# TRACE MINERAL SUPPLEMENTATION IN CATTLE: IMPLICATIONS IN HEIFER DEVELOPMENT, REPRODUCTIVE PERFORMANCE, AND FETAL PROGRAMMING

BY

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DISSERTATION

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## ABSTRACT

Minerals play numerous roles in the ruminant animal's body and are included as components of bone, tissues, body fluids, enzymes, and hormones. Grazing cattle primarily receive trace minerals through forages; however, these sources often do not meet the trace mineral requirements of cattle due to variation in soil composition. In these instances, producers commonly supplement trace minerals through free-choice mineral and salt blocks or protein/energy supplements fortified with trace minerals to ensure optimal cattle performance and health. The interactions that trace minerals can have on animal production are complex and multiple factors can impact an animal's response to mineral supplementation. The objective of this dissertation was to evaluate alternative trace mineral supplementation strategies and the effects these minerals may have on heifer development, reproductive success, fetal growth, and long term productivity of beef cattle.

Recently data have suggested that organic or chelated trace minerals may improve growth, reproduction, and health traits in ruminants. To evaluate the effect of supplementing two different chelated trace mineral sources on reproductive performance of beef cows, 204 spring-calving, Angus and Simmental × Angus cows [body weight (**BW**) = 649 ± 129 kg] were utilized. Cows received 1 of 2 glycine ligand chelated trace minerals, both formulated to replace 50% of the Cu, Mn, and Zn inorganic trace mineral (**MAAC**; MAAC, Novus International; **TRAX;** B-Traxim 2C, Pancosma). Liver mineral concentrations were not different ($P \geq 0.11$) regardless of treatment. Liver metallothionein (**MT**)/actin expression was not different ($P \geq 0.24$) at trial initiation or at breeding. Interestingly, TRAX cattle did have greater ($P = 0.03$) MT/actin expression comparted to MAAC cattle at the time of final pregnancy confirmation. There was no effect ($P = 0.91$) of supplementation on artificial insemination (**AI**) conception rate

(MAAC=72.2% and TRAX=71.2%). Interestingly, overall pregnancy rate was greater ($P = 0.03$) for TRAX (98.4%) compared to their MAAC (90.1%) counterparts. Supplementing beef cows with B-Traxim 2C prior to breeding improved overall pregnancy rates but did not alter BW or trace mineral status.

Injectable trace minerals offer another unique way to supplement trace minerals. Three experiments were conducted at separate locations to determine the effects of a trace mineral injection (**TMI**), Multimin 90, on heifer performance and reproduction. In Exp. 1, (spring-born, Angus, n = 93, BW = 428 ± 45.2 kg), Exp. 2 (spring-born, Angus × Simmental, n = 120, BW = 426 ± 54.0 kg), and Exp. 3 (fall-born, commercial Angus, n = 199, BW = 345 ± 39.7 kg) heifers were assigned to 1 of 2 treatments: a control, saline injection, or TMI at a dose of 1 mL/68 kg BW. Injections were given 33 d prior to breeding at the initiation of a 14-d controlled internal drug release (**CIDR**)-prostaglandin protocol. In Exp.1 pregnancy rates to timed AI and overall pregnancy rates were similar ($P \geq 0.74$) regardless of treatment. During Exp. 2, there was a tendency ($P = 0.07$) for TMI heifers to have an increased AI pregnancy rate (62% vs. 45%) compared with control heifers despite no difference ($P = 0.51$) in overall pregnancy rate. In Exp. 3, there were no differences ($P \geq 0.50$) in AI and overall pregnancy rates.

An additional experiment was conducted to determine the effects of repeated TMI on heifer development and reproductive performance. Commercial Angus heifers (n = 290; 199 ± 34.3 kg; 221 ± 22 d of age) were administered an injectable trace mineral (**MM;** Multimin90) or saline (**CON**) given subcutaneously, post-weaning at 221, 319, 401, and 521 ± 22 d of age. Plasma Mn and Zn concentrations did not differ ($P \geq 0.54$). However, MM heifers had greater ($P \leq 0.01$) plasma and liver concentrations of Cu and Se compared to CON. Interestingly, MM decreased ($P = 0.02$) liver Zn concentrations compared to CON, and there was no difference ($P =$

0.60) in liver Mn. Antral follicle count and ovarian size did not differ ($P \geq 0.51$) due to treatment. Throughout development, number of heifers cycling was lesser ($P < 0.01$) for MM than CON heifers. However, there was no difference ($P \geq 0.19$) in reproductive tract scores (**RTS**), AI pregnancy rates, or overall pregnancy rates. Commercial Angus heifers (n = 190; 315 ± 49.3 kg) from the previous experiment, that were confirmed pregnant, were utilized to determine the effects of trace mineral injections during gestation on heifer and subsequent calf performance. Treatments were maintained and subsequent injections were given 205, 114, and 44±26 d prepartum. Data were reported from 174 calves (n = 87 calves/treatment). Multimin heifers tended ($P = 0.08$) to have greater initial liver Se and tended to have decreased ($P = 0.08$) initial liver Zn compared to CON. At calving, MM cows had increased ($P \leq 0$ .01) liver Cu and Se. There was no difference ($P \geq 0.47$) in Julian calving date, calving percent or unassisted births. Calf birth BW was lesser ($P = 0.02$) for MM than CON calves and MM calves had greater ($P = 0.03$) liver Cu concentrations at birth compared to CON. Despite MM cows having increased ($P < 0.01$) milk production, calf weaning BW and average daily gain (**ADG**) were not different ($P \geq 0.87$). Additionally, calf morbidity and mortality were not different ($P \geq 0.43$) between treatments. Calf mineral status was not different ($P \geq 0.57$) at the time of weaning regardless of treatment; however, MM cows had decreased (P = 0.03) liver Zn. Multimin cows had decreased ($P = 0.05$) AI pregnancy rates, yet, there was no difference ($P = 0.34$) in overall pregnancy rate.

Twenty-four commercial Angus steers (BW = 204 ± 19 kg; 12 MM steers and 12 CON) from the previous experiment, were utilized to determine the effects of maternal supplementation with an injectable trace mineral on the inflammatory response of calves subjected to a lipopolysaccharide (**LPS**) challenge at the initiation of a 42 d receiving period. Initial plasma Zn

tended ($P = 0.06$) to be greater for MM steers. However, there was no difference ($P \geq 0.31$) in trace mineral status or serum cortisol at any other time. Total area under the curve (**TAUC**) for body temperature was lesser ($P > 0.01$) for MM steers. Basal LPS binding protein (**LBP**) concentrations and TAUC for LBP tended ($P \leq 0.10$) to be greater for MM steers. Peak concentration of interleukin-1β (**IL-1β**) tended ($P = 0.09$) to be reached earlier for CON steers. However, there was no difference ($P \geq 0.15$) in glucose, insulin, and interleukin-6 (**IL-6**), concentrations regardless of treatment. Additionally, calf performance and feed efficiency were not different ($P \geq 0.17$) between treatments except ADG from d 28 – 42, which was greater ($P = 0.03$) for CON steers.

In summary, additional injectable trace mineral supplementation in developing beef heifers resulted in varied reproductive responses even when provided adequate trace mineral. Supplementing an injectable trace mineral during heifer development and gestation did result in increased cow milk production. However, there was no effect on overall cow pregnancy rates or pre-weaning calf health or performance. Additionally, maternal supplementation resulted in altered body temperature and LBP production in subsequent calves when exposed to an inflammatory challenge. Due to the difficulty of assessing trace mineral status of an entire herd, supplementing trace minerals through an injection may be a viable way to ensure a consistent, adequate trace mineral supply to heifers for optimal development, reproductive success, and subsequent offspring performance. While mineral status and reproductive responses across these experiments were variable, it is important to note that injectable trace minerals do not appear to incur any negative impacts on beef heifer reproductive success or subsequent calf performance.

## DEDICATION

In loving memory of my mom. You touched my life with grace and laughter. This work is dedicated to the strong, brave, wonderful woman you were and that I one day aspire to be.

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*"Commit thy works unto the Lord, and thy thoughts shall be established"* - Proverbs 16:3

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# CHAPTER 1

# LITERATURE REVIEW

## INTRODUCTION

The USDA/NASS (2017) reports that as of January 1, 2017 both the number of beef heifers and beef cows had increased since 2016, with an estimated 6.42 million head of replacement beef heifers and 31.2 million head of beef cows in the United States. The development of these heifers to productive beef cows represents a substantial economic impact to the producer, and proper development can ultimately impact a heifer's reproductive success and longevity within the herd. Diet and plane of nutrition during development have been linked to physiological changes that result in attainment of puberty in heifers. While maximizing pregnancy rates are a key component of heifer development, profitability and sustainability should also be considered. Funston (2012) reviewed aspects of developing replacement heifers and concluded that systems should result in a sound, low-cost pregnant female and that development costs were a major determinant of lifetime cow profitability.

Pregnancy rates in heifers can be impacted by nutrition (Larson, 2007), post weaning growth rate (Dufour, 1975), and age and weight at puberty (Wiltbank et al., 1966). Additionally, heifers that calve earlier in the first calving season have been shown to have greater lifetime productivity compared to their counterparts calving later in the season (Lesmeister et al., 1973). Nutritional demands of heifers continue to be increased during gestation because nutrients are partitioned between her own continued growth and the development of the fetus. Deficiency of key nutrients for extended periods during this time can have lasting negative impacts on heifer development, calf viability and long term reproductive success.

Seventeen minerals are required in beef cattle and these minerals can be classified as either macro- or microminerals. Minerals play numerous roles in the ruminant animal's body and are included as components of bone, tissues, body fluids, enzymes, and hormones. Microminerals are commonly referred to as trace minerals and include Cr, Co, Cu, I, Fe, Mn, Mo, Ni, Se, and Zn (NASEM, 2016). Trace minerals are present in the body in very low concentrations are required in milligram or microgram amounts. Grazing cattle primarily receive trace minerals through forages, however these sources often do not meet the trace mineral requirements of cattle due to variation in soil composition (Smart et al., 1981). Additionally, the bioavailability of these trace mineral sources can vary due to interactions with other minerals and feed components within the gastrointestinal tract (Spears, 2003). In these instances, producers commonly supplement trace minerals through free-choice mineral and salt blocks or protein/energy supplements fortified with trace minerals (Arthington et al., 2014) to ensure optimal cattle performance and health. The interactions that trace minerals can have on animal production are complex and multiple factors can impact an animal's response to mineral supplementation. Four key trace minerals, Cu, Mn, Se, and Zn have been the focus of recent literature and further research is needed to elucidate the effects these minerals may have on heifer development, reproductive success, fetal growth, and long term productivity.

## ASSESSING TRACE MINERAL STATUS IN RUMINANTS

Assessment of trace mineral status in ruminants is crucial for determining if a nutrient deficiency exists and to estimate the reserves an animal may have available for biological processes. Assessment of status also allows producers and researchers to determine the success and efficacy of mineral supplementation. The most common and well-studied methods of assessing mineral status include assessing mineral concentrations in the blood (whole blood,

plasma, and serum) or concentrations in the liver (Kincaid, 2000). To date measuring trace elements in body tissues can be quite difficult; however, modern analytical techniques are allowing for increased accuracy and making assessment practical from a diagnostic standpoint (Herdt and Hoff, 2011). Additionally, with increased precision and modern analytical techniques, appropriate ranges for trace mineral concentrations in bovine tissue and blood have been established (**Table 1**).

Utilizing blood to assess trace mineral status can involve testing specific components of blood (plasma, serum, or whole blood) for total mineral concentrations or it may involve the evaluation of activities of specific proteins and enzymes that require trace minerals. Total mineral concentrations are typically assessed using either inductively coupled plasma spectroscopy (ICP) or mass spectroscopy (MS). Both methods can be completed quickly with extreme sensitivity and precision. Additionally, these methods can be utilized to assess a wide array of minerals at a relatively low cost making them a particle method. Contrastingly, assessing specific enzyme or specific cofactors that contain trace minerals are very specific and sometimes can be utilized for diagnostic purposes; however, individual tests can be quite expensive and require intensive labor for analysis. Both methods of assessing trace mineral status come with immense limitations in the ruminant animal as factors other than nutrition have been shown to manipulate blood mineral and enzyme concentrations. Inflammation, physiological state of the animal, presence of antagonists, and even sampling contamination can all impact blood mineral parameters, confounding results and minimizing the impact of nutritional influence (Herdt and Hoff, 2011).

Alternatively, hepatic tissue more commonly represents the status of several trace elements in ruminants and may more consistently represent nutritional changes (Kincaid, 2000).

As little as 50 – 75 mg of wet liver tissue can reliably provide mineral concentrations. Samples can be collected using a 14-G Tru-Cut needle (Products Group International, Lyons, CO, USA) or utilizing a bone marrow biopsy probe via the methods of Engle and Spears (2000). Both processes allow sample to be quickly obtained with minimal to no risk to the patient. Samples can then be analyzed utilizing ICP and are reported on a DM basis. While hepatic mineral concentration is a more reliable indicator of when animals fall within a deficient, marginal, or adequate range, it still may not provide a functional measure of status when all concentrations are considered adequate (Herdt and Hoff, 2011). For example, liver Cu concentrations allow few conclusions to be drawn between a heifer with 150 mg/kg of liver Cu versus a heifer with 300 mg/kg of liver Cu, other than that both heifers have adequate Cu nutrition. Zinc further confounds this issue, as there are no clear storage pools of Zn in the body (Herdt and Huff, 2011). While liver Zn concentration will decline with dietary deficiency, liver Zn concentrations are rarely reflective of Zn intake (Herdt and Huff, 2011). Ultimately, further research is needed to help better assess trace mineral status of ruminant animals and to understand the impacts of minute changes in tissue mineral concentration, even when animals are considered within an adequate range.

## COPPER, MANGANESE, SELENIUM, AND ZINC: BASAL REQUIREMENTS, PHYSIOLOGICAL FUNCTIONS, AND REPRODUCTIVE IMPACTS IN BEEF CATTLE

### *Copper*

The importance of Cu and the roles it plays in many biological functions is well recognized within the literature. However, the requirements for Cu in the ruminant diet can be

quite variable ranging anywhere from 4 mg/kg to 15 mg/kg (NASEM, 2016). This variability is largely driven by the dietary concentration of Mo and S. The NASEM recommends beef cattle diets contain 10 mg/kg of Cu when S levels do not exceed 0.25% and Mo remains under 2 mg/kg. Copper status can be challenged when interactions between Cu, Mo, and S occur in the rumen and cause the formation of S-Mo compounds called thiomolybdates. Thiomolybdates can be present in multiple forms, dithiomolybdates, trithiomolybdates, and tetrathiomolybdates; which scavenge Cu and depreciate the available concentration for biochemical processes (Suttle, 1991). The ruminant's vulnerability to Cu deficiency is chiefly determined by the availability of Cu in the rumen and deficiencies are further exacerbated by the interaction between Mo and S.

Copper is a key component in the formation of many Cu-containing proteins and is required for the function of several enzymes within the body (Hurley and Doane, 1989). Perhaps the most notable of which is Cu, Zn superoxide dismutase (SOD) which catalyzes the breakdown of toxic superoxide radicals to hydrogen peroxide (Tainer et al., 1983). Copper is also a component of ceruloplasmin which serves as a copper transport protein (Hsieh and Frieden, 1975). Blakley and Hamilton (1985) determined that ceruloplasmin activity is closely correlated to both serum Cu concentrations and plasma Cu concentrations. Copper can also be found bound to albumin and other low molecular weight compounds, most notably amino acids such as histidine (DiSilvestro and Cousins, 1983; Hurley and Doane, 1989). Research has also shown that copper is an essential part of cytochrome c oxidase, which is an essential component of the electron transport chain (Hsieh and Frieden, 1975). However, research has yet to define how these Cu forms may be taken up by the reproductive tissues.

A variety of clinical symptoms can occur with Cu deficiency including infertility, anemia, and suppression of immune function (Underwood and Suttle, 1999). Hypocuprosis,

which is noted by an abnormally low level of Cu in the blood is closely associated with reproductive disorders in cattle and the most common symptoms include prenatal mortality and early embryonic loss (Hidiroglou, 1979). In cases of severe Cu deficiency, anestrus commonly occurs thus a decline in fertility is one of the earliest signs of Cu deficiency in cows (Hignett, 1960; Hidiroglue, 1979). Blakemore and Venn (1950) reported that heifers suffering from hypocupraemia first exhibited signs of decreased appetite followed by an interference in normal breeding; however, the administration of Cu sulfate induced normal breeding patterns. Additionally, Hunter (1977) selected five dairy herds of cattle ($n$ = 463), where blood samples indicated a marginal copper deficiency was present. Coprin, a copper glycinate injection was given to every other cow to calve, giving a random distribution of treatment and control cattle. Cows treated with Cu glycinate showed a 19% increase in first service conception rate. Allcroft and Parker (1949) noted similar results that feeding Cu deficient cows (blood-Cu levels 0.02 – 0.04 mg/mL), grazing Cu-deficient pastures, 2 g of Cu sulphate daily markedly improved condition and infertility issues associated with depressed estrus.

Contrastingly, Muehlenbein et al. (2001) reported that feeding supplemental Cu to cows (either organic or inorganic) did not improve 60-d pregnancy rates or the health and performance of the calves when compared with cows that did not receive supplementation. This experiment was conducted over 2 years utilizing 195 crossbred first calf heifers, and 144 crossbred cows fed a hay based diet and supplemented with either 0 mg Cu (Control), 200 mg Cu from $CuSO_4$, or 100 mg Cu from AvailaCu. Overall, Cu supplementation had no effect on the transfer of Cu or IgG to the calf and had no effect on calf health or performance even though both sources of supplementation increased liver Cu levels in cows. Even the control cattle, which were marginal in liver Cu status (Cu = 49 ± 11.9 mg/kg) at calving, produced calves that performed at normal

levels and rebred at greater levels. Other work feeding 99 prepubertal heifers a control grass hay diet with supplemental Cu at 8 ppm or Cu deficient diet (3.2 ppm) of grass hay diet with increased levels of Mo (8 ppm) and S (0.3%) also showed no difference in Cu,Zn-SOD activitiy, onset of puberty, or first-service conception rate (Arthington and Corah, 1993). However, heifers fed the Cu deficient diet did have decreased liver Cu concentrations and decreased ceruloplasmin activity. Suggesting additional research is needed to clarify the complex relationship between Cu status, conception rates, and calf performance.

Copper is also an important component in the conversion of arachidonic acid to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Maddox (1973) reported that the presence of $Cu^{+2}$ in sheep vesicular tissue stimulates this conversion and also may selectively inactivate prostaglandin $E_2$ ($PGE_2$) synthetase which then simultaneously decreases $PGE_2$ production. Interestingly, Cu has also been shown to facilitate $PGE_2$ action on LH releasing hormone neurons. Using the median eminence area of male rats, Barnea et al. (1985) showed that Cu enhances the binding of $PGE_2$ to its receptor and thus enhances the release of LH releasing hormone. Suggesting that Cu may play an important role in not only the production of $PGF_{2\alpha}$ and $PGE_2$ but also the role these hormones play in LH release. Further research is needed to determine Cu role in prostaglandin production may be contributing to the suboptimal reproductive performance commonly noted with Cu deficiencies in previous research.

*Manganese*

Manganese requirements for reproduction are considerably greater than that needed for normal growth. The NASEM (2016) states that the requirement for growing and finishing cattle is approximately 20 mg Mn/kg diet. However, this level of supplementation still may not support maximal growth, only normal skeletal development. Breeding cattle requirements are much

greater at 40 mg Mn/kg of diet (NASEM, 2016). The requirements for Mn can also be altered based on interactions with other minerals, as Ca, P, and Fe have all been shown to antagonize Mn and thus could lead to Mn deficiency via indirect effects of these minerals' interactions (Hidiroglou and Knipfel, 1981).

Manganese serves as a component or cofactor of many enzymes including pyruvate carboxylase and phosphoenolpyruvate carboxykinase which functions in gluconeogenesis, arginase which functions in the urea cycle, and farnesyl pyrophosphate synthetase which functions in cholesterol synthesis (Leach and Harris, 1997). Perhaps most notably Mn is required for the function of Mn-SOD which is primarily of mitochondrial origin (Weisiger and Fridovich, 1972; Leach and Harris, 1997). Manganese-SOD is an antioxidant enzyme important for the prevention of oxygen toxicity and autoimmune disease (Leach and Harris, 1997). Manganese also activates glycosyl transferases which are important for glycoprotein biosynthesis. Glycosyl transferases play a critical role in proteoglycan formation which is a component of cartilage tissue and important for skeletal development (Leach and Harris, 1997). Due to these critical roles, Mn deficiencies can produce a number of striking symptoms in both the dam and the neonate.

Manganese deficiency can commonly result in a suppression of estrus, reduced conception rates, increased incidences of abortions, and abnormally small birth weights (Hidiroglou, 1979). Early work by Rojas et al. (1965) showed that while Mn deficiency in Hereford cows did not affect weight gain or feed efficiency, cows on a low Mn diet (15.8 ppm Mn) required more services to establish pregnancy. Interestingly though, these cows did seem to exhibit regular estrous cycles. Additionally, calves, from cows fed a low Mn diet, had increased incidences of deformities. Wilson (1966) studied Mn deficiencies across multiple herds and

noted the main clinical signs of deficiency were infertility, extended periods of anestrus, or irregular returns to estrus. Conception rates of cattle with Mn deficiencies are commonly noted at only 35 – 40% and follicular development is often poor.

More recently, Hansen et al. (2006b) reported that when beef heifers were supplemented a minimum of 15.8 mg of Mn/ kg of diet DM, measures of reproductive performance were not significantly affected, including the onset of estrus and conception. However, further work showed that though these heifers reached normal estrus and had established pregnancies, the calves born from heifers receiving only 15.8 mg of Mn/ kg of diet DM had decreased birth weights than their counterparts born from heifers supplemented 50 mg of Mn/ kg of diet DM. Additionally, calves born from heifers with low Mn supplementation showed various signs of Mn deficiency, including swollen joints, disproportionate dwarfism, and superior brachygnathish (Hansen et al., 2006a). Suggesting that gestating heifers may require additional Mn to overcome Mn deficiencies in calves.

While the precise role Mn plays in the reproductive process remains unknown, research suggests that Mn may influence certain endocrine organs and their activity. Hidiroglou et al. (1977) utilized 16 Holstein cows, 8 of which were exhibiting normal estrous cycles and 8 which were diagnosed with ovarian cysts to determine the Mn concentrations of ovarian tissues. While there was no difference in blood Mn levels between the two groups, the cows without cystic ovaries had numerically greater Mn concentration in the corpora lutea (1515 ± 954 ng/g fresh tissue and 697 ± 461 ng/g fresh tissue for normally cycling cows and cystic cows, respectively). Additionally, for normally cycling cows, Mn concentrations were significantly greater (384 ± 99 ng/g fresh tissue) in the cortical stroma, where ovarian follicles are contained in various stages of development, than in cows with cystic ovaries (133 ± 24 ng/g fresh tissue). The authors noted

that even though cows did not appear to have a Mn deficiency, cystic ovaries is a common cause of infertility in cattle and in some cases cystic ovaries will respond to treatment with colloidal Mn (Tutt, 1934). Other work in sheep has noted that when cycling Shropshire x Suffolk ewes were injected intravenously with radioactive Mn the greatest uptake of radioactivity was found in the ovary and largely as a component of the corpus luteum and the Graafian follicle (Hidiroglou, 1975). This data suggests the ovary is an active target for Mn uptake, specifically during the estrous cycle and that Mn may play a physiological role in normal cyclic function.

The role Mn plays in reproductive disorders is not fully understood. Research has hypothesized that Mn may play a key role in both cholesterol and steroid synthesis (Benedict et al., 1965; Davis et al., 1990). Benedict et al. (1965) noted that Mn linearly increased farnesyl pyrophosphate synthetase activity, which is responsible for catalyzing the formation of farnesyl pyrophosphate. Farnesyl pyrophosphate is precursor for cholesterol which can then be converted to steroids, and steroid hormones. Furthermore, Davis et al. (1990) noted that manganese deficiency in rats decreased plasma and HDL cholesterol levels and hypothesized that this may have resulted from decreased cholesterol synthesis or secretion. This data further supports the hypothesis that Mn my play and important role in cholesterol synthesis.

*Selenium*

Selenium has been recognized as a dietary essential micromineral since 1957 (Ammerman and Miller, 1974), and the current requirement for beef cattle can be met by 0.1 mg Se/kg (NASEM, 2016). Perhaps more than other minerals, Se also comes with a major concern for toxicity. The National Research Council (1980) estimates the maximum tolerable level for Se to be 2 mg/kg and signs of acute toxicity includes blind staggers, diarrhea, ataxia, and death from respiratory failure. Both toxicity and deficiency occur naturally due to the wide varieties in

natural Se concentrations of animal feeds. Still, there are also additional factors that can affect the ruminant animal's requirement for selenium. Most notably of which is the interrelation between vitamin E and Se which can exert a sparing effect on each other; however, lipids, S, S-containing amino acids, and Ca have also been noted to have interactions with Se (Ammerman and Miller, 1974; NASEM, 2016).

Selenium plays an important role in numerous biochemical functions. Selenium is capable of modifying the expression of at least 30 selenoproteins, which are formed when selenoamino acids are incorporated into proteins in the place of S residues (Sunde, 1997; Beckett and Arthur, 2005). Selenium is a component of selenocysteine which has been called the 21$^{st}$ amino acid and Se can also replace the S in methionine to form selenomethionine (Daniels, 1996). The first known role for Se was in glutathione peroxidase, which serves as an antioxidant and converts hydrogen peroxide to water to prevent biological systems from oxidative damage (Rotruck et al., 1973). Selenium is also an integral component of type I iodothyronine 5'-deiodinase which is responsible for converting thyroxine (T4) to the active thyroid hormone, triiodothyronine (T3; Arthur, 1991). Researchers have further speculated that Se status may also exacerbate the problems associated with iodine deficiency and changes in thyroid hormone (Arthur, 1991). The consequences of selenium deficiency in ruminants is therefore likely driven not only by the inability to metabolize peroxisomes, but also impairment of thyroid hormone which can result in decreased growth.

Pronounced selenium deficiency in ruminants has been noted to reduce growth and cause nutritional muscular dystrophy (white muscle disease) in young animals and reduce reproductive performance in older animals. From a reproductive standpoint Se may have various roles. For example, Segerson et al. (1976) reported that Se supplementation to 36 beef heifers increased

both the number of fertile ova and the percent fertility. Interestingly, the authors speculated that this may be due to the lack of transport of sperm to the site of fertilization, as no spermatozoa were within or surrounding the zona pellucida. Selenium has also been shown to assist with uterine involution (Harrison et al., 1986). Harrison et al. (1986) utilized 78 dairy cows and treated them with either 0.1 mg/kg of Se via an i.m. injection 21 d pre calving or with no additional Se and reported that Se treated cows reached minimum uterine size in cows exhibiting metritis significantly faster than control cows also exhibiting metritis. Other work by Harrison et al. (1984) has noted that not only does Se exert a role in uterine involution, but also may effect incidences of cystic ovaries in dairy cows. Utilizing 78 dairy cows treated with either 0.1 mg/kg Se via an intramuscular injection or no additional Se, the authors noted that only 60% of Se treated cows exhibited metritis and only 19% exhibited cystic ovaries, whereas 84% of the non-treated cows exhibited metritis, and 47% exhibited cystic ovaries. Additionally, Se-deficient animals are consistently noted to have decreased sperm production and poor sperm quality (Becket and Arthur, 2005).

Selenium supplementation has been noted in several incidences to minimize the number of retained placentas within herds, especially when the prevalence of retained placentas was already increased. Julien and Conrad (1976) increased Se in diets of Se deficient dairy cows and reduced the overall incidence of retained placentas from 38% to 0%. Other work by Eger et al. (1985) noted that injecting multiparous and primiparous Holstein cows with Se ranging from 2.3 to 23 mg 3 weeks prepartum reduced the incidences of retained placentas by almost half. Additionally, the authors noted that lesser doses of Se (2.3 to 4.6 mg) tended to be the most effective and that Se alone was as effective as the combination of Se and vitamin E. It should be

noted though that the herds utilized in this trial had a history of 17% retained placentas in primiparous animals and 28% in multiparous animals in the previous 12 months.

Contrastingly, Moeini et al. (2009) injected Holstein heifers with either 0, 20, or 40 mL of Se and vitamin E supplements (each mL contained 0.5 mg Se and 50 IU of $_{D, L}$ – alpha-tocopheryl acetate) four and two weeks pre calving and noted no incidences of retained placentas regardless of treatment. However, the authors did note that heifers receiving 20 or 40 mL of Se and vitamin E exhibited fewer days open and that heifers receiving 40 mL also required fewer number of services per conception compared to the control heifers supplemented with 0 mL Se and vitamin E. Also, heifer supplemented with 40 mL of Se and vitamin E had increased milk production at 8 weeks of lactation and decreased somatic cell counts compared to controls. It is important to note that previous research suggests that differences in retained placentas are typically only noted in herd where incidence of retained placentas was already increased, so though this data does not note differences in retained placenta, and it appears that Se is still offering reproductive benefits to these dairy heifers.

The exact mechanism by which Se is manipulating reproductive performance is not known. However, researchers have speculated that Se may be incorporated into glutathione peroxidase where it protects the ova from oxidative damage thus positively impacting fertilization (Hurley and Doane, 1989). Additionally, Se may influence uterine contractions that assist with moving sperm towards the oviduct, which presumably would increase fertility. Segerson et al. (1980) elucidated that Se is not only more important than vitamin E in influencing uterine motility and contraction velocity, but also that Se supplementation increased the total number of uterine contractions and the velocity of contractions. Interestingly, Se treatment also resulted in contractions migrating toward the oviduct that were two times that of

the un-supplemented control animals. Interestingly, data has also shown that Se in the form of glutathione peroxidase may modulate the conversion of arachidonic acid to prostaglandins (Reddanna et al., 1989), suggesting glutathione peroxidase may have additional roles beyond that of simple antioxidant defense. Harrison and Conrad (1984) supplemented dairy cows with 5 mg of Se/d for 10 days and noted that selenium concentration in whole blood, plasma, liver, and the ovary increased compared to un-supplemented cattle. Also, Se dependent glutathione peroxidase activity was significantly increased in the luteal tissue of the ovary, which the authors speculate may suggest a linear relationship between Se and Se dependent glutathione peroxidase in ovary, uterus, and adrenal tissues.

*Zinc*

Literature first established Zn as an essential mineral for laboratory animals in the 1930's (Miller, 1981). The NASEM (2016) recommended requirement for zinc in beef cattle diets is currently 30 mg Zn/kg of diet. However, it is important to note that this may not be adequate to meet the requirements for milk production and reproduction as these requirements are less clearly defined. Zinc toxicity is not usually of a concern in the ruminant animal, except in instances when galvanized containers are used, specifically in acidic conditions (Miller, 1970). Though the amount of Zn necessary to cause toxicity is much greater than the requirements, the National Research Council (1980) defines the maximum tolerable concentration for Zn as 500 mg/kg of diet.

Zinc deficiency has been shown to contribute to multiple symptoms and reduced efficiency in cattle. Miller and Miller (1962) fed male Holstein calves low zinc diets with no zinc supplementation to induce deficiency and described the syndromes and effects on calves. Deficiency was first noted after 11 weeks of age and symptoms included inflammation around

the nose and mouth, unthrifty appearance, poor hair coat quality, stiffness of the joints, dry scaly skin, gnashing of teeth, alopecia, parakeratosis, decreased testicular development, decreased average daily gain, and decreased feed intake. The authors also noted that the addition of 260 ppm of Zn to deficient calves' diets reversed all conditions listed above with the exception of the underdeveloped testicles. While these conditions of deficiency may not occur under practical feeding conditions, it is important to note the severe consequences that result from Zn deficiency. Additionally, it is possible that even borderline deficiencies, which are more likely to occur under common production practices, may contribute to decreased performance and efficiency.

Zinc is the most abundant intracellular trace mineral and is only second to iron in overall abundance in the body (Herdt and Hoff, 2011). The functions of Zn are numerous and perhaps more notable than any other trace mineral. Zinc is required for over 2,000 transcription factors and almost every metabolic or signaling pathway is dependent of a zinc requiring protein (Suttle, 2010). Some of the most notable functions of Zn include being required in Cu/Zn superoxide dismutase (Tainer et al., 1983) and zinc finger domains in DNA binding proteins (Berg, 1990). The four most important functions of Zn may be those which limit health and production of the ruminant animal: Gene expression, appetite control, fat absorption, and antioxidant defense (Suttle, 2010).

Little is known regarding how Zn may play a role in the function of reproductive tissues. Though the role Zn plays in male reproduction and development has been well studied, minimal work has been conducted in regards to Zn deficiencies and reproductive performance in the cow. Campbell and Miller (1998) utilized dairy heifers and supplemented them with either 0 or 0.8 g of Zn and noted that days to first estrus was reduced and days to first AI tended to be reduced for heifers supplemented with Zn. However, Zn supplementation had no effect on AI conception;

however, the authors speculated that Zn may still improve reproductive performance. Other work in sheep has noted that when ewes are supplemented 0, 50, 100, or 150 ppm of Zn oxide all ewes receiving Zn exhibited increased incidence of estrus and significantly increased pregnancy rates and lambing rates (Abdel Monem and El-Shahat, 2011). Zinc supplementation also resulted in a shorter onset of estrus comparted to their un-supplemented counterparts. Additionally, when ewes were supplemented with 100 or 150 ppm of Zn laparoscopic examination revealed they had greater population of large follicles and greater ovulation rates than those treated with 50 or 0 ppm of Zn.

Apgar and Fitzgerald (1985) reported the effects of feeding 30 primiparous Finn cross ewes either a low Zn diet (<1 ppm Zn; n = 15) or a supplemental Zn diet (20 ppm Zn; n = 15) for 22 weeks. Ewes fed the unsupplemented Zn diet had decreased plasma Zn compared to the supplemented ewes. Additionally, the authors reported that of the unsupplemented ewes, one was not pregnant, three aborted, one resorbed, one delivered mummified twins, two delivered malformed lambs, and only 3 lambs were healthy enough to survive. Signs of deficiency were also noted in the ewes not receiving Zn as they exhibited cracked, bleeding and rough scaly skin. This data suggests that low Zn throughout pregnancy has severe negative impacts on reproduction and that poor Zn status may help to explain reproductive problems.

Zinc has been recognized for several decades as indispensable for sexual functions in the male animal, and deficiencies can result in various malformations and negative effects on fertility (Hidiroglou, 1979). There are several hypothesis in regards to how Zn may interact with male reproduction, including being an essential component of enzymes involved in steroid production, influencing gonadotropic hormones either indirectly through the pituitary or directly in the gonads and prostate glands (Hurley and Doane, 1989). During cases of Zn deficiency,

retarded testicular development has been noted, as well as atrophy of tubular epithelium and decreased Zn concentration of the testis, epididymis, and dorsolateral prostate (Hidiroglou, 1982). However, many of the roles Zn plays in the male reproductive system are complex and scarcely understood.

