could be due to differences in mineral combination or method of administration, or CASA technology. In the current study, there were treatment effects on BCF, the number of times that the curvelinear path of sperm intersects the

average path (Amann and Waberski, 2014). It is uncertain why MIN treatment affected BCF in both yr and VSL in yr 2; however, it did not affect any other CASA components.

Heifer Fertility

There were no differences between CON and MIN treatments for heifer BCS, age at puberty, AI pregnancy rate or day of calving season ($P > 0.05$). In a design similar to the current study, Brasche (2014) injected virgin, Angus-crossbred heifers 33 d prior to AI with a 0.57 mL/ 45.5 kg of BW injectable solution containing 60 mg/mL Zn, 15 mg/mL Cu, 10 mg/mL Mn and 5 mg/mL Se to observe effects on pregnancy rate. Heifers were synchronized to a 14 d CIDR-PG-AI or a 5 d Co-Synch plus CIDR protocol (Brasche, 2014). For the 14 d CIDR-PG-AI, a CIDR was inserted 33 d prior to artificial insemination and removed 14 d later. Prostaglandin was injected 16 d after CIDR removal and heifers were inseminated 73 h later. For the 5 d Co-Synch plus CIDR protocol, a CIDR was inserted 7 d prior to AI and an injection of gonadotropin-releasing-hormone (**GnRH**) was given. The CIDR was removed 5 d later and a PG injection was given. A second PG injection was given 5.6 h later, and heifers were inseminated 55 h after the last PG injection. Similar to heifers in the current study, Brasche (2014) reported that heifers synchronized to the 14 d CIDR-PG-AI protocol and injected with trace minerals did not have improved pregnancy rate to AI. However, the author also reported that when data were combined from heifers synchronized to both protocols, heifers injected with trace minerals had higher pregnancy rates (93.0%) compared to control heifers (83.0%).

Lactating crossbred beef cows maintained on pasture in Florida also had greater pregnancy rates after one 5 mL injection of Cu, Zn, Se and Mn (Arthington et al., 2014a). However, the authors determined this improvement was not influenced by mineral injection but rather an increase in BCS. The BCS was determined at time of injection and therefore not

influenced by mineral treatment (Arthington et al., 2014a). In the current study, BCS was not affected by mineral injection nor did it influence pregnancy rate.

Vanegas et al. (2004) evaluated the effects of 1 or 2 doses of an injectable trace mineral solution containing 10 mg/mL Cu, 20 mg/mL Zn, 5 mg/mL Se and 20 mg/mL Mn on first service conception rate in dairy cows. Single injections were administered to cows 38 to 45 d in lactation, while cows receiving the double dose were injected 2 to 3 wk prior to calving and again at 38 to 45 d in lactation. The authors found that injecting cows with one 5 mL dose of trace minerals before breeding had no benefit on first service conception rates (Vanegas et al., 2004). Additionally, the authors reported that a 2 dose regimen, 1 injection before calving and 1 before breeding, resulted in decreased conception rates compared to control cows. These results are inconsistent with the current study, in which a 2 dose regimen, 1 injection at weaning and one 33 d prior to breeding, did not have a detrimental effect on first service conception to AI. These differences could be due to differences in dosage or concentration of trace minerals injected, time of injection or class of cattle in which treatment was administered.

Consistent with the current study, Grings et al. (1999) reported that neither heifer pregnancy rates nor age at puberty were affected by dietary supplementation of Cu, Zn and Mn. The authors indicated that heifers were sired by Hereford, Limousin or Piedmontese bulls, and age at puberty was not affected by mineral treatment but instead influenced by sire breed or age of dam. While age at puberty in heifers was not affected by mineral supplementation in the current study, breed effects were not studied because all heifers were progeny of Angus bulls and cows.

In agreement with results from the current study, injectable Cu, Zn, Se and Mn administered 30 d prior to breeding in crossbred beef cows had no influence on reproductive

performance over a 2-yr period (Whitworth et al., 2014). The authors gave the same injectable minerals as the current study; however, they were administered at approximately twice the dosage. Moreover, the authors noted that cows had additional access to ad libitum minerals, possibly providing adequate mineral supplementation (Whitworth et al., 2014). Heifers in the current study were fed additional dietary trace minerals in their daily total mixed ration, also suggesting their mineral intake may have been adequate for performance. This conclusion is uncertain because initial and final mineral status were unknown for both the Whitworth et al. (2014) experiment and the current study. Nevertheless, it appears that injectable minerals do not benefit reproductive performance in yearling beef heifers that are not known to be deficient for these trace minerals.

Overall, results from the current study suggest that there is no benefit to yearling bull or heifer fertility, or newly weaned calf performance or morbidity, when supplementing cattle of unknown mineral status with injectable trace minerals. Previous research has indicated that mineral supplementation improved performance in deficient cattle but not in cattle with adequate mineral concentrations (Engle et al., 1995; Genther and Hansen, 2014). Unfortunately, initial mineral status of cattle in the current study was unknown. Additionally, cattle in the current study received minerals in their daily ration, making it plausible that minimum mineral requirements were met by cattle in each treatment.

