Spring 2015

Effect of a Trace Mineral Injection on Beef Cattle Performance

Carmen J. Brasche

University of Nebraska-Lincoln, cjbrasche@gmail.com

Follow this and additional works at: http://digitalcommons.unl.edu/animalscidiss
Part of the Meat Science Commons, and the Other Animal Sciences Commons

http://digitalcommons.unl.edu/animalscidiss/106

This Article is brought to you for free and open access by the Animal Science Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Theses and Dissertations in Animal Science by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
EFFECT OF A TRACE MINERAL INJECTION ON BEEF CATTLE PERFORMANCE

by

Carmen J. Brasche

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor
Mary E. Drewnoski

Lincoln, Nebraska

May, 2015
Adequate trace mineral status is important in beef cow/calf and replacement heifer systems because trace minerals play vital roles in reproduction, immunity and growth. Many experiments have investigated the use of a trace mineral injection containing Cu, Mn, Se and Zn (TMI) on growth and immunity of beef cattle. However, very few have investigated TMI use on reproductive performance. Therefore, five experiments, a two year cow/calf experiment, and four replacement heifer experiments were designed to investigate the use of a TMI on reproductive performance of cows and heifers, calf growth and trace mineral status of cows, heifers, and calves. The use of the TMI at pre-calving and pre-breeding did not consistently improve reproductive performance of heifers and cows with good trace mineral statuses that were receiving supplemental trace minerals. There were no differences \( P \geq 0.36 \) in the AI pregnancy rate [48 and 38% control (CON) vs 44 and 39% TMI, year 1 and year 2, respectively] or overall pregnancy rate (93 and 93 % CON vs 93 and 90% TMI, year 1 and year 2, respectively) of cows receiving a TMI when compared to control cows during the two year trial. In experiment 1 beef heifers (Angus×Hereford×Simmental) were bred to fixed time AI, TMI heifers had increased overall pregnancy rates (83 vs 92%, CON vs TMI, respectively; \( P = 0.02 \)). In experiment 2 beef heifers were AI bred to synchronization
response, TMI had no effect (78 vs 83%, CON vs TMI, respectively, \( P = 0.46 \)).

Conception rates to AI and overall pregnancy were not affected by TMI use in two experiments using Black Angus beef heifers being developed in a dry lot fed adequate trace minerals in a total mixed ration. Growth of suckling calves measured as birth weight, average daily gain, actual weaning weight, and 205 d adjusted weight were not influenced by TMI. However, trace mineral status of all groups of cattle were increased with use of TMI. In heifers liver Cu and Se were increased \( (P \leq 0.02) \) 25 d post injection.

The use of a TMI was effective in increasing \( (P \leq 0.05) \) liver concentration of Cu and Se in cows prior to breeding 15-18 days after receiving injections. In calves, the use of the TMI enhanced \( (P \leq 0.05) \) plasma concentrations of Mn, Se and Zn at branding and increased liver concentrations of Cu and Se at weaning. In conclusion these series of experiments demonstrate that use of a TMI can increase trace mineral status of animals. However, influences on performance are dependent on the trace mineral status of the animals and increases in performance are not seen in animals with adequate status.

Key Words: beef cow/calf, beef heifer, conception, growth, injectable trace mineral
ACKNOWLEDGMENTS

I would like to thank my family for supporting me throughout the years in all of the decisions and goals that I have made and achieved. I would like to thank my mom and dad, Mike and Lisa, for instilling in me the value of an education and believing that I could do anything in life that I set my mind to. My dad, Mike, for teaching me the value of hard work and that things do pay off for those who work for it. I would like to thank my mom, Lisa, for showing me how women can become leaders in a primarily male driven industry and that being tough can get you through almost anything. My brother and sister for being a great support system and teaching me to pursue my dreams without hesitation. I want to thank my fiancé Alan. For his help and support as I have gone through this graduate student journey and the endless late nights of editing my papers, presentations and studying for exams.