Different levels of Zn have been shown to positively impact both quantitative and qualitative attributes of semen. Kumar et al. (2006) utilized 16 crossbred bulls and supplemented them with either 0, 35 or 70 ppm Zn from Zn sulfate to assess the effects of Zn on semen quality. After 6 months of Zn supplementation, mean ejaculate volume, semen volume, live sperm, and motility were greater for Zn-supplemented groups compared to their control counter parts. Additionally, all Zn-supplemented bulls had improved sperm functional ability compared to the control bulls. The bulls supplemented with 70 ppm also had greater testosterone concentrations in blood serum compared to the control group. The authors noted that Zn supplementation appeared to positively impact numerous attributes of semen when supplemented for at least 6 months.

Contrastingly, Pitts et al. (1966) noted the effects of Zn deficiency on reproduction in Holstein bulls. Bulls were either severely Zn deficient, or supplemented with adequate amounts of Zn in the diet for 21 weeks. The deficiency did result in decreased testicle size compared to the supplemented bulls. However, once the deficiency was removed, by 64 weeks, there was no difference in testicle size. Additionally, there were no effects of treatment on sperm concentration, total volume of semen produced, total number of spermatozoa produced, and percentage motile. Thus in contrast to the previous experiment Zn had little to no effect on reproductive performance. However, it is important to note that in this experiment supplementation only lasted 21 weeks, whereas in previously mentioned experiments

supplementation occurred for a minimum of 24 weeks before any differences were noticed. While, it is clear that Zn plays a key role in male reproduction and performance, more research is needed to elucidate the mechanisms and intricate roles Zn plays in both the male and female animal. Additionally, further research needs to be conducted to better determine optimal dietary amounts of Zn required for maximum reproductive performance.

## FETAL PROGRAMMING AND MATERNAL TRACE MINERAL SUPPLEMENTATION

The concept of developmental or fetal programming suggests that a maternal stimulus that alters the fetal environment could have long-term effects on the offspring (Funston et al., 2010). Growth and development of a fetus can be influenced by multiple factors including genetics, environment, maternal maturity, and nutrition (Wu et al., 2006). Furthermore, the restriction of fetal growth not only reduces survival of the neonate but also can have long term negative effects on body composition, growth, feed efficiency, health, and performance (Wu et al., 2006). While the effects of maternal macro nutrient restriction are perhaps the most studied and have been reviewed numerous times in the literature (Funston et al., 2010; Robinson et al., 1995; Wu et al., 2006), little research has been conducted evaluating the effects of maternal trace mineral supplementation on subsequent calf performance and health.

### *Placental Transfer of Trace Minerals*

Copper status of the cow may be impacted by the demand for Cu by the fetus as Gooneratne and Christensen (1988) noted that fetal liver Cu concentrations are markedly greater (202 mg/kg of Cu and 391 mg/kg of Cu at d 30-59 of pregnancy and at d 240-270 of pregnancy, respectively) at all stages of pregnancy than dam liver Cu concentrations (50.7 mg/kg of Cu and

18.8 mg/kg of Cu at d 30-59 of pregnancy and at d 240-270 of pregnancy, respectively). The authors also noted that fetuses had a greater liver Cu level when the dam's liver Cu was greater than 25 mg/kg DM than fetuses from dams that had a liver Cu level less than 25 mg/kg DM. Suggesting additional importance in adequate Cu supplementation in pregnant cattle to maintain fetal Cu status. Small (1996) utilized 26 Hereford cross multiparous cows and primparous heifers to further investigate the effect parturition may have on serum mineral concentration in beef cattle and noted that serum Cu levels were lesser (Serum Cu = 7.44 µmol/ L) at parturition than at 7 d prior to or 7 d post calving (Serum Cu = 9.05 and 10.33 µmol/ L, respectively).

Other work has noted that dam's plasma Cu concentrations may actually decrease in the weeks leading up to parturition. Xin et al. (1993) treated 18 multiparous Holstein cows with three levels of dietary Cu, 5.5 ppm of Cu (control), 10 ppm of Cu, and 20 ppm of Cu, and assessed the changes of Cu concentrations in the blood and liver from 8 weeks prepartum to 8 weeks postpartum. Plasma Cu concentrations exhibited a quadratic response as a function to week, with the least plasma Cu concentration occurring 5 weeks prior to parturition; however liver Cu concentrations declined continuously and the least concentration was noted at parturition. Interestingly, supplementing either 10 or 20 ppm of seemed to mitigate this dramatic decrease in cow liver Cu concentration. The authors also speculated that this decrease in Cu concentration noted in the last period of pregnancy may largely be driven by the demand of the fetal liver. Work by Widdowson et al. (1974) showed increased concentrations of ceruloplasmin in maternal serum and noted no Cu in the form of ceruloplasmin in the fetus. The authors presumed that only non-ceruloplasmin fractions pass to the fetus and that it is only after birth that the neonate synthesizes ceruloplasmin for itself.

Manganese plays a clear and important role in neonate development, however little research has been conducted in regards to how Mn passes across the placenta and to the fetus in ruminants. A majority of the work centering on Mn placental transfer has occurred in swine. Gamble et al. (1971) issued 15 Duroc gilts intravenous injections of radioactive Mn and slaughtered gilts at 3, 6, 120, and 168 h after dosing to measure the radioactive Mn concentration in the placenta, placental fluids, and fetuses. By 3 h post Mn administration the placenta contained 96% of the radioactive Mn. However at 6 h post administration the placenta contained only 82% of the radioactive Mn and by 120 h post administrations the placenta contained only 12% of the radioactive Mn while the fetuses contained 87% and the placental fluids contained 0.3%. This suggests that Mn is rapidly taken up by the placenta and transferred to the fetus and that placental fluids likely contribute very little to this process. Hansard (1972) completed a similar experiment comparing the placental transfer of Mn in swine, cattle, and sheep and reported that placental transfer rate of radioactive Mn was faster in ruminants than for swine. Also, the authors reported that 15% of the radioactive Mn was absorbed by the cows, and of that 70% of the Mn was ultimately deposited in the fetus after 168h. Though research in ruminants is limited these data further suggest Mn uptake by the fetus is rapid and this placental transfer likely plays an important role in fetus development.

Though the mechanism still remains unknown, research has also suggested that Se may efficiently pass through the placenta to the neonate. Van Saun et al. (1989) utilized 101 pregnant dairy cows to determine the relationship between maternal and fetal Se status. The authors noted that fetal liver Se concentration was greater than corresponding liver Se concentration of the dam, which they suggest supports the hypothesis of efficient placental transfer of Se. Additionally, Pavlata et al. (2003) noted that in herds of cattle both adequate and deficient in Se

status that no correlation exists between cow blood Se concentrations and colostrum Se concentrations, suggesting that colostrum is not a suitable medium for assessing Se status and additionally that colostrum may play a minimal role in Se transfer to the calf.

Additionally, little research has been conducted in regards to Zn and placental transfer to the fetus, studies have been conducted investigating the differences in plasma Zn concentrations of both pregnant and non-pregnant cattle over time. Dufty et al. (1977) reported that little difference in plasma Zn was noted in non-pregnant Hereford heifers at different stages of the estrus cycle. Contrastingly, pregnant animal's plasma Zn status remained relatively stable until late pregnancy and then decreased as much as 15.9%. The authors speculated that these differences could be a response to the stress of parturition and the onset of lactation. Still, further work is needed to determine the influence Zn has on the performance of cattle during pregnancy and how Zn is transported and impacts the fetus.

*Maternal Trace Mineral Supplementation*

In an effort to assess the effects of maternal Cu supplementation on calf health and performance, Muehlenbein et al. (2001) utilized 195 crossbred beef cows supplemented with either a control, containing no supplemental Cu, an inorganic Cu (200 mg Cu from $CuSO_4$), or an organic Cu (100 mg Cu from AvailaCu). Treatments were administered late gestation, approximately 60 d prior to calving. Liver biopsies were collected from calves 10 d post calving and no difference was reported for liver Cu concentrations regardless of treatment. It is important to note that dam liver Cu concentrations were also not different at the time of calving regardless of treatment. Additionally, there was no difference in colostrum or milk Cu concentrations. Similarly, there were no differences in calf growth or health from calving until the time of weaning. Interestingly though, the authors noted that in the first year of the experiment cows in

the control group had decreased IgG concentrations in colostrum compared to those cows supplemented with inorganic Cu. The authors also reported that in year one, calves from the dams supplemented with organic Cu had the greatest serum IgG concentrations even though their dam's IgG concentration in colostrum was not different from controls. However, this response was reversed in the second year with calves from dams supplemented with organic Cu exhibiting the lowest IgG serum concentrations. The authors did note that while all calves' serum IgG concentrations were considered within normal ranges the lack of significant health differences across treatments made drawing conclusions regarding the overall effect of Cu supplementation on calf immunity challenging.

Jacometo et al. (2015) also assessed the effects of maternal organic trace mineral supplementation during late gestation on the growth and the immune system of calves. Multiparous Holstein cows (n = 40) were administered an oral bolus once daily of either inorganic or organic Zn (75 mg/kg), Mn (65 mg/kg), Cu (11 mg/kg), and Co (1 mg/kg), 30 d prior to parturition. At the time of birth all calves were fed and managed similarly allowing any differences to be attributed solely to maternal mineral supplementation. Calf performance and evaluation of inflammation markers were assessed in the first 21 d of age. Supplementation of maternal organic trace minerals had no effect on calf BW. Interestingly, calves from cows supplemented with inorganic trace minerals exhibited increased glucose, paraoxonase, and myeloperoxidase which are markers of inflammation and oxidative stress, respectively. Treatment however had no effect on plasma Cu, Mn, Fe, and Zn concentrations. Maternal supplementation with inorganic trace minerals also resulted in calves with increased expression of inflammatory mediators including myeloid differentiation primary response gene (MYD88), nuclear factor of kappa light polypepetide gene enhancer in B-cells (NFKB), TNF receptor-

associated factor 6 (TRAF6), and Interleukin-1 receptor-associated kinase 1 (IRAK1). Additionally, these calves also exhibited increased expression of miR-155 and miR-125b suggesting these changes in the calf inflammatory response are being modulated via changes in miRNA expression. Ultimately the authors hypothesized that maternal supplementation with organic trace minerals, which may have a greater absorption efficiency, could alter the immune response of neonate calves. However, further research would need to be conducted utilizing inflammatory challenges to assess both potential impacts on neonate immune response and long term health of the calf.

As previously discussed, Se plays a key role in glutathione peroxidase activity and may be of particular importance during times of oxidative stress (Rotruck et al., 1973). Gunter et al. (2003) assessed the effects of maternal Se supplementation and source of supplementation on both the performance and the glutathione peroxidase activity of their calves. Treatments consisted of no supplemental Se, 26 mg of Se/kg of free choice mineral as sodium selenite, or 26 mg of Se/kg of free choice mineral as seleno-yeast and were supplemented in the last 60 d of gestation to commercial beef cows. Calf birth weight and total BW gain calculated at the time of weaning did not differ across treatments. As expected cows receiving any type of Se supplementation had greater blood Se concentrations compared to those receiving no supplementation at the time of calving. Additionally, those cows receiving the seleno-yeast supplementation had greater blood Se concentrations compared to those supplemented with sodium selenite. This remained consistent with calves from seleno-yeast supplemented cows exhibiting the highest blood Se concentrations and the highest glutathione peroxidase activity in erythrocytes at birth. Interestingly, at 3 months of age calves nursing cows consuming seleno-yeast still maintained higher glutathione peroxidase activity and glutathione peroxidase activity

of calves from control and selenium selenite cows were lower and not different. These data suggest that calves from dams supplemented with seleno-yeast may have an increased capacity to handle oxidative stress. It is important to note that the authors did not address whether calves could have access to the free choice mineral as they grew old enough to consume solid feed. By 3 months of age it is likely the calves may have consumed some of the free choice mineral supplement if they had access, which would confound the results of this experiment.

Fetal programming is complex and can be influenced by numerous factors. The concept of trace mineral supplementation potentially altering fetal growth and ultimately long term health and performance of calves is a novel approach with limited research. While few (Gunter et al., 2003, Jacometo et al., 2015) have noted a potential benefit in immune or inflammatory response of young neonates research has not been conducted to determine if these benefits would maintain throughout the life of that calf. Additionally, current research only has focused on mineral supplementation in late gestation. While trace minerals may be of particular importance during the last 2 month of gestation as 75% of fetal growth occurs during this time (Funston et al., 2010), they may also be important during early fetal development when differentiation, organogenesis, vascularization, and placental growth occur. Alterations to maternal nutrition during early gestation may have impacts on not only future growth of the fetus but may also impact future performance and health of the offspring. Ultimately, research needs to be conducted to determine the role trace mineral supplementation may play in the complex process of fetal programming.

## ALTERNATIVE TRACE MINERAL SUPPLEMENTATION

Minerals play a vital role in ruminant nutrition and trace minerals such as copper, manganese, selenium and zinc have been shown to play a critical role in numerous biochemical

processes and are key components of a ruminant animal's health and productivity (Suttle, 2010). In beef production, forage is a primary dietary component; however, mineral composition of forage can vary widely due to differences in soil composition, resulting in insufficient supply of some minerals relative to the requirement (Smart et al., 1981). Additionally, the bioavailability of trace minerals in supplemental trace mineral sources can vary and interactions with other minerals and feed components within the gastrointestinal tract may alter the mineral status of a ruminant (Spears, 2003). When diets include these mineral antagonists, trace minerals that are not available or soluble in the rumen, such as chelated or hydroxy-bound trace minerals, have the potential to improve the status of the animal (Rabiee et al., 2010). Also, trace minerals that completely bypass the gastrointestinal tract, such as injectable trace minerals, may also improve status by avoiding complex ruminal interaction and absorption competition (Pogge et al., 2012).

*Organic trace minerals*

Traditionally, trace minerals have been supplemented to the beef cattle diet as inorganic salts; for example, sulfates, chlorides, oxides, and carbonates. However, recently data has suggested that organic or chelated trace minerals may improve growth, reproduction, and health traits in ruminants (Rabiee et al., 2010). There are several commercially available organic trace mineral supplements; however they vary in regards to the type of ligand or ligands used to form the metal complex or chelate. Most organic minerals fall into three different classifications, complexes, chelates or proteinates. The Association of American Feed Control Officials provides definitions for the various types of organic mineral products, which are shown in **Table 2**. While not all metal complexes are classified as chelates, chelation refers to the complex formed between a ligand and a metal ion (Spears, 1996). Kratzer and Vohra (1986) state that for a mineral to be classified as a chelate the ligand or chelating agent must meet two requirements: 1)

a minimum of two functional groups (oxygen, nitrogen, hydroxyl, amino) which are each capable of donating a pair of electrons to combine with a metal and 2) form a heterocyclic ring structure with the metal.

The primary development of organic trace minerals centers on the component that they are more bioavailable to the ruminant animal than traditional inorganic sources. There are several justifications as to why these organic trace minerals may be more available; including, the organic sources of trace minerals are more similar than the inorganic sources to the forms that trace minerals naturally occur in the body so the animal would not have to convert inorganic sources to more usable organic forms. This is of particular importance as almost all trace minerals that are in the body are in an organic complex or chelate and not as free inorganic forms (Spears, 1996). Therefore, utilization of an inorganic metal is entirely reliant on the animal's ability to convert them to organic, biologically active forms. Also, the metal chelate or complex may be more stable in the digestive tract which could prevent the formation of complexes with other dietary components and prevent antagonisms from forming with other minerals resulting in a greater absorption rate (Spears, 1996). Additionally, trace minerals that naturally occur in feeds also exist primarily in an organic form as a chelate or a complex.

Recently, research has focused on the effectiveness of supplementing organic trace minerals and their effects on reproductive performance. In a meta-analysis by Rabiee et al. (2010), twenty research papers and reports were analyzed comparing the effects of organic trace mineral supplementation versus traditional inorganic supplementation. Meta-regression analysis showed that supplementing cows with organic trace minerals reduced the number of days open and the number of services per conception in lactating dairy cows. Additionally, organic trace mineral supplementation increased milk production by 0.93 kg per day in dairy cows. Suggesting

that organic trace mineral supplementation could improve reproduction and production parameters in lactating dairy cattle.

Other work by Ahola et al. (2004) reported the effects of supplementing organic and inorganic sources of Cu, Zn, and Mn on mineral status and reproduction in beef cattle. Crossbred beef cows were utilized over 2 years (178 in year 1; 148 in year 2) and assigned 1 of 3 treatments: a control with no supplemented Cu, Zn or Mn, an organic trace mineral with 50% organic and 50% inorganic Cu, Zn, and Mn, or an inorganic trace mineral with 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$. After year 1, all supplemented cows (organic and inorganic) had greater liver Cu, Zn, and Mn concentrations compared to control cattle, and cows supplemented with organic trace minerals had greater liver Cu concentrations than cows supplemented with inorganic minerals. Also in year 1, there was a tendency for organic supplemented cows to have greater pregnancy rates than inorganic supplemented cows. In year 2, kilograms of calf weaned per cow exposed tended to be greater in organic than inorganic supplemented cows. These results suggest that supplementation and source of trace mineral may affect mineral status of cows, pregnancy rates, and kilograms of calf weaned.

Toni et al. (2007) noted similar results when feeding 180 Holstein cows either Cu, Mn, and Zn from an inorganic source, or replacing those trace minerals with an organic source. Feeding organic trace minerals increased BCS and decreased culling rate compared to inorganic supplemented cows. Additionally, organic supplementation tended to increase first service conception rate. Further supporting that replacing inorganic trace minerals with an organic source could improve performance of cattle. Organic trace minerals may also result in increased colostrum immunoglobulins and decreased calf mortality (Formigoni et al., 2011). Two hundred and ninety-six pregnant Holstein cows were utilized to assess the effect of feeding a control

treatment of all inorganic trace minerals or Cu, Mn, and Zn supplemented as 500 g/kg inorganic and 500 g/kg organic trace minerals. Colostrum from cows receiving organic trace minerals contained greater concentrations of immunoglobulins compared to colostrum from inorganic supplemented cows. Interestingly, organic supplementation had no effect on the concentration of trace minerals in the colostrum. However, calf mortality at calving was lesser in cows fed organic trace minerals versus their inorganic fed counterparts.

George et al. (1997) reported that organic trace minerals may also improve the performance and immune function of heifers in the feedlot. Crossbred heifer calves ($n$ = 105) were supplemented with 1 of 3 treatments: an inorganic Zn, Mn, Cu, and Co trace mineral source, an organic Zn, Mn, Cu, and Co source, or an organic Zn, Mn, Cu, and Co source supplemented 3 times the amount for 14 d and then reduced to normal amounts for the remainder of the feeding period. There were no differences noted across treatments for DMI, ADG, or feed efficiency over the 42 d trial. However, organic supplemented heifers did exhibit increased secondary PI-3 antibody titer response post-vaccination, and antibody titer response to IBRV vaccination was improved compared to inorganic supplemented calves. Additionally, calves fed organic trace minerals supplemented at increased concentrations resulted in a better skin swelling response at 12, 24, and 48 h postinjection to intradermal PHA, and these calves had a 17.2% reduction in the incidence of respiratory disease compared with the inorganic supplemented heifers and heifers supplemented organic trace minerals at normal levels. In this trial, feeding elevated organic trace minerals showed significant improvements in humoral and cell-mediated immunity when compared to the inorganic supplemented calves. Organic trace minerals may also enhance the immune response of early lactation dairy cows when compared to cows supplemented with inorganic sources (Nemec et al., 2012). Twenty-five Holstein cows were

supplemented with either organic chelated forms of Cu, Mn, and Zn, or with inorganic sulfate forms of Cu, Mn, and Zn and after 8 weeks of supplementation all cows received a rabies vaccination to assess immune response. Neutrophil function was unaffected by treatment. However, rabies antibody titer was 2.8 times greater from cattle supplemented with organic trace minerals versus their inorganic supplemented counterparts. No differences were noted on milk production, milk composition, or plasma minerals. This data suggests that organic trace minerals may also improve the immune response of lactating dairy cattle.

Contrastingly, others have reported no benefit to supplementing organic trace minerals versus inorganic trace minerals. Lamb et al. (2008) supplemented heifers 1 of 3 diets: a control diet with no added mineral, an organic mineral supplement, or an inorganic mineral supplement. These treatments were supplemented for 23 d to assess the effects on follicular response, ovulation, and embryo production when heifers were superovulated. The mean number of embryos recovered was similar across all treatment, and there was no difference in the number of degenerate or transferable embryos. The authors concluded that the source of trace mineral did not significantly alter embryo quality or number when heifers were fed a well-balanced diet that met all trace mineral requirements. It is also important to note that these trace mineral supplements were only provided for 23 d which may not have been long enough to elicit a response. However, Hackbart et al. (2010) supplemented organic and inorganic trace minerals to lactating dairy cows for up to 5 months and also saw no difference in follicular dynamics, embryo quality and liver or luteal trace mineral concentrations. The authors still speculated that organic trace mineral feeding may be required for a longer period of time before any biological effects could be observed. Additionally, they also noted that even though the organic trace minerals may be more biologically available based on other research, in this trial control cattle

were still fed NASEM (2016) recommended levels of trace minerals indicating cattle were not deficient and that these complex processes may not be improved by increasing trace mineral concentrations above what is considered physiologically optimal. Finally, it is important to note that for both trials, cattle were prepared for AI via synchronization and perhaps if cattle had ovulated naturally without the use of exogenous hormones, supplementation may have had a greater impact on follicular dynamics and ovarian measures.

Despite all the literature suggesting that organic trace minerals offer advantages in bioavailability either due to superior solubility or to the unique chemical structure of the compound, little research actually addresses the structural stability or solubility of organic trace mineral supplements. Brown and Zeringue (1994) examined the solubility of chelates, complexes and proteinates and utilized gel filtration chromatography to determine if the metals that were solubilized from these products remained in a complex with amino acids. Utilizing 15 different organic trace minerals from 5 commercial manufactures (2 Cu proteinates, 2 Cu amino acid chelates, 2 Zn proteinates, 2 Zn chelates, 2 Mn proteinates, and 2 Zn chelates) the authors assessed the solubility of metals at both a pH of 5 and a pH of 2. At a pH of 5 all the products, with the exception of two (one Cu proteinate and one Cu lysine complex) were from 92 to 99.4% soluble at 0.125 mg/mL of product concentration. At a pH of 2, solubility of all products tested was almost 100%, suggesting metals from organic trace minerals would be expected to be completely solubilized in the abomasum before reaching absorptive sites in the small intestine. This greater degree of solubility suggests a potential advantage for these products over inorganic mineral sources which offer limited solubility in the digestive tract. Interestingly, gel filtration chromatography indicated that once these organic trace metals were solubilized they were no longer bound to proteinaceous ligand or were bound so weakly that dissociation occurred under

the conditions of gentle gel filtration. Suggesting that once organic trace minerals are solubilized they would not be absorbed or metabolized differently than the soluble inorganic sources of trace minerals. This data suggests that the difference in bioavailability reported between organic and inorganic trace minerals is likely a component of the increased solubility of organic trace minerals, nearly 100% in an acidic environment, and not in the form in which these trace minerals are absorbed.

*Injectable trace minerals*

Injectable trace minerals offer another unique way to supplement trace minerals that allows minerals to bypass the gastrointestinal tracts and avoids competition for absorption at the intestinal level. Once trace minerals are injected into the animal, the minerals circulate throughout the body and will incorporate into cells as needed, while the remaining mineral is transported to the liver where it is either excreted from the body or bound to proteins for long term storage (Suttle, 2010). Increased mineral status of an animal could be of particular importance at times of increased stress and when biological needs are increased, such as shipping or breeding. Additionally, when animals are sick or stressed they often stop drinking and DMI is depressed (Dantzer, 2006), resulting in a decreased trace mineral intake at times when trace minerals may be most vital. Additionally, injectable trace minerals offer an advantage compared to traditional oral supplement methods in that they provide a targeted delivery of a specific amount of trace minerals to individual animals. This eliminates the variability associated with fluctuation in voluntary intake noted among cattle provided free choice mineral (Arthington and Swenson, 2004).

Recently, Pogge et al. (2012) demonstrated that trace mineral injections are an effective way to improve the trace mineral status of calves, particularly Cu and Se. Twenty Simmental and

Angus steers were blocked by breed and given either Multimin90 (Multimin USA, Fort Collins, CO) or saline at a dose of 1 mL/45kg BW. Jugular blood samples were collected before injection and at 8 and 10 h post-injection and then again 1, 8, and 15 d post-injection. Additionally, liver biopsies were collected 3 d pre-injection and 1, 8, and 15 d post-injection. Plasma Zn, Mn, and Se were greater post-injection for Multimin treated steers compared to their saline treated counterparts. Also, liver concentrations of Cu, Zn, and Se were greater in Multimin treated steers post-injection compared with saline treated steers. Overall, this data shows that through a 15 d sampling period, an injectable trace mineral increases liver concentrations of Cu, Zn, and Se, and suggests that injectable trace minerals may be an adequate way to increase mineral status of cattle.

*Injectable trace minerals – Reproduction*

Injectable trace minerals also may exhibit a positive effect on reproductive performance of heifers. Sales et al. (2011) utilized 826 crossbred heifers from five different farms in Brazil to assess the effect of an injectable trace mineral (Multimin; 100 mg Zn, 100 mg Mn, 50 mg Cu, and 25 mg Se) on pregnancy rate of heifer synchronized for timed embryo transfer. Injectable trace minerals were given subcutaneously 17 d prior to timed embryo transfer. Injectable trace minerals did not increase the number of heifers successfully synchronized when compared to control cattle (82.1% and 83.1% for Multimin and control cattle, respectively). However, heifers that received injectable trace minerals had 1.58 fold and 1.72 fold increased pregnancy rates at 23 and 48 days after timed embryo transfer compared to the control group, respectively. This data suggests that even though injectable trace minerals did not increase the number of heifers successfully synchronized, supplementation did increase conception rates and the chance of embryo survival at 23 and 48 days after timed embryo transfer.

Other work by Brasche et al. (2014a) has noted that injectable trace minerals may also have a positive impact on the reproductive performance of virgin beef heifers. One hundred and nine Angus-crossbred heifers were randomly assigned to treatments in a 2×2 factorial, receiving either a trace mineral injection or no injection and one of two synchronization protocols: a 14d CIDR-PG protocol or a 5d Co-synch plus CIDR protocol. Trace mineral injections were given 33 d prior to AI to assess the overall effects on pregnancy rate as well as conception to AI. There was no difference between conception rates within the 5d CO-Synch plus CIDR protocol (66.0% and 52.0% for control and trace mineral injection, respectively) or within the 14d CIDR-PG protocol (55.0% and 75.0% for control and trace mineral injection, respectively). There was also no interaction between trace mineral injection and synchronization protocol for overall pregnancy rate. Interestingly though, trace mineral supplementation did have a significant effect on overall pregnancy rate determined 105 d post AI, with heifers receiving trace mineral injections (93.0%) having greater pregnancy rates compared to the unsupplemented control cattle (83.0%) after AI and bull exposure. Kirchhoff and Fike (2015) noted similar results when they supplemented 82 Angus, Hereford, and Simmental heifers with either an injectable trace mineral or sterilized saline to serve as a control 4 weeks prior to breeding. These heifers were also supplemented with a TMR that included trace minerals at NASEM (2016) recommended levels. Heifers receiving an injectable trace mineral (51.28%) had greater pregnancy rates to fixed time AI compared to control heifers (25.58%). Interestingly, the authors also measured estrous behavior with heat detection patches and even though there were differences in pregnancy rates, there was no difference between the percentage of heifers exhibiting estrous behavior, as indicated by a red estrous detection patch, regardless of treatment (30.77% and 47.50% for injectable trace mineral and control treatments, respectively). These data suggest that injectable

trace minerals prior to breeding may increase pregnancy rates in heifers. However, it is unknown if this is a component of a specific mineral or a result of the entire cohort of supplemented injectable trace minerals.

Machado et al. (2013) also noted that injectable trace minerals may have a positive impact on udder health and reproduction performance. Utilizing 1,416 Holstein cows from 3 dairy farms the authors assessed the effects of an injectable trace mineral (300 mg Zn, 50 mg Mn, 25 mg Se, and 75 mg Cu) administered at 230 and 260 d of gestation and again 35 d postpartum on health, reproductive performance, and milk production. All cattle also received a TMR which supplied 2-6 times the NASEM (2016) requirement for trace minerals. Cattle receiving trace mineral injections had decreased somatic cell count scores when compared to control cattle. Additionally, the incidence of subclinical mastitis was lesser for the trace mineral supplemented cattle (8.0%) comparted to control cattle (10.4%). Also, cattle receiving injectable trace minerals had decreased incidence of endometritis (28.6% and 34.2% for trace mineral supplemented and control cattle, respectively) and decreased incidence of stillbirth. However, treatment had no effective on other reproductive performance parameters or milk production. It is important to note that in this trial cattle were already supplemented above NASEM (2016) recommendations prior to injectable trace mineral supplementation, suggesting these cattle already had adequate trace mineral status. Additionally, further research is needed to determine which trace mineral (Cu, Mn, Se, or Zn) provided the benefits noted in these experiments or if it was the combination of several or all supplemented minerals.

Often times when trace mineral status of cattle is adequate or when cattle are in a minimal stress environment, little benefit can be noted from injectable trace minerals. Brasche et al. (2014b) utilized 174 beef cows to assess the effects of either an injectable trace mineral 72 or

80 h post CIDR removal or no injection on reproductive performance. The authors noted that liver concentrations of the trace minerals measured (Cu, Se, Mn, and Zn) in both treatments were considered adequate at all-time points. Though, cows supplemented with trace minerals did have greater liver Se concentrations, there was no difference in liver Mn, Zn, or Cu compared to control cattle. Also, there was no difference in cow BCS between treatments, and injectable trace minerals had no effect on reproductive performance including conception to AI and overall conception rates. While these cows were in adequate status it is also important to note that the trace mineral injection was administered following CIDR removal, which may have been too early to allow a positive response to be noted from the injection. Previous research has shown improvement in reproductive performance when trace mineral injections are administered approximately 4 weeks prior to the time of breeding.

In other work Vanegas et al. (2004) assessed the effect of an injectable trace mineral supplement on first-service conception rate of dairy cows in in an intensively managed herd. Eight hundred and thirty-five dairy cows from a commercial dairy received either 1 injection of trace mineral supplement 45 d in lactation, 2 injections of trace mineral 3 weeks prior to calving and again 45 d in lactation, or no injection, which served as the control group. Conception rates were not different between cattle receiving one injection (26.8%) and the control cattle (27.5%). Interestingly, cows receiving two injections of trace mineral actually had decreased conception rates compared to their control counterparts (21.5% and 31.5% for 2 injections and control cattle, respectively). In this intensively managed herd, a two dose regiment of injectable trace mineral actually decreased reproductive performance and a single shot of injectable trace mineral offered no beneficial effects on conception rates. It is unknown from a physiological standpoint why trace minerals actually resulted in decreased conception rates and more research is needed to

evaluate the effects of trace mineral injections under other management strategies where possible deficiencies are preset.

*Injectable trace minerals – Health and immunity*

Research has recently been conducted to assess the effects of injectable trace minerals on calf performance and health. Arthington et al. (2014) utilized 150 Brangus-crossbred calves (*n* = 75/treatment) to assess the effects of injectable trace minerals on trace mineral status and performance. Treatments consisted of a subcutaneous injection of trace mineral or saline administered at birth and then again at 100 and 200 d of age. Twelve heifers per treatment were selected when the calves were weaned, and transported 1,600 km to assess acute phase proteins and health status. Injectable trace mineral had no effect on calf BW gain however it did result in greater concentration of liver Cu and Se compared to saline treated calves. Overall ADG, PRBC antibody titers, and liver Se concentration were greater for heifers receiving the injectable trace mineral compared to the saline injected heifers. These data suggest that injectable trace minerals may also result in an increased humoral response and a heightened APP response to stress of weaning and transportation. Teixeira et al. (2014) noted similar results when 790 Holstein heifer calves were either given an injectable trace mineral supplement or no injection to assess the effects on calf immunity, health and growth. Blood samples were collected after birth to assess glutathione peroxidase activity, superoxide dismutase activity, haptoglobin, and neutrophil and monocyte function. At 14 d of age calves treated with injectable trace minerals had greater glutathione peroxidase activity compared to non-treated calves. Also, calves treated with trace minerals had reduced incidence of pneumonia (41.7% and 49.7% for trace mineral treated and control cattle, respectively) and diarrhea (41.7% and 49.1% for trace mineral treated and control cattle, respectively) compared to control calves. No effects were observed on average daily gain

of calves or survivability, as well as serum superoxide dismutase activity and haptoglobin concentrations, regardless of treatment.

In other work, Berry et al. (2000) noted that crossbred bull calves (n = 60) receiving 3 mL of an injectable trace mineral (Multimin90) had increased average daily gain, improved feed conversion, and tended to be treated for sickness less than control animals. The bulls utilized in this experiment were assembled from sale barns across the state of Oklahoma and treatments were assigned upon arrival to the research facility, suggesting these cattle had the potential to be highly stressed and potentially exhibited diverse trace mineral backgrounds. This data suggests that an injectable trace minerals may improve performance and health in receiving cattle however trace mineral status and markers of inflammatory stress were not monitored in this experiment.

Genther-Schroeder and Hansen (2015) went on to access the effect of an injectable trace mineral on the inflammatory response, growth and carcass characteristics of beef steers following transit stress. Ninety-eight weaned steers were administered either a trace mineral injection or a saline injection. Twenty-eight days after treatment administration half the steers from each treatment were transported 20 h and the other half of the steers were returned to their pens for 20 h with feed and water restriction. Cattle received another trace mineral or saline injection on d 113 of the trail at the beginning of the finishing period. During the transit period shipping resulted in increased serum IL-8 concentrations in saline treated cattle. Interestingly, steers that received the injectable trace mineral had decreased ADG compared to controls during the 14 d transit period. However, the trace mineral injection had no effect on overall growing period or finishing period average daily gain. Additionally, the administration of a trace mineral injection also had no effect on the inflammatory response or plasma trace mineral concentrations.

These steers were assessed for trace mineral status and were well within adequate range at the initiation of the experiment. Since trace minerals were not limiting for cattle in this experiment, this may help explain the minimal effects noted from supplementing cattle with an injectable trace mineral. These data collectively suggest that not only may trace minerals play a viable role in the reproductive success of cattle, but may also be crucial for calf health and development, particularly at times of increased stress.