Moreover, published literature implicates that the effects of injectable trace minerals in cattle vary dependent on timing of injection, number of injections, class of cattle utilized, additional mineral supplemented, and initial mineral status. Nevertheless, it is known that minerals are vital to proper health, growth and reproduction (Underwood and Suttle, 1999); therefore, further research should be conducted to better understand the influence of injectable

mineral sources on yearling bull and heifer fertility, and feedlot performance and morbidity of newly weaned calves.

Table 3.1. Ingredient composition and proximate analysis of the basal diet fed to developing heifers, and bulls, by yr (DM basis)

	Yr 1 Bulls		Yr 2 Bulls		Heifers
Item	Receiving	Developing	Receiving	Developing	Developing
Ingredient	(%)	(%)	(%)	(%)	(%)
Alfalfa hay, ground	15.29	50.90	6.34	27.65	16.92
Wheat straw, ground	8.73	-	22.66	-	18.26
Corn silage	37.65	11.12	13.42	15.03	33.48
Cracked corn	12.91	28.54	22.25	-	-
Whole corn	-	-	-	42.72	17.83
Dried distillers grain	23.63	5.65	29.91	9.73	10.00
Supplement[1]	1.79	3.79	5.43	4.86	3.50
Dried distillers grain, %	45.48	-	-	-	-
Ground corn, %	31.11	-	-	-	-
Urea, %	8.03	-	-	-	-
Salt, %	5.61	-	-	-	-
Limestone, %	5.56	-	-	-	-
Soybean oil, %	2.00	-	-	-	-
Dyna-K, %	0.80	-	-	-	-
Fe sulfate, %	0.38	-	-	-	-
Zn sulfate, %	0.36	12.58	12.58	12.58	12.58
Cu sulfate, %	0.15	2.77	2.77	2.77	2.77
Co sulfate, %	-	0.05	0.05	0.05	0.05
Mn sulfate, %	-	8.81	8.81	8.81	8.81
P, %	-	75.65	75.65	75.65	75.65
I, %	0.08	0.08	0.08	0.08	0.08
Se, %	0.03	0.04	0.04	0.04	0.04
Co carbonate, %	0.03	-	-	-	-
Vitamin A, %	0.10	0.005	0.005	0.005	0.005
Vitamin D, %	-	0.0001	0.0001	0.0001	0.0001
Vitamin E, %	0.01	0.01	0.01	0.01	0.01
Monensin, g/kg	200.00	0.45	0.45	0.45	0.45
Proximate analysis					
DM, %	54.91	59.12	70.88	68.71	67.97
CP, %	14.25	13.03	15.94	16.10	32.03
NE_G, Mcal/kg	1.04	0.88	1.17	1.15	0.97
NE_M, Mcal/kg	1.76	1.61	1.90	1.87	1.68
TDN, %	70.66	65.26	75.32	74.63	67.98
ADF, %	26.16	33.67	19.69	20.65	29.21

[1] Yr 1 receiving bull ration = dry mineral supplement; Yr 1 developing bull ration, both yr 2 bull rations and heifer ration = 30% CP liquid supplement.

Table 3.2. Least squares means for BW and ADG of yearling Angus bulls given injectable trace minerals at weaning (d 0) and again on d 64 (yr 1) or d 110 (yr 2)

	Treatment[1]			*P* =		
Item	CON	MIN	SEM	Trt	Time	Trt x Time
Yr 1						
n	9	22				
BW, kg						0.39
Initial (d 0)	298.0	317.7	4.78	0.01	-	
Final (d 144)	488.0	481.8	5.07	0.47	<0.0001	
Overall ADG, kg	1.11	1.11	0.064	0.99	<0.0001	
Yr 2						
n	18	17				
BW, kg						0.23
Initial (d 0)	306.5	306.9	5.70	0.96	-	
Final (d 144)	498.0	489.3	5.73	0.62	<0.0001	
Overall ADG, kg	1.18	1.10	0.050	0.30	<0.0001	

[1]Treatments: CON = Control, no injection; MIN = mineral injection of 60 mg/mL Zn, 15 mg/mL Cu, 10 mg/ml Mn and 5 mg/mL Se (MultiMin®, MultiMin USA, Fort Collins, CO). First injection administered at 1 mL/45.4 kg of BW and second injection at 1 mL/68.0 kg of BW.

Table 3.3. Least squares means for Angus steers BW and ADG from weaning to harvest after receiving injectable trace minerals at weaning (d 0)

	Treatment[1]			
Item	CON	MIN	SEM	*P* =
n	50	55		
BW, kg				
d 0	276.7	273.6	5.33	0.68
d 62[2]	327.1	290.2	12.27	0.04
d 123	476.4	471.7	6.27	0.60
d 195 or 216	610.1	585.7	17.96	0.34
Days on feed[3], d	158.0	153.9	1.64	0.07
Overall ADG[4], kg/d	1.99	1.98	0.006	0.55
Total gain[4], kg	297.9	300.9	2.79	0.45

[1] Treatments: CON = Control, no injection; MIN = mineral injection of 60 mg/mL Zn, 15 mg/mL Cu, 10 mg/ml Mn and 5 mg/mL Se (MultiMin®, MultiMin USA, Fort Collins, CO). First injection administered at 1 mL/45.4 kg of BW and second injection at 1 mL/68.0 kg of BW.
[2] Finishing phase began and lasted through d 195 or 216.

[3] Days on finishing ration only; 146 or 170 d total

[4]Calculated only for finishing phase from d 62 to d 195 or 216.

Table 3.4. Least squares means for ADG, G:F, age at puberty and pregnancy rate in developing Angus heifers given injectable trace minerals at weaning (d 0) and again on d 135, which was 33 d prior to synchronized AI

	Treatment[1]			
Item	CON	MIN	SEM	*P* =
n	60	47		
Initial BW(d 0), kg	281.6	280.4	3.90	0.83
Final BW (d 84), kg	360.9	356.4	4.83	0.51
Overall ADG (d 0 to 84), kg/d	0.93	0.93	0.0004	0.69
DMI (d 0 to 84), kg/d	7.87	7.81	0.16	0.80
Overall G:F (d 0 to 84)	0.12	0.12	0.004	0.43
BCS at breeding[2]	6.0	5.9	0.08	0.12
Age at puberty[3], d	318.1	312.5	3.51	0.26
Pregnant to AI[4], %	66.4	70.9	0.06	0.59
Season long pregnancy rate[5], %	94.4	97.4	0.03	0.50
Day of calving season,[6] d	18.9	17.7	2.70	0.74

[1] Treatments: CON = Control, no injection; MIN = mineral injection of 60 mg/mL Zn, 15 mg/ mL Cu, 10 mg/ml Mn and 5 mg/mL Se (MultiMin®, MultiMin USA, Fort Collins, CO). First injection administered at 1 mL/45.4 kg of BW and second injection at 1 mL/68.0 kg of BW.
[2] Analyzed using the 9-point scale, where 1=thin and 9=obese (Richards et al., 1986).
[3]Age at puberty determined by estrus detection patches (Estrotect™ Heat Detector, Rockway Inc., Spring Valley, WI) applied on day 0 and recorded every 11 d as activated, not activated or missing.
[4] Heifers were estrus synchronized and inseminated using a 14 d CIDR-PG-AI protocol, in which a CIDR was inserted 33 d prior to artificial insemination and removed 14 d later. Prostaglandin was injected 16 d after CIDR removal and heifers were inseminated 3 d later (d 168). Pregnancy to AI was determined via ultrasound at 56 d post AI.
[5] Season long pregnancy rate was determined via rectal palpation 178 d post AI.
[6] d 0 = date first calf was born among heifers.

Table 3.5. Least squares means for morbidity rate and diagnosis in yearling Angus bulls given injectable trace minerals at weaning (d 0) and again on d 64 (yr 1) or d 110 (yr 2)[1]

	Treatment[2]			
Item	CON	MIN	SEM	*P* =
n	27	39		
No treatment, %	75.6 (19/27)[3]	63.0 (26/39)	0.08	0.27
Treated one time, %	24.4 (8/27)	36.9 (13/39)	0.08	0.27
Lameness[4], %	37.5 (3/8)	53.9 (7/13)	0.16	0.49
Respiratory[4], %	37.5 (3/8)	38.5 (5/13)	0.16	0.97
Other[4], %	25.0 (2/8)	7.7 (1/13)	0.11	0.29
Treated two times, %	4.9 (2/27)	12.0 (4/39)	0.05	0.32
Lameness[4], %	0.0 (0/2)	50.0 (2/4)	0.30	0.31
Respiratory[4], %	100.0 (2/2)	25.0 (1/4)	0.52	0.12
Other[4], %	0.0 (0/2)	25.0 (1/4)	0.26	0.54
Treated three times, %	3.7 (1/27)	2.6 (2/39)	0.03	0.79
Respiratory[4], %	0.0 (0/1)	50.0 (1/2)	0.60	0.67
Other[4], %	100.0 (1/1)	50.0 (1/2)	0.03	0.79

[1] No treatment x yr interaction ($P = 0.45$) thus data were pooled across yr.
[2] Treatments: CON = Control, no injection; MIN = mineral injection of 60 mg/mL Zn, 15 mg/ mL Cu, 10 mg/ml Mn and 5 mg/mL Se (MultiMin®, MultiMin USA, Fort Collins, CO). First injection administered at 1 mL/45.4 kg of BW and second injection at 1 mL/68.0 kg of BW.
[3] (n treated/ total n)
[4] Diagnosed reason for treatment.