## CONCLUSION

With the number of beef heifers in the United States increasing in recent years and the development of these heifers representing a substantial economic impact to the producer, resources need to be dedicated to determining low cost ways to generate sound, functional females. Trace minerals are just one of these avenues, as they play important roles in the ruminant animal and are critical for optimal production, health, and for numerous biochemical processes. While even assessing trace mineral status of ruminants currently remains challenging, the importance of trace mineral supplementation cannot be ignored. Trace minerals have been shown to improve growth, reproduction, and health traits across numerous production settings. Additionally, supplementation of trace minerals during gestation may also improve fetal development and could potentially have long term effects on the offspring.

Mineral supplementation offers many additional and unique challenges. Minerals are primarily provided to ruminants in forage, however when these concentrations are inadequate, free choice supplementation is typically provided. The bioavailability of these sources can then be effected by complex ruminal and gastrointestinal interactions with other minerals and feed components. Organic and injectable trace minerals offer two novel approaches to trace mineral supplementation. While it is clear that trace minerals are critical for ruminant health and

performance more research is needed to elucidate the optimal form of supplementation as well as how these various forms and amounts of trace minerals can effect reproductive performance, health, and overall productivity of ruminants.

## TABLES

**Table 1.1.** Criteria for classification of cattle mineral status[1]

| Classification | Liver concentration (DM basis) | Blood concentration |
|---|---|---|
| Copper | | |
| Adequate | 125 – 600 mg/kg | 0.7 – 0.9 mg/L of plasma |
| Marginal | 33 – 125 mg/kg | 0.5 – 0.7 mg/L of plasma |
| Deficient | <33 mg/kg | 0.2 – 0.5 mg/L of plasma |
| Manganese | | |
| Adequate | >13 mg/kg | 6 – 70 µg/L of serum |
| Marginal | 7 – 13 mg/kg | 20 – 60 µg/L of serum |
| Deficient | <7 mg/kg | <20 µg/L of serum |
| Selenium | | |
| Adequate | 1.25 – 2.5 mg/kg | 210 – 1200 µg/L of whole blood |
| Marginal | 0.6 – 1.25 mg/kg | 60 – 200 µg/L of whole blood |
| Deficient | 0.1 – 0.5 mg/kg | <60 µg/L of whole blood |
| Zinc | | |
| Adequate | 25 – 200 mg/kg | 0.8 – 1.4 mg/L of plasma |
| Marginal | 25 – 40 mg/kg | 0.5 – 0.8 mg/L of plasma |
| Deficient | <20 – 40 mg/kg | 0.2 – 0.4 mg/L of plasma |

[1]Adapted from Kincaid, 2000.

**Table 1.2.** Definitions of various organic mineral products according to the Association of American Feed Control Officials[1]

| |
|---|
| 57.150 Metal amino acid complex – The product resulting from complexing of a soluble metal salt with an amino acid(s). |
| 57.142 Metal amino acid chelate – The product resulting from the reaction of a metal ion from a soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three (preferably two) moles of amino acids to form coordinate covalent bonds. The average weight of the hydrolyzed amino acids must be approximately 150 and the resulting molecular weight of the chelate must not exceed 800. |
| 57.23 Metal proteinate – The product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein. |
| 57.29 Metal polysaccharide complex – The product resulting from complexing of a soluble salt with polysaccharide solution. |

[1]From Spears, 1996.

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# CHAPTER 2

## EFFECT OF SUPPLEMENTATION OF CHELATED TRACE MINERALS ON REPRODUCTIVE PERFORMANCE OF BEEF CATTLE

**ABSTRACT:** To evaluate the effect of supplementing two different chelated trace mineral sources on reproductive performance of beef cows, 204 spring-calving, Angus and Simmental × Angus cows (BW = 649±129 kg) were utilized. Cows received 1 of 2 glycine ligand chelated trace minerals, both formulated to replace 50% of the Cu, Mn, and Zn inorganic trace mineral (**MAAC**; MAAC, Novus International; **TRAX;** B-Traxim 2C, Pancosma). Cows were housed at two locations, and a complete randomized design was used. Cows were stratified by body weight (**BW**), age, and body condition score (**BCS**) across treatment. Treatments were applied in two stages. During the dry lot phase, cows were provided a TMR, targeted to provide 113.4 g of trace mineral·cow$^{-1}$·d$^{-1}$. Cows were then moved to pasture on d 31 and mineral was provided free choice. Body weight and BCS were collected at the trial initiation, breeding, and AI and final pregnancy confirmation. Liver samples were collected at trial initiation, AI synchronization, and final pregnancy confirmation for trace mineral and metallothionein (**MT**) analysis. On d 32 and 42, cyclicity was determined based on plasma progesterone levels. On d 42, cows were enrolled in the 7-d Co-Synch + controlled internal drug insert and timed-AI protocol. Following AI, cows were exposed to one bull/pen for a 44 d breeding season. Conception rates to AI and overall pregnancy rates were determined on d 91 and d 147, respectively. Cow BW did not differ ($P \geq 0.17$) across all time points, and BCS was not different ($P \geq 0.50$) at initiation, breeding, or final pregnancy confirmation. However, there was a tendency ($P = 0.07$) for BCS to be greater for TRAX cattle at the time of AI confirmation. Liver mineral concentrations were not different ($P \geq 0.11$) regardless of treatment. Liver MT/actin expression was not different ($P \geq 0.24$) at trial

initiation or at breeding. Interestingly, TRAX cattle did have greater ($P$ = 0.03) MT/actin expression comparted to MAAC cattle at the time of final pregnancy confirmation. There was no difference ($P$ = 0.69) across treatments in percent of cows exhibiting estrous cyclicity, and there was no effect ($P$ = 0.91) of supplementation on AI conception (MAAC=72.2% and TRAX=71.2%). Interestingly, overall pregnancy rate was greater ($P$ = 0.03) for TRAX (98.4%) compared to their MAAC (90.1%) counterparts. Supplementing beef cows with B-Traxim 2C prior to breeding improved overall pregnancy rates but did not alter BW or trace mineral status.

## INTRODUCTION

Traditionally, trace minerals have been supplemented to the beef cattle diet as inorganic salts; for example, sulfates, chlorides, oxides, and carbonates. Recent data have suggested that organic or chelated trace minerals may improve growth, reproduction, and health traits in ruminants (Ahola et al., 2004; Hansen et al., 2008; Rabiee et al., 2010). In a meta-analysis by Rabiee et al. (2010), twenty research papers and reports were analyzed comparing the effects of organic trace mineral supplementation versus traditional inorganic supplementation. Meta-regression analysis reported that supplementing cows with organic trace minerals reduced the number of days open and the number of services per conception in lactating dairy cows. Additionally, Ahola et al. (2004) noted that organic trace mineral supplementation increased pregnancy rates and liver mineral concentrations in beef cattle compared to those supplemented inorganic sources. These data suggest that organic trace mineral supplementation could improve reproduction parameters in cattle.

There are several commercially available organic trace mineral supplements; however, they vary in regards to the type of ligand or ligands used to form the metal complex or chelate. Most organic minerals fall into three different classifications, complexes, chelates or proteinates.

While not all metal complexes are classified as chelates, chelation refers to the complex formed between a ligand and a metal ion (Spears, 1996). To date neither the Association of American Feed Control Officials (AAFCO, 2000) nor the Association of American Feed Control Officials (AOAC, 1995) have developed a definitive method to assess the degree of chelation associated with trace minerals bound to organic ligands. This strength of chelation may differ between products even bound to similar ligands and could ultimately impact the bioavailability of these commercially available organic trace mineral products. Specifically, glycine chelates have been shown to be as much as 157% more bioavailable than sulfate bound trace minerals (Hansen et al., 2008). However, as glycine chelated trace minerals become more commercially available, to our knowledge, no research has been conducted comparing these novel products. Therefore, the current study was to evaluate reproductive performance and trace mineral status of beef cattle when provided two different chelated trace mineral sources.

## MATERIALS AND METHODS

***Animals and experimental design.*** To evaluate the effect of supplementing two different chelated trace mineral sources on reproductive performance of beef cows, 204 spring-calving, Angus and Simmental × Angus cows (BW = 649 ± 129 kg) were utilized. Cows received 1 of 2 glycine ligand chelated trace minerals, both formulated to replace 50% of the Cu, Mn, and Zn inorganic trace mineral (**MAAC**; MAAC, Novus International; **TRAX;** B-Traxim 2C, Pancosma). Cattle were maintained at two locations, the Beef Cattle and Sheep Field Laboratory in Urbana, IL or the Orr Agricultural Research and Demonstration Center in Baylis, IL. A complete randomized design was used with cows stratified by weight, age, and BCS across treatment into 8 pens per location with 12-13 cows/pen. Treatments were applied in 2 stages based on housing of the animals. During the dry lot phase cows were supplemented with a TMR

(Table 1) targeted to provide 113.4 g of trace mineral·cow$^{-1}$·d$^{-1}$ for 30 d, and once cows were moved to pasture, mineral was provided free choice (MAAC = 16.3% distillers grains, 19% salt, 20.3% Ca as calcium carbonate, 9.8% Mg as magnesium oxide, 2.3% K as potassium chloride, 0.5% I as ethylenediamine dihydroiodide, 0.3% Se, 0.3% Fe as iron sulfate, 0.003% Co as cobalt carbonate, 0.86% Cu as copper sulfate and copper glycinate [MAAC, Novus International], 2.05% Mn as manganese oxide and manganese glycinate [MAAC, Novus International], and 1.33% Zn as zinc oxide and zinc glycinate [MAAC, Novus International]; TRAX = 16.3% distillers grains, 19% salt, 20.3% Ca as calcium carbonate, 9.8% Mg as magnesium oxide, 2.3% K as potassium chloride, 0.5% I as ethylenediamine dihydroiodide, 0.3% Se, 0.3% Fe as iron sulfate, 0.003% Co as cobalt carbonate, 0.76% Cu as copper sulfate and copper glycinate [B-Traxim 2C, Pancosma], 0.89% Mn as manganese oxide and manganese glycinate [B-Traxim 2C, Pancosma], and 0.83% Zn as zinc oxide and zinc glycinate [B-Traxim 2C, Pancosma]). Cows were housed in dry lots from the initiation of the trial until d 30, when they were moved to pasture and grazed red clover (*Trifolium pretense*), white clover (*Trifolium repens*), and endophyte-infected fescue (*Festuca arundinacea*) pastures (59.97% NDF, 32.35% ADF, and 13.45% CP Beef Cattle and Sheep Field Laboratory in Urbana, IL; 55.78% NDF, 30.95% ADF, and 13.77% CP Orr Agricultural Research and Demonstration Center in Baylis, IL) for the remainder of the trial.

On d 42, cows were enrolled in the 7-d Select-Synch + controlled internal drug release (CIDR; Pfizer Animal Health, New York, NY) insert and timed-AI protocol (Johnson et al., 2013). Following AI, cows remained on pasture for the remainder of the breeding season. Ten d following AI cows were exposed to one bull per pen that had previously passed a breeding soundness exam, for a 44 d breeding season. Artificial insemination conception rates were

collected 40 d after AI and overall pregnancy rates were determined on d 147. Artificial insemination conception and overall pregnancy rates were determined by a trained technician via ultrasonography (Aloka 500 instrument, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

***Sample collection and analytical procedures.*** Body weight and BCS [emaciated = 1; obese = 9; as described by Wagner et al. (1988)] were collected at the initiation of the trial (d -1 and d 0), at CIDR removal (d 51), at AI pregnancy confirmation (d 91) and at final pregnancy confirmation (d 147 and d 148). Feed samples were collected every two weeks for analysis during the dry lot phase and forage samples were collected every two weeks after cattle were moved to pasture. Individual feed ingredients were collected monthly and composited and dried at 55° C for a minimum of 3 days and ground through a 1 mm screen using a Wiley mill (Arthur, H. Thomas, Philadelphia, PA) for nutrient composition analysis. Forage samples were composited and dried similarly. Ground feed samples were analyzed for CP (Leco TruMac, LECO Corporation, St. Joseph, MI), NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY), and crude fat using an Ankom XT10 fat extractor (Ankom Technology, Macedon, NY). Ground forage samples were also analyzed for NDF, ADF, and CP. Forage and feed samples were also sent to a commercial lab where they were subjected to nitric acid digestion and inductively coupled plasma spectroscopy analysis for complete minerals (method 975.03: AOAC, 1988; The Ohio State University, Service Testing and Research Lab, Wooster, OH).

Blood samples were collected on d 32 and d 42 to determine cyclicity based on blood progesterone levels. Blood was collected via jugular venipuncture into 10 mL serum blood collection vacuum tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ). Blood was allowed

to clot at room temperature before being centrifuged at 1,300 × *g* for 20 min at 5°C. Serum was stored at -20°C for subsequent progesterone analysis. Serum progesterone concentration was analyzed using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA) validated by Reis et al. (2015). The intrassay CV for all samples was 3.6%. Estrous cyclicity was defined as when one blood sample contained >2 ng/mL of progesterone or when both samples contained >1 ng/mL of progesterone.

Liver samples for trace mineral determination and metallothionein (MT) analysis were collected at the initiation of the trial (d 0), at synchronization (d 42) and at final pregnancy confirmation (d 147) from 32 cows (16 per treatment; 2 per pen; the same cow was sampled on all dates). Within a pen, 2 cows that were most similar to pen average body weight and BCS were selected for sampling. Liver biopsies were collected using the method of Engle and Spears (2000) with the modification that all cows were given 5 mL of Lidocaine Injectable-2% (MWI, Boise, ID). Biopsy samples for trace mineral analysis were placed on ice and transported to the laboratory where they were frozen at -20°C until further analysis. Samples for metallothionein analysis were frozen in liquid nitrogen and stored at -80°C until their subsequent analysis. Liver samples for trace mineral analysis were sent to a commercial lab where they were subjected to nitric acid digestion and inductively coupled plasma spectroscopy analysis for complete minerals (method 975.03: AOAC, 1988; The Ohio State University, Service Testing and Research Lab, Wooster, OH). Liver tissue was stored in RNAlater® (Life Technologies, Grand Island, NY) at 4°C for 24 h and then at -20°C until total RNA isolation. Total RNA was isolated from ~40 mg liver samples using Trizol® (Life Technologies). One µg of total RNA, oligo dT and M-MLV Reverse Transcriptase from Thermo Fisher Scientific were used to synthesize cDNA according to the manufacturers' instructions. Bovine MT forward primer (5'- CTGCTCCTGCCCCAC -3'),

reverse primer (5'-CAGCCCTGGGCACAC -3'), and probe (5'-FAM-AGATGTCCCTCCTGCAAGAAGA-BHQ1-3)' were ordered from Fluoresentric. Cattle Actin forward primers (5'-CAGCACAATGAAGATCAAGATCATC-3'), reverse primer (5'-CGGACTCATCGTACTCCTGCTT-3') and probe (FAM-TCGCTGTCCACCTTCCAGCAGATGT- BHQ) as described previously (Viarouge et. al. 2015) were ordered from Integrated DNA Technologies. All primers/probe were verified for the efficiency (100% ± 10%) and linearity (r2 ³ 0.99) of amplification. Levels of mRNA were measured by quantitative PCR using Applied Biosystems® SYBR® Green PCR Master Mix (Life Technologies) and a 7500 Fast Real-Time PCR System. The relative mRNA levels of MT were first normalized to housekeeping gene *actin* and then normalized to a common control sample to minimize variation between PCR plates.

***Statistical Analysis.*** Data were analyzed as a completely randomized design using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). All binary data were analyzed using the GLIMMIX procedure of SAS. Location was included as a fixed effect and pen was included as a random effect. Values determined at day 0 for mineral analysis were used in a covariate analysis for all variables. Pen served as the experimental unit for all analysis. Significance was declared at $P \leq 0.05$, and tendencies were declared from $0.05 < P \leq 0.10$. Means reported in tables are least squares means ± SEM.

## RESULTS

Cow BW did not differ ($P \geq 0.17$; Table 2) regardless of treatment across all time points, and BCS was not different ($P \geq 0.50$) at initiation, breeding, or final pregnancy confirmation. However, there was a tendency ($P = 0.07$) for BCS to be greater for TRAX cows at the time of AI confirmation compared to their MAAC counterparts. Liver Cu, Mn, and Zn were not different

($P \geq 0.11$; Table 3) regardless of treatment. Additionally, there were no differences ($P \geq 0.24$; Figure 1) at trial initiation or at breeding in liver MT/actin expression between MAAC and TRAX cattle. Interestingly, TRAX cattle did have greater ($P = 0.03$) MT/actin expression comparted to MAAC cattle at the time of final pregnancy confirmation. There was also no difference ($P = 0.69$) between treatments in percent of cows exhibiting estrous cyclicity. Additionally, there was no effect ($P = 0.91$) of supplementation on AI conception. However, overall pregnancy rate was greater ($P = 0.03$) for TRAX cattle compared to their MAAC counterparts.

## DISCUSSION

Cow BW was not different at any time point, however TRAX cows tended to have a greater BCS at the time of AI confirmation compared to their MAAC counterparts. This significant difference in BCS was driven by a very small standard error (SEM = 0.10) and this difference is likely physiologically insignificant. To our knowledge, no other work has compared 2 organic trace mineral glycinate sources in beef cattle. Some researchers have assessed performance parameters of livestock supplemented organic trace minerals with differing ligands. Schlegel et al. (2008) reported increased body weight gain in piglets supplemented Fe, Cu, Mn, and Zn glycinates (B-TRAXIM 2C, Pancosma) versus soy based chelates. While challenging to draw comparisons in experiments utilizing swine, this further suggests that glycinate sources of organic trace minerals are more available to be utilized by the animal for important biological processes.

In the present trial liver Cu, Mn, and Zn were not different regardless of treatment, and there were no differences at trial initiation or at breeding in liver MT/actin expression between MAAC and TRAX cattle. Interestingly, TRAX cattle did have greater MT/actin expression comparted to

MAAC cattle at the time of final pregnancy confirmation. Metallothionein is a highly conserved protein that serves multiple functions within the body, including maintaining homeostatic control of trace mineral absorption and metabolism, metal-transfer, and metal storage (Bremner and Beattie, 1990). Additionally, MT synthesis is induced by these metals such as Cu, Mn, and Zn that it binds (Bremner and Beattie, 1990). Even though liver mineral concentrations were not altered by supplementation, cattle supplemented TRAX had increased liver MT/actin expression at the time of final pregnancy confirmation suggesting a potential increased bioavailability of TRAX compared to MAAC. This delay in response of MT/actin expression may also be due to the delayed synthesis of MT in response to dietary trace minerals, as MT is highly dependent on time and the amount of trace mineral consumed (Cao et al., 2000).

There was no difference between treatments in percent of cows exhibiting estrous cyclicity. Additionally, there was no effect ($P = 0.91$) of supplementation on AI conception. Overall pregnancy rate averaged 94.3%, and was greater for TRAX (98.4%) cattle compared to their MAAC (90.1%) counterparts. This increased overall pregnancy rate could be driven by an increase in trace mineral available for biological processes as suggested by the increased MT/actin expression at the time of final pregnancy confirmation. In other work supplementing sows crystalline glycine chelates (B-TRAXIM 2C, Pancosma), Durosoy and Fuchs (2008) reported a 10% increase in pregnancy rate compared to sows supplemented an organic chelate of amino acids from hydrolyzed soya protein. These data suggest that while organic trace minerals offer an increased bioavailability to inorganic sources, not all organic sources are chelated similarly and thus may also vary in there bioavailability.

Supplementation of two glycine chelated trace mineral sources resulted in minimal to no effects on cow BW, BCS, and liver mineral concentrations. However, MT/actin expression at the

time of final pregnancy confirmation was increased and greater overall pregnancy rates were noted with TRAX supplemented cattle. These data suggest that B-Traxim 2C (Pancosma) may be more bioavailable and supplementing beef cows with this product prior to breeding may improve overall pregnancy rates but without altering BW or BCS.

## TABLES AND FIGURE

**Table 2.1**. Ingredient composition of cow diets (% DM basis)

| | Inclusion, % DM | | | |
|---|---|---|---|---|
| | Urbana[1] | | Orr[2] | |
| Item | MAAC[3] | TRAX[4] | MAAC[3] | TRAX[4] |
| Ingredient, % | | | | |
| Corn silage | 77.5 | 77.5 | 57.5 | 57.5 |
| Corn stalks | - | - | 20 | 20 |
| MDGS[5] | 20 | 20 | 20 | 20 |
| MAAC supplement[6] | 2.5 | - | 2.5 | - |
| TRAX supplement[7] | - | 2.5 | - | 2.5 |
| Analyzed nutrient content | | | | |
| CP, % | 10.1 | 10.1 | 10.0 | 10.0 |
| NDF, % | 44.3 | 44.3 | 46.3 | 46.3 |
| ADF, % | 23.5 | 23.5 | 23.5 | 23.5 |
| Crude fat, % | 3.7 | 3.7 | 4.1 | 4.1 |
| S, % | 0.21 | 0.21 | 0.20 | 0.20 |
| Cu, mg/kg | 18.0 | 23.7 | 23.5 | 30.0 |
| Mn, mg/kg | 38.8 | 49.3 | 62.0 | 65.0 |
| Zn, mg/kg | 37.4 | 39.7 | 45.9 | 47.4 |

[1]Beef Cattle and Sheep Field Laboratory in Urbana, IL

[2]Orr Agricultural Research and Demonstration Center in Baylis, IL

[3] MAAC is glycine ligand, chelated trace mineral (MAAC) produced by Novus International (Saint Charles, MO) and was formulated to replace 50% of the inorganic Cu, Mn, and Zn in the diet.

[4]TRAX is glycine ligand, chelated trace mineral (B-Traxim 2C) produced by Pancosma (Geneva, Switzerland) and was formulated to replace 50% of the inorganic Cu, Mn, and Zn in the diet.

[5]Modified distillers grains with solubles.

[6]Supplement contained 60.394% ground corn, 39.432% trace mineral salt (5% distillers grains, 25% salt, 1% liquid molasses, 16.8% Ca as calcium carbonate, 3.4% Mg as magnesium oxide, 4.3% K as potassium chloride, 0.07% I as ethylenediamine dihydroiodide, 0.3% Se, 0.002% Co as cobalt carbonate, 0.86% Cu as copper sulfate and copper glycinate (MAAC, Novus International)], 2.05% Mn as manganese oxide and manganese glycinate (MAAC, Novus International)], and 1.33% Zn as zinc oxide and zinc glycinate (MAAC, Novus International)].

[7] Supplement contained 60.394% ground corn, 39.432% trace mineral salt (5% distillers grains, 25% salt, 1% liquid molasses, 16.8% Ca as calcium carbonate, 3.4% Mg as magnesium oxide, 4.3% K as potassium chloride, 0.07% I as ethylenediamine dihydroiodide, 0.3% Se, 0.002% Co as cobalt carbonate, 0.76% Cu as copper sulfate and copper glycinate (B-Traxim 2C, Pancosma)], 0.89% Mn as manganese oxide and manganese glycinate (B-Traxim 2C, Pancosma)], and 0.83% Zn as zinc oxide and zinc glycinate (B-Traxim 2C, Pancosma)].

**Table 2.2.** Influence of trace mineral supplementation type on cow BW, BCS, and reproduction

| | Treatment | | | |
|---|---|---|---|---|
| Item | MAAC[1] | TRAX[2] | SEM | *P*-value |
| BW, kg | | | | |
| Initial | 651 | 647 | 9.0 | 0.73 |
| Breeding | 672 | 674 | 8.5 | 0.87 |
| AI pregnancy confirmation | 651 | 667 | 8.0 | 0.17 |
| Final pregnancy confirmation | 643 | 658 | 7.7 | 0.18 |
| BCS | | | | |
| Initial | 6.0 | 6.0 | 0.08 | 0.88 |
| Breeding | 6.3 | 6.3 | 0.08 | 0.98 |
| AI pregnancy confirmation | 5.9 | 6.1 | 0.10 | 0.07 |
| Final pregnancy confirmation | 5.9 | 6.0 | 0.09 | 0.50 |
| Reproduction | | | | |
| Estrous cyclicity[3], % | 73.1 | 75.8 | - | 0.69 |
| AI conception, % | 72.2 | 71.2 | - | 0.91 |
| Overall pregnancy, % | 90.1 | 98.4 | - | 0.03 |

[1]MAAC is glycine ligand, chelated trace mineral (MAAC) produced by Novus International (Saint Charles, MO) and was formulated to replace 50% of the inorganic Cu, Mn, and Zn in the diet.
[2]TRAX is glycine ligand, chelated trace mineral (B-Traxim 2C) produced by Pancosma (Geneva, Switzerland) and was formulated to replace 50% of the inorganic Cu, Mn, and Zn in the diet.
[3]Cyclicity was defined as when one blood sample contained >2 ng/mL of progesterone or when both samples contained >1 ng/mL of progesterone

**Table 2.3.** Influence of trace mineral supplementation type on cow mineral status

| | Treatment | | | |
|---|---|---|---|---|
| Item | MAAC[1] | TRAX[2] | SEM | *P*-value |
| Liver mineral,[3] mg/kg | | | | |
| Breeding | | | | |
| Cu | 368.4 | 366.7 | 14.44 | 0.93 |
| Mn | 10.58 | 10.59 | 0.249 | 0.97 |
| Zn | 120.7 | 107.7 | 5.32 | 0.11 |
| Final Pregnancy confirmation | | | | |
| Cu | 306.1 | 282.0 | 30.06 | 0.58 |
| Mn | 10.01 | 10.08 | 0.339 | 0.88 |
| Zn | 121.9 | 121.0 | 10.40 | 0.95 |

[1]MAAC is glycine ligand, chelated trace mineral (MAAC) produced by Novus International (Saint Charles, MO) and was formulated to replace 50% of the inorganic Cu, Mn, and Zn in the diet.
[2]TRAX is glycine ligand, chelated trace mineral (B-Traxim 2C) produced by Pancosma (Geneva, Switzerland) and was formulated to replace 50% of the inorganic Cu, Mn, and Zn in the diet.
[3]Values determined at d 0 for mineral analysis were used in a covariate analysis.

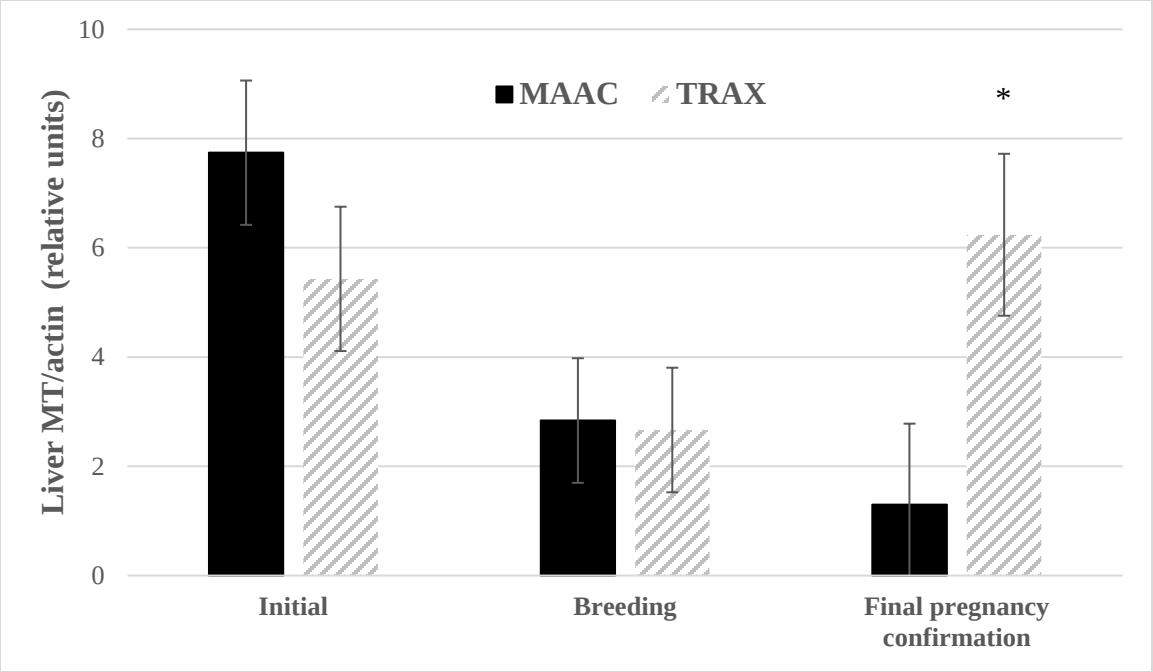


**Figure 2.1.** The effect of trace mineral supplementation type on cow liver metallothionein (MT)/actin mRNA expression normalized to a common control. MAAC is glycine ligand, chelated trace mineral (MAAC) produced by Novus International (Saint Charles, MO). TRAX is glycine ligand, chelated trace mineral (B-Traxim 2C) produced by Pancosma (Geneva, Switzerland). Both supplements were formulated to replace 50% of the inorganic Cu, Mn, and Zn in the diet. * indicates differences ($P$ = 0.03).

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# CHAPTER 3

## EFFECT OF AN INJECTABLE TRACE MINERAL AT THE INITIATION OF A 14 DAY CIDR PROTOCOL ON HEIFER PERFORMANCE AND REPRODUCTION[1]

**ABSTRACT:** Three experiments were conducted at separate locations to determine the effects of a trace mineral injection (**TMI**), Multimin 90, on heifer performance and reproduction. In Exp. 1, (spring-born, Angus, n = 93, body weight [**BW**] = 428 ± 45.2 kg), Exp. 2 (spring-born, Angus × Simmental, n = 120, BW = 426 ± 54.0 kg), and Exp. 3 (fall-born, commercial Angus, n = 199, BW = 345 ± 39.7 kg) heifers were stratified by BW within experiment and assigned to 1 of 2 treatments: a control, saline injection, or TMI at a dose of 1 mL/68 kg BW. Free choice mineral, containing Cu, Mn, Se, and Zn formulated to meet or exceed NRC recommendations, was supplemented to heifers. Injections w[1]ere given 33 d prior to breeding at the initiation of a 14-d controlled internal drug release (**CIDR**)-prostaglandin protocol. There was no difference ($P \geq 0.37$) in BW during Exp. 1. Additionally, there was no difference ($P \geq 0.52$) in body condition score (**BCS**) at initiation or at artificial insemination (**AI**) and final pregnancy confirmation in Exp. 1; however, a greater ($P = 0.03$) BCS was noted for control heifers at breeding. Pregnancy rates to timed AI and overall pregnancy rates were also similar ($P \geq 0.74$) regardless of treatment. During Exp. 2, BCS and BW did not differ ($P \geq 0.44$) across treatments. There was a tendency ($P = 0.07$) for TMI heifers to have an increased AI pregnancy rate (62% vs. 45%) compared with control heifers despite no difference ($P = 0.51$) in overall pregnancy rate. In Exp. 3, BW was not different ($P \geq 0.39$) across all time points. Also, BCS did not differ ($P \geq 0.45$) at initiation, AI, or final pregnancy conformation. Interestingly, there was a tendency ($P = 0.10$) for

[1]R.S. Stokes, A.R. Ralph, A.J. Mickna, W.P. Chapple, A.R. Schroeder, F.A. Ireland, and D. W. Shike. 2017. Effect of an injectable trace mineral at the initiation of a 14 day CIDR protocol on heifer performance and reproduction. Transl. Anim. Sci. 1:458-466. doi:10.2527/tas2017.0050.

TMI heifers to have an increased BCS at the time of breeding compared with control heifers. However, there were no differences ($P \geq 0.50$) in AI and overall pregnancy rates. In 1 of 3 experiments, an injectable trace mineral administered 33 d prior to the breeding season in conjunction with a 14-d CIDR protocol, tended to increased AI conception rates of heifers even when adequate trace mineral supplement was provided. The variable response observed across experiments may be caused by differences in breed, calving season, mineral sources, and management strategies.

**Key words:** artificial insemination, beef heifer, injectable trace mineral, pregnancy rate, reproduction

## INTRODUCTION

Trace minerals such as copper, manganese, selenium, and zinc play critical roles in biochemical processes and are key components of a ruminant animal's health and productivity (Suttle, 2010). Grazing cattle primarily receive trace minerals through forages; however, these sources often do not meet cattle requirements due to variation in soil composition (Smart et al., 1981). In these instances, producers commonly supplement through free-choice mineral, salt blocks fortified with trace minerals, or protein/energy supplements fortified with trace minerals (Arthington et al., 2014). Bioavailability of trace mineral sources can vary due to interactions with other minerals and feed components within the gastrointestinal tract (Spears, 2003). An injectable trace mineral provides the opportunity to supplement trace minerals that completely bypass the gastrointestinal tract and thus avoid the complex ruminal interactions. Also, a multi-element injectable trace mineral allows for targeted delivery of a specific amount to individual animals (Arthington et al., 2014) and eliminates the variability associated with voluntary intake of free choice mineral (Arthington and Swenson, 2004).

Pogge et al. (2012) recently demonstrated trace mineral injections are an effective way to increase the trace mineral status of calves, particularly Cu and Se. Increased mineral status may be of particular importance when biological needs are increased, such as breeding. In some research, an injectable trace mineral has improved reproductive performance. Sales et al. (2011) reported injectable trace mineral supplementation increased conception rates and chance of embryo survival after timed embryo transfer. Additionally, Kirchhoff (2015) reported an injectable trace mineral increased heifer artificial insemination (**AI**) conception rate. Multimin 90 is labeled for administration 30 d prior to breeding, which coincides with the initiation of a 14-d controlled internal drug release (**CIDR**) prostaglandin (**PG**) protocol. From a practical management standpoint, this allows for the administration of injectable trace mineral without any additional handling. Therefore, the objective of these experiments was to assess the effects of an injectable trace mineral administered at the initiation of a 14-d CIDR protocol on heifer performance and reproduction.

## MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois (IACUC #16046) and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animal in Agricultural Research and Teaching (FASS, 2010).