Table 3.6. Least squares means for Angus steer morbidity rate and diagnosis from weaning to harvest after receiving injectable trace minerals at weaning (d 0)

	Treatment[1]			
Item	CON	MIN	SEM	*P* =
n	50	55		
No treatment, %	36.0 (18/50)[2]	41.8 (23/55)	0.07	0.55
Treated one time, %	30.0 (15/50)	30.9 (17/55)	0.06	0.92
Lameness[3], %	20.0 (3/15)	11.8 (2/17)	0.09	0.54
Respiratory[3], %	66.7 (10/15)	82.4 (14/17)	0.11	0.32
Other[3], %	13.3 (2/15)	5.9 (1/17)	0.07	0.49
Treated two times, %	2.0 (1/50)	7.3 (4/55)	0.03	0.21
Respiratory[3], %	0.0 (0/1)	50.0 (2/4)	0.43	0.50
Other[3], %	100.0 (1/1)	50.0 (2/4)	0.43	0.50
Treated three times, %	4.0 (2/50)	3.6 (2/55)	0.03	0.92
Respiratory[3], %	0.0 (0/2)	50.0 (1/2)	0.35	0.42
Other[3], %	100.0 (2/2)	50.0 (1/2)	0.35	0.42

[1] Treatments: CON = Control, no injection; MIN = mineral injection of 60 mg/mL Zn, 15 mg/ mL Cu, 10 mg/ml Mn and 5 mg/mL Se (MultiMin®, MultiMin USA, Fort Collins, CO). First injection administered at 1 mL/45.4 kg of BW and second injection at 1 mL/68.0 kg of BW.
[2] (n treated/ total n)
[3] Diagnosed reason for treatment.

Table 3.7. Least squares means for morbidity rate and diagnosis in developing Angus heifers given injectable trace minerals at weaning (d 0) and again on d 135, which was 33 d prior to synchronized AI

	Treatment[1]			
Item	CON	MIN	SEM	*P* =
n	60	47		
Never treated, %	91.6 (55/60)[2]	76.7 (36/47)	0.05	0.03
Treated one time, %	6.7 (4/60)	14.9 (7/47)	0.04	0.17
Lameness[3], %	25.0 (1/4)	28.6 (2/7)	0.22	0.91
Respiratory[3], %	75.0 (3/4)	71.4 (5/7)	0.22	0.91
Treated two or more times, %	1.7 (1/60)	6.4 (4/47)	0.03	0.21
Lameness[3], %	0.0 (0/0)	25.0 (1/4)	0.38	0.69
Other[3], %	100.0 (1/1)	75.0 (3/4)	0.38	0.69

[1] Treatments: CON = Control, no injection; MIN = mineral injection of 60 mg/mL Zn, 15 mg/mL Cu, 10 mg/ml Mn and 5 mg/mL Se (MultiMin®, MultiMin USA, Fort Collins, CO). First injection administered at 1 mL/45.4 kg of BW and second injection at 1 mL/68.0 kg of BW.
[2] (n treated/ total n)
[3] Diagnosed reason for treatment.

Table 3.8. Least squares means for breeding soundness exam traits in yearling Angus bulls given injectable trace minerals at weaning (d 0) and again on d 64 (yr 1) or 110 (yr 2), which corresponded to 80 (yr 1) or 38 d (yr 2) prior to breeding soundness exams

	Treatment[1]			
Traits	CON	MIN	SEM	*P* =
Yr 1				
n	9	22		
Scrotal circumference, cm	33.6	34.9	0.58	0.11
Sperm motility, %	65.6	69.3	2.15	0.24
Normal sperm, %	77.8	81.5	4.18	0.54
Sperm with primary defects, %	16.9	14.8	32.21	0.97
Sperm with secondary defects, %	5.2	4.2	0.94	0.45
Yr 2				
n	18	17		
Scrotal circumference, cm	34.8	35.7	0.60	0.29
Sperm motility, %	63.8	66.6	1.76	0.27
Normal sperm, %	75.0	73.5	3.25	0.74
Sperm with primary defects, %	20.1	19.2	3.15	0.85
Sperm with secondary defects, %	4.9	7.3	0.92	0.08

[1] Treatments: CON = Control, no injection; MIN = mineral injection of 60 mg/mL Zn, 15 mg/mL Cu, 10 mg/ml Mn and 5 mg/mL Se (MultiMin®, MultiMin USA, Fort Collins, CO). First injection administered at 1 mL/45.4 kg of BW and second injection at 1 mL/68.0 kg of BW.

Table 3.9. Least squares means for Computer-Assisted Sperm Analysis traits in yearling Angus bulls given injectable trace minerals at weaning (d 0) and again on d 64 (yr 1) or 110 (yr 2), which corresponded to 80 (yr 1) or 38 d (yr 2) prior to breeding soundness exams