### *Experiment 1*

***Animals and Experimental Design.*** Spring-born, Angus heifers (Year 1: initial body weigh [BW] = 430 ± 489.9 kg, $n$ = 40; Year 2: initial BW = 425 ± 40.5 kg, $n$ = 53) housed at the University of Illinois Beef Cattle and Sheep Field Laboratory in Urbana, IL were utilized to

assess the effects of an injectable trace mineral (Multimin 90, Multimin USA, Fort Collins, CO) on heifer reproduction and performance over a 2-year period. Heifers were stratified by BW and assigned to 1 of 2 treatments: either an injection with sterilized saline (CON1) or an injection of Multimin 90 (MM1) administered subcutaneously at a dose of 1 mL/68 kg BW. The Multimin 90 contained 60 mg/mL of zinc as zinc oxide, 10 mg/mL of manganese as manganese carbonate, 5 mg/mL of selenium as sodium selenite, and 15 mg/mL of copper as copper carbonate. Prior to the start of the trial, heifers were weaned and developed on a diet consisting of roughage, corn co-products, and supplement. Heifers were then adapted to a total mixed ration (**TMR**, Table 1) that included both inorganic and organic trace mineral, and remained on the same diet through the initiation of the trial. In year 1 heifer initial BW was collected on d 0 (April 2015) and heifers were enrolled in a 14-d CIDR (Pfizer Animal Health, New York, NY) insert – PG and timed AI protocol (Mallory et al., 2012). On d 19 heifers were transported to pasture where they grazed 70% endophyte-infected fescue (*Festuca arundinacea*), and 30% red (*Trifolium pretense*) and white clover (*Trifolium repens*) pastures (60.91% NDF, 32.28% ADF, and 13.04% CP). While on pasture, heifers had access to free choice mineral [19.7% limestone, 19.7% trace mineral salt (8.5% Ca as calcium carbonate, 5% Mg as magnesium oxide and magnesium sulfate, 7.6% K as potassium chloride, 6.7% Cl as potassium chloride, 10% S as S8, prilled, 0.5% Cu as copper sulfate and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN), 2% Fe as iron sulfate, 3% Mn as manganese sulfate and Availa-4, 3% Zn as zinc sulfate and Availa-4, 278 mg/kg Co as Availa-4, 250 mg/kg I as calcium iodate, 150 mg/kg Se as sodium selenite, 2,205 KIU/kg VitA as retinyl acetate, 662.5 KIU/kg VitD as cholecalciferol, 22,047.5 IU/kg VitE as DL-α-tocopheryl acetate, and less than 1% crude protein, fat, crude fiber, salt), 14.2% salt, 40.6% monocalcium phosphate, 4.2% dried molasses, 1.4% zinc sulfate, and 0.01% iodine].

Heifers were weighed, body condition scored (**BCS**), and AI on d 33. Ten d following AI, heifers were exposed to 1 bull that had previously passed a breeding soundness exam, for a 64 d breeding season. Artificial insemination conception rates, BCS, and BW were collected on d 70 and final BCS, weights, and overall pregnancy confirmation were collected on d 130. A trained technician determined artificial insemination conception and overall pregnancy rates via ultrasonography (Aloka 500, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

In year 2, heifers were managed similarly with the following exceptions. At the initiation of the trial heifer BCS was measured. Ten d following AI heifers were divided into 2 groups with equal treatment representation in each group and exposed to 1 bull/group that had previously passed a breeding soundness exam. On d 60 (17 d after heifers had been exposed to bulls) 1 bull had to be removed from the study, so heifers were again co-mingled and placed with 1 bull that had passed a breeding soundness exam. Final BCS, BW, and pregnancy confirmation were collected on d 145.

***Sample Collection and Analytical Procedures.*** For nutrient composition analysis, individual feed ingredients were collected monthly and composited and dried at 55° C for a minimum of 3 days and ground through a 1 mm screen using a Wiley mill (Arthur, H. Thomas, Philadelphia, PA). Forage samples were collected from pastures on a monthly basis and composited and dried similarly. Ground feed samples were analyzed for CP (Leco TruMac, LECO Corporation, St. Joseph, MI), NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY), and crude fat using an Ankom XT10 fat extractor (Ankom Technology, Macedon, NY). Ground forage samples were also analyzed for NDF, ADF, and CP. Forage and feed samples were also sent to a commercial lab where they were subjected to nitric acid

digestion and inductively coupled plasma spectroscopy analysis for complete minerals (method 975.03: AOAC, 1988; The Ohio State University, Service Testing and Research Lab, Wooster, OH).

***Statistical Analysis.*** Body weight and BCS were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Artificial insemination rates and overall pregnancy rates were analyzed using the GLIMMIX procedure of SAS. Final pregnancy confirmation data included bull in the model; however, this was not significant ($P = 0.57$), so bull was removed from the model. Heifer was considered the experimental unit for all measures. The model included the fixed effect of treatment and the random effect of year. Significance was declared at $P \leq 0.05$, and tendencies were declared from $0.05 < P \leq 0.10$. Means reported in tables are least squares means ± SEM.

***Experiment 2***

***Animals and Experimental Design.*** To determine the effects of an injectable trace mineral on heifer reproduction and performance over a 2 year period, spring-born, Angus × Simmental heifers (Year 1: initial BW = 411 ± 48.1 kg, $n = 65$; Year 2: initial BW = 441 ± 59.8 kg, $n = 55$) were utilized. Heifers were either 0.5 Angus and 0.5 Simmental or 0.625 Angus and 0.375 Simmental. Cattle were stratified by BW into 2 groups and randomly assigned either a sterilized saline injection (CON2) or a Multimin 90 injection (MM2) at a rate of 1 mL/68 kg BW. In year 1, prior to the start of the trial, heifers were housed at the University of Illinois Beef Cattle and Sheep field Laboratory in Urbana, IL and fed a diet consisting of alfalfa haylage, corn silage, corn co-products, and supplement. Heifers were then adapted to a TMR (Table 1) and remained on this diet until d -13, which included both an organic and inorganic trace mineral supplement. On d -13 heifers were transported to the Orr Agricultural Research and Demonstration Center in

Baylis, IL. Heifers were housed in soil surface pens and offered a TMR and an inorganic trace mineral supplement (Table 2). Intake averaged 7.1 kg of DM/d per heifer across treatments. At the initiation of the study (April, 2015), individual heifer BW were measured, treatments applied, and heifers were enrolled in a 14-d CIDR-PG and timed AI protocol. On d 33 heifers were weighed, BCS, and timed AI. Immediately following timed AI heifers were transported to pasture for the duration of the breeding season. Heifers grazed pastures with an average coverage area of 70% endophyte-infected fescue (*Festuca arundinacea*) and 30% red clover (*Trifolium pretense*) and white clover (*Trifolium repens*, 9.05% NDF, 32.98% ADF, and 11.96% CP) for the remainder of the study and were given access to free choice mineral (12% Ca as calcium carbonate, 8% P, 18% salt, 11% Mg as magnesium sulfate, 90 mg/kg I as calcium iodate, 108,862 IU/kg vitamin A, 18,144 IU/kg vitamin $D_3$, 454 IU/kg vitamin E, and 5600 mg/kg of chlortetracycline [Aureomycin; Alpharma Inc. Animal Health, Bridgewater, NJ]). Ten days following AI, a bull was placed with each pen of heifers for a 45 d breeding season. Artificial insemination conception rates, BW, and BCS were determined on d 70. Overall pregnancy rate, BW, and BCS were collected on d 144. A trained technician determined artificial insemination conception and overall pregnancy rates via ultrasonography (Aloka 500 instrument, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

In year 2, heifers were managed similarly with the following exceptions. The University of Illinois Beef Cattle and Sheep field Laboratory in Urbana, IL was used to house heifers from weaning until d 10 of the trial. On d 10, heifers were transported to the Orr Agricultural Research and Demonstration Center in Baylis, IL and were housed in soil surface pens and received a similar diet and trace mineral supplement (Table 2). Average heifer DMI was 8.5 kg/d per heifer across treatments. At the time of CIDR removal, all heifers' tail heads were painted and paint

scores (1 = completely gone, 2 = partially gone, and 3 = untouched) were collected at the time of breeding. Heifers were managed in 2 groups and 10 d following AI were exposed for a 46 day breeding season to 1 bull per group that had previously passed a breeding soundness exam. Overall pregnancy rate, BW, and BCS were collected on d 145.

***Sample Collection and Analytical Procedures.*** Feed ingredients and forage samples were collected and analyzed as described in Exp. 1.

***Statistical Analysis.*** Data were analyzed as described in Exp. 1 with the following exception that group was included as a fixed effect. Treatment distributions of tail paint scores were determined using PROC GLIMMIX of SAS.

***Experiment 3***

***Animals and Experimental Design.*** Fall-born, commercial Angus (*n* = 199, initial BW = 345 ± 39.7 kg) were utilized to determine the effects of an injectable trace mineral on heifer BW, BCS, and reproductive performance. Heifers were fed and managed at the Dixon Springs Agricultural Research Center, Simpson, IL. Body weight and sire were used to stratify cattle. Heifers were utilized previously in another study and thus heifers were also stratified by previous treatment (Kordas et al., 2017). On d 0 (October, 2015), initial BW and BCS were collected, heifers were synchronized with a 14-d CIDR-PG timed AI protocol as previously described by Mallory et al. (2012), and administered 1 of 2 treatments: a control sterile saline injection (CON3) or a Multimin 90 injection (MM3) both administered at a rate of 1 mL/68 kg of BW. Heifers were placed on pastures following AI, managed as a single group, and grazed 70% endophyte-infected fescue (*Festuca arundinacea*) and 30% red clover (*Tri-folium pretense*) pastures (60.53% NDF, 34.41% ADF, and 9.81% CP). Heifers were offered a supplement consisting of 50% soybean

hulls (62.53% NDF, 45.74% ADF, and 11.26% CP) and 50% corn gluten feed pellets (33.65% NDF, 9.19% ADF, and 23.77% CP) at a rate of 2.7 kg/heifer per d. Free choice loose mineral (Renaissance Nutrition, Roaring Springs, PA; 0.16% S, 17.88% Ca as calcium carbonate, 2.99% P as monocalcium phosphate, 24.5% salt, 9.35% Na, 5.74% Mg as magnesium oxide, 0.06% K, 2,214 mg/kg Fe as iron oxide, 2,013 mg/kg Mn as manganese hydroxychloride [Intellibond M, Micronutrients Inc., Indianapolis, IN], 2,511 mg/kg Zn as hydroxyl zinc [Intellibond Z, Micronutrients Inc.], 1,001 mg/kg Cu as tribasic copper chloride [Intellibond C, Micronutrients Inc.], 27 mg/kg Co as cobalt carbonate, 36 mg/kg I as calcium iodate, 26 mg/kg Se as sodium selenite, 110,178 IU/kg vitamin A, 3,084 IU/kg vitamin D, 545 IU/kg vitamin E, and 1,179 mg/kg of chlortetracycline) was offered ad libitum to heifers.

On d 30, heat detection patches (Estrotect Heat Detectors, Rockway Inc., Spring Valley, WI) were placed on all heifers. On d 33 heifers were weighed, BCS, AI and heat patches were visually scored from 0 to 3 (0 = missing, 1 = fully activated, 2= partially activated, and 3 = not activated). Nine days following AI, heifers were exposed to 5 yearling bulls that had all passed breeding soundness exams, for a 71 d breeding season. Due to limited pasture forage availability, on d 71 heifers were offered free choice grass hay (68.7% NDF, 39.9% ADF, and 6.01% CP) for the remainder of the trial. On d 76 individual BW, BCS, and AI pregnancy conception rates were collected. Overall conception rates and final BW and BCS were collected on d 153. A trained technician determined AI conception and overall pregnancy rates via ultrasonography (Aloka 500 instrument, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

***Sample Collection and Analytical Procedures.*** Feed ingredients and forage samples were collected and analyzed as described in Exp. 1.

***Statistical Analysis.*** Heifer BW and BCS were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Heifer AI conception and overall pregnancy rates were analyzed using the GLIMMIX procedure of SAS. Treatment distributions of heat patch scores were determined using PROC GLIMMIX of SAS. The model included the fixed effects of treatment. Treatment effects were considered significant at $P \leq 0.05$ and tendencies were noted at $0.05 < P \leq 0.10$.

## RESULTS

### *Experiment 1*

All heifers received the same diet (Table 1) or pasture regardless of treatment. There was no difference ($P \geq 0.37$, Table 3) for heifer BW at any time point. Also, there was no difference ($P \geq 0.52$) for heifer BCS at the initiation of the trial, AI pregnancy confirmation, or final pregnancy confirmation regardless of treatment. Interestingly, CON1 heifers had greater ($P = 0.03$) BCS at breeding than their MM1 treated counterparts. Pregnancy rates to timed AI and overall pregnancy rates were similar for CON1 and MM1 treated heifers ($P \geq 0.74$, Figure 1).

### *Experiment 2*

All heifers received the same diet (Table 2) or pasture regardless of treatment. Heifer BW and BCS did not differ across all time points ($P \geq 0.44$, Table 4). Additionally, the distribution of tail paint scores was similar ($P \geq 0.90$) across treatments. There was a tendency ($P = 0.07$, Figure 2) for MM2 heifers to have greater AI pregnancy rates (62.1%) compared with their CON2 (45.2%) counterparts. However, by the time of overall pregnancy confirmation there was no difference ($P = 0.51$) for pregnancy rates regardless of treatment and overall pregnancy rate averaged 82.7%.

***Experiment 3***

Heifer BW did not differ across all time points ($P \geq 0.39$, Table 5). Additionally, heifer BCS did not differ ($P \geq 0.45$) at the initiation of the trail or at AI and final pregnancy confirmation. However, there was a tendency ($P = 0.10$) for MM3 heifers to have a greater BCS at the time of breeding than their CON3 counterparts. There were also no differences ($P \geq 0.50$) across treatments, in the distribution of heat patch scores for Exp. 3. Pregnancy rates to timed AI and overall pregnancy rates were also similar for both CON3 and MM3 treated heifers ($P \geq 0.50$, Figure 3).

## DISCUSSION

For all experiments, trace mineral injection did not affect heifer BW at any time point. Also, heifer BCS was similar at initiation, AI pregnancy confirmation, and final pregnancy confirmation regardless of treatment across these experiments. Since diet and pasture availability were the same for both treatments, regardless of experiment, it is likely this did not impact the results. These results are consistent with Gadberry and Baldridge (2013), where BW and BCS did not differ for Angus cows receiving either an injectable trace mineral or no injectable trace mineral prior to breeding. However, the effects of injectable trace mineral supplementation on cow BW and BCS has been inconsistent across literature. Arthington et al. (2014) reported in beef heifers, those administered an injectable trace mineral tended to have greater ADG compared with heifers given saline. Still, it is important to note the heifers utilized by Arthington et al. (2014) were administered treatments at weaning and heifers remained on study through breeding, for a total of 177 d. In the current experiments, treatments were administered 33 d prior to breeding, at estrus synchronization, and heifers remained on study until the time of

final pregnancy confirmation, making it challenging to draw comparisons across these experiments.

Interestingly, in Exp. 1, CON1 heifers tended to have a greater BCS at the time of breeding than their MM1 counterparts. Contrastingly in Exp. 3, CON3 heifers tended to have a lesser BCS than MM3 heifers at the time of breeding. It is important to note these differences in BCS were significant due to a small standard error (0.172 and 0.048 for Exp. 1 and Exp. 3, respectively) and it is likely this difference is physiologically insignificant. Still, other authors have noted decreased performance in heifers treated with an injectable trace mineral after stress due to shipping or transportation (Arthington et al., 2014). Arthington et al. (2014) hypothesized this performance decrease is due to an increase in Cu-dependent acute phase protein ceruloplasmin, which can affect nutrient metabolism and animal growth (Johnson, 1997), and could be greater in cattle with an increased Cu status. From a management standpoint, it is unlikely the heifers in the current experiments, had been subjected to any stress that would have increased these acute phase proteins. However, stress was not assessed in this trial so, it is unknown if this may have explained the BCS changes noted at breeding.

In both Exp. 1 and Exp. 3, pregnancy rates to timed AI and overall pregnancy rates were similar for both treatments. In an intensively managed dairy herd fed a TMR that met NRC requirements for trace mineral supplementation, no difference was reported in first-service conception rate when a single injection of trace mineral was administered prior to breeding (Vanegas et al., 2004). While it was physiologically unclear why this may have occurred, this data could suggest additional trace mineral supplementation in a herd provided with NRC recommended levels of trace minerals provides no beneficial effects. The heifers in the current

study were provided trace mineral at or above NRC recommendations, suggesting they likely had an adequate mineral status.

It is important to note heifers in Exp. 3 weighed 348 kg at breeding, approximately 53% of the herd's 650 kg average mature BW. The NRC (2016) suggests Bos Taurus heifers should reach puberty at approximately 60% of mature weight. Additionally, Vera et al. (1993) noted heifers in a nutritionally restrictive environment will have an even greater percentage of mature BW at puberty than noted above. Mature cows also in a restrictive environment often weigh less than cows of a similar genotype not maintained in a restrictive environment (Pahnish et at., 1983). These heifers were more nutritionally challenged and thus below their expected percent of mature BW at breeding. The authors speculated the greatest response in trace mineral supplementation would be noted in Exp. 3. However, pregnancy rates to timed AI and overall pregnancy rates were similar across treatments, and there were no differences in the distribution of heat patch scores for Exp. 3. This data suggests all heifers responded similarly to estrus synchronization and there was no difference in the number of heifers exhibiting standing estrus at the time of AI. This is comparable to work by Brasche et al. (2015) who reported no effect of trace mineral injection on overall pregnancy rates or overall AI conception of commercial and purebred Angus heifers synchronized using a 14-d CIDR-PG protocol. Gadberry and Baldridge (2013) also noted no effect on pregnancy rate when Angus cows were treated with either an injectable trace mineral or no injection. Although the authors speculated the greatest response would be noted in this experiment, as heifers were less than the recommended 60% of mature BW, control heifer still had acceptable AI pregnancy rates (66%). Other researchers have reported favorable reproductive success when heifers were bred at 50-55% of mature BW (Funston et al., 2012; Gunn et al., 2015).

Exp. 2 heifers treated with a trace mineral injection (TMI) tended to have greater AI pregnancy rates compared with their control counterparts. However, overall pregnancy rates were similar regardless of treatment. Heifers that conceive to AI will not only have an increased probability of weaning more calves in their lifetime but also heavier calves (Burris and Priode, 1958). Additionally, when more heifers conceive to AI, this can result in a more uniform calf crop and a shortened breeding season (Dziuk and Bellows, 1983). Even though all heifers had access to free choice trace mineral during the grazing period and were provided with an organic trace mineral as part of a TMR from weaning until beginning the grazing period, a favorable response ($P = 0.07$) in AI pregnancy rates due to the trace mineral injection was still noted. Since offering free choice mineral can result in inconsistent consumption and erratic intake, it is possible the trace mineral status of MM2 heifers was more optimal than CON2 heifers. However, it is important to note the distribution of tail paint scores was similar across treatments, suggesting all heifers responded similarly to estrus synchronization, and thus conception differences were not due to differences in synchronization response. Interestingly, Kirchhoff (2015) reported a similar increase in pregnancy rate to AI when beef heifers were treated with an injectable trace mineral and noted no differences across treatment in estrous behavior as indicated by estrous detection patches. Mundell et al. (2012) also noted comparable results when heifers were treated with either a trace mineral injection or with sterile saline; heifers that received the trace mineral injection had greater fixed time AI conception rates and overall pregnancy rates were similar between treatments. These data suggest an injectable trace mineral may increase AI conception rates even if dietary trace minerals are meeting heifer's requirements.

However, as with performance parameters, the effects of an injectable trace mineral on reproductive performance has been inconsistent. Brasche et al. (2014a) treated Angus heifers with either a trace mineral or sterile saline injection 33 d prior to AI and reported greater AI and overall pregnancy rates for heifers receiving a trace mineral injection. These differences were noted despite the trace mineral injection having no effect on the liver concentration of Mn, Cu, or Zn. Though, Brasche et al. (2014a) did report an increase in liver Se concentrations of heifers treated with a trace mineral injection. In contrast, Daugherty et al. (2002) improved the Cu status of cows treated with trace mineral injection and vitamin E; however, saw no effect on the conception rate of beef cows compared to saline-treated cows. Brasche et al. (2014b) also noted no difference in AI or overall pregnancy rates when cows were given either a trace mineral or saline injection at breeding. While it is unknown what may be driving this variability across experiments it is possible other factors such as breed differences, management strategies, nutrient status, and potential mineral antagonists are playing a complex role in assessing reproductive performance in cattle supplemented with an injectable trace mineral.

The cattle utilized in Exp. 2 were predominantly Simmental influenced cattle, while those utilized in Exp. 1 were Angus, suggesting breed differences may account for some variability noted. Particularly with Cu, differences have been reported in how Simmental cattle absorb (Ward et al., 1995) and excrete (Gooneratne et al., 1994) this trace mineral, suggesting Simmental may have greater Cu requirements compared with Angus. Additionally, data has suggested Simmental cattle may have differences in liver Mn excretion compared with Angus cattle (Pogge et al., 2012). This data suggests the cattle utilized in Exp. 2, which the literature suggests were genetically already predisposed to a greater trace mineral requirement, could have had an altered trace mineral status at the initiation of the trial, allowing the TMI to improve

heifer mineral status at breeding, when trace minerals are of utmost importance. This could help to explain why increased AI conception rates were noted for MM2 heifers over their CON2 counterparts. Unfortunately, since trace mineral status was not assessed in this trial, we are unable to conclude if these Simmental heifers did have a greater need for the trace minerals provided from the trace mineral injection.

Heifers utilized in Exp. 2 were developed in a drylot and then moved to an early grazing season pasture at breeding. The NRC (2016) suggests this management strategy can result in BW loss and greater instances of reproductive failure. The heifers in Exp. 2 lost 13.5 kg from the initiation of the trial to breeding (33 d), suggesting the negative energy balance immediately prior to breeding could have decreased reproductive performance. It is possible that the additional Cu, Mn, Se, and Zn provided to the MM2 treated heifers could have played an important role in maintaining reproductive success. Trace minerals play a crucial role in a ruminant animal's productivity (Suttle, 2010) and can play important roles in hormone synthesis (Paterson and Engle, 2005), which is vital for reproductive success. However, further research is needed to elucidate how trace minerals alter or improve embryo survival when cattle are in a negative energy balance at breeding.

In conclusion, under the conditions of these 3 experiments, an injectable trace mineral administered 33 d prior to the breeding season, in certain management settings, resulted in increased AI conception rates of heifers even when provided adequate trace mineral supplement. Due to the difficulty of assessing trace mineral status of an entire herd, supplementing trace minerals through an injection may be a viable way to ensure a consistent, adequate trace mineral supply to heifers for optimal reproductive performance. The reproductive response across these 3 experiments was variable. However, it is important to note that injectable trace minerals do not

appear to incur any negative impacts on heifer performance or reproductive success. Further research is required to substantiate this hypothesis and to further understand the response differences elicited by injectable trace mineral supplementation across herds.

## TABLES AND FIGURES

**Table 3.1**. Ingredient composition of heifer diets (% DM basis) for Exp. 1

| | Inclusion, %DM | |
|---|---|---|
| Item | Year 1 | Year 2 |
| Ingredient, % | | |
| Corn silage | 72 | 60 |
| MDGS[1] | 13 | 15 |
| Treated corn stalks[2] | 10 | - |
| Grass hay | - | 20 |
| Supplement[3] | 5 | 5 |
| Analyzed nutrient content | | |
| CP, % | 10.1 | 9.9 |
| NDF, % | 51.1 | 50.7 |
| ADF, % | 28.9 | 27.7 |
| Crude fat, % | 2.9 | 3.5 |
| S, % | 0.18 | 0.20 |
| Cu, mg/kg | 4.8 | 13.6 |
| Mn, mg/kg | 29.3 | 29.1 |
| Zn, mg/kg | 21.9 | 36.6 |

[1]Experiment 1 determined the effects of a TMI on spring born Angus × Simmental heifer performance and reproduction.

[2]Modified distillers grains with solubles.

[3]Corn stalks were treated with Silage SAVOR Plus (Kemin Industries, Inc., Des Moines, IA) at 0.5 kg $Mg^{-1}$ applied at bagging.

[4]Supplement contained 87.7% ground corn, 8.9% limestone, 1.8% trace mineral salt [8.5% Ca as calcium carbonate, 5% Mg as magnesium oxide and magnesium sulfate, 7.6% K as potassium chloride, 6.7% Cl as potassium chloride, 10% S as S8, prilled, 0.5% Cu as copper sulfate and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN), 2% Fe as iron sulfate, 3% Mn as manganese sulfate and Availa-4, 3% Zn as zinc sulfate and Availa-4, 278 mg/kg Co as Availa-4, 250 mg/kg I as calcium iodate, 150 mg/kg Se as sodium selenite, 2,205 KIU/kg VitA as retinyl acetate, 662.5 KIU/kg VitD as cholecalciferol, 22,047.5 IU/kg VitE as DL-α-tocopheryl acetate, and less than 1% crude protein, fat, crude fiber, salt], 0.1% Rumensin 90 (198 g monensin/kg, Rumensin 90; Elanco Animal Health, Greenfield, IN), and 1.5% fat.

**Table 3.2**. Ingredient composition of heifer diets (% DM basis) for Exp. 2[1]

| | Inclusion, %DM | |
|---|---|---|
| Item | Year 1 | Year 2 |
| Ingredient, % | | |
| Corn silage | 24.7 | 59.0 |
| MDGS[2] | 14.14 | 20.0 |
| Grass hay | 61.16 | 16.0 |
| Supplement[3,4] | - | 5.0 |
| Analyzed nutrient content | | |
| CP, % | 12.1 | 10.5 |
| NDF, % | 53.9 | 41.1 |
| ADF, % | 33.2 | 21.0 |
| Crude fat, % | 2.3 | 4.2 |
| S, % | 0.27 | 0.22 |
| Cu, mg/kg | 6.4 | 7.0 |
| Mn, mg/kg | 40.2 | 67.2 |
| Zn, mg/kg | 30.0 | 37.8 |

[1]Experiment 2 determined the effects of a TMI on spring born Angus × Simmental heifer performance and reproduction.

[2]Modified distillers grains with solubles.

[3]In year 1 supplement was top dressed at a rate of 0.11 kg/heifer per d (23.4% Ca as calcium carbonate, 15.7% salt, 1.0% Mg as magnesium oxide, 3,500 mg/kg of Zn as zinc sulfate, 3,350 mg/kg of Cu as copper sulfate, 26.4 mg/kg of Se as sodium selenite, 181,437 IU/kg of vitamin A, and 181 IU/kg of vitamin E).

[4]Supplement contained 87.7% ground corn, 8.9% limestone, 1.8% trace mineral salt [8.5% Ca as calcium carbonate, 5% Mg as magnesium oxide and magnesium sulfate, 7.6% K as potassium chloride, 6.7% Cl as potassium chloride, 10% S as S8, prilled, 0.5% Cu as copper sulfate and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN), 2% Fe as iron sulfate, 3% Mn as manganese sulfate and Availa-4, 3% Zn as zinc sulfate and Availa-4, 278 mg/kg Co as Availa-4, 250 mg/kg I as calcium iodate, 150 mg/kg Se as sodium selenite, 2,205 KIU/kg VitA as retinyl acetate, 662.5 KIU/kg VitD as cholecalciferol, 22,047.5 IU/kg VitE as DL-α-tocopheryl acetate, and less than 1% crude protein, fat, crude fiber, salt], 0.1% Rumensin 90 (198 g monensin/kg, Rumensin 90; Elanco Animal Health, Greenfield, IN), and 1.5% fat.

**Table 3.3.** Influence of an injectable trace mineral on heifer BW and BCS over 2 consecutive years in Exp. 1

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | CON1 | MM1 | SEM | *P*-value |
| n, heifer | 47 | 46 | | |
| BW, kg | | | | |
| Initial | 429 | 426 | 4.6 | 0.72 |
| Breeding | 425 | 424 | 6.4 | 0.92 |
| AI pregnancy confirmation | 433 | 418 | 6.5 | 0.37 |
| Final pregnancy confirmation | 437 | 436 | 16.2 | 0.78 |
| BCS | | | | |
| Initial | 5.4 | 5.4 | 0.07 | 0.52 |
| Breeding | 5.7 | 5.5 | 0.17 | 0.03 |
| AI pregnancy confirmation | 5.5 | 5.5 | 0.22 | 0.86 |
| Final pregnancy confirmation | 5.5 | 5.6 | 0.22 | 0.64 |

[1]Control (CON1) cattle received a sterilized saline solution at 1 mL/68 kg BW, and Multimin 90 (MM1) cattle received injectable trace mineral at 1 mL/68 kg BW.

**Table 3.4.** Influence of an injectable trace mineral on heifer BW, BCS, and tail paint score over 2 consecutive years in Exp. 2

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | CON2 | MM2 | SEM | *P*-value |
| n, heifer | 60 | 60 | | |
| BW, kg | | | | |
| Initial | 427 | 426 | 15.1 | 0.97 |
| Breeding | 413 | 413 | 10.3 | 0.96 |
| AI pregnancy confirmation | 432 | 427 | 9.3 | 0.44 |
| Final pregnancy confirmation | 434 | 435 | 27.5 | 0.81 |
| BCS | | | | |
| Initial | 5.5 | 5.5 | 0.09 | 0.58 |
| Breeding | 5.8 | 5.8 | 0.09 | 0.78 |
| AI pregnancy confirmation | 5.6 | 5.6 | 0.14 | 0.80 |
| Final pregnancy confirmation | 5.5 | 5.5 | 0.17 | 0.58 |
| Tail paint score[2,3], % | | | | |
| 1 | 25 | 23 | - | 0.90 |
| 2 | 75 | 74 | - | 0.95 |
| 3 | - | - | - | - |

[1]Control (CON2) cattle received a sterilized saline solution at 1 mL/68 kg BW, and Multimin 90 (MM2) cattle received injectable trace mineral at 1 mL/68 kg BW.
[2]Tail paint scores were visually assessed at the time of breeding (1 = completely gone, 2 = partially gone, 3 = untouched).
[3]Tail paint scores were not collected in year 1.

**Table 3.5**. Influence of an injectable trace mineral on heifer BW, BCS, and heat patch scores in Exp. 3

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | CON3 | MM3 | SEM | *P*-value |
| n, heifer | 99 | 100 | | |
| BW, kg | | | | |
| Initial | 344 | 344 | 2.8 | 0.93 |
| Breeding | 347 | 348 | 2.9 | 0.79 |
| AI pregnancy confirmation | 346 | 348 | 3.1 | 0.76 |
| Final pregnancy confirmation | 374 | 370 | 3.3 | 0.39 |
| BCS | | | | |
| Initial | 5.4 | 5.4 | 0.05 | 0.95 |
| Breeding | 5.6 | 5.7 | 0.05 | 0.10 |
| AI pregnancy confirmation | 5.0 | 5.1 | 0.05 | 0.45 |
| Final pregnancy confirmation | 4.9 | 4.9 | 0.05 | 1.00 |
| Heat Patch Score[2], % | | | | |
| 1 | 58 | 59 | - | 0.89 |
| 2 | 17 | 20 | - | 0.59 |
| 3 | 25 | 21 | - | 0.50 |

[1]Control (CON3) cattle received a sterilized saline solution at 1 mL/68 kg BW, and Multimin 90 (MM3) cattle received injectable trace mineral at 1 mL/68 kg BW.

[2] Heat patches were visually scored at time of breeding from 0 – 3 (0 = missing, 1 = fully activated, 2= partially activated, 3 = not activated). No heifers were missing heat patches at the time of breeding.

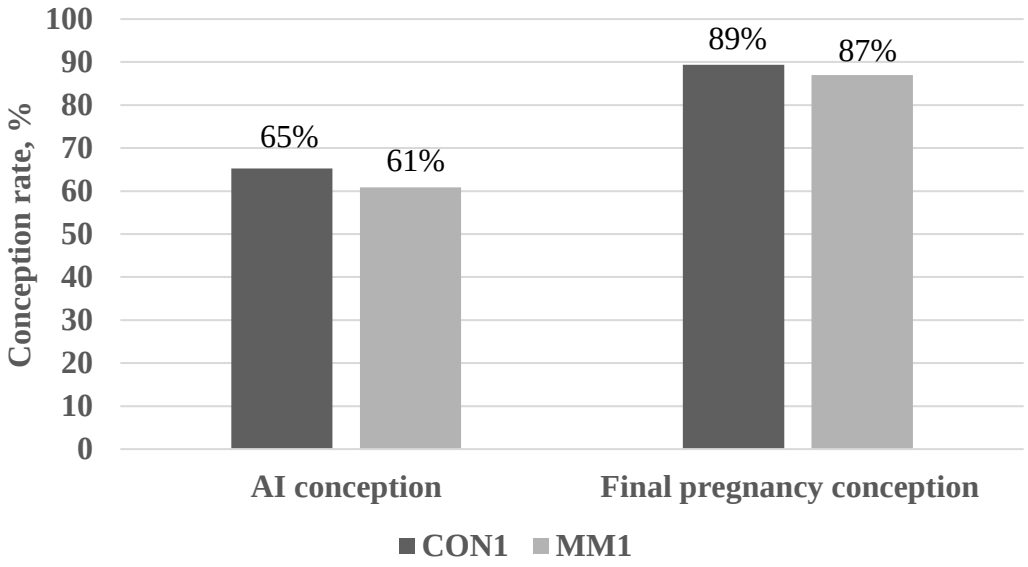


**Figure 3.1.** The effect of an injectable trace mineral (Multimin 90, MM1) or sterilized saline solution (CON1) at the initiation of a synchronization protocol on heifer AI and final pregnancy rates over 2 consecutive years in Exp. 1. Pregnancy rates to timed AI and overall pregnancy rates were similar for CON1 and MM1 treated heifers ($P \geq 0.74$).

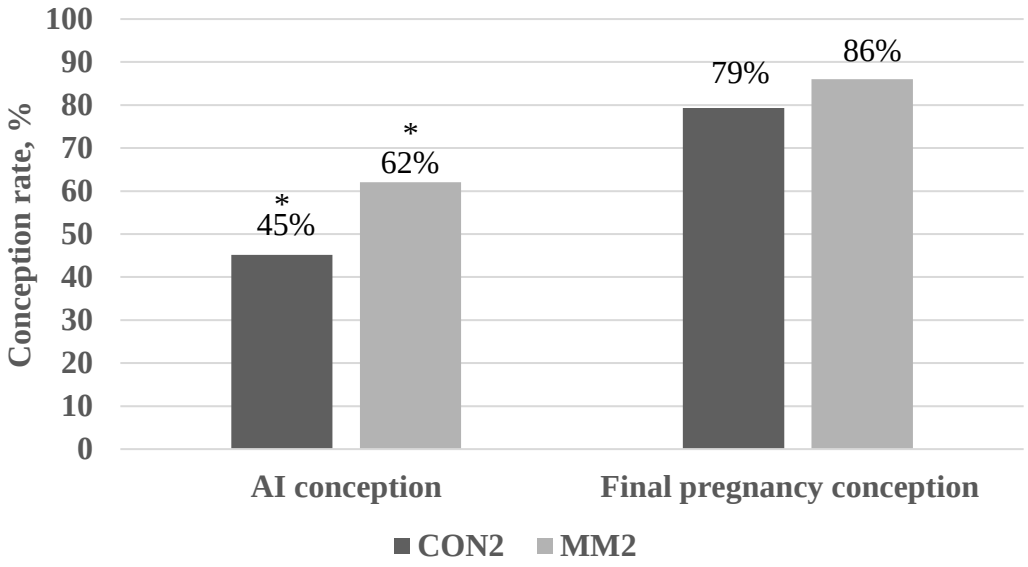


**Figure 3.2.** The effect of an injectable trace mineral (Multimin 90, MM2) or sterilized saline solution (CON2) at the initiation of a synchronization protocol on heifer AI and final pregnancy rates over 2 consecutive years in Exp. 2. * indicates a tendency ($P = 0.07$) for MM2 AI conception rates to be greater than CON2 heifers. Overall pregnancy rates were similar for CON2 and MM2 treated heifers ($P = 0.51$).