	Treatment[1]			
Traits	CON	MIN	SEM	*P* =
Yr 1				
n	9	22		
Motile sperm, %	73.1	63.9	6.28	0.32
Progressive sperm, %	56.0	48.7	5.45	0.36
Sperm path velocity, µm/s	33.66	33.86	2.08	0.95
Sperm progressive velocity, µm/s	35.64	34.27	23.25	0.97
Sperm track speed, µm/s	53.08	56.33	4.25	0.60
Sperm lateral amplitude, µm	2.62	2.62	0.20	0.99
Sperm beat frequency, Hz	18.01`	15.05	1.21	0.10
Sperm straightness, %	12.4	13.5	1.16	0.51
Sperm linearity, %	18.7	16.5	0.97	0.13
Sperm volume, mL	4.09	4.25	0.57	0.85
Concentration, $x10^6$/mL	462.3	410.9	67.12	0.60
Yr 2				
n	18	17		
Motile sperm, %	35.2	29.3	6.43	0.52
Progressive sperm, %	33.0	26.2	5.78	0.41
Sperm path velocity, µm/s	97.45	86.10	8.20	0.34
Sperm progressive velocity, µm/s	94.15	75.80	6.10	0.04
Sperm track speed, µm/s	152.33	138.97	10.39	0.37
Sperm lateral amplitude, µm	4.83	5.45	0.32	0.18
Sperm beat frequency, Hz	38.93	31.67	1.52	< 0.01
Sperm straightness, %	91.2	88.5	1.15	0.12
Sperm linearity, %	66.0	59.6	13.92	0.77
Sperm volume, mL	4.10	4.72	0.47	0.37
Concentration, $x10^6$/mL	447.4	340.6	68.95	0.28

[1] Treatments: CON = Control, no injection; MIN = mineral injection of 60 mg/mL Zn, 15 mg/ mL Cu, 10 mg/ml Mn and 5 mg/mL Se (MultiMin®, MultiMin USA, Fort Collins, CO). First injection administered at 1 mL/45.4 kg of BW and second injection at 1 mL/68.0 kg of BW.

LITERATURE CITED

Ahola, J.K., L.R. Sharpe, K.L Dorton, P.D Burns, T.L Stanton, and T.E. Engle. 2005. Effects of lifetime copper, zinc and manganese supplementation and source on performance, mineral status, immunity and carcass characteristics of feedlot cattle. Prof. Anim. Sci. 21:305-317.

Amann, R.P., and D. Waberski. 2014. Computer-assisted sperm analysis (CASA): Capabilities and potential developments. Therio. 81:5-17.

Arthington, J.D., P.G.M.A. Martins, P. Moriel, and L. Havenga. 2014a. Effects of injectable trace minerals at the start of the breeding season on attainment of pregnancy in commercial beef cows. ADSA-ASAS-CSAS Joint Annual Meeting, Kansas City Convention Center. Kansas City, MO. p.1469 (Abstr.)

Arthington, J.D., P. Moriel, P.G.M.A. Martins, G.C. Lamb, and L.J. Havenga. 2014b. Effects of trace mineral injections on measures of performance and trace mineral status of pre- and postweaned beef calves. J. Anim. Sci. 92:2630-2640.

Bansal, A.K., and G.S. Bilaspuri. 2008. Effect of manganese on bovine sperm motility, viability and lipid peroxidation *in vitro.* Anim. Reprod. 5:90-96.

Bartle, J.L., P.L. Senger, and J.K. Hillers. 1980. Influence of injected selenium in dairy bulls on blood and semen selenium, glutathione peroxidase and seminal quality. Bio. Reprod. 23:1007-1013.

Berry, B. A., W. T. Choat, D. R. Gill, C. R. Krehbiel, and R. Ball. 2000. Efficacy of Multimin™ in improving performance and health in receiving cattle. Anim. Sci. Rep. Oklahoma

Agric. Exp. Stn. Accessed March 15, 2015. http://www.ansi.okstate.edu/research/2000rr/12.htm.

Brasche, C.J., J.B. Hall, and M.E. Drewnoski. 2014. Effect of an injectable trace mineral on pregnancy rate in virgin heifers when synchronized using 5 day Co-Synch plus CIDR or 14 day CIDR-PG protocol. ADSA-ASAS-CSAS Joint Annual Meeting. Kansas City Convention Center, Kansas City, MO. p. 926. (Abstr.)

Engle, T.E., C.F. Nockels, C.V. Kimberling, R.E. Toombs, R.S. Yemm, D.L. Weaber, D.L. and A.B. Johnson. 1995. The effect of feeding organic and inorganic zinc on biochemical parameters in zinc-deficient calves. Proc., West. Sect. Am. Soc. Anim. Sci. 46:471-474.

Gadberry, M.S., and K. Simon. 2012. Response of beef cows, not exposed to mineral supplements, to an injectable trace mineral supplement. Univ. of Arkansas Anim. Sci. Rep. Fayetteville, AR.

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

Genther, O.N., and S.L. Hansen. 2014. Effects of dietary trace mineral supplementation and a multi-element trace mineral injection on shipping response and growth performance of beef cattle. J. Anim. Sci. 92:2522-2530.

Grings, E.E., R.B. Staigmiller, R.E. Short, R.A. Bellows, and M.D. MacNeil. 1999. Effects of stair-step nutrition and trace mineral supplementation on attainment of puberty in beef heifer of three sire breeds. J. Anim. Sci. 77:810-815.

Kegley, E.B., K.P. Coffey, and J.T. Richeson. 2011. Effects of trace mineral injection 28 days before weaning on calf health, performance and carcass characteristics. Univ. of Arkansas Anim. Sci. Rep. Fayetteville, AR.