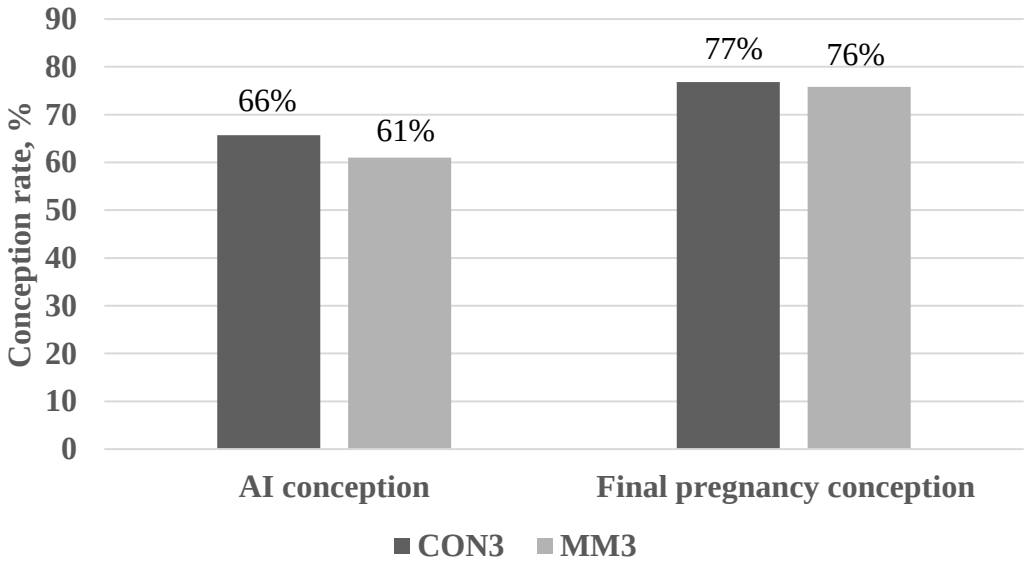


**Figure 3.3.** The effect of an injectable trace mineral (Multimin 90, MM3) or sterilized saline solution (CON3) at the initiation of a synchronization protocol on heifer AI and final pregnancy rates in Exp. 3. Pregnancy rates to timed AI and overall pregnancy rates were also similar for both CON3 and MM3 treated heifers ($P \geq 0.50$).

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# CHAPTER 4

## EFFECT OF REPEATED TRACE MINERAL INJECTIONS ON BEEF HEIFER DEVELOPMENT AND REPRODUCTIVE PERFORMANCE[2]

**ABSTRACT:** To determine the effects of repeated trace mineral injections on heifer development and reproductive performance, commercial Angus heifers (*n*=290; 199±34.3 kg; 221±22 d of age) were utilized in a completely randomized design. Heifers were stratified by body weight (**BW**) and were administered an injectable trace mineral (**MM;** Multimin90) or saline (**CON**) given subcutaneously, post-weaning at 221, 319, 401, and 521±22 d of age. Throughout development, heifers grazed endophyte-infected fescue, red clover pastures and were supplemented with corn distillers grains (2.7 kg·heifer$^{-1}$·d$^{-1}$) and given access to free choice inorganic minerals. Heifer BW and body condition scores (**BCS**) were collected at trial initiation and 4-7 week intervals thereafter. Hair coat scores (**HCS**) and respiration rates (n=30 heifers/treatment) were collected at 269, 310, and 361±22 d of age. Blood and liver samples were collected at trial initiation and estrous synchronization from 30 heifers/treatment to determine trace mineral status. At 319, 372, and 421±22 d of age, antral follicle count and ovarian size were determined via ultrasonography. Two blood samples from all heifers were collected 10 d apart, concurrent with ultrasound dates, for cyclicity determination. Estrous synchronization was initiated, and reproductive tract scores (**RTS**) were collected at 421±22 d of age, and heifers were bred via artificial insemination (**AI**) at 430±22 d of age. Heifer BW, BCS, and HCS did not differ ($P \geq 0.12$) throughout development, except at 268±22 d of age when BCS was greater ($P = 0.03$) for MM than CON heifers. Respiratory rates were greater ($P = 0.05$) for MM than CON

[2]R.S. Stokes, M.J. Volk, F.A. Ireland, P.J. Gunn, and D.W. Shike. 2018. Effect of repeated trace mineral injections on beef heifer development and reproductive performance. J. Anim. Sci. doi:10.1093/jas/sky253.

heifers at 269±22 d of age but did not differ ($P \geq 0.66$) at 310 and 361±22 d of age. Plasma Mn and Zn concentrations did not differ ($P \geq 0.54$). However, MM heifers had greater ($P \leq 0.01$) plasma and liver concentrations of Cu and Se compared to CON. Interestingly, MM decreased ($P = 0.02$) liver Zn concentrations compared to CON, and there was no difference ($P = 0.60$) in liver Mn. Antral follicle count and ovarian size did not differ ($P \geq 0.51$) due to treatment. Throughout development, number of heifers cycling was lesser ($P < 0.01$) for MM than CON heifers. However, there was no difference ($P \geq 0.19$) in RTS, AI pregnancy rates, or overall pregnancy rates. Supplementing an injectable trace mineral increased heifer Cu and Se status; however, no effect was noted on ovarian development or pregnancy rates.

**Key Words:** antral follicle count, copper, injectable trace mineral, reproduction, selenium, zinc

## INTRODUCTION

Trace minerals such as copper, manganese, selenium, and zinc play a critical role in numerous biochemical processes and are key components of a ruminant animal's health and productivity (Suttle, 2010). An injectable platform offers a unique way to supplement trace minerals in a manner that may circumvent the gastrointestinal tract, thus avoiding antagonists and competition for absorption at the intestinal level (Pogge et al., 2012). Once trace minerals are injected into the animal, the minerals circulate throughout the body and incorporate into cells as needed, while the remaining mineral is transported to the liver where it is either excreted or bound to proteins for long-term storage (Suttle, 2010). Increased mineral status of an animal could be of particular importance at times when biological needs are increased, including when animals are growing or breeding. Additionally, injectable trace minerals offer an advantage compared to traditional oral supplement methods in that they provide a targeted delivery of a specific amount of trace minerals to individual animals. This injection eliminates the variability

associated with fluctuation in voluntary intake noted among cattle provided free choice mineral (Arthington and Swenson, 2004).

Multimin90 (Multimin USA, Fort Collins, CO) is an injectable trace mineral that is labeled for administration every 90 d in heifers, and in particular, the 4 weeks prior to breeding. Recently, literature has focused on the administration of an injectable trace mineral pre-breeding to both heifers and cows with inconsistent results noted (Vanagas et al., 2004; Brasche, 2015; Gonzalez-Maldonado et al., 2017; Stokes et al., 2017). Moreover, the effects of utilizing an injectable trace mineral every 90 d on heifer development has yet to be reported. Therefore, the objective of this experiment was to evaluate the effects of repeated trace mineral injections on heifer growth, mineral status, and reproductive development and performance.

## MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois (IACUC #16046) and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animal in Agricultural Research and Teaching (FASS, 2010).

### *Animals and Experimental Design.*

Two hundred and ninety fall-born, commercial Angus heifers (199 ± 34.3 kg) were utilized in a stratified randomized design to determine the effects of repeated trace mineral injections on heifer development and reproductive performance. Heifers were stratified at 221 ± 22 d of age by body weight (**BW**) and body condition score (**BCS**) and assigned 1 of 2 treatments: 1) an injectable trace mineral (**MM;** Multimin90; Multimin USA, Fort Collins, CO) or 2) a sterilized physiological saline injection (**CON;** Fig. 1). The Multimin90 contained 60

mg/mL of zinc, 10 mg/mL of manganese, 5 mg/mL of selenium, and 15 mg/mL of copper. Injections were administered subcutaneously and at label dose. Specifically, administration occurred post-weaning at 221 and 319 ± 22 d of age at a rate of 1 mL/45 kg BW and at 401 and 521 ± 22 d of age at a rate of 1 mL/68 kg BW. Heifers were housed at the Dixon Springs Agricultural Center in Simpson, IL and grazed endophyte-infected fescue (*Festuca arundinacea*; 81% percent endophyte infected tillers) and red clover (*Tri-folium pretense*) pastures (8.7% ash; Fig. 2) and were supplemented with corn distillers grains (2.7 $kg \cdot heifer^{-1} \cdot day^{-1}$; 35.1% neutral detergent fiber (**NDF**), 11.2% acid detergent fiber (**ADF**), 13.32% fat, and 30.3% crude protein (**CP**). Heifers were also given access to free choice inorganic trace minerals (Renaissance Nutrition, Roaring Springs, PA; 0.24% S, 21.37% Ca as calcium carbonate, 2.99% P as monocalcium phosphate, 24.5% salt, 9.35% Na, 5.84% Mg as magnesium oxide, 0.06% K, 2,214 mg/kg Fe as iron oxide, 2,000 mg/kg Mn as manganous oxide, 2,500 mg/kg Zn as zinc oxide, 1,500 mg/kg Cu as copper sulfate, 27 mg/kg Co as cobalt carbonate, 36 mg/kg I, 26 mg/kg Se as sodium selenite, 110,179 IU/kg vitamin A, 3,084 IU/kg vitamin D, 545 IU/kg vitamin E, and 1,179 mg/kg of chlortetracycline). Throughout the experiment heifers consumed 97.6 $g \cdot heifer^{-1} \cdot d^{-1}$ of free choice mineral, with a targeted consumption of 85 $g \cdot heifer^{-1} \cdot d^{-1}$. One heifer from the CON treatment was removed from trial at 289 ± 22 d of age for a chronic respiratory infection. At approximately 360 d of age, heifers across all pastures were observed attempting to suckle each other. One heifer from the MM treatment was removed at 359 ± 22 d of age for a mastitis infection, and an additional 8 heifers (3 CON and 5 MM) were removed at 383 ± 22 d of age for mastitis infections or poor performance. An additional heifer from the MM treatment was identified as a freemartin and removed at the time of breeding.

***Sample Collection and Analytical Procedures***

Heifer BW and BCS [emaciated = 1; obese = 9; as described by Wagner et al. (1988)] were collected at trial initiation (221 ± 22 d of age), 268, 309, 362, 401, 430 ± 22 d of age, artificial insemination (**AI**) pregnancy confirmation (466 ± 22 d of age), and final pregnancy confirmation (536 ± 22 d of age). Hair coat scores (**HCS**; 1 to 5, in which 1 = slick and 5 = unshed) were also recorded at trial initiation and 268, 309, and 362 ± 22 d of age from the same farm technician. Sixty heifers (30 per treatment) that were most similar to average initial (221 ± 22 d of age) BW and BCS were selected for additional sampling and were utilized throughout all observation points. Respiration rates were collected from these 60 heifers at 269, 310, and 361 ± 22 d of age (June 22, 2016, August 2, 2016, and September 22, 2016, respectively).

For nutrient composition analysis, samples of distillers grains were collected monthly and composited and dried at 55° C for a minimum of 3 days and ground through a 1 mm screen using a Wiley mill (Arthur, H. Thomas, Philadelphia, PA). Forage samples were collected at grazing height from pastures on a monthly basis and composited and dried similarly. Ground distillers samples were analyzed for CP (Leco TruMac, LECO Corporation, St. Joseph, MI), NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY), and crude fat using an Ankom XT10 fat extractor (Ankom Technology, Macedon, NY). Ground forage samples were also analyzed for NDF, ADF, and CP using the same analytical procedures previously described. Total ergot alkaloid analysis of forages was conducted in a commercial laboratory (Agrinostics Limited, Co., Watkinsville, GA).

### *Blood and Liver Sampling and Analysis*

Blood samples were collected from all heifers at trial initiation and 268, 309, and 362 ± 22 d of age (June 21, 2016, August 1, 2016, and September 23, 2016, respectively) for prolactin analysis. Blood was collected via jugular venipuncture into a 10-mL serum blood collection vacuum tube (Becton, Dickinson, and Co., Franklin Lakes, NJ). Blood was allowed to clot for 2 h at room temperature before being centrifuged at 1,300 × *g* for 20 min at 5°C. Serum was stored at -20°C for subsequent prolactin analysis. Serum was analyzed for prolactin analysis via a radioimmunoassay as described by Bernard et al. (1993) at the University of Tennessee (Knoxville, TN). The intra- and inter-assay CV for all prolactin analysis were 6.06% and 5.29%, respectively and the sensitivity across assays was 0.05 ng/mL.

Liver and blood samples were collected from the same predetermined 60 heifers for trace mineral determination at the initiation of the trial and at breeding (422 ± 22 d of age). Liver biopsy samples were collected using the method of Engle and Spears (2000) with the modification that all heifers were given an intradermal 5 mL injection of Lidocaine Injectable-2% (MWI, Boise, ID) at the site of the biopsy. Biopsy samples were transported to the laboratory on ice where they were frozen at -20°C until further analysis. Blood was collected at the time of liver biopsy via jugular venipuncture into $K_{2EDTA}$ vacuum tubes (10 mL; Becton, Dickinson, and Co., Franklin Lakes, NJ). Blood was centrifuged at 1,300 × *g* for 20 min at 5°C and plasma was stored at -20°C for subsequent trace mineral analysis. Liver samples for Cu, Mn, Se, and Zn analysis were sent to a commercial lab where they were subjected to nitric acid digestion and inductively coupled plasma spectroscopy analyses for complete minerals (method 975.03: AOAC, 1988; The Ohio State University, Service Testing and Research Lab, Wooster, OH). Plasma samples were sent to Michigan State University Diagnostic Center for Population and

Animal Health (East Lansing, MI) and concentrations of Cu, Mn, Se, and Zn were analyzed using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc., Santa Clara, CA) via procedures described previously (Wahlen et al., 2005).

***Reproductive Development***

Antral follicle counts and ovarian length and width were measured from the 60 predetermined heifers via transrectal ultrasonography (Ibex$^{TM}$ Portable Ultrasound, variable MHz linear array transducer, E.I. Medical Imaging, Loveland, CO) at 319, 372, and 421 ± 22 d of age (August 11, 2016, October 3, 2016, and November 21, 2016, respectively). The number and location of all antral follicles ≥ 3 mm in diameter were recorded for each ovary. Two blood samples from all 290 heifers were collected 10 d apart to determine percent of heifers cycling at approximately ten (309 and 319 ± 22 d of age), twelve (362 and 372 ± 22 d of age), and fourteen (411 and 421 ± 22 d of age) months of age, concurrent with ultrasound dates. Samples were collected via jugular venipuncture in 10 mL $K_{2EDTA}$ vacuum tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ) and immediately placed on ice. Samples were centrifuged at 1,300 × *g* for 20 min at 5°C, and plasma was stored at -20°C until analyzed. Heifers were considered cycling when a single plasma sample contained ≥ 2 ng/mL of progesterone, or when both samples collected 10 d apart contained ≥ 1 ng/mL of progesterone as previously described by Gunn et al. (2015). Plasma progesterone concentration was analyzed using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA) validated by Reis et al. (2015). The average intra-assay CV for all progesterone samples was 3.6% and the sensitivity across assays was 0.2 ng/mL

***Estrous Synchronization and Breeding***

At 421 ± 22 d of age reproductive tract scores (**RTS**; 1 = immature to 5 = luteal phase; Anderson et al., 1991) were assigned to all heifers. Concurrently, heifers were enrolled in a 7-d CO-Synch + controlled internal drug release (**CIDR**) insert and timed-AI protocol. At protocol initiation, heifers received an intravaginal progesterone insert (CIDR; Pfizer Animal Health, New York, NY) and were administered 100 µg of GnRH (Factrel; Zoetis, Parsippany, NJ). Seven days later, the CIDR was removed, and heifers were administered 25-mg dose of prostaglandin (Lutalyse; Pfizer Animal Health). Fifty-four hours following CIDR removal heifers were bred via timed-AI and administered 100 µg of gonadotropin-releasing hormone (Cystorelin; Merial, Duluth, GA). Both sire and AI technician were stratified across treatments. Heat detection patches (Estrotect Heat Detectors; Rockway Inc., Spring Valley, WI) were placed on all heifers at the time of CIDR removal and visually scored from 0 – 3 (0 = not activated, 1 = partially activated, 2 = fully activated, and 3 = missing) at time of AI. Immediately following AI heifers were combined into 2 groups with an equal representation of each treatment and placed on pasture. Ten days following AI, heifers were placed with 8 bulls (which passed breeding soundness exams; 4 bulls/group) for a 96-d breeding season. At 466 ± 22 d of age, AI conception rates were collected by a trained technician via ultrasonography (Aloka 500 instrument, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array). Overall pregnancy rates were determine at 536 ± 22 d of age by a trained technician via rectal palpation or ultrasonography (Aloka 500 instrument, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

***Statistical Analysis.***

Body weight, BCS, HCS, respiration rates, prolactin, antral follicle counts, ovarian length and width, and plasma and liver mineral data were analyzed as a completely randomized design using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). All binary data, including progesterone, distribution of RTS, distribution of heat patch scores, AI conception, and overall pregnancy rates were analyzed using the GLIMMIX procedure of SAS. The model included the fixed effect of treatment and group. The model for cyclicity, RTS, heat patch score, AI conception rates, and overall pregnancy rates included age as a covariate. Body weight, BCS, HCS, respiration rates, prolactin, progesterone, antral follicle counts, and ovarian length and width were analyzed as repeated measures with the fixed effects of treatment, group, day, and the interaction between treatment and time. For all variables, the unstructured covariance structure was utilized, as it resulted in the smallest Akaike information criterion. Day was the repeated effect for all repeated measures and age at time of ultrasound was utilized as a covariate for antral follicle counts and ovarian length and width. Liver and plasma trace mineral values determined at d 0 were used as a covariate in the analysis of trace mineral status at breeding. Initial liver Mn and Cu and plasma Se were significant ($P \leq 0.05$) when utilized as a covariate, however to maintain consistency across the model, all initial liver and plasma values were included as covariates. Animal served as the experimental unit for all analyses. Treatment effects were considered significant at $P \leq 0.05$ and tendencies were noted at $0.05 < P \leq 0.10$. Means reported in tables are least squares means ± SEM.

## RESULTS AND DISCUSSION

### *Heifer Performance*

Heifer BW and BCS did not differ ($P \geq 0.27$; Fig. 3) throughout development. However, results across literature have been inconsistent regarding the effects of an injectable trace mineral on cattle BW and BCS. Mundell et al. (2012) supplemented cows grazing native range with an injectable trace mineral 30 d prior to fixed time AI and noted no difference in BW and BCS regardless of treatment. Similarly, Gadberry and Baldridge (2013) administered Angus cows an injectable trace mineral prior to calving and again prior to breeding and noted no effect on cow BW or BCS. Other work utilizing weaned heifers noted an increase in average daily gain (**ADG**) over 177 d when heifers were administered an injectable trace mineral (Arthington et al., 2014). Ultimately, differences in time of administration of treatment, differences in breeds, and differences in nutrient availability make drawing comparisons across these experiments challenging.

Forage samples for ergot alkaloid concentrations were collected monthly, with the lowest concentrations noted in July and peak concentrations occurring in May, September, and October (May = 2088 µg/kg; June = 921 µg/kg; July = 330 µg/kg; August = 787 µg/kg; September = 2275 µg/kg). Heifer hair coat scores (Fig. 4), respiration rates and prolactin concentrations (Fig. 5) did not differ ($P \geq 0.30$) between treatments throughout the experiment. However, there was an effect of day ($P < 0.01$) on prolactin concentrations, likely driven by all heifers having decreased serum prolactin concentrations at 362 ± 22 d of age. This decrease in serum prolactin coincided with a substantial increase in pasture total ergot alkaloid concentration (1192 µg/kg). Similarly, Gadberry and Baldbridge (2013) also noted no difference in hair coat scores when supplementing calves with an injectable trace mineral 130 d after birth. However, these calves

were not grazing endophyte infected fescue. Previous research has noted that cattle grazing endophyte infected tall fescue had decreased plasma and liver Cu concentrations (Saker et al., 1998; Stoszek et al. (1979). Both Saker et al (1998) and Stoszek et al. (1979) reported that cattle grazing endophyte infected fescue had plasma Cu concentrations below 0.5 mg/kg and Stoszek et al. (1979) reported liver Cu concentrations below 50 mg/kg. These plasma and liver Cu concentrations would classify these grazing cattle Cu deficient according to Kincaid (2000). In the current study, MM heifers had greater ($P \leq 0.01$; Table 1) plasma and liver concentrations of Cu compared to CON. Stoszek et al. (1979) also noted increases in both plasma and liver Cu, with Cu status changing from deficient to adequate levels, when beef heifers grazing tall fescue were supplemented with either 400 or 1,000 mg/Cu as $CuSO_4$ compared to steers that received no Cu supplementation; however, no level of Cu supplementation affected heifer ADG even when Cu status was improved. Stoszek et al. (1979) did not evaluate respiration, HCS, and prolactin levels. Saker et al. (1998) noted similar increases in Cu plasma concentrations when steers grazing tall fescue received a Cu oxide bolus; however, performance parameters were not measured. While it is well-established endophyte infected fescue impacts Cu status of animals, it is still unknown whether additional Cu supplementation may improve resistance to fescue toxicity in cattle. More research is needed to clarify the relationship between endophyte infected fescue and Cu supplementation and the effect this may have on fescue toxicity symptoms in cattle.

Plasma and liver Mn concentrations did not differ ($P \geq 0.54$) between MM and CON heifers. Differences in storage and metabolism of Mn may explain why no differences were noted. Manganese that is stored in the liver is often not indicative of dietary intake (Kincaid, 2000) and other authors have reported minimal (Hansen et al., 2006; Pogge et al., 2012) to no

(Arthington et al., 2014) response in liver and plasma Mn based on Mn supplementation. Multimin heifers had greater ($P \leq 0.01$) plasma and liver concentrations of Se compared to CON. Even though all heifers were receiving free choice trace mineral, based on liver Se concentrations, all heifers were considered marginally Se deficient at the initiation of the trail (0.70 and 0.63 ± 0.091 mg/kg for CON and MM respectively) using recommended classification by Kincaid (2000). By the time of breeding CON heifers were still considered to be marginally Se deficient based on liver Se concentrations (0.98 ± 0.143 mg/kg); however, MM heifers had reached an adequate Se status (1.53 ± 0.143 mg/kg). Because all heifers were grazing similar pasture and provided the same free choice trace mineral, this difference in Se status is likely attributed to the trace mineral injection.

Plasma Zn did not differ ($P = 0.94$) between CON and MM heifers; however, liver Zn concentrations were greater ($P = 0.02$) for CON than MM heifers. The literature has been inconsistent regarding the effects of an injectable trace mineral on liver Zn concentrations. Work by Brasche (2015) reported no effect of an injectable trace mineral (Multimin90) when mineral status was measured 24 d post injection on liver Zn concentrations in Angus-crossbred beef heifers, and Pogge et al. (2012) reported an increase in liver Zn concentration when steers were supplemented with an injectable trace mineral. Both plasma and liver Zn concentrations may be variable based on immune status and age (Kincaid, 2000), suggesting these may not be accurate indicators of Zn status.

***Reproductive Development and Performance***

To our knowledge, no other study has compared the effects of an injectable trace mineral source on ovarian morphology and follicular development in beef heifers. For all measures of

heifer ovarian morphology, treatment by day was not significant ($P \geq 0.45$; Table 2) and day was significant ($P \leq 0.01$). No differences due to treatment ($P \geq 0.51$) were detected in antral follicle count and ovarian size. This is similar to results by Lamb et al. (2008) who noted no effect of additional mineral supplementation on number of follicles, number of corpora lutea, or number of unovulated follicles in Angus heifers. It is important to note heifers utilized by Lamb et al. (2008) were supplemented with 1 of 3 free choice mineral treatments: no additional mineral, an organic mineral, or inorganic form of both macro and micro minerals. Heifers in that study did not receive an injectable trace mineral and mineral status was not assessed in the experiment (Lamb et al., 2008), thus making it a challenge to draw conclusions across these experiments. Other work has assessed the effects of providing Holstein cows post-calving an inorganic or organic form of Cu, Mn, Co, and Zn in a concentrate based pelleted premix and noted no effects on first-wave follicular dynamics, luteal measures, or embryo quality (Hackbart et al., 2010). Gonzalez-Maldonado et al. (2017) supplemented over conditioned dairy cows with an injectable trace mineral prior to estrus synchronization and noted no differences in follicle population or diameter of preovulatory follicles when measured at the time of CIDR removal. As multiple mechanisms are responsible for mediating the effects of fertility, follicular growth, and reproductive performance, more research is needed to determine the specific role trace minerals may play in these functions.

Copper, Mn, Se, and Zn are vital for ruminant health and nutrition, and deficiencies in trace minerals has resulted in the suppression of estrus, reduced conception rates, and overall decreased reproductive performance (Hidiroglou, 1979; Sunde, 1997; Suttle, 2010). Interestingly though, there was not a treatment by day interaction on percent of heifers cycling, there was a main effect of both treatment ($P < 0.01$; Figure 6) and day ($P < 0.01$) with fewer MM heifers

cycling compared to CON heifers. It is unknown if additional trace mineral supplementation is driving this overall decrease in cyclicity noted in the MM heifers, or if heifers were randomly assigned to the MM treatment that were predisposed to reach puberty at a later time point. Minimal research has been conducted to determine the role trace minerals may play in the attainment of puberty in beef heifers. Grings et al. (1999) noted no effect on age of puberty when supplementing dietary Cu, Zn, and Mn to beef heifers. However, Grings et al. (1999) also did not report a difference in plasma Cu concentrations and they did not collect liver mineral data, making drawing conclusion regarding the role trace minerals may play in cyclicity in beef heifers challenging. Additionally, utilizing blood measures such as plasma or serum for trace mineral status determination may provide little insight as often times homeostatic mechanisms can limit changes in circulating concentrations of trace minerals until reserves become substantially depleted (Miller, 1975).

Both treatments had the greatest number of cycling heifers at 10 months of age and exhibited a decrease in number of cycling heifers at 12 months of age and only a slight increase in number of heifers cycling at 14 months of age. While unexpected, the lesser percent of heifers cycling at 12 months of age does coincide with the marked decrease in BCS and prolactin concentration and an increase in respiration rates at 12 months of age, suggesting heifers may have been in a negative energy balance and environmentally stressed prior to breeding. Ultimately, further research is needed to elucidate why MM treated heifers exhibited an overall decrease in the percentage of heifers attaining cyclicity.

Foreshadowed by the difference in number of heifers obtaining cyclicity, there was a tendency ($P = 0.09$; Table 3) for MM heifers to have an increased number of inactivated heat patches. However, there were no differences ($P \geq 0.38$) between treatments in the number of

partially or fully active heat patches. There was also no effect ($P \geq 0.19$) of treatment on RTS distributions. Assuming that heifers receiving a RTS of a 4 or 5 would be cycling and ready to breed, reproductive tract scores did closely coincide with the number of heifers cycling at 14 months of age. Thirty-six percent of CON heifers and 31% of MM heifers would classify as cycling based on RTS and according to progesterone data 31% and 26% of CON and MM heifers, respectively were cycling at the time of breeding. Despite the differences noted in number of cycling heifers and the tendency for heat patch scores to be different, there were no differences in ($P \geq 0.36$) both AI conception and overall pregnancy rate. These results were consistent with Stokes et al. (2017) who reported in two out of three experiments no difference in AI or overall pregnancy rates when beef heifers were supplemented an injectable trace mineral (Multimin90) prior to estrous synchronization. However, the effects of an injectable trace mineral on reproductive performance have been largely inconsistent across the literature. In an additional experiment, Stokes et al. (2017) reported a tendency for heifers administered an injectable trace mineral (Multimin90) prior to breeding to have increase AI conception rates. Despite this difference in AI conception, no difference was noted in overall pregnancy rates (Stokes et al., 2017). Trace mineral status was not assessed in any of these experiments making it challenging to determine if these effects on reproductive performance were due to differences in mineral status.

Other authors have also noted inconsistent results throughout the literature when assessing the effects of an injectable trace mineral on heifer reproductive performance. Brasche (2015) noted no differences in AI pregnancy rates when Angus-crossbred heifers (n = 109) were supplemented with an injectable trace mineral (Multimin90) 30 d prior to breeding however there was a tendency for overall pregnancy rates to be increased (92.7% = injectable trace

mineral; 83.3% = control). Liver mineral status was assessed 170 d post injection and there was no difference in Cu, Se, or Zn liver concentrations for these heifers. However, there was a tendency for liver Mn concentration to be greater for control (11.5 mg/kg) than heifers supplemented with an injectable trace mineral (10.5 mg/kg). In a subsequent experiment by Brasche (2015) no difference was noted in AI or overall pregnancy rates when Angus crossbred heifers (n = 112) were supplemented with an injectable trace mineral (Multimin90) 30 d prior to breeding. Heifers receiving the injectable trace mineral also had increased liver Cu (371 and 292 mg/kg for trace mineral and control supplemented cattle, respectively) and Se (2.3 and 1.5 mg/kg for trace mineral and control supplemented cattle, respectively) compared to their control counterparts 24 d following the injection (Brasche, 2015). However, both liver Cu and Se status were considered adequate for these control heifers according to Kincaid (2000), and thus the lack of differences in reproductive performance was not remarkable.

Vanegas et al. (2004) conducted two experiments administering either one or two doses of an injectable trace mineral (Multimin90) to dairy cows approximately 3 weeks pre-calving and noted that one dose provided no differences in first service conception rates. Two doses administered 60 d apart significantly decreased the first service conception rate compared to controls. These dairy cows were also being supplemented with a basal diet containing 0.3 mg/kg of dietary Se and 20 mg/kg of Cu. The NRC (2001) requirements for these cattle were estimated to be 0.3 mg/kg of Se and 11 mg/kg of Cu. However, no liver or blood samples were collected for evaluation of trace mineral status that may help explain these results. The inconsistency noted across literature could be driven by multiple factors including time of administration, breed, previous mineral status, and other nutrient, breeding, or environmental factors.

Both the AI and overall pregnancy rates reported in this experiment were lesser than pregnancy rates of heifers in this herd from previous years. It is important to note that a substantial amount of heifers were retained as replacements for this experiment that in a normal setting would have been removed from the herd. This may have negatively impacted the reproductive success of the whole group as under normal management conditions the younger, lighter weight heifers that are less likely to conceive, would have been removed. As a component of having younger, light weight heifers, these heifers averaged 302 kg at the time of breeding, only 54% of their dams average mature BW of 556 kg. The Nutrient Requirements of Beef Cattle (2016) suggests Bos Taurus heifers attain puberty at approximately 60% of mature weight. However, a review by Funston et al. (2012) reported that heifers targeted to 55% of mature weight had similar calf production rates as heifers developed to heaver target weight of 60 or 65%. However, cyclicity and pregnancy data from the current experiment would support that these heifers may have needed to reach a greater targeted percent of mature body weight for optimal reproductive success. Additionally, at approximately 360 d of age, a few heifers were observed attempting to suckle each other and began lactating. Likely driven by this behavior, 14 heifers (7 CON heifers and 7 MM heifers) were subsequently treated for mastitis. Of these heifers, 6 (1 CON and 5 MM heifers) were then removed at 383 ± 22 d of age from trial. The initiation of lactation involves numerous hormones including prolactin, glucocorticoids, and estrogen (Tucker, 2000) all of which could have substantial impacts on normal heifer growth and reproductive maturity.

Repeated supplementation of an injectable trace mineral to developing heifers resulted in an increase in Cu and Se status. However, minimal effects on heifer growth and symptoms of fescue toxicosis were noted. Interestingly, heifers administered the injectable trace mineral did

have decreased attainment of cyclicity. However, this ultimately did not affect AI or overall pregnancy rates. These data suggest that additional mineral supplementation in the form of an injectable trace mineral may increase trace mineral status; however, no improvement in developing heifer performance or reproductive success was observed.

## TABLES AND FIGURES

**Table 4.1**. Influence of an injectable trace mineral supplementation on heifer mineral status

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value |
| Plasma mineral | | | | |
| Initial[2] | | | | |
| Cu, mg/L | 0.80 | 0.80 | - | - |
| Mn, µg/L | 1.64 | 2.33 | - | - |
| Se, µg/L | 57.1 | 60.5 | - | - |
| Zn, mg/L | 0.95 | 0.98 | - | - |
| Breeding[3] | | | | |
| Cu, mg/L | 0.71 | 0.85 | 0.037 | 0.01 |
| Mn, µg/L | 1.64 | 1.73 | 0.100 | 0.54 |
| Se, µg/L | 65.8 | 75.1 | 1.42 | <0.01 |
| Zn, mg/L | 0.94 | 0.93 | 0.037 | 0.94 |
| Liver mineral, mg/kg | | | | |
| Initial[2] | | | | |
| Cu | 167.3 | 221.5 | - | - |
| Mn | 11.02 | 11.53 | - | - |
| Se | 0.70 | 0.63 | - | - |
| Zn | 115.9 | 113.8 | - | - |
| Breeding[3] | | | | |
| Cu | 108.0 | 175.8 | 10.06 | <0.01 |
| Mn | 9.85 | 9.98 | 0.167 | 0.60 |
| Se | 0.98 | 1.53 | 0.143 | <0.01 |
| Zn | 110.5 | 103.8 | 1.91 | 0.02 |

[1]Control cattle received a sterilized saline solution, and Multimin 90 (MM) cattle received injectable trace mineral at approximately 90 d intervals.
[2]Initial values served as covariates in analysis for respective parameters at subsequent sampling dates.
[3]Values determined at d 0 were used as a covariate in the analysis of trace mineral status at breeding.

**Table 4.2.** Influence of injectable trace mineral supplementation on heifer ovarian morphology

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value[2] |
| Left antral follicles[3] | | | | 0.92 |
| 10 months | 6.9 | 7.1 | 0.49 | |
| 12 months | 8.2 | 8.8 | 0.50 | |
| 14 months | 9.4 | 8.8 | 0.49 | |
| Right antral follicles[3] | | | | 0.51 |
| 10 months | 7.0 | 7.5 | 0.55 | |
| 12 months | 9.0 | 9.1 | 0.55 | |
| 14 months | 8.5 | 8.8 | 0.55 | |
| Total antral follicles[3] | | | | 0.96 |
| 10 months | 13.9 | 14.1 | 0.91 | |
| 12 months | 17.0 | 17.3 | 0.91 | |
| 14 months | 17.8 | 17.1 | 0.91 | |
| Average ovarian length[3], mm | | | | 0.89 |
| 10 months | 22.4 | 22.1 | 0.58 | |
| 12 months | 23.0 | 23.1 | 0.58 | |
| 14 months | 25.8 | 25.8 | 0.58 | |
| Average ovarian height[3], mm | | | | 0.71 |
| 10 months | 12.6 | 12.9 | 1.01 | |
| 12 months | 13.0 | 13.1 | 0.47 | |
| 14 months | 13.7 | 14.0 | 0.98 | |

[1]Control cattle received a sterilized saline solution, and Multimin 90 (MM) cattle received injectable trace mineral at approximately 90 d intervals.