Kirchhoff, A.A., K.E. Fike, and R. Breiner. 2015. An injectable trave mineral supplement in yearling bulls causes short term increase in circulating trace mineral leverls but does not improve sperm quality. Kansas Ag. Exp. Stn. Rep. 1:1. Topeka, KS.

Knazicka, Z., J. Lukacova, A. Gren, G. Formicki, P. Massanyi, and N. Lukac. 2013. Relationship between level of copper in bovine seminal plasma and spermatozoa motility. J. Microbio. Biotech. Food Sci. 2(1):1351-1362.

Kumar, N., R.P. Verma, L.P. Singh, V.P. Varshney, R.S. and Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* x *Bos taurus*) bulls. Reprod. Nutr. Dev. 46:663-675.

National Research Council (NRC). 1996. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington D.C. (2000):54-74.

Rhoads, A.R., T.L. Stanton, T.E. Engle, and C.V. Kimberling. 2003. Effects of concentration and source of trace minerals on performance, immunity, mineral and lipid metabolism, and carcass characteristics of beef steers. Prof. Anim. Sci. 19:150-158.

Richards, M.W., J.C. Spitzer, and M.B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300-306.

Richeson, J. T., and E. B. Kegley. 2011. Effect of supplemental trace minerals from injection on health and performance of highly stressed, newly received beef heifers. Prof. Anim. Sci. 27:461–466.

Rowe, M.P., J.G. Powell, E.B. Kegley, T.D. Lester, C.L.Williams, R.J. Page, and R.W. Rorie. 2011. Influence of organic versus inorganic trace mineral supplementation on bull semen quality. Univ. of Arkansas Anim. Sci. Dept. Rep. Fayetteville, AR.

Segerson, E.C., and B.H. Johnson. 1980. Selenium and reproductive function in yearling bulls. J. Anim. Sci. 51:395-401.

Spears, J.W. 1996. Organic trace minerals in ruminant nutrition. Anim. Sci. Feed Technol. 58:151–163.

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

Underwood, E.J., and N.F. Suttle. 1999. The Mineral Nutrition of Livestock (3rd ed.). CABI Publishing, Wallingfor, UK.

Vanegas, J.A., J. Reynolds, and E.R. Atwill. 2004. Effects of an injectable trace mineral supplement on first-service conception rate of dairy cows. Dairy Sci. 87:3665–3671.

Whitworth, W.A., T.G. Montgomery, A.J. Hood, and M.S. Gadberry. 2014. Effects of injectable trace mineral supplementation on reproductive rates in *Bos indicus* cattle. Univ. of Arkansas Anim. Sci. Rep. Fayetteville, AR. p. 42-43.

APPENDIX I

SAS Code for Chapter II

Code for analyzing heifer ADG:

```
proc sort data=adg;
by Tag Pen Trt;
proc transpose data=adg out=TrData;
by Tag Pen Trt;
run;
proc print;
data TrData;
set TrData;
adg=Col1;
Time=_Name_;
if trt in (2,3) then Rs='No ';
if trt in (1,4) then Rs='yes ';
if trt in (1,3) then Rx='No ';
if trt in (2,4) then Rx='Yes ';
run;
proc print;
proc glimmix;
class Trt Pen Tag Time;
model adg= Trt|Time/ddfm=KR;
random Pen(Trt) Tag(Pen*Trt) Time*Pen(Trt);
lsmeans Trt|Time / pdiff;
lsmeans trt*time / plot=meanplot (sliceby=trt join);
slice Trt*Time/sliceby=Time pdiff;

proc mixed;
class Rs Rx Pen Tag Time;
model adg= Rs|Rx|Time/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx) Time*Pen(Rs*Rx);
lsmeans Rs|Rx|Time / pdiff;
lsmeans trt*time / plot=meanplot (sliceby=trt join);
slice Rs*Rx*Time/sliceby=Time pdiff;

proc mixed;
class Pen Rs Rx;
model IBW= Rs|Rx;
Random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
Lsmeans Rs|Rx / pdiff;
run;
proc mixed;
class Pen Rs Rx;
model FBW=Rs|Rx;
Random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
```

```
Lsmeans Rs|Rx / pdiff;
run;
```

Code for analyzing heifer ADG by period:

```
proc mixed;
class Rs Rx Pen Tag;
model week1= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week2= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week3= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week4= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week6adj= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week7= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week8= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;
```

Code for analyzing heifer DMI:

```
proc sort data=dmi;
by Pen Trt;
proc transpose data=dmi out=TrData;
by Pen Trt;
run;
proc print;
data TrData;
set TrData;
dmi=Col1;
Time=_Name_;
if trt in (2,3) then Rs='No ';
if trt in (1,4) then Rs='yes ';
if trt in (1,3) then Rx='No ';
if trt in (2,4) then Rx='Yes ';
run;
proc print;
proc glimmix;
class Trt Pen Time;
model dmi= Trt|Time/ddfm=KR;
random Pen(Trt) Time*Pen(Trt);
lsmeans Trt|Time / pdiff;
lsmeans trt*time / plot=meanplot (sliceby=trt join);
slice Trt*Time/sliceby=Time pdiff;

proc mixed;
class Rs Rx Pen Time;
model dmi= Rs|Rx|Time/ddfm=KR;
random Pen(Rs*Rx) Time*Pen(Rs*Rx);
lsmeans Rs|Rx|Time / pdiff;
```

Code for analyzing heifer DMI by period:

```
proc mixed;
class Rs Rx Pen ;
model week1= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen ;
model week2= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) ;
lsmeans Rs|Rx / pdiff;
```

```
proc mixed;
class Rs Rx Pen ;
model week3= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen ;
model week4= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen ;
model week5= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen;
model week6= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen ;
model week7= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen;
model week8= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen;
model week9= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen;
model week10= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
```

```
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen;
model week11= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx);
lsmeans Rs|Rx / pdiff;
```

Code for analyzing heifer feed efficiency:

```
proc mixed;
class Rs Rx Pen Tag eff;
model eff= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;
```

Code for analyzing heifer reproductive performance:

```
proc glimmix;
class Tag Pen Rs Rx;
model AIbred= Rs|Rx Sire /ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc glimmix;
class Tag Pen Rx Rs;
model Seasonlong= Rx|Rs /ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rx|Rs / pdiff;

proc mixed;
class Pen Rs Rx;
model BCS=Rs|Rx;
Random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
Lsmeans Rs|Rx / pdiff;
run;

proc mixed;
class Pen Rs Rx;
model AgeofPub=Rs|Rx;
Random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
Lsmeans Rs|Rx / pdiff;
run;

proc glimmix;
```

```
class Tag Pen Rx Rs;
model daysinseason= Rx|Rs /ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rx|Rs / pdiff;
```

Code for analyzing heifer exit velocity by period:

```
proc mixed;
class Rs Rx Pen Tag;
model week1= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week2= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week3= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week4= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week5= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week6= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week7= Rs|Rx/ddfm=KR;
```

```
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;

proc mixed;
class Rs Rx Pen Tag;
model week8= Rs|Rx/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx);
lsmeans Rs|Rx / pdiff;
```

Code for analyzing heifer overall velocity:

```
proc glimmix;
class Trt Pen Tag Time;
model velocity= Trt|Time/ddfm=KR;
random Pen(Trt) Tag(Pen*Trt) Time*Pen(Trt);
lsmeans Trt|Time / pdiff;
lsmeans trt*time / plot=meanplot (sliceby=trt join);
slice Trt*Time/sliceby=Time pdiff;

proc print;
proc glimmix;
class Rs Rx Pen Tag Time;
model velocity= Rs|Rx|Time/ddfm=KR;
random Pen(Rs*Rx) Tag(Pen*Rs*Rx) Time*Pen(Rs*Rx);
lsmeans Rs|Rx|Time / pdiff;
```

SAS Code for Chapter III

Code for analyzing bull ADG for yr 1 and yr 2 with repeated measures:

```
proc mixed;
class Tag MMtrt day;
model ADG= IBW day|MMtrt  /ddfm=KR;
repeated day/subject=tag(MMtrt) type=sp(pow) (day) r rcorr;
random tag;
lsmeans day|MMtrt / pdiff;
```

Code for analyzing bull BW gain for yr 1 and yr 2 with repeated measures:

```
proc mixed;
class Tag MMtrt day;
model BW= IBW MMtrt|day  /ddfm=KR;
repeated day/subject=tag(mmtrt) type=sp(pow) (day) r rcorr;
random tag;
lsmeans MMtrt|day / pdiff;
```

Code for analyzing bull ADG and BW gain for yr 1 without repeated measures:

```
proc mixed;
class Tag MMtrt;
model wwd0= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model wt64d= MMtrt wwd0 /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model wt113d= MMtrt wwd0 /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model wt144d= MMtrt wwd0 /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model adg064= MMtrt wwd0 /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model adg64113= MMtrt wwd0 /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model adg113144= MMtrt wwd0/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
```

```
model overalladg= MMtrt wwd0 /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

Code for analyzing bull ADG and BW gain for yr 2 without repeated measures:

```
proc mixed;
class Tag trt;
model InWtKg= Trt /ddfm=KR;
random tag;
lsmeans trt / pdiff;

proc mixed;
class Tag trt;
model d64Wt= Trt Sire  /ddfm=KR;
random tag;
lsmeans trt / pdiff;

proc mixed;
class Tag trt;
model ADGd064= Trt Sire /ddfm=KR;
random tag;
lsmeans trt / pdiff;

proc mixed;
class Tag trt;
model d110Wt= Trt /ddfm=KR;
random tag;
lsmeans trt / pdiff;

proc mixed;
class Tag trt;
model d113Wt= Trt /ddfm=KR;
random tag;
lsmeans trt / pdiff;

proc mixed;
class Tag trt;
model ADG64113= Trt AgeAtBSE /ddfm=KR;
random tag;
lsmeans trt / pdiff;

proc mixed;
class Tag trt;
model FinalWtKg= Trt Sire InWtKg /ddfm=KR;
random tag;
```