[2] For all measures of heifer ovarian morphology treatment by day was not significant ($P \geq 0.45$) and day was significant ($P \leq 0.01$). Therefore, only the overall effect of treatment is reported.

[3]Measurements include total number of antral follicles observed via ultrasonography at approximately ten (319 ± 22 d of age) twelve (372 ± 22 d of age), and fourteen (421 ± 22 d of age) months of age

**Table 4.3.** Influence of injectable trace mineral supplementation on heifer reproductive performance and pregnancy diagnosis.

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value |
| Reproductive tract score[2], % | | | | |
| 1 | - | - | - | - |
| 2 | 19 | 26 | - | 0.19 |
| 3 | 34 | 36 | - | 0.73 |
| 4 | 18 | 17 | - | 0.83 |
| 5 | 18 | 14 | - | 0.33 |
| Heat patch score[3], % | | | | |
| 0 | 44 | 54 | - | 0.09 |
| 1 | 28 | 24 | - | 0.42 |
| 2 | 24 | 20 | - | 0.38 |
| 3 | - | - | - | - |
| AI pregnancy rate, % | 30 | 37 | - | 0.36 |
| Overall pregnancy rate, % | 75 | 74 | - | 0.78 |

[1]Control cattle received a sterilized saline solution, and Multimin 90 (MM) cattle received injectable trace mineral at approximately 90 d intervals.
[2]Reproductive tract scores were determined at the time of estrus synchronization initiation from 0 – 5 (1 = immature to 5 = luteal phase)
[3]Heat patches were visually scored at time of breeding from 0 – 3 (0 = not activated, 1 = partially activated, 2 = fully activated, 3 = missing).

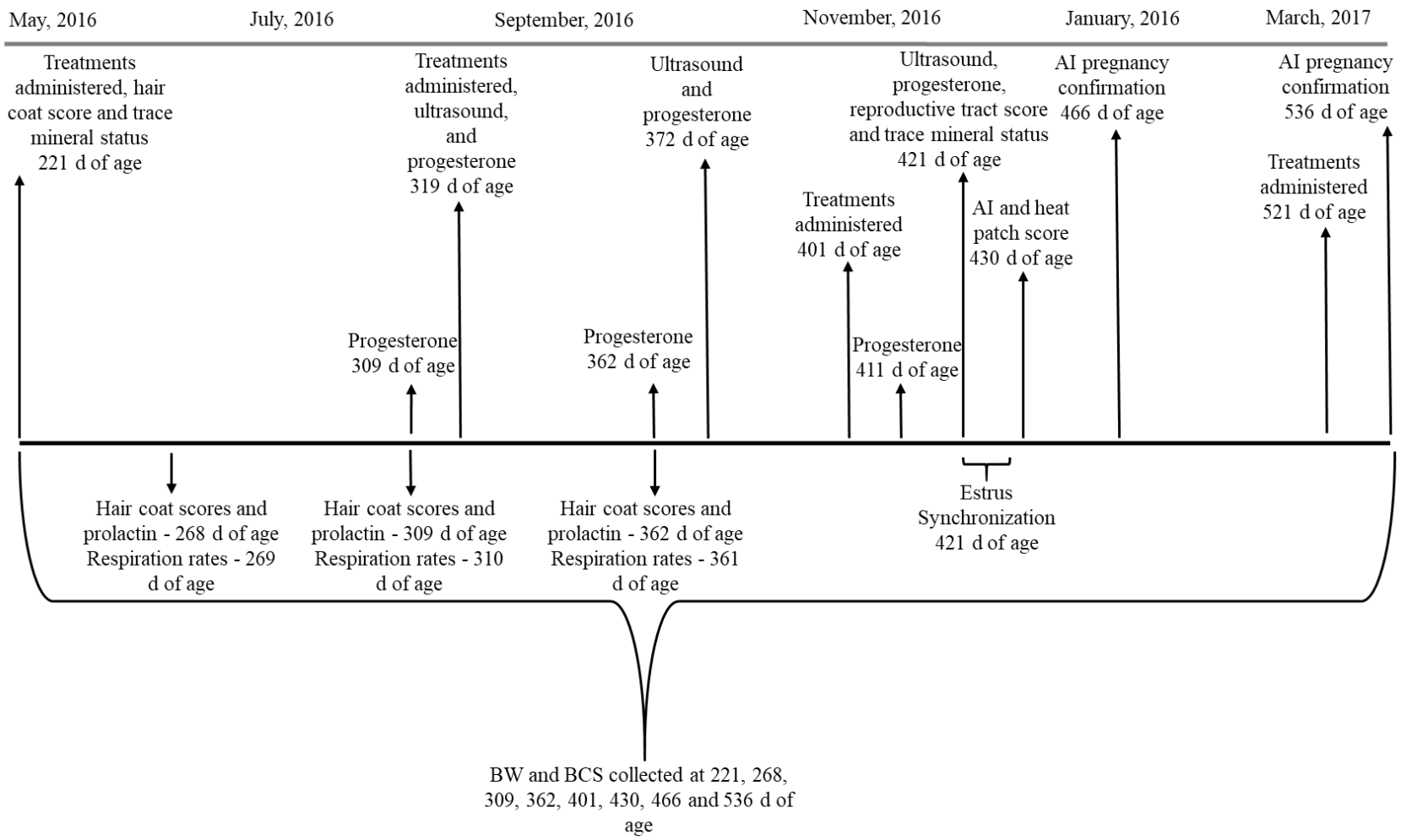


**Figure 4.1.** Experimental timeline.

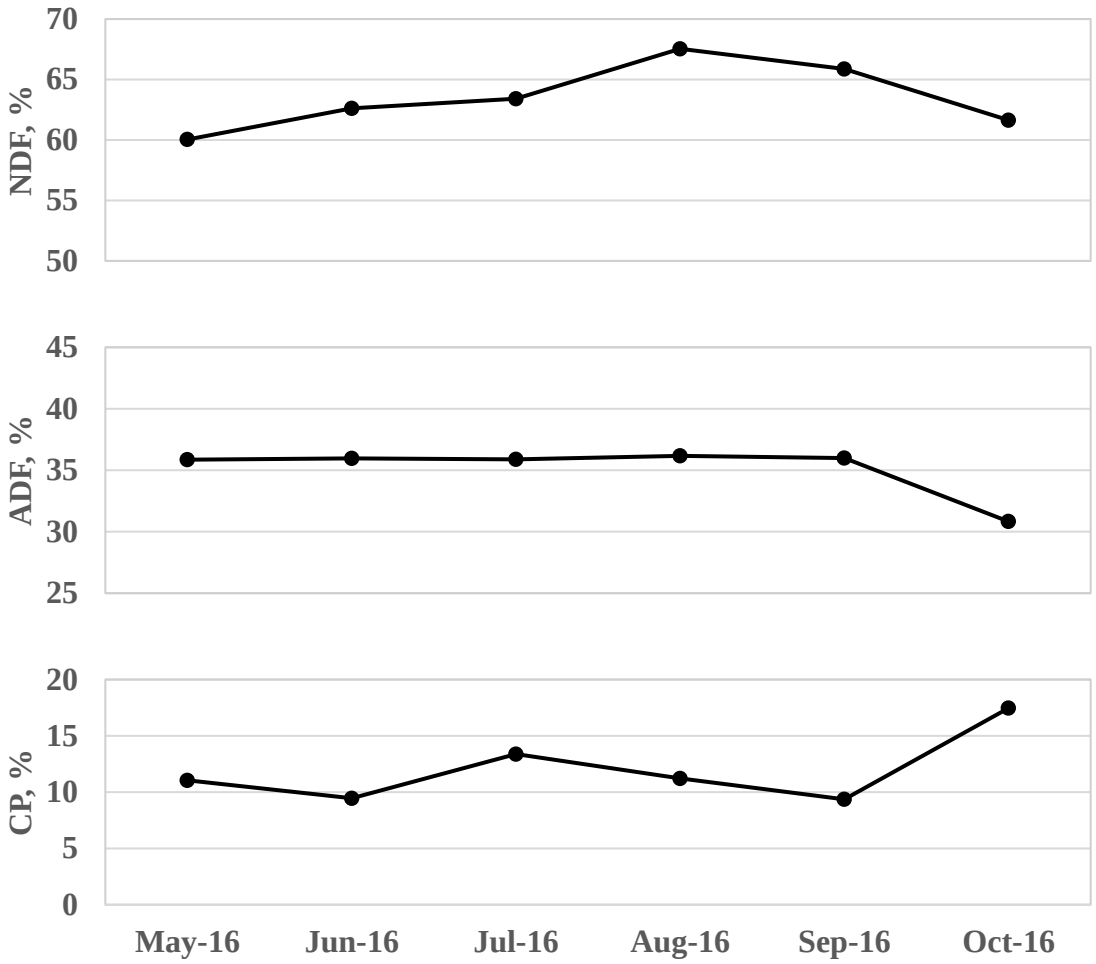


**Figure 4.2.** Forage quality [percentage neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP)] of endophyte-infected fescue (*Festuca arundinacea*) and red clover (*Tri-folium pretense*) pastures from May 2016 to October 2016. Samples were collected as cattle rotated pastures and were composited on a monthly basis.

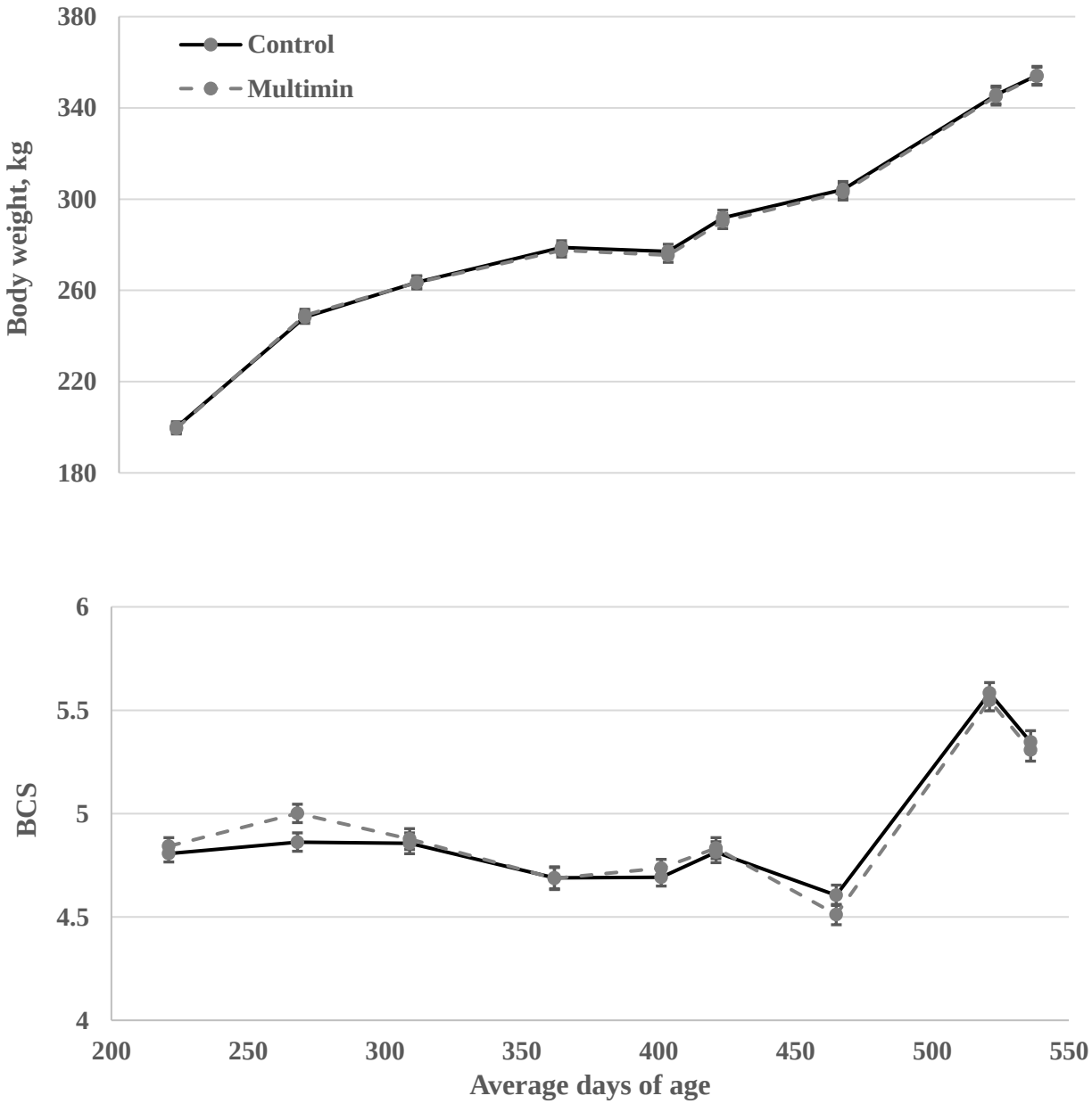


**Figure 4.3.** Effect of an injectable trace mineral (Multimin 90) on heifer BW and BCS. Control cattle received a sterilized saline solution, and Multimin 90 (Multimin) cattle received injectable trace mineral at approximately 90 d intervals. For BW, treatment by day were not significant ($P$ = 0.58), the main effects of treatment and day were ($P$ = 0.90) and ($P$ < 0.01), respectively. For BCS, treatment by day were not significant ($P$ = 0.27), the main effects of treatment and day were ($P$ = 0.83) and ($P$ < 0.01), respectively.

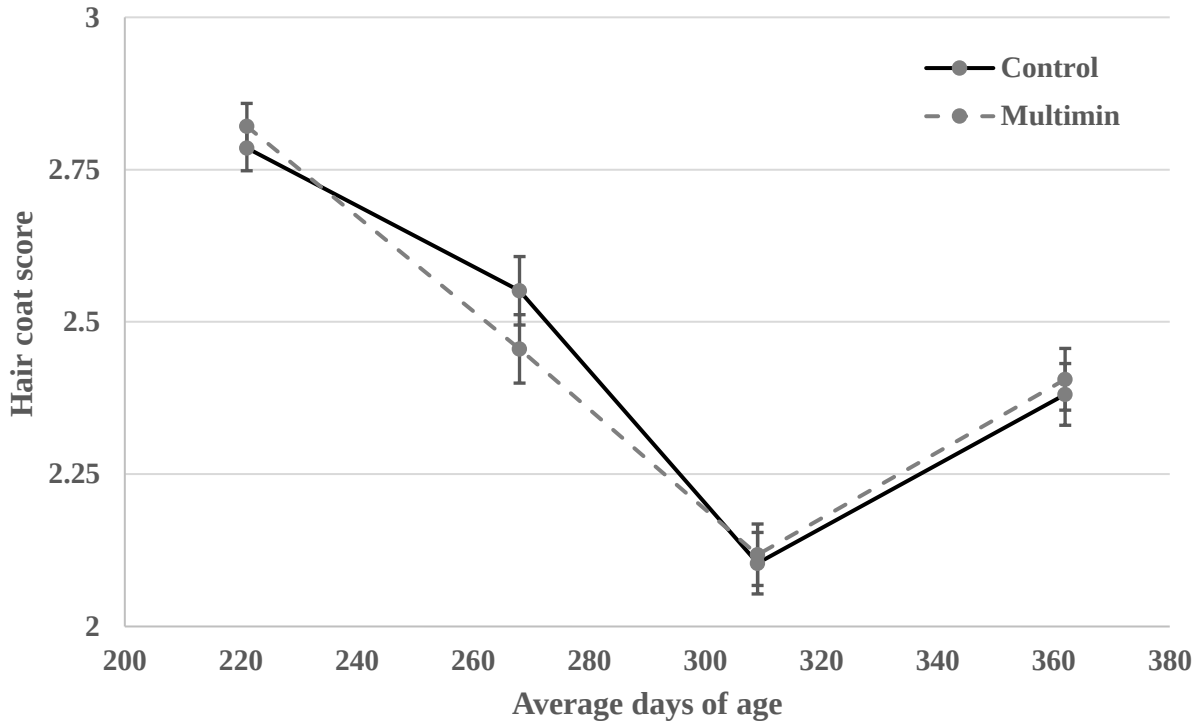


**Figure 4.4.** Effect of an injectable trace mineral (Multimin 90) on heifer hair coat score (1 to 5, in which 1 = slick and 5 = unshed). Control cattle received a sterilized saline solution, and Multimin 90 (Multimin) cattle received injectable trace mineral at approximately 90 d intervals. Treatment by day was not significant ($P = 0.58$), and the main effects of treatment and day were ($P = 0.90$) and ($P < 0.01$), respectively.

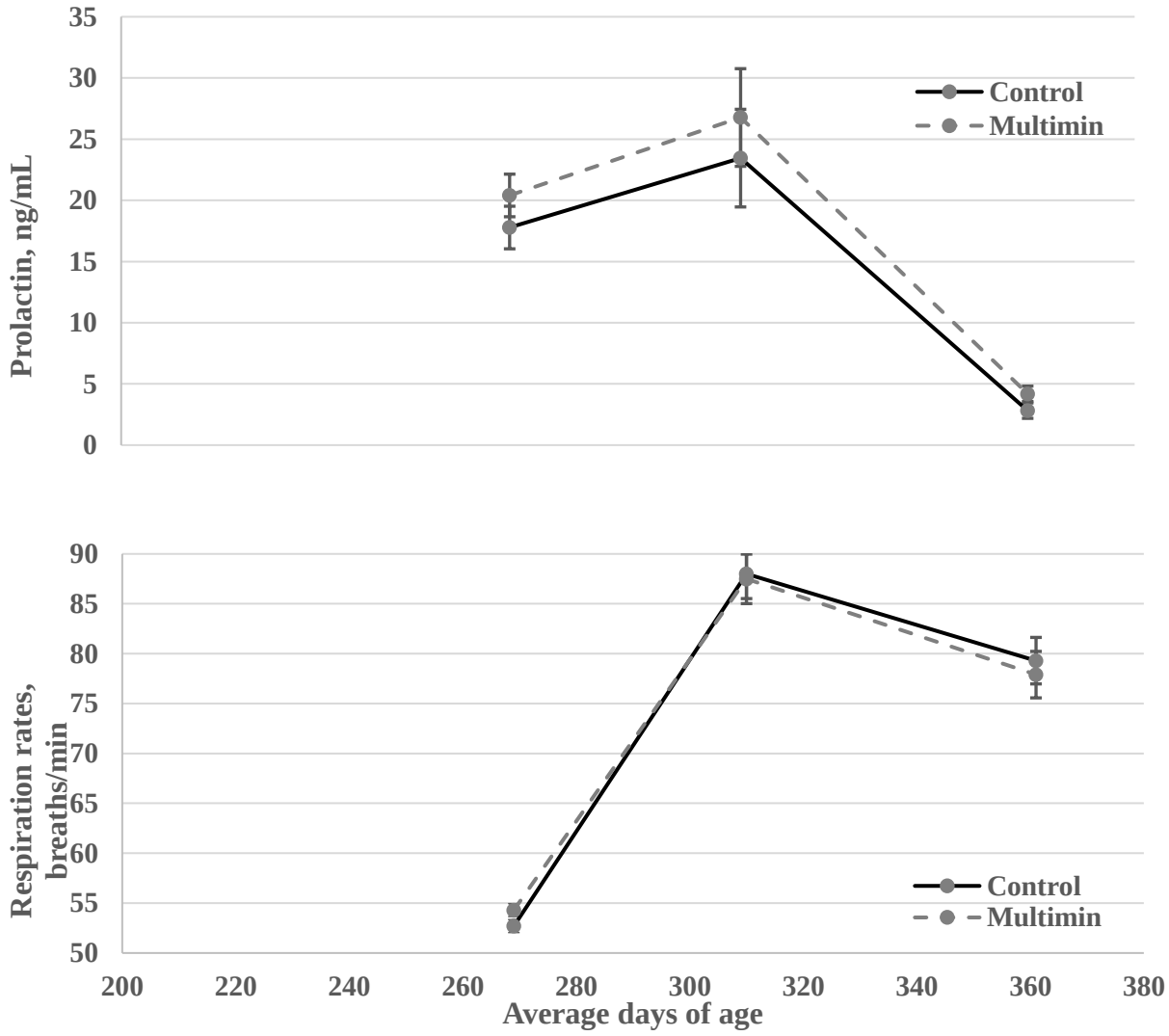


**Figure 4.5.** Effect of an injectable trace mineral (Multimin 90) on heifer (n = 60; 30 heifers/treatment) prolactin concentrations and respiration rates (breaths/min). Control cattle received a sterilized saline solution, and Multimin 90 (Multimin) cattle received injectable trace mineral at approximately 90 d intervals. For serum prolactin concentration, treatment by day was not significant ($P = 0.86$), and the main effects of treatment and day were ($P = 0.30$) and ($P < 0.01$), respectively. For respiration rates, treatment by day was not significant ($P = 0.61$), and the main effects of treatment and day were ($P = 0.94$) and ($P < 0.01$), respectively.

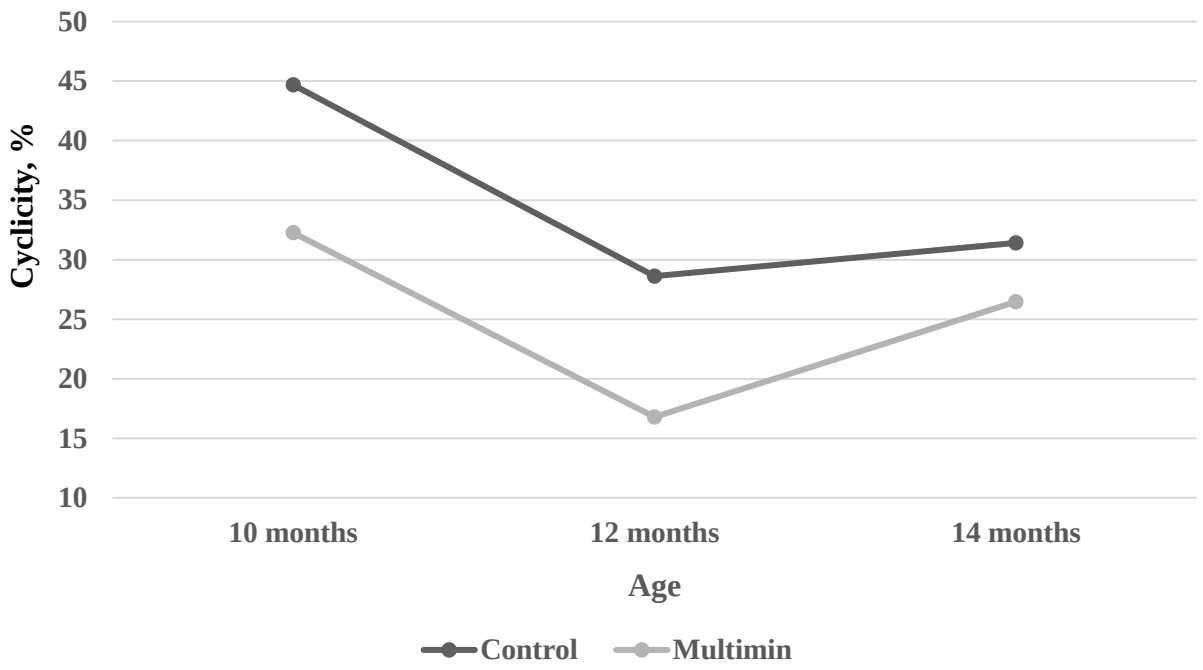


**Figure 4.6.** Effect of an injectable trace mineral (Multimin 90) on percent of heifers reaching cyclicity. Control cattle received a sterilized saline solution, and Multimin 90 (Multimin) cattle received injectable trace mineral at approximately 90 d intervals. Cyclicity was defined as when one blood sample contained ≥2 ng/mL of progesterone or when both samples contained ≥1 ng/mL of progesterone. Measurements taken at approximately ten (309 and 319 ± 22 d of age), twelve (362 and 372 ± 22 d of age), and fourteen (411 and 421 ± 22 d of age) months of age. Treatment by day was not significant ($P = 0.45$), and the main effects of treatment and day were ($P < 0.01$) and ($P < 0.01$), respectively.

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# CHAPTER 5

## INFLUENCE OF REPEATED TRACE MINERAL INJECTIONS DURING GESTATION ON BEEF HEIFER AND SUBSEQUENT CALF PERFORMANCE

**ABSTRACT:** Commercial Angus heifers (n=190; 315±49.3 kg) were utilized to determine the effects of trace mineral injections during gestation on heifer and subsequent calf performance. Heifers received 3 previous subcutaneous trace mineral (**MM;** Multimin90; n=93) or saline (**CON;** n=97) injections approximately 90 d apart. These treatments were maintained and subsequent injections were given 205, 114, and 44±26 d prepartum. Heifers were provided free-choice, inorganic minerals. Heifer body weights (**BW**) and body condition scores (**BCS**) were collected at trial initiation (296±26 d prepartum) and 5-10 week intervals thereafter. Liver samples were collected at trial initiation, 5 and 176±3 d postpartum from a subset of cows to determine trace mineral status. Milk production was assessed on 80 cow-calf pairs (40/treatment) at 71±15 d postpartum. Cows were artificially inseminated (**AI**) 82 d postpartum then exposed to bulls for 38 d. Data were reported from 174 calves (n=87 calves/treatment). Calf liver samples were collected 5 and 147±3 d postpartum to determine trace mineral status. Calf weaning BW was collected at 159±26 d postpartum. Calf performance including calving date, birth BW, weaning BW, average daily gain (**ADG**), and health were collected. Heifer BW and BCS did not differ ($P \geq 0.72$) throughout the experiment. Multimin heifers tended ($P=0.08$) to have greater initial liver Se and tended to have decreased ($P=0.08$) initial liver Zn compared to CON. At calving, MM cows had increased ($P \leq 0.01$) liver Cu and Se. There was no difference ($P \geq 0.47$) in Julian calving date, calving percent or unassisted births. Calf birth BW was lesser ($P=0.02$) for MM than CON calves and MM calves had greater ($P=0.03$) liver Cu concentrations at birth compared to CON. Despite MM cows having increased ($P<0.01$) milk production, calf weaning

BW and ADG were not different ($P \geq 0.87$). Additionally, calf morbidity and mortality were not different ($P \geq 0.43$) between treatments. Calf mineral status was not different ($P \geq 0.57$) at the time of weaning regardless of treatment; however, MM cows had decreased (P=0.03) liver Zn. Multimin cows had decreased ($P=0.05$) AI pregnancy rates, yet, there was no difference ($P=0.34$) in overall pregnancy rate. Supplementing an injectable trace mineral during heifer development and gestation increased cow milk production and resulted in decreased AI pregnancy rates. There was no effect on overall pregnancy rates or pre-weaning calf health or performance.

**Key Words:** beef calf, beef cow, fetal programming, reproduction, injectable trace mineral

## INTRODUCTION

Fetal programming is complex and can be influenced by numerous factors. The concept of trace mineral supplementation potentially altering fetal growth and ultimately long-term health and performance of calves is a novel concept with limited research. Current research has primarily focused on organic trace mineral supplementation in late gestation (Gunter et al., 2003, Jacometo et al., 2015). While trace minerals may be of particular importance during the last 2 month of gestation as 75% of fetal growth occurs during this time they may also be important during early fetal development when differentiation, organogenesis, vascularization, and placental growth occur (Funston et al., 2010). Alterations to maternal nutrition during early gestation may have impacts on not only future growth of the fetus but may also impact future performance and health of the offspring.

Injectable trace minerals may be advantageous when compared to traditional oral supplement methods in that they provide a targeted delivery of specific amounts of trace

minerals to individual animals. This eliminates the variability associated with fluctuation in voluntary intake noted among cattle provided free choice mineral (Arthington and Swenson, 2004). Ultimately, research needs to be conducted to determine the role injectable trace mineral supplementation may play in the complex process of fetal development. Therefore, the objective of this experiment was to evaluate the effects of repeated trace mineral injections during gestation on beef heifer and subsequent calf performance.

## MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois (IACUC #16046) and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animal in Agricultural Research and Teaching (FASS, 2010).

### *Animals and Experimental Design.*

To determine the effects of repeated trace mineral injections on gestating heifer and subsequent calf performance, 190 Angus × Simmental heifers (315 ± 49.3 kg) were utilized. The development and reproductive performance of these heifers was previously reported by Stokes et al. (2018). Heifers received 3 previous subcutaneous trace mineral (**MM;** Multimin90; Multimin USA, Fort Collins, CO) or sterilized physiological saline (**CON**) injections approximately 90 d apart. These treatments were maintained and subsequent injections were given 205, 114, and 44 ± 26 d prepartum. Heifers were artificially inseminated (**AI**; 296 ± 26 d prepartum; 11/30/2016). All heifers were confirmed pregnant (93 MM and 97 CON heifers) by either AI (43 MM and 46 CON heifers) or clean-up bull (50 MM and 51 CON heifers).

Cattle were housed at the Dixon Springs Agricultural Center in Simpson, Illinois and grazed endophyte-infected fescue (*Festuca arundinacea*) and red clover pastures [*Tri-folium pretense;* 64% neutral detergent fiber (**NDF**), 36% acid detergent fiber (**ADF**), and 10.5% crude protein (**CP**)]. Pasture groups were rotated under the discretion of trained University of Illinois research personnel based on visual appraisal of forage availability. Cattle were supplemented with corn distillers grains (2.7 kg·heifer$^{-1}$·d$^{-1}$; 43% NDF, 11% ADF, 10.5% fat, and 28.4% CP) from trial initiation until 90 ± 26 d postpartum. At this time cattle were provided a total mixed ration (**TMR**) for the remainder of the experiment consisting of corn silage, mixed grass hay, corn distillers grains, and soybean hull pellets (54% NDF, 34% ADF, 2.7% fat, and 10.7% CP). Additionally, heifers were given access to free choice inorganic trace minerals (Renaissance Nutrition, Roaring Springs, PA; 0.24% S, 21.37% Ca as calcium carbonate, 2.99% P as monocalcium phosphate, 24.5% salt, 9.35% Na, 5.84% Mg as magnesium oxide, 0.06% K, 2,214 mg/kg Fe as iron oxide, 2,000 mg/kg Mn as manganous oxide, 2,500 mg/kg Zn as zinc oxide, 1,500 mg/kg Cu as copper sulfate, 27 mg/kg Co as cobalt carbonate, 36 mg/kg I, 26 mg/kg Se as sodium selenite, 110,179 IU/kg vitamin A, 3,084 IU/kg vitamin D, ad 545 IU/kg vitamin E) throughout the experiment. Four cows (2 CON and 2 MM) were removed from trial due to death or poor performance. All analysis included cow performance data until the date they were removed from study.

***Sample Collection and Analytical Procedures***

Cattle body weights (**BW**) and body condition scores [**BCS**; emaciated = 1; obese = 9; as described by Wagner et al. (1988)] were collected at trial initiation (296), 260, 205, 190, 114, and 44 ± 26 d prepartum and 53, 82, 123, 174 ± 26 d postpartum. Thirty-eight heifers that mineral status had previously been determined (Stokes et al., 2018) on and their subsequent

calves were utilized for additional sampling. Liver and blood samples were collected from these heifers 8 d prior to the start of the experiment (308 ± 3 d prepartum), and 5 and 176 ± 3 d postpartum and from their calves 5 ± 3 d post calving for trace mineral determination. At the time of calving an additional 30 cows (15/treatment) and their AI sired bull calves were selected for additional blood and liver biopsies. Blood and liver biopsies were collected from calves 5 ± 3 d and 147 ± 3 d postpartum. Liver biopsy samples were collected using the method of Engle and Spears (2000) with the modification that all heifers were given an intradermal 5 mL of Lidocaine Injectable-2% (MWI, Boise, ID) at the site of the biopsy. Following the biopsy, samples were transported to the laboratory on ice and were frozen at -20°C for subsequent trace mineral analysis. Blood was collected at the time of biopsy via jugular venipuncture into trace element serum vacuum tubes (6.0 mL; Becton, Dickinson, and Co., Franklin Lakes, NJ). Blood samples were centrifuged at 1,300 × *g* for 20 min at 4°C and plasma was stored at -20°C for subsequent trace mineral analysis. Milk samples were collected at 69 ± 3 d postpartum for trace mineral analysis. Milk samples were collected using a method previously described by Clements et al. (2017) with the modification that cows were administered 1 mL/cow oxytocin intramuscularly (MWI Animal Health, Boise, ID) to stimulate milk let down. Cows were then hand milked to obtain a 50-mL sample. Following collection samples were stored on ice and transported to the laboratory where they were spun at 218 × *g* for 10 min at 4°C. The skim portion was removed and stored at 2°C for 24 h until shipped for analysis. Blood, liver, and milk samples were shipped to Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI) and concentrations of Cu, Mn, Se, and Zn were analyzed using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc., Santa Clara, CA) via procedures described previously (Wahlen et al., 2005).

Milk production was assessed on 80 cow-calf pairs (40/treatment) at 71 ± 15 d postpartum via the weigh-suckle-weigh technique as described by Beal et al. (1990), with age and sex of calf equally represented across treatments. Cows were enrolled in a 7-d CO-Synch + controlled internal drug release (**CIDR**) procedure (Johnson et al., 2013) 73 ± 26 d postpartum and artificially inseminated (**AI**) 82 ± 26 d postpartum. Sire and AI technician were stratified across treatments. Ten d following AI, cows were placed with 6 bulls (3 bulls/pasture) that had previously passed a breeding soundness exam for a 38 d breeding season. First service AI conception rates and overall pregnancy rates were determined at 123 and 174 ± 26 d postpartum by a trained technician via ultrasonography (Aloka 500 instrument, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

Calf BW was collected using a hand scale within 48 h of birth. All calves were weighed at weaning (159 ± 26 d postpartum) and calf overall average daily gain (**ADG**) was calculated from birth to weaning. Prior to weaning calves were vaccinated with Bovishield Gold FP5 VL5 HB (Zoetis, Florham Park, NJ), Covexin 8 (Merck Animal Health, Madison, NJ), and Pulmo-Guard MpB (AgriLabs, St. Joseph, MO). Calf health was monitored throughout the experiment by trained university farm personnel. Data were reported from 174 calves (87 calves/treatment). Twenty-three calves (11 CON and 12 MM) were removed throughout the experiment due to death or chronic illness. All analysis included calf performance data until the date they were removed from study.

For nutrient composition analysis, feed and forage samples were collected monthly and composited and dried at 55° C for a minimum of 3 days and ground through a 1 mm screen using a Wiley mill (Arthur, H. Thomas, Philadelphia, PA). Ground samples were analyzed for CP (Leco TruMac, LECO Corporation, St. Joseph, MI), NDF and ADF using an Ankom 200 Fiber

Analyzer (Ankom Technology, Macedon, NY), and crude fat using an Ankom XT10 fat extractor (Ankom Technology, Macedon, NY).

***Statistical Analysis.***

Cow and calf BW, BCS, calf Julian birth date, calf ADG, milk production, and plasma, liver, and milk mineral were analyzed using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effects of treatment and pasture. Cow BW and BCS were analyzed as repeated measures with the fixed effects of treatment, pasture, day, and the interaction between treatment and day. For all repeated variables, the unstructured covariance structure was utilized, as it provided the smallest Akaike information criterion. Day was the repeated effect for all repeated measures. All binary data, including calving percent, percent of unassisted births, calf morbidity and mortality, and AI and overall pregnancy rates were analyzed in the GLIMMIX procedure of SAS. The model included the fixed effect of treatment and pasture for all binary variables. Technician and AI sire were not significant for AI pregnancy rates and thus were removed from the model. Animal served as the experimental unit for all analyses. Treatment effects were considered significant at $P \leq 0.05$ and tendencies were noted at $0.05 < P \leq 0.10$. Means reported in tables are least squares means ± SEM.

## RESULTS AND DISCUSSION

Body weight and BCS were not different ($P \geq 0.49$; Fig. 1) throughout the experiment between MM and CON cows. During development, Stokes et al. (2018) also reported these heifers had no difference in BW and BCS despite the increased Cu and Se status of Multimin treated heifers. Additionally, in other work by Stokes et al. (2017), in one of three experiments there was no difference in BW and BCS between heifers receiving an injection of Multimin or

sterilized saline. However, in the other two experiments, one noted an increased BCS for control cattle at the time of breeding and the other reported an increased BCS for Multimin treated cattle at the time of breeding. Gadberry and Baldridge (2013) also noted no difference in BW or BCS when Angus cows were administered two dose of an injectable trace mineral, one prior to calving and another prior to breeding. However, these experiments were short term and only assessed trace mineral supplementation one time, prior to breeding, and trace mineral status was not assessed.

Initial liver Cu and Mn were not different ($P \geq 0.11$; Table 1) between treatments, but liver Se tended ($P = 0.08$) to be greater for MM cows compared to CON. Multimin supplemented cows also tended ($P = 0.08$) to have decreased liver Zn and had decreased ($P = 0.04$) plasma Zn. Similar results were noted in these heifers during development, with Multimin treated heifers having decreased liver Zn prior to breeding (Stokes et al., 2018). Blood measures may provide little information regarding trace mineral status as circulating concentrations of trace minerals are often regulated by homeostatic mechanisms until reserves become substantially depleted (Miller, 1975). Additionally, adequate markers of Zn status have yet to be established as both plasma and liver concentrations can vary based on immune status and age (Kincaid, 2000). Despite these initial differences in Zn concentrations, there was no difference ($P \geq 0.81$) in liver or plasma Zn at the time of calving. There was also no difference ($P \geq 0.45$) in plasma or liver Mn at the time of calving.

At calving, plasma and liver Cu and liver Se concentrations were greater ($P \leq 0.01$) for MM than CON cows and plasma Se concentrations tended ($P = 0.08$) to be greater for MM than CON. At the time of calving liver Cu concentrations of MM cows had decreased by 28%, and CON cows had decreased liver Cu concentrations by almost 80% compared to initial status. This

change in liver Cu status resulted in CON cattle being classified as deficient according to Kincaid (2000). Though dramatic, this decrease in Cu status at calving was expected as status of the cow is impacted by the demand for Cu from the fetus. Small (1996) utilized 26 Hereford cross multiparous cows and primparous heifers to investigate the effect parturition had on serum Cu concentration and noted that Cu concentrations were lesser (Serum Cu = 7.44 µmol/ L) at parturition than at 7 d prior to or 7 d post calving (Serum Cu = 9.05 and 10.33 µmol/ L, respectively). Xin et al. (1993) utilized 18 multiparous Holstein cows supplemented with three levels of dietary Cu, 5.5 mg/kg of Cu, 10 mg/kg of Cu and 20 mg/kg of Cu, and assessed the changes of Cu concentrations in the blood and liver from 8 weeks prepartum to 8 weeks postpartum. Liver Cu concentrations declined continuously in these cattle with the least concentration noted at parturition. Additionally, as with the cows utilized in the present experiment, supplementing additional Cu at either 10 or 20 mg/kg seemed to mitigate the drastic decrease in cow liver Cu concentrations.

At birth calf plasma Cu, Mn, Se, and Zn were not different ($P \geq 0.17$; Table 2) between treatments. Additionally, liver Mn and Zn were not different ($P \geq 0.78$) in calves at the time of birth. However, foreshadowed by the difference in maternal Cu and Se status, calves from MM supplemented dams also had increased ($P \leq 0.03$) liver Cu and Se concentrations at birth. Additionally, calves from both treatments had over two times the liver Cu concentration compared to that of their dams. This difference was even greater in work by Gooneratne and Christensen (1988), who noted that fetal liver Cu concentrations were markedly greater (202 mg/kg of Cu and 391 mg/kg of Cu at d 30-59 of pregnancy and at d 240-270 of pregnancy, respectively) at all stages of pregnancy than dam liver Cu concentrations (50.7 mg/kg of Cu and 18.8 mg/kg of Cu at d 30-59 of pregnancy and at d 240-270 of pregnancy, respectively).

Final trace mineral status was determined approximately 176 d postpartum, and at this point cows had not been supplemented with an injectable trace mineral for 220 d. Likely due to the amount of time since supplementation, plasma Cu and Mn and liver Cu, Mn, and Se were not different ($P \geq 0.31$) between treatments. Plasma and liver Zn were lesser ($P = 0.03$) in MM than CON cows. This is consistent with previous data from these heifers, that even when supplemented, Multimin treated heifers had decreased liver Zn concentrations compared to controls at the time of breeding (Stokes et al., 2018). Interestingly, MM cows tended to have decreased ($P = 0.07$) plasma Se concentrations compared to controls at the time of weaning. Though there tended to be differences in plasma Se both treatment groups did have adequate Se status. Little research has been conducted regarding trace mineral status of cattle following the withdrawal or removal from a supplementation program. Stockdale and Gill (2011) supplemented dairy cows with either 20, 30, 40, or 60 mg of Se from Se yeast for 6 weeks and monitored blood and milk Se concentrations for 21 weeks following the withdrawal from supplementation. Blood and milk Se concentrations were markedly increased by week 6 of supplementation, however by 21 weeks after supplementation Se concentrations were not different. Stockdale and Gill (2011) stopped assessing Se status at this point and so it is unclear if they would have seen similar results at 25 wk post supplementation, with supplemented cows having decreased plasma Se concentrations. Despite these differences in cow mineral status at the time of weaning, calf liver mineral concentrations were not different ($P \geq 0.57$) regardless of maternal treatment.

Cow milk production and milk mineral composition were collected at 71 ± 15 d postpartum and 69 ± 3 d postpartum, respectively. Dams supplemented with MM had 1.57 kg/d greater ($P < 0.01$; Table 3) 24 h milk production compared to their CON counterparts (6.13 and

4.56 kg/d for MM and CON, respectively). Machado et al. (2013) administered Holstein dairy cows a trace mineral injection and noted no differences in milk production. In a meta-analysis, Rabiee et al. (2010) reported that organic trace minerals supplemented to dairy cows increased milk production by 0.93 kg/d. However, due to the variability in breed of cattle and type of mineral supplementation it makes drawing conclusions across these experiments challenging. Despite differences in milk production, milk mineral composition was not different ($P \geq 0.62$) between treatments. Milk Cu concentrations were below detectable limits, but this is commonly noted as milk Cu concentrations are typically as low as 0.1 – 0.2 mg/L (Lonnerdal et al., 1981).

Calving percent, Julian calving date, and percent unassisted births were not different ($P \geq 0.47$) between CON and MM supplemented dams. However, calf birth BW was lesser ($P = 0.02$; Table 4) for calves from MM supplemented dams than their CON counterparts. Even though calf BW gain has been shown to be driven by milk production (Clutter and Nielsen, 1987) and MM dams had greater milk production, calf weaning BW and ADG were not different ($P \geq 0.87$) between treatments. Dams and calves were comingled across treatments to minimize difference due to pasture variation and this may have allowed calves to cross-nurse between treatments, potentially explaining the lack of difference noted in calf ADG. There were also no differences ($P \geq 0.43$) between treatments in calf morbidity and mortality. Arthington et al. (2014) reported that beef calves administered an injectable trace mineral had an increased mineral status, greater humoral response to a novel antigen, and a heightened acute phase protein response when subjected to transportation stress, suggesting direct administration of an injectable trace mineral may improve calf health. While no differences in health parameters were noted in the present study, the incidence of morbidity was lower than previously reported years (Clements et al.,

2017; morbidity = 27%) and if the health of these calves in the present experiment had been more challenged perhaps differences would have been noted.

To our knowledge, no other experiments have assessed the effect of injectable trace minerals on subsequent calf performance. Other authors have assessed the impact of various organic and inorganic trace mineral supplementation strategies on subsequent calf health and performance. Jacometo et al. (2015) supplemented dams a daily oral bolus of either inorganic or organic Zn (75 mg/kg), Mn (65 mg/kg), Cu (11 mg/kg), and Co (1 mg/kg), 30 d prior to parturition and noted no effect on calf birth BW. However, treatment also had no effects on calf plasma mineral concentrations. Similarly, Gunter et al. (2003) also noted no difference in calf birth BW or total BW gain when dams were provided either no supplement, 26 mg of Se/kg of free choice mineral as sodium selenite, or 26 mg of Se/kg of free choice mineral as seleno-yeast the last 60 d of gestation. The calves and their dams receiving the seleno-yeast supplementation also exhibited the greatest blood Se concentration. Muehlenbein et al. (2001) also noted no difference in calf growth or health from the time of calving to weaning regardless of dams being supplemented with either no supplemental Cu, an inorganic Cu (200 mg Cu from $CuSO_4$), or an organic Cu (100 mg Cu from AvailaCu). However, Cu status of dams and calves were not different regardless of treatment in this experiment (Muehlenbein et al., 2001). Ultimately, more research is needed to understand the complex role trace minerals may be playing in utero and how they may ultimately affect the long term health and productivity of cattle.

Interestingly, MM cows had decreased ($P = 0.05$) AI pregnancy rates (53%) compared to CON cows (67%). However, there was no difference ($P = 0.34$) in overall pregnancy rates. This is in contrast to the primiparous AI pregnancy rate for these females, which was not different between treatments (Stokes et al., 2018). In other work, Stokes et al. (2017) noted increased AI

pregnancy rates in Simmental × Angus heifers administered an injectable trace mineral 30 d prior to breeding. However, Vanegas et al. (2004) reported a significant decrease in first service conception rate when administering dairy cows two doses of an injectable trace mineral, 60 d apart prior, to breeding. Liver and blood samples were not collected in either of these experiments to help explain these results. However, this difference in AI pregnancy rate, noted in the present experiment, could have been driven by the differences noted in milk production, with MM cows having increased 24 h milk production. Both suckling behavior and milk yield can affect the activity of the hypothalamus and ovaries ultimately extending the anestrous period post calving and inhibiting follicular development (Montiel and Ahuja, 2005). Likely all cows were exhibiting estrus later into the breeding season and would have then been bull bred, potentially explaining the lack of differences in overall pregnancy rate. Ultimately though, estrus and suckling behavior were not assessed in this experiment and this data may have helped explain differences noted in cow reproductive performance.

Repeated trace mineral injections during gestation resulted in an increased Cu status of both dam and calf at birth. Additionally, cows supplemented with an injectable trace mineral had increased milk production, which may have contributed to decreased AI pregnancy rates. However, overall pregnancy rates were not different regardless of treatment. Despite calves from dams treated with an injectable trace mineral having a decreased birth BW there was no effect on calf pre-weaning health or performance. These data suggest that repeated trace mineral injections during gestation may increase trace mineral status and milk production. However, this resulted in no improvement in beef calf health and performance. Additional research will be required to determine how these repeated trace mineral injections during gestation may impact the health of

calves when stressed or challenged, and how this change in gestational supplementation may impact long term calf performance.

## TABLES AND FIGURE

**Table 5.1.** Influence of an injectable trace mineral supplementation on dam mineral status

| Item | Treatment[1] | | SEM | *P*-value |
|---|---|---|---|---|
| | Control | MM | | |
| Plasma mineral | | | | |
| Initial[2] | | | | |
| Cu, mg/L | 0.75 | 0.84 | 0.047 | 0.19 |
| Mn, µg/L | 1.75 | 1.63 | 0.126 | 0.52 |
| Se, µg/L | 68.4 | 75.8 | 1.74 | 0.52 |
| Zn, mg/L | 1.04 | 0.90 | 0.045 | 0.04 |
| Calving[3] | | | | |
| Cu, mg/L | 0.55 | 0.86 | 0.037 | <0.01 |
| Mn, µg/L | 3.51 | 2.71 | 0.738 | 0.45 |
| Se, µg/L | 57.6 | 62.0 | 1.75 | 0.08 |
| Zn, mg/L | 0.79 | 0.77 | 0.033 | 0.83 |
| Weaning[4] | | | | |
| Cu, mg/L | 0.83 | 0.80 | 0.033 | 0.47 |
| Mn, µg/L | 5.62 | 4.89 | 1.430 | 0.72 |
| Se, µg/L | 92.7 | 87.4 | 1.96 | 0.07 |
| Zn, mg/L | 1.11 | 0.99 | 0.036 | 0.03 |
| Liver mineral, mg/kg | | | | |
| Initial[2] | | | | |
| Cu | 132.3 | 175.3 | 18.18 | 0.11 |
| Mn | 9.98 | 10.03 | 0.258 | 0.91 |
| Se | 1.06 | 1.55 | 0.190 | 0.08 |
| Zn | 111.0 | 103.6 | 2.81 | 0.08 |
| Calving[3] | | | | |
| Cu | 25.7 | 126.9 | 10.14 | <0.01 |
| Mn | 9.73 | 9.71 | 0.298 | 0.96 |
| Se | 0.98 | 1.42 | 0.048 | <0.01 |
| Zn | 120.5 | 123.5 | 8.58 | 0.81 |
| Weaning[4] | | | | |
| Cu | 180.9 | 216.8 | 23.94 | 0.31 |
| Mn | 11.56 | 11.66 | 0.389 | 0.86 |
| Se | 1.63 | 1.60 | 0.082 | 0.81 |
| Zn | 127.3 | 112.9 | 4.43 | 0.03 |

[1]Control cattle received a sterilized saline solution, and Multimin 90 (MM) cattle received injectable trace mineral at approximately 90 d intervals.

[2]308 ± 3 d prepartum; samples were collected from 22 MM and 16 CON heifers

[3]5 ± 3 d postpartum; samples were collected from 34 MM and 30 CON cows

[4]176 ± 3 d postpartum; samples were collected from 20 MM and 13 CON cows

**Table 5.2.** Influence of maternal injectable trace mineral supplementation on calf mineral status

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value |
| Plasma mineral | | | | |
| Birth[2] | | | | |
| Cu, mg/L | 0.60 | 0.61 | 0.035 | 0.92 |
| Mn, µg/L | 1.63 | 1.98 | 0.174 | 0.17 |
| Se, µg/L | 48.2 | 50.2 | 2.89 | 0.63 |
| Zn, mg/L | 0.91 | 0.90 | 0.065 | 0.91 |
| Liver mineral, mg/kg | | | | |
| Birth[2] | | | | |
| Cu | 182.6 | 302.2 | 21.28 | <0.01 |
| Mn | 9.09 | 9.30 | 0.524 | 0.78 |
| Se | 1.13 | 1.38 | 0.079 | 0.03 |
| Zn | 274.1 | 269.7 | 29.67 | 0.92 |
| Weaning[3] | | | | |
| Cu | 108.2 | 103.8 | 15.77 | 0.85 |
| Mn | 8.77 | 9.02 | 0.698 | 0.66 |
| Se | 1.22 | 1.41 | 0.223 | 0.57 |
| Zn | 125.1 | 123.0 | 5.99 | 0.81 |

[1]Control dams received a sterilized saline solution, and Multimin 90 (MM) dams received injectable trace mineral at approximately 90 d intervals.
[2]5 ± 3 d postpartum; samples were collected from 33 MM and 27 CON calves
[3]147 ± 3 d postpartum; samples were collected from 15 MM and 14 CON calves

**Table 5.3.** Influence of an injectable trace mineral on cow calving, milk production, milk mineral composition, and subsequent reproduction

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value |
| Calving | | | | |
| Calving, % | 91 | 94 | - | 0.47 |
| Calving date, Julian d | 266 | 264 | 2.8 | 0.61 |
| Unassisted birth, % | 98 | 97 | - | 0.65 |
| Milk production,[2] kg/d | 4.56 | 6.13 | 0.346 | <0.01 |
| Milk composition[3] | | | | |
| Cu,[4] mg/L | - | - | - | - |
| Mn, µg/L | 2.25 | 2.20 | 1.348 | 0.98 |
| Se, µg/L | 23.1 | 23.9 | 1.44 | 0.80 |
| Zn, mg/L | 16.5 | 17.0 | 0.78 | 0.62 |
| Artificial insemination pregnancy rate, % | 67 | 53 | - | 0.05 |
| Overall pregnancy rate, % | 96 | 93 | - | 0.34 |

[1]Control dams received a sterilized saline solution, and Multimin 90 (MM) dams received injectable trace mineral at approximately 90 d intervals.
[2]71 ± 15 d postpartum
[3]69 ± 3 d postpartum
[4]Copper milk concentrations were below detectable limits

**Table 5.4.** Influence of maternal injectable trace mineral supplementation on calf performance and health

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value |
| Birth body weight, kg | 30.5 | 28.7 | 0.55 | 0.02 |
| Weaning body weight, kg | 164.6 | 163.9 | 2.78 | 0.87 |
| Average daily gain, kg | 0.81 | 0.81 | 0.015 | 0.90 |
| Morbidity, % | 9.4 | 6.0 | - | 0.43 |
| Mortality, % | 10.5 | 11.7 | . | 0.80 |

[1]Control dams received a sterilized saline solution, and Multimin 90 (MM) dams received injectable trace mineral at approximately 90 d intervals.

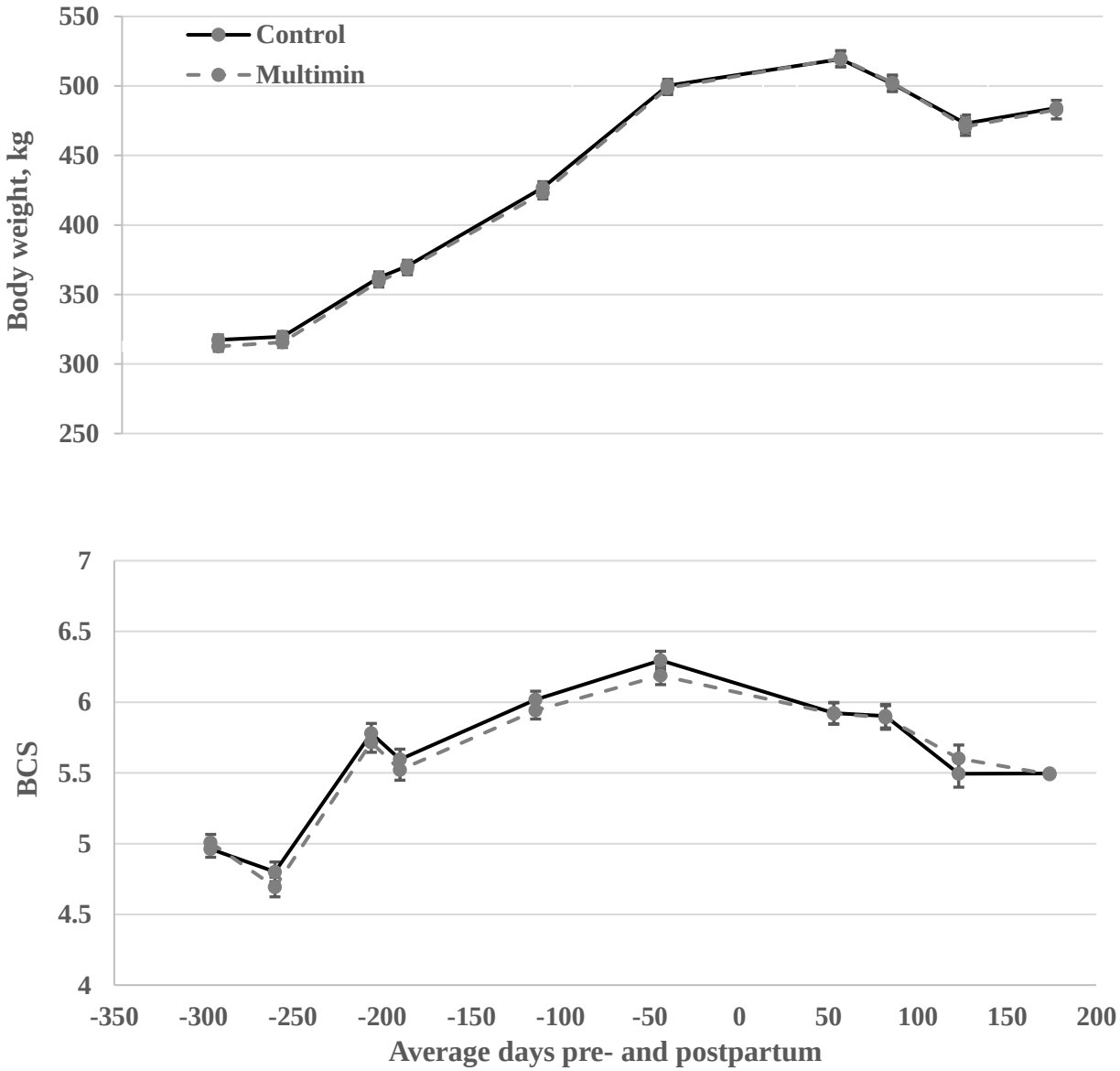


**Figure 5.1.** Effect of an injectable trace mineral (Multimin 90) on heifer BW and BCS at trial initiation (296), 260, 205, 190, 114, and 44 ± 26 d prepartum and 53, 82, 123, 174 ± 26 d postpartum. Day 0 represents average calving date. Control cattle received a sterilized saline solution, and Multimin 90 (Multimin) cattle received injectable trace mineral at approximately 90 d intervals. For BW, treatment by day was not significant ($P = 0.90$), the main effects of treatment and day were ($P = 0.72$) and ($P < 0.01$), respectively. For BCS, treatment by day was not significant ($P = 0.49$), the main effects of treatment and day were ($P = 0.55$) and ($P < 0.01$), respectively.

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# CHAPTER 6

# EFFECTS OF MATERNAL SUPPLEMENTATION WITH AN INJECTABLE TRACE MINERAL ON SUBSEQUENT CALF PERFORMANCE AND INFLAMMATORY RESPONSE

**ABSTRACT:** Newly weaned commercial Angus steers [body weight (**BW**) = 204 ±19 kg; n = 24; 12 steers from dams administered an injectable trace mineral (**MM**; Mulimin90) and 12 steers from control (**CON**) dams] were utilized to determine the effects of maternal supplementation with an injectable trace mineral on the inflammatory response of calves subjected to a lipopolysaccharide (**LPS**) challenge at the initiation of a 42 d receiving period. On d -2 calves were weaned and the following day shipped 220 miles to the Beef Cattle and Sheep Field Laboratory in Urbana, IL. Calf BW was collected at trial initiation and subsequently ever 14 d. Dry matter intake was collected daily and average daily gain (**ADG**) and feed efficiency were assessed. On d 0 calves were administered an intravenous LPS challenge. Body temperature and blood samples were collected from steers prior to LPS administration (0 h) and again at 0.5, 1, 2, 3, 4, 5, and 6 h. Blood samples were analyzed for trace mineral and cortisol at 0 and 2 h and glucose, insulin, LPS binding protein (**LBP**), interleukin-1β (**IL-1β**), and interleukin-6 (**IL-6**) at 0, 0.5, 1, 2, 3, 4, 5, and 6 h. Initial plasma Zn tended ($P = 0.06$) to be greater for MM steers. However, there was no difference ($P \geq 0.31$) in trace mineral status or serum cortisol at any other time. Total area under the curve (**TAUC**) for body temperature was lesser ($P > 0.01$) for MM steers. Basal LBP concentrations and TAUC for LBP tended ($P \leq 0.10$) to be greater for MM steers. Peak concentration of IL-1β tended ($P = 0.09$) to be reached earlier for CON steers. However, there was no difference ($P \geq 0.15$) in glucose, insulin, and IL-6 concentrations regardless of treatment. Additionally, calf performance and feed efficiency were

not different ($P \geq 0.17$) between treatments except ADG from d 28 – 42, which was greater ($P = 0.03$) for CON steers. Maternal supplementation with an injectable trace mineral tended to improve steer plasma Zn status at 0 h and tended to increase basal concentrations of LBP and overall LBP production when steers were administered an LPS challenge. Additionally, MM steers exhibited a more favorable change in body temperature following LPS administration. However, additional injectable trace mineral supplementation of dams during gestation had minimal to no effect on cytokine production and overall calf performance and efficiency.

**Key words:** beef calf, fetal programming, immune challenge, inflammation, injectable trace mineral

## INTRODUCTION

Upon arrival to a feedlot, calves experience extreme stress which coincides with greater susceptibility to an immune challenge. In addition to transportation stress, other compounding stressors may include weaning and comingling. Bovine respiratory disease or "shipping fever" and digestive problems, such as sub-acute ruminal acidosis, are two of the leading causes of morbidity and mortality in feedlot cattle (USDA, 2011), and both are associated with gram negative bacterial infections. Additionally, as the use of antibiotics to treat these costly diseases comes under more scrutiny, and as the livestock industry works to diminish the use of antibiotics, the need for alternative therapies to both prevent and minimize infections becomes critical.

The concept of developmental or fetal programming suggests that a maternal stimulus that alters the fetal environment could have long-term effects on the offspring (Funston et al., 2010). Fetal growth and development can be influenced by genetics, environment, maternal

maturity, and nutrition (Wu et al., 2006). While the effects of maternal macro nutrient restriction are the most studied and have been extensively reviewed (Funston et al., 2010; Wu et al., 2006), the effects of maternal trace mineral supplementation on subsequent calf performance and health are minimally studied. Trace minerals play a critical role in numerous biochemical processes and are a key component of an animal's health and productivity. Recent research has focused on the positive impacts of trace mineral nutrition on the inflammatory response in beef cattle (Berry et al., 2000; Arthington et al., 2014; Genther-Schroeder and Hansen, 2015). Therefore the objective of this study was to determine the effect of maternal injectable trace mineral supplementation (Cu, Mn, Se, and Zn) on subsequent calf performance and health. We proposed that maternal supplementation with an injectable trace mineral during gestation would improve the trace mineral status of subsequent progeny and positively impact the inflammatory response of growing calves when subjected to an lipopolysaccharide (**LPS**) challenge.

## MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois (IACUC #18007) and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animal in Agricultural Research and Teaching (FASS, 2010).

### *Animals and Experimental Design.*

Newly weaned commercial Angus steers [body weight (**BW**) = 204 ±19 kg; age = 183 ± 9 d; n = 24; 12 steers from dams administered an injectable trace mineral (**MM;** Multimin90; Multimin USA, Fort Collins, CO) and 12 steers from control (**CON**) dams] were utilized to determine the effects of maternal supplementation with an injectable trace mineral on the

inflammatory response of calves subjected to a LPS challenge. Dam treatments and previous calf performance and mineral status were reported by Stokes et al. (2018a, 2018b). In brief, dams were administered a subcutaneous saline or trace mineral injections post-weaning at 221, 319, 401, and 521 ± 22 d of age. Treatments were maintained and administered 205, 114, and 44 ± 26 d prepartum. At birth calves were administered injections of vitamin A, D, and E. Calves were vaccinated and booster vaccines of Bovishield Gold FP5 VL5 HB (Zoetis, Florham Park, NJ), Covexin 8 (Merck Animal Health, Madison, NJ), and Pulmo-Guard MpB (AgriLabs, St. Joseph, MO) were administered at 121 and 159 ± 26 d of age, respectively. Dams and their calves were housed at the Dixon Springs Agricultural Center in Simpson, IL and were given access to free choice inorganic trace minerals. On d -14, prior to weaning, dams and calves were given access to GrowSafe bunks (GrowSafe System Ltd., Airdrie, AB, Canada) to allow for calf adaptation to bunks. Diets consisted of 33% dried distillers grains with solubles, 17% soybean hull pellets, 25% shelled corn, 20% ground corn, and 5% supplement [66% neutral detergent fiber (**NDF**), 51% acid detergent fiber (**ADF**), and 11.7% crude protein (**CP**)] On d -2 calves were weaned, and the following day they were shipped approximately 220 miles to the Illinois Beef Cattle and Sheep Field Laboratory in Urbana, IL. Upon arrival, calves were weighed and assigned to pens, with an equal representation of each treatment within pen (6 calves/pen; 3 MM calves and 3 CON calves/pen). Steers were housed in barns on slatted concrete floors covered by interlocking rubber matting. Pens were constructed of 5.08 cm galvanized steel tubing and were 4.88 × 4.88 m in dimension. Calves were given ad libitum access to a receiving ration (Table 1) from d -1 to d 7 and were then transferred to a growing diet for the remainder of the 42 d trial. Calves were weighed every 14 d and individual dry matter intake (**DMI**) was collected daily via GrowSafe bunks to assess average daily gain (**ADG**) and feed efficiency (**G:F**). Calf health was monitored

daily by trained farm personnel for the 42 d receiving period. Four calves died and were removed from trial following the completion of the LPS challenge. All data from these calves were included in analysis until the time of removal.

***Sample Collection and Analytical Procedures***

Upon arrival calves were fitted with commercially available jugular catheters (AniCath L/A 16g × 13mm; Millpledge Veterinary, Clarborough, Nottinghamshire, United Kingdom). Following insertion, catheters were flushed with a minimum of 6 mL sterile heparinized saline (1000 µg/mL) to prevent overnight clotting. Catheters were secured to the neck with polypropylene suture (Ethicon US, LLC, Blue Ash, OH) and covered with a patch between sample collections. Fifteen h following arrival (d 0), calves were weighed and administered an intravenous LPS (0.5 µg/kg of BW of LPS from *Escherichia coli* O111:B4; Sigma-Aldrich, St. Louis, MO) challenge. Following administration of LPS, catheters were flushed with 8 mL of sterile saline to ensure all LPS was cleared from the catheter. Blood samples were collected prior to LPS administration (0 h) and again at 0.5, 1, 2, 3, 4, 5, and 6 h post LPS administration. Following blood collection, catheters were flushed with a minimum of 6 mL heparinized saline (1000 µg/mL) to prevent clotting. At each of these time points rectal temperatures were collected from steers. To ensure blood was drawn at the proper time from all steers, each pen of steers went to a different on site handling facility for blood collection.

Blood samples from all 8 time points were collected and analyzed for interleukin 1β (**IL-1β**), interleukin 6 (**IL-6**), glucose, insulin, and LPS binding protein (**LBP**). Additional blood samples were collected at 0 and 2 h for cortisol and trace mineral analysis. Blood was collected into serum vacutainer tubes (10.0 mL; Becton, Dickinson, and Co., Franklin Lakes, NJ) for analysis of interleukin 1β (**IL-1β**), interleukin 6 (**IL-6**) and cortisol. Blood was collected in

$K_{2EDTA}$ vacuum tubes (10 mL; Becton, Dickinson, and Co., Franklin Lakes, NJ) for glucose, insulin, and LBP analysis. Serum was allowed to clot for 2 h following collection. Samples were centrifuged at 1,300 × *g* for 20 min at 5°C, and plasma and serum were stored at -80°C until analysis. Blood for trace mineral analysis was collected into trace element vacutainers (6.0 mL; Becton, Dickinson, and Co., Franklin Lakes, NJ), and tubes were spun 1300 × *g* for 10 min at 25°C. Plasma was removed and stored at -20°C until further analysis.

Serum IL-1β and IL-6 concentrations were analyzed using a commercially available Bovine ELISA reagent kits (Bovine IL-1β ELISA reagent kit; Invitrogen Corporation, Grand Island, NY; intra-assay CV = 3.08 and inter-assay CV = 7.31; Bovine IL-6 ELISA reagent kit; Thermo Scientific, Rockford, IL, intra-assay CV = 4.29 and inter-assay CV = 6.05). Samples for cortisol analysis were sent to the University of Illinois Veterinary Diagnostic Laboratory and were tested on an Immulite 1000 (Siemens Healthineers, Erlangen Germany). Glucose concentrations were determined using the Glucose LiquiColor Procedure (No. 1070; Stanbio Laboratory, Boerne, TX; intra-assay CV = 2.03 and inter-assay CV = 1.60) and Insulin concentrations were analyzed using a commercially available bovine insulin ELISA kit (Bovine Insulin Elisa, Alpco, Salem, NH; intra-assay CV = 3.56 and inter-assay CV = 3.51). Concentrations of LBP were determined using a human LBP ELISA kit (Multispecies reactive; Cell Sciences, Newburyport, MA; intra-assay CV = 4.55 and inter-assay CV = 12.66). Plasma samples for trace mineral analysis were sent to Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI) and concentrations of Cu, Mn, Se, and Zn were analyzed using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc., Santa Clara, CA) via procedures described previously (Wahlen et al., 2005).

Feed ingredients were collected every 14 d for nutrient composition analysis. Samples were dried at 55° C for a minimum of 3 days and ground through a 1 mm screen using a Wiley mill (Arthur, H. Thomas, Philadelphia, PA). Samples were analyzed for CP (Leco TruMac, LECO Corporation, St. Joseph, MI), NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY), and crude fat using an Ankom XT10 fat extractor (Ankom Technology, Macedon, NY).

***Statistical Analysis.***

Blood samples collected at 0 h, prior to administration of the LPS challenge, were utilized as the baseline concentration. Peak concentrations and peak time were calculated for IL-1B, IL-6, glucose, insulin, and LBP. Additionally, the total area under the curve (**TAUC**) was calculated using the trapezoidal method and actual concentration values as described by Cardoso et al. (2011).

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as a stratified randomized design. The model included the fixed effects of treatment, pen, and handling facility. Steer served as the experimental unit for all analyses. Significance was declared at $P \leq 0.05$ and tendencies were noted at $0.05 < P \leq 0.10$. Means reported in tables are least squares means ± SEM.

## RESULTS AND DISCUSSION

Due to the segmented nature of the beef industry, cattle entering the feedlot are commonly exposed to numerous stressors including weaning, transportation, and comingling. These stressors result in cattle that are more susceptible to respiratory and metabolic disorders. Challenging calves with LPS offered a repeatable mechanism to initiate an inflammatory

response in a way that mimics a gram negative bacterial infection that stressed feedlot cattle may commonly encounter. Previous liver mineral for these steers was reported at the time of weaning by Stokes et al. (2018a). While mineral status was not different in these calves at the time of weaning, calves from CON dams did have marginally deficient liver Se concentrations, while MM calves fell within the adequate range according to Kincaid (2000). This marginal deficiency was present even though all cow/calf pairs had access to free choice trace mineral, though individual calf intake were not able to be estimated. Additionally, these calves were consuming milk from their dams, which would serve as an additional source of trace mineral.