```
lsmeans trt / pdiff;

proc mixed;
class Tag trt;
model ADG113148= Trt /ddfm=KR;
random tag;
lsmeans trt / pdiff;

proc mixed;
class Tag trt;
model OverallADG= Trt Sire /ddfm=KR;
random tag;
lsmeans trt / pdiff;
```

Code for analyzing bull CASA results in yr 1:

```
proc mixed;
class Tag MMtrt;
model mot= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model prog= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model vap= MMtrt|sire /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model vsl= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model vcl= MMtrt|sire /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

```
proc mixed;
class Tag MMtrt;
model alh= MMtrt|sire/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model bcf= MMtrt|sire /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model str= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model lin= MMtrt|sire/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model vol= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model conc= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

Code for analyzing bull CASA results in yr 2:

```
proc mixed;
class Tag MMtrt;
model mot= MMtrt SC /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
```

```
model prog= MMtrt SC /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model vap= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model vsl= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model vcl= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model alh= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model bcf= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model str= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model lin= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

```
proc mixed;
class Tag MMtrt;
model vol= MMtrt SC /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model conc= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

Code for analyzing bull BSE results in yr 1:

```
proc mixed;
class Tag MMtrt;
model sc= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model mot= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model norm= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model pri= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model sec= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

Code for analyzing bull BSE results in yr 2:

```
proc mixed;
class Tag MMtrt;
model sc= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model mot= MMtrt SC /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model norm= MMtrt SC /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model pri= MMtrt SC  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model sec= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

Code for analyzing bull motility correlation yr 1:

```
proc corr;
var Gmot sire age finalwt sc fmot;
run;

proc corr spearman;
var Gmot sire age finalwt sc fmot;
run;
```

Code for analyzing bull motility correlation yr 2:

```
proc corr;
var Gmot sire age finalwt sc fmot;
run;
```

```
proc corr spearman;
var Gmot sire age finalwt sc fmot;
run;
```

Code for analyzing bull morbidity:

```
proc glimmix;
class Tag MMtrt;
model zerotrt= MMtrt year /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model onetrt= MMtrt year /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model twotrt= MMtrt year /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model threetrt= MMtrt /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T1Lame= MMtrt /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T1Resp= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T1Other= MMtrt /ddfm=KR;
random tag;
```

```
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T2Lame= MMtrt /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T2Resp= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T2Other= MMtrt /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T3Lame= MMtrt /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T3Resp= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T3Other= MMtrt /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;
```

Code for analyzing heifer reproductive performance:

```
proc glimmix;
class Tag MMtrt;
model AIBRED= MMtrt Sire/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

```
proc glimmix;
class Tag MMtrt;
model SLPREG= MMtrt sire/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model DaysinSeason= MMtrt /ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc mixed;
class Tag MMtrt RxRsTrt;
model BCS= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt RxRsTrt;
model ageofpuberty= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

Code for analyzing heifer feedlot performance:

```
 proc mixed;
class Tag MMtrt RxRsTrt;
model initialwtkg= MMtrt  /ddfm=KR;
random tag pen;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt RxRsTrt;
model finalwtkg= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt RxRsTrt;
model overalladg= MMtrt initialwtkg finalwtkg /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
```

```
class Tag MMtrt RxRsTrt;
model avgdmi= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt RxRsTrt;
model overallgf= MMtrt RxRsTrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

Code for analyzing heifer morbidity:

```
proc glimmix;
class Tag MMtrt;
model zerotrt= MMtrt initialwt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model onetrt= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model twotrt= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model threetrt= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t1lame= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T1resp= MMtrt/ddfm=KR;
```

```
random tag;
lsmeans MMtrt / pdiff;


proc glimmix;
class Tag MMtrt;
model t1other= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t2lame= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T2resp= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t2other= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;
```

Code for analyzing steer performance:

```
proc mixed;
class Tag MMtrt;
model wwkg= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model initialwt= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model midwt= MMtrt /ddfm=KR;
random tag;
```

```
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model finalwt= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model daysonfeed= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model totalgain= MMtrt initialwt finalwt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc mixed;
class Tag MMtrt;
model adg= MMtrt finalwt daysonfeed totalgain  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

Code for analyzing steer morbidity:

```
proc glimmix;
class Tag MMtrt;
model zerotrt= MMtrt  /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model onetrt= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model Twotrt= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;
```

```
proc glimmix;
class Tag MMtrt;
model Threetrt= MMtrt /ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t1lame= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T1resp= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t1other= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t2lame= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T2resp= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t2other= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t3lame= MMtrt/ddfm=KR;
random tag;
```

```
lsmeans mmtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model T3resp= MMtrt/ddfm=KR;
random tag;
lsmeans MMtrt / pdiff;

proc glimmix;
class Tag MMtrt;
model t3other= MMtrt/ddfm=KR;
random tag;
lsmeans mmtrt / pdiff
```