Prior to LPS administration, plasma Cu, Mn, and Se were not different ($P \geq 0.55$; Fig. 1) between treatments. However, there was a tendency for increased ($P = 0.06$) plasma Zn concentrations in MM steers. Small decreases in plasma Zn concentrations may be a result of early deficiency (Underwood and Suttle, 1999). However, there are no clear storage pools of Zn in the body and numerous other factors have been shown to manipulate blood mineral concentrations including the physiological state of the animal and presence of antagonists (Herdt and Hoff, 2011). Two hours post LPS administration there were no differences ($P \geq 0.31$) in plasma Cu, Mn, Se, or Zn concentrations, regardless of treatment. Despite these lack of differences, both treatments noted an over 70% decrease in plasma Mn concentrations and about a 30% decrease in plasma Zn concentrations. The decrease in plasma Zn was expected and has previously been reported in cattle when administered an LPS challenge (Zebeli et al., 2012). Interleukin-6 has been shown to mediate this change in circulating Zn concentrations by partitioning Zn to the liver (Savlov et al., 1962). This repartitioning of Zn may deprive infecting microbes of this essential trace element, inhibiting growth and replication without impacting host immunity (Sugarman, 1983). Though less understood, research has hypothesized that Mn may

also be sequestered to allow resistance to bacterial infections (Kehl-Fie and Skaar, 2010). Manganese is also a component of Mn superoxide dismutase, an antioxidant, and sequestering Mn may be a form of nutritional immunity that allows the host to mount an antibacterial immune response via oxidative stress (Weiss and Carver, 2018).

Steer serum cortisol concentrations were not different ($P \geq 0.43$; Fig. 2) between treatments prior to LPS administration, or 2 h post LPS administration. Unstressed cattle typically exhibit plasma cortisol levels ranging from 1 to 18 ng/mL (Rhynes and Ewing, 1973; Lefcourt et al., 1993). Based on this basal range of cortisol, initial cortisol concentrations of steers was already elevated prior to administration of LPS. This was not surprising though as these steers had been recently weaned, shipped, processed through the chute, and fitted with jugular catheters prior to the collection of this initial sample. Two hours following the LPS challenge cortisol concentrations rose to over 70 ng/mL. This is comparable to work by Carroll et al. (2009) who reported that steers exposed to an LPS challenge reached serum cortisol concentrations of 99 ng/mL and peak concentration was reached 3.4 hours post challenge. Increased circulating cortisol may serve as an effector molecule for the innate immune system and may stimulate early physiological responses following an LPS challenge (Carroll et al., 2009).

Base rectal body temperatures were relatively elevated prior to LPS administration, however there was no difference ($P = 0.13$; Table 2) between treatments. Peak temperature and time of peak temperature were not different ($P \geq 0.49$) regardless of treatment. Interestingly, MM steers had a decreased ($P < 0.01$) TAUC for rectal body temperature compared to CON steers. These data suggest that once reaching peak body temperature, MM steers were able to recover and return to baseline temperatures more efficiently. Jacometo et al. (2015)

supplemented gestating Holstein cows with inorganic or organic Zn, Mn, Cu, and Co and noted that calves from organic supplemented dams tended to have lower rectal temperatures 7 and 21 d after birth. This change was noted despite claves having no difference in plasma Cu, Mn, Fe, or Zn. However, Genther-Schroeder and Hansen (2015) noted that in weaned steers experiencing shipping stress, rectal temperature was not affected by increased mineral status.

Both the action of cytokines and fever have been shown to modulate feed intake and the activity of glucose-responsive neurons in the hypothalamus (Hori et al., 1991). However, base concentration, peak concentration, time of peak concentration, and TAUC were not different ($P \geq 0.15$) for both glucose and insulin, regardless of treatment. As expected with immune challenged animals, a quick rise (43 and 45 min to peak concentration for CON and MM steers, respectively) in circulating glucose was noted to fuel the upregulation of immune cells (Spurlock, 1997). Even moderate infections can result in a 150 - 200% increase in the rate of gluconeogenesis in the infected host (Lochmiller and Deerenberg, 2000). This rise in plasma glucose concentration was then followed by an increase in plasma insulin. This repartitioning of nutrients for immune related processes is a metabolically costly process and ultimately a key obstacle for animals to achieve maximal growth and efficiency (Kvidera et al., 2017).

The biological activity of LPS circulating in the blood stream can be mediated by LBP, which accelerates the binding of LPS to CD14, a macrophage receptor that stimulates phagocytosis (Fenton and Golenbock, 1998). Not only did MM steers tend ($P = 0.09$) to have increased base concentrations of LBP, they also tended ($P = 0.10$) to exhibit a greater TAUC for LBP concentrations following the administration of an LPS challenge. Peak concentration of LBP and time of peak concentration were similar ($P \geq 0.33$) for both treatments. Fenton and Golenbock (1998) revealed that LBP is required for a rapid inflammatory response and survival

post infection when mice are exposed to *Salmonella*, a gram negative bacteria containing LPS. The increased production of LBP by immune challenged MM steers in the present experiment may have resulted in expedited activation of the acute immune response and may have facilitated a more rapid clearance of LPS from the host.

The cytokines IL-1β and IL-6 are endogenous pyrogens that mediate fever by stimulating acute phase proteins and activating the hypothalamic-pituitary-adrenal axis of the brain (Kozak et al., 1998). Despite differences between treatments for body temperature, there were no differences ($P \geq 0.42$; Table 3) in base concentration, peak concentration, and TAUC for IL-1β and no differences ($P \geq 0.27$) in base concentration, peak concentration, time of peak concentration, and TAUC for IL-6. There was however a tendency ($P = 0.09$) for CON steers to reach peak concentration of IL-1β earlier than MM steers. The difference noted between body temperature and these endogenous pyrogens may be explained by data from Kozak et al. (1998), who reported that in mice IL-1β and IL-6 may not be required for an LPS induced fever. Kozak et al. (1998) hypothesized that tumor necrosis factor-α (**TNF-α**), may instead play a role in fever induction. Unfortunately, TNF-α was not assessed in this study and therefore it is unknown if this may have been driving the differences noted in steer body temperature.

Likely driven by the minimal differences noted in immune parameters, calf performance, including BW, ADG, DMI, and G:F, were not different ($P \geq 0.17$; Table 4) at any time point except for d 28 – 42, when ADG was greater ($P = 0.03$) for CON than MM calves. This interim 28 – 42 d ADG represents a short time and only single day BW were collected for these interim BW. Though this single ADG time point is different, the more meaningful representation of this data would be reflected by the lack of difference in overall ADG. Jacometo et al. (2015) also noted no difference in calf BW or mineral status from birth to 8 weeks of age when dams were

supplemented organic or inorganic trace minerals during gestation. Similarly, Muehlenbein et al. (2001) supplemented gestating beef cows inorganic or organic copper and noted no difference in calf birth BW or weaning BW and no difference in calf serum Cu concentrations. These experiments both supplemented organic trace minerals to dams, and the calves in these experiments were overall heathy and not subjected to health or immune challenges. However, these data collectively suggest that additional mineral supplementation to gestating dams may not improve subsequent calf growth and performance.

The present data demonstrate how maternal supplementation with an injectable trace mineral affects subsequent calf growth and immune response following an LPS induced immune challenge. Supplementing an injectable trace mineral to gestating dams tended to improve steer plasma Zn status at the time of weaning and steers from MM supplemented dams tended to have increased basal concentrations of LBP and greater overall LBP production when administered an LPS challenge. Additionally, steers from dams supplemented an injectable trace mineral exhibited a more favorable change in body temperature following LPS administration. However, additional injectable trace mineral supplementation of dams during gestation had minimal to no effect on nutrient partitioning, cytokine production, and overall calf performance and efficiency. Additional research is needed to determine the role maternal trace mineral supplementation may be playing in LBP production as well as the mechanisms that may be altering body temperature during inflammation.

## TABLES AND FIGURES

**Table 6.1**. Ingredient and nutrient composition of calf diets (% dry matter basis)

| | Inclusion | |
|---|---|---|
| Item | Receiving[1] | Growing[2] |
| Ingredient, % | | |
| High moisture corn | - | 15 |
| Modified distillers grains | 40 | 15 |
| Corn silage | - | 35 |
| Hay | 50 | 25 |
| Supplement | 10 | 10 |
| Analyzed nutrient content | | |
| Crude Protein, % | 16.6 | 10.9 |
| NDF,[3] % | 52.5 | 42.1 |
| ADF,[4] % | 26.4 | 21.3 |
| Crude Fat, % | 5.6 | 3.3 |

[1]Receiving diet was provided from d -1 to 7.
[2]Growing diet was provided from d 8 to 42.
[3]Neutral detergent fiber
[4]Acid detergent fiber

**Table 6.2.** Influence of maternal injectable trace mineral supplementation on calf body temperature and blood metabolites over a 6 h period following the administration of a lipopolysaccharide immune challenge

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value |
| Body temperature | | | | |
| Base temperature,[2] C | 39.5 | 39.3 | 0.12 | 0.13 |
| Peak temperature, C | 40.6 | 40.6 | 0.10 | 0.90 |
| Peak time, min | 163 | 140 | 22.5 | 0.49 |
| TAUC[3] | 25356 | 21480 | 876.0 | <0.01 |
| Glucose | | | | |
| Base concentration,[2] mg/dL | 86.6 | 91.8 | 2.45 | 0.15 |
| Peak concentration, mg/dL | 124.4 | 118.7 | 6.59 | 0.55 |
| Peak time, min | 43 | 45 | 6.5 | 0.79 |
| TAUC[3] | 27445 | 28396 | 909.1 | 0.47 |
| Insulin | | | | |
| Base concentration,[2] mg/dL | 1.06 | 0.64 | 0.249 | 0.25 |
| Peak concentration, mg/dL | 4.71 | 7.54 | 1.368 | 0.16 |
| Peak time, min | 125 | 125 | 4.7 | 1.00 |
| TAUC[3] | 8.2 | 8.2 | 1.66 | 1.00 |
| LBP[4] | | | | |
| Base concentration,[2] µg/mL | 2.50 | 4.68 | 0.855 | 0.09 |
| Peak concentration, µg/mL | 11.24 | 11.32 | 0.591 | 0.92 |
| Peak time, min | 210 | 245 | 25.1 | 0.33 |
| TAUC[3] | 3488 | 4359 | 350.0 | 0.10 |

[1]Control dams received a sterilized saline solution, and Multimin90 (MM) dams received injectable trace mineral at approximately 90 d intervals during gestation.
[2]Value determined at time 0, prior to the administration of the lipopolysaccharide challenge
[3]Total area under the curve
[4]Lipopolysaccharide binding protein

**Table 6.3.** Influence of maternal injectable trace mineral supplementation on cytokine production over a 6 h period following the administration of a lipopolysaccharide immune challenge

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value |
| IL-1β[2] | | | | |
| Base concentration,[3] pg/mL | 12.13 | 6.29 | 4.981 | 0.42 |
| Peak concentration, pg/mL | 125.3 | 125.6 | 25.03 | 0.99 |
| Peak time, min | 195 | 225 | 11.9 | 0.09 |
| TAUC[4] | 19979 | 22990 | 5276.5 | 0.69 |
| IL-6[5] | | | | |
| Base concentration,[3] pg/mL | 1867.6 | 1107.0 | 779.04 | 0.50 |
| Peak concentration, pg/mL | 15391 | 16810 | 1293.9 | 0.45 |
| Peak time, min | 205 | 245 | 24.8 | 0.27 |
| TAUC[4] | 2313951 | 2898703 | 371270 | 0.28 |

[1]Control dams received a sterilized saline solution, and Multimin90 (MM) dams received injectable trace mineral at approximately 90 d intervals during gestation.
[2]Interleukin-1β
[3]Value determined at time 0, prior to the administration of the lipopolysaccharide challenge
[4]Total area under the curve
[5]Interleukin-6

**Table 6.4.** Influence of maternal injectable trace mineral supplementation on calf performance during a 42 d receiving period following the administration of a lipopolysaccharide immune challenge

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | MM | SEM | *P*-value |
| Body weight, kg | | | | |
| d 0 | 207 | 201 | 5.9 | 0.48 |
| d 14 | 217 | 213 | 7.4 | 0.71 |
| d 28 | 224 | 218 | 7.3 | 0.55 |
| d 42 | 252 | 239 | 8.0 | 0.25 |
| Average daily gain, kg | | | | |
| d 0 - 14 | 0.76 | 1.29 | 0.277 | 0.17 |
| d 14 - 28 | 0.51 | 0.68 | 0.350 | 0.73 |
| d 28 - 42 | 1.99 | 1.48 | 0.150 | 0.03 |
| d 0 - 42 | 1.09 | 0.97 | 0.148 | 0.59 |
| Dry matter intake, kg | | | | |
| d 0 - 14 | 6.33 | 5.42 | 0.646 | 0.34 |
| d 14 - 28 | 10.72 | 9.63 | 0.657 | 0.25 |
| d 28 - 42 | 13.20 | 11.86 | 0.741 | 0.22 |
| d 0 - 42 | 10.05 | 9.42 | 0.516 | 0.40 |
| G:F[2] | | | | |
| d 0 - 14 | 0.116 | 0.192 | 0.0444 | 0.22 |
| d 14 - 28 | 0.070 | 0.054 | 0.0292 | 0.69 |
| d 28 - 42 | 0.158 | 0.133 | 0.0178 | 0.32 |
| d 0 - 42 | 0.106 | 0.111 | 0.0114 | 0.75 |

[1]Control dams received a sterilized saline solution, and Multimin90 (MM) dams received injectable trace mineral at approximately 90 d intervals during gestation.
[2]Feed efficiency is reported as gain to feed (G:F)

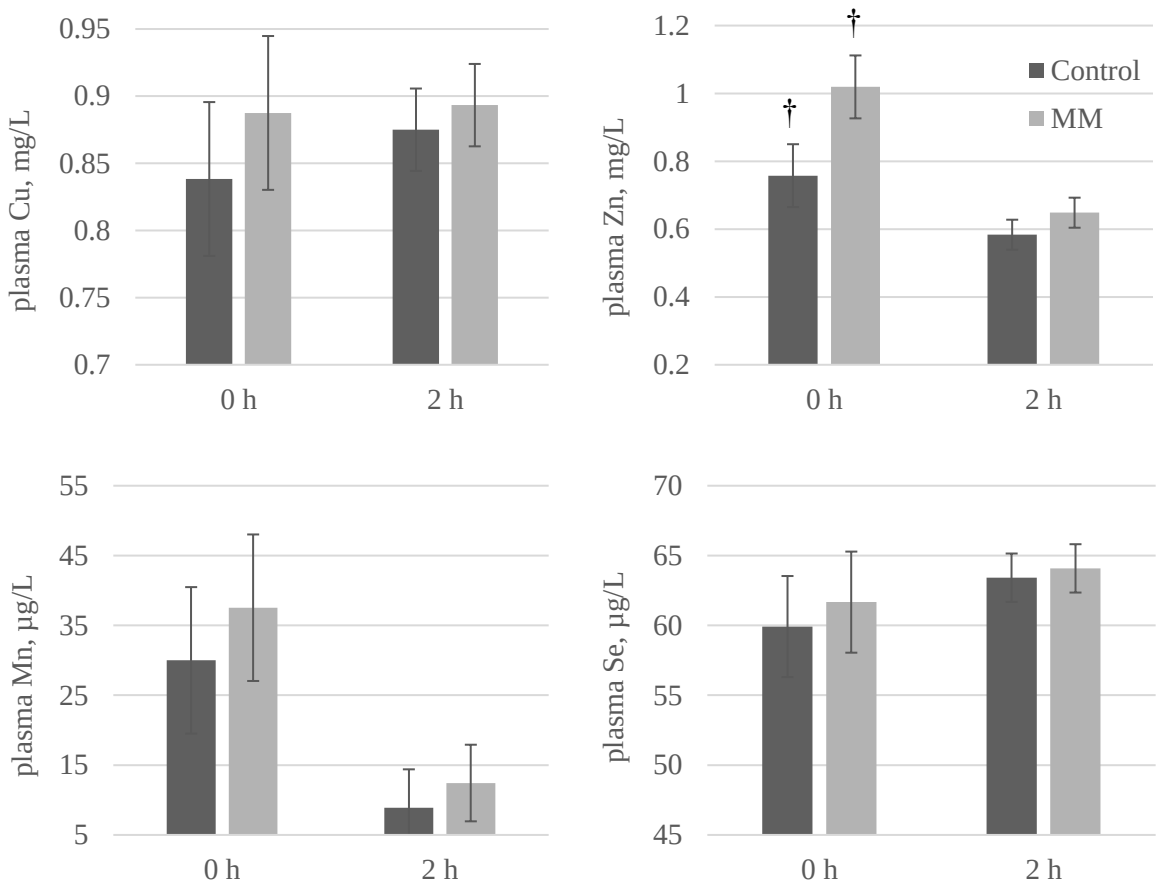


**Figure 6.1.** Influence of maternal injectable trace mineral supplementation on calf plasma mineral status following the administration of a lipopolysaccharide immune challenge. Control dams received a sterilized saline solution, and Multimin90 (MM) dams received injectable trace mineral at approximately 90 d intervals during gestation. Time 0 h was collected prior to the administration of the lipopolysaccharide challenge. At 0 h there was a tendency for MM calves to have greater ($P = 0.06$) plasma Zn concentrations compared to control. However trace mineral status was not different ($P \geq 0.31$) between treatments at any other time point.

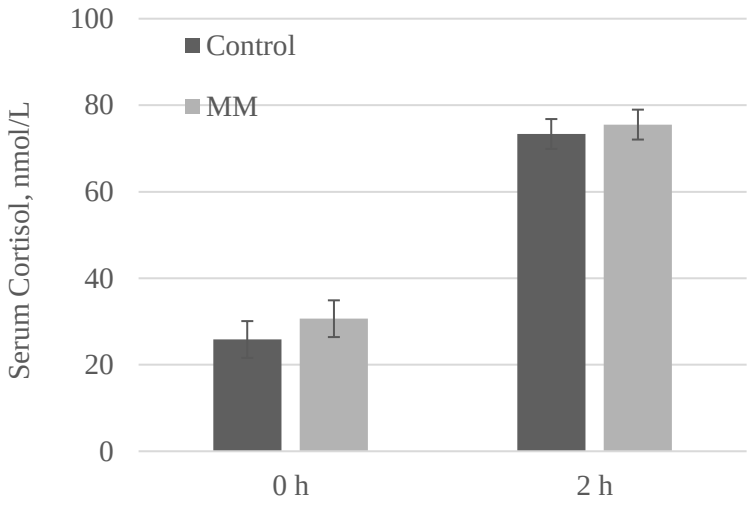


**Figure 6.2.** Influence of maternal injectable trace mineral supplementation on calf serum cortisol concentration following the administration of a lipopolysaccharide immune challenge. Control dams received a sterilized saline solution, and Multimin90 (MM) dams received injectable trace mineral at approximately 90 d intervals during gestation. Time 0 h was collected prior to the administration of the lipopolysaccharide challenge. Serum cortisol was not different ($P \geq 0.43$) between treatments at any time point.

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# CHAPTER 7

# CONCLUSIONS

The development of heifers to productive beef cows represents a substantial economic impact to the producer, and proper development can ultimately impact a heifer's reproductive success and longevity within the herd. Diet and plane of nutrition during development have been linked to physiological changes that result in attainment of puberty in heifers. While maximizing pregnancy rates are a key component of heifer development, profitability and sustainability should also be considered. Trace minerals such as copper, manganese, selenium, and zinc play a critical role in numerous biochemical processes and are key components of a ruminant animal's health and productivity. Increased mineral status of an animal could be of particular importance at times when biological needs are increased, including when animals are growing or breeding.

The present data demonstrate variable results for performance and reproductive success when additional trace mineral supplementation was provided to developing and gestating beef heifers. Repeated administration with an injectable trace mineral is a viable way to improve heifer trace mineral status, specifically Cu and Se. Due to the difficulty of assessing trace mineral status of an entire herd, supplementing trace minerals through an injection may ensure a consistent, adequate trace mineral supply to heifers for optimal reproductive performance. While the responses measured across these experiments were variable, it is important to note that injectable trace minerals do not appear to incur any negative impacts on heifer performance or overall reproductive success.

Furthermore, the concept of developmental or fetal programming suggests that a maternal stimulus that alters the fetal environment could have long-term effects on the offspring. Fetal

growth and development can be influenced by genetics, environment, maternal maturity, and nutrition. Maternal supplementation with an injectable trace mineral during gestation may alter calf body temperature and LBP production when exposed to LPS induced immune challenge. However, this maternal supplementation had minimal to no effects on nutrient partitioning, cytokine and acute phase protein production, and overall calf performance and efficiency. Additional research is needed to determine the role maternal trace mineral supplementation may be playing in LBP production as well as the mechanisms that may be altering body temperature during inflammation.

## APPENDIX A

## EFFECTS OF MATERNAL SUPPLEMENTATION WITH AN INJECTABLE TRACE MINERAL ON SUBSEQUENT CALF PERFORMANCE AND INFLAMMATORY RESPONSE – REPEATED MEASURES ANALYSIS

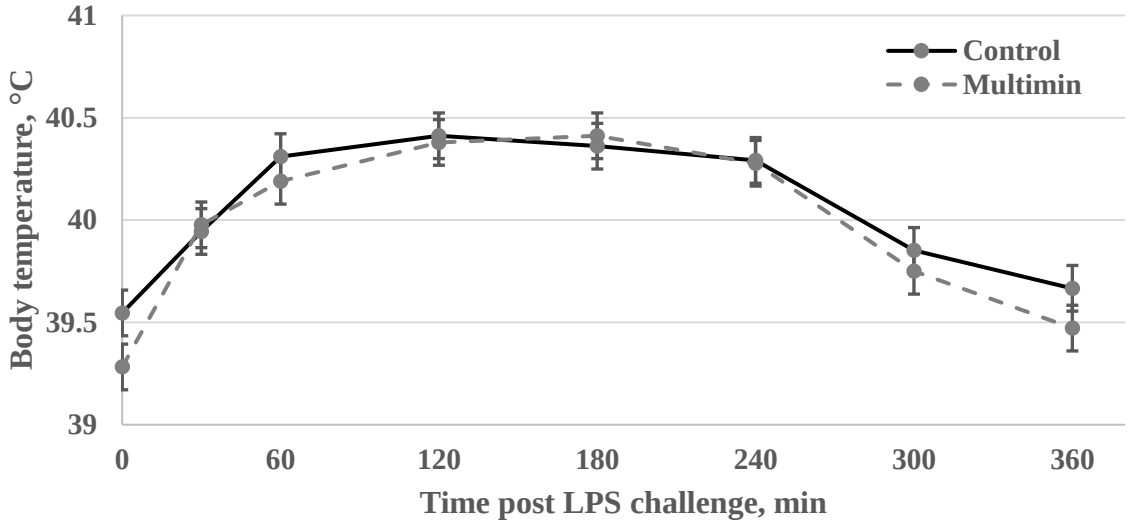


**Figure A.1.** Influence of maternal injectable trace mineral supplementation on calf body temperature following the administration of a lipopolysaccharide (LPS) immune challenge. Control dams received a sterilized saline solution, and Multimin dams received injectable trace mineral at approximately 90 d intervals during gestation. Time 0 h was collected prior to the administration of the lipopolysaccharide challenge. Treatment by time was not significant ($P$ = 0.48) and the main effects of treatment and time were ($P$ = 0.41) and ($P$ < 0.01), respectively.

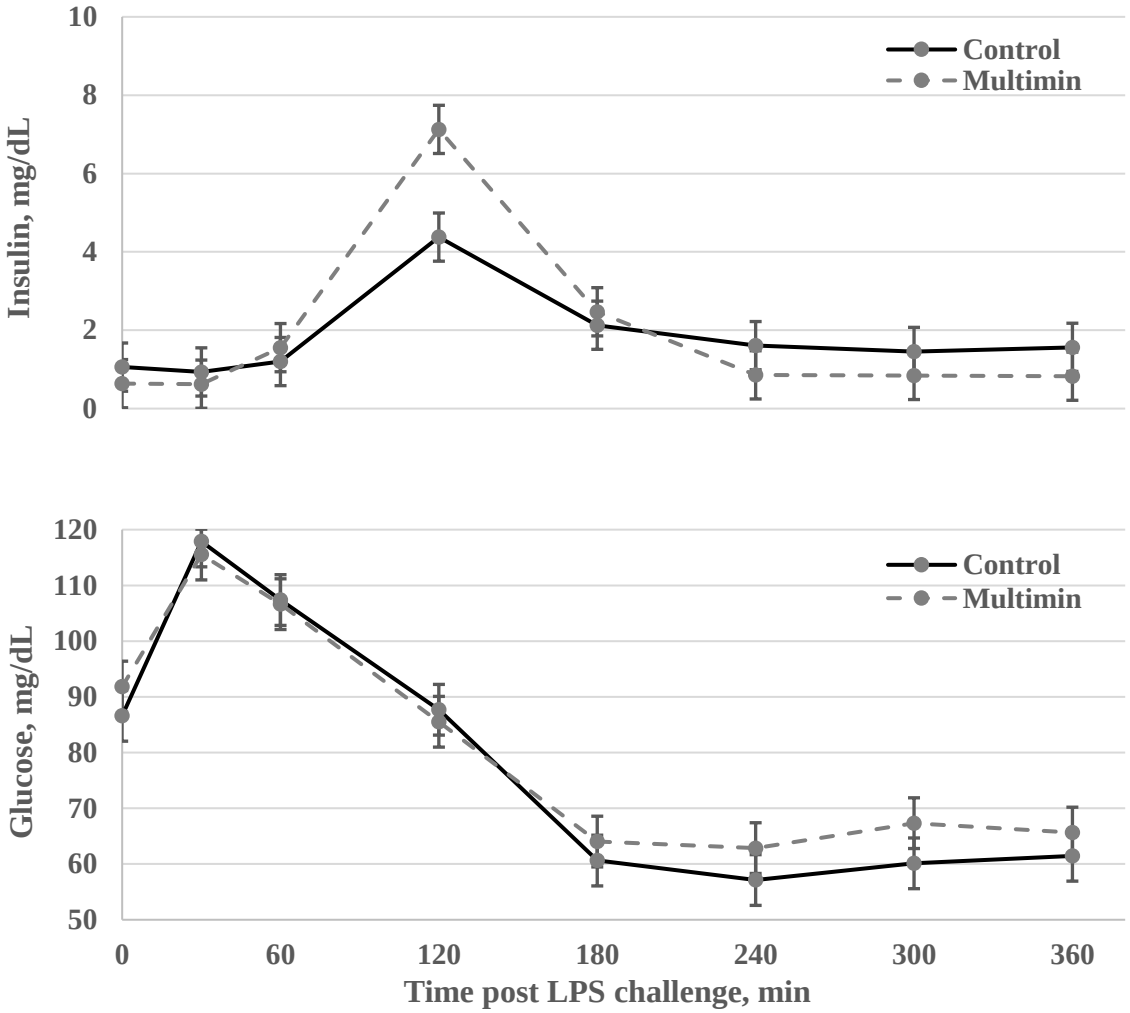


**Figure A.2.** Influence of maternal injectable trace mineral supplementation on calf plasma glucose and insulin concentrations following the administration of a lipopolysaccharide (LPS) immune challenge. Control dams received a sterilized saline solution, and Multimin dams received injectable trace mineral at approximately 90 d intervals during gestation. Time 0 h was collected prior to the administration of the lipopolysaccharide challenge. For glucose, treatment by time was not significant ($P = 0.93$) and the main effects of treatment and time were ($P = 0.45$) and ($P < 0.01$), respectively. For insulin, treatment by time tended to be different ($P = 0.09$) and the main effects of treatment and time were ($P = 0.85$) and ($P < 0.01$), respectively.

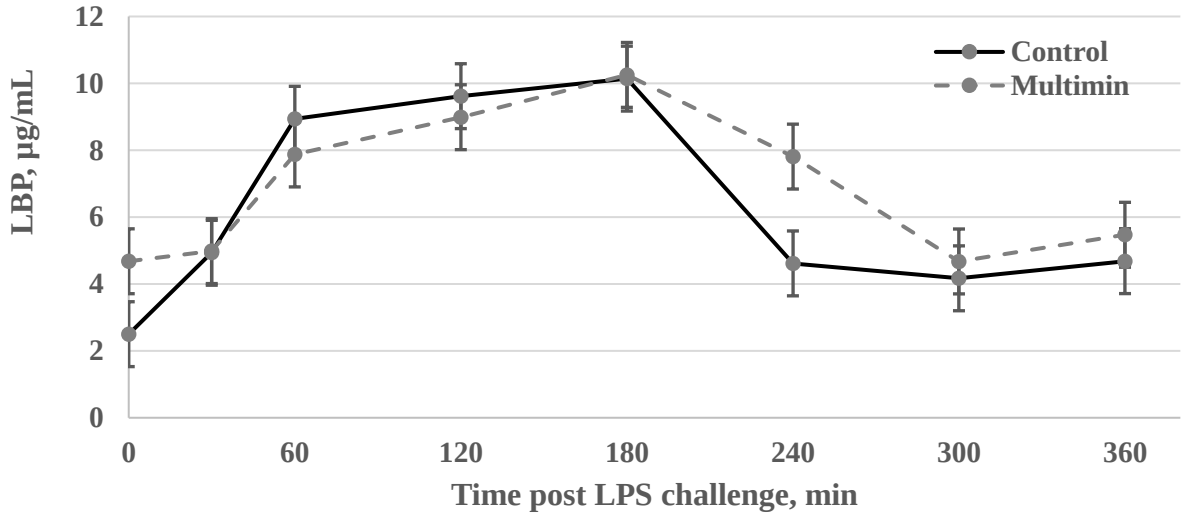


**Figure A.3.** Influence of maternal injectable trace mineral supplementation on calf plasma lipopolysaccharide (LPS) binding protein (LBP) concentration following the administration of a LPS immune challenge. Control dams received a sterilized saline solution, and Multimin dams received injectable trace mineral at approximately 90 d intervals during gestation. Time 0 h was collected prior to the administration of the lipopolysaccharide challenge. Treatment by time was not significant ($P = 0.37$) and the main effects of treatment and time were ($P = 0.26$) and ($P < 0.01$), respectively.

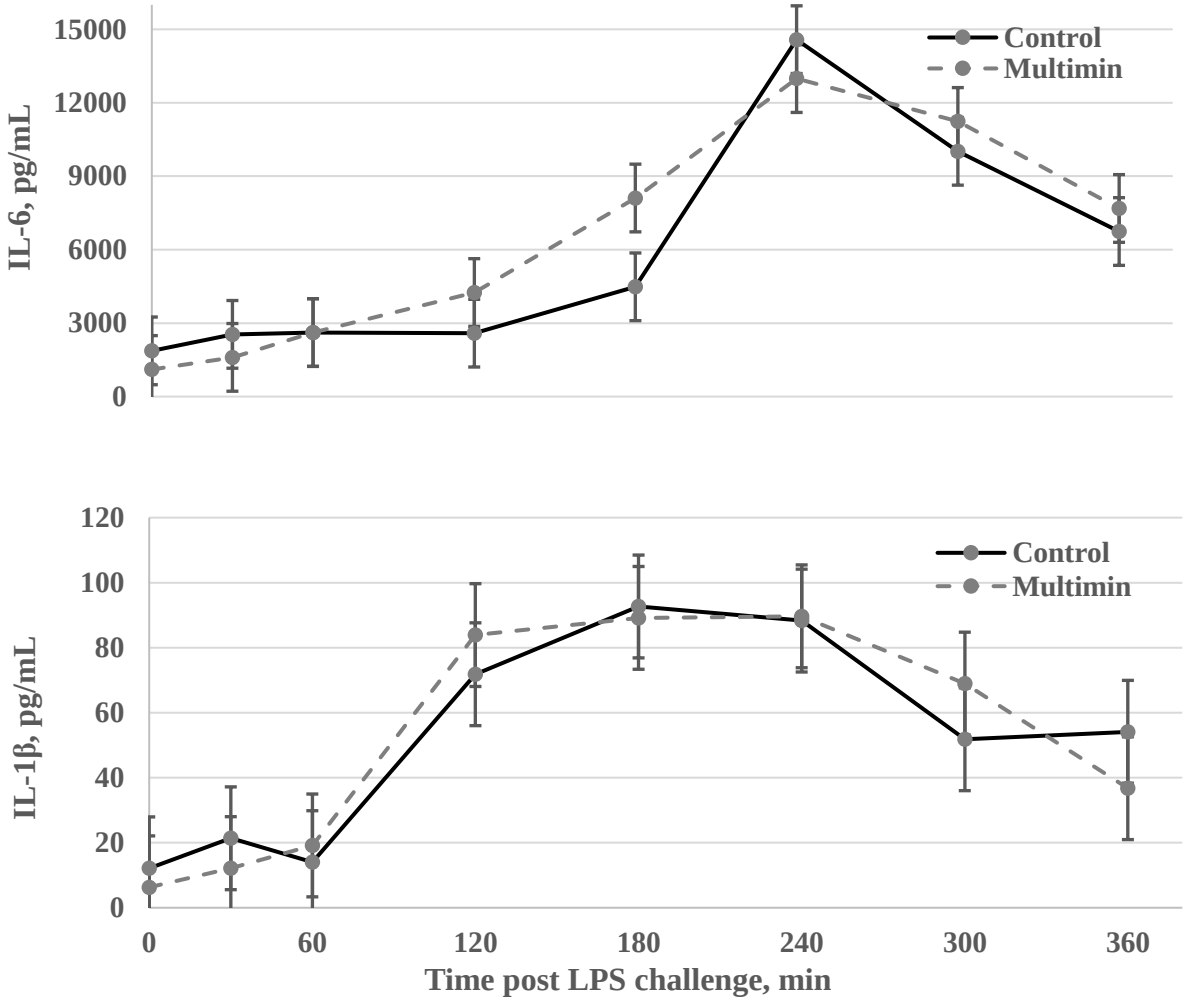


**Figure A.4.** Influence of maternal injectable trace mineral supplementation on calf interleukin-1β (IL-1β) and interleukin-6 (IL-6) concentrations following the administration of a lipopolysaccharide (LPS) immune challenge. Control dams received a sterilized saline solution, and Multimin dams received injectable trace mineral at approximately 90 d intervals during gestation. Time 0 h was collected prior to the administration of the lipopolysaccharide challenge. For IL-1β, treatment by time was not significant ($P = 0.88$) and the main effects of treatment and time were ($P = 1.00$) and ($P < 0.01$), respectively. For IL-6, treatment by time was not significant ($P = 0.59$) and the main effects of treatment and time were ($P = 0.51$) and ($P < 0.01$), respectively.