My time here at the University of Nebraska as well as my time at the University of Idaho has been a life changing experience. I would like to thank Dr. Mary Drewnoski for allowing me the opportunity to attend graduate school; if it weren’t for her guidance none of this would have been possible. Also, thank you for the guidance throughout my master’s program. You have helped me to step out of my comfort zone and grow as an individual. Also, I would like to thank Dr. John Hall for your assistance I have greatly appreciated your mentorship along my journey. Lastly, I would like to thank my fellow graduate students from both the University of Idaho and University of Nebraska. My graduate career experience has been unique and I think the graduate students I have met at both universities have helped me develop into the individual I am today.
# Table of Contents

Chapter I. Introduction ......................................................................................... 1

Chapter II. Review of Literature ........................................................................ 3

  Trace mineral nutrition of cattle ....................................................................... 3

  Role of Cu, Mn, Se and Zn in reproduction, growth and immunity ............... 4

    Copper ........................................................................................................ 4

    Manganese ................................................................................................. 7

    Selenium ..................................................................................................... 9

    Zinc ........................................................................................................... 12

Trace mineral deficiencies of forage based production ................................. 14

  Copper ........................................................................................................ 16

  Manganese ................................................................................................. 18

  Selenium ..................................................................................................... 19

  Zinc ........................................................................................................... 20

Trace mineral status indices ............................................................................. 21

  Copper ........................................................................................................ 21

  Manganese ................................................................................................. 22

  Selenium ..................................................................................................... 22

  Zinc ........................................................................................................... 23

Method of Mineral Supplementation ................................................................. 23

  Free Choice Mineral .................................................................................... 24

  Trace Mineral Boluses .................................................................................. 30

  Trace Mineral Injection ............................................................................... 34

Conclusion ........................................................................................................ 40
Chapter III. Effect of trace mineral injection on performance and trace mineral status of beef cows and calves grazing irrigated pasture

Abstract

Introduction

Materials and Methods

Results and Discussion

Literature Cited

Tables

Chapter IV. Effect of trace mineral injection on pregnancy rate of beef heifers

Abstract

Introduction

Materials and Methods

Results and Discussion

Literature Cited

Tables

Chapter V. Effect of trace mineral injection on pregnancy rate of heifers when synchronized using the 14 day CIDR-PG protocol

Abstract

Introduction

Materials and Methods

Results and Discussion

Literature Cited

Tables
CHAPTER I

Introduction

Historically, cattle producers believed that cattle contained “nutritional wisdom” meaning that they would consume anything such as dirt, bones and wood in order to meet their bodies nutrient requirements (Olson, 2001). Unfortunately for producers, this is not true and cattle cannot intuitively know when they are deficient in nutrients or more specifically trace minerals to meet requirements for optimal performance. And so development of supplemental trace minerals for cattle is common practice for cattle producers. Trace minerals can be supplemented in a multitude of ways such as free choice minerals, indwelling ruminal boluses, oral drenching, added to a total mixed ration and of interest in this paper via an injection. Supplementation is advised in cattle production because of trace minerals roles within the body for synthesis of hormones and enzymes as well as formation of bone and development of the central nervous system. Subclinical deficiencies in trace minerals have been linked to decreased growth, immunity and impaired reproductive performance.

Deficiencies can arise in two ways: primary deficiencies occur when dietary intake of trace minerals do not meet the requirements of the animal or as a secondary deficiency which occurs when intake may meet requirements, but antagonisms reduce the availability of the mineral to the animal. Use of injectable trace minerals is a beneficial way to avoid secondary deficiencies as they avoid antagonisms that occur in the rumen. Injectable trace minerals can also be a suitable way of supplementation to avoid primary
deficiencies because they insure each animal is receiving trace minerals during times of
greater requirement such as during breeding or high stress.

Grazing cattle are typically supplemented trace minerals via a free choice
supplement because it is easy to supplement. The issue with free choice supplementation
is that cattle consume these supplements based on their taste for salt rather than their
body’s nutritional needs. For this reason deficiencies can arise within a herd of cattle,
leaving some cattle deficient in trace minerals that are required in the body. However,
delivery of trace minerals via an injection again ensures that all animals are receiving
trace minerals at times when there requirements may be higher such as at breeding or pre-
calving in cows. Trace mineral injections can be used in addition to free choice
supplementation as they are not stored in the body for extended periods of time as some
may be used immediately or excreted from the body a few days after being injected.
CHAPTER II

Review of Literature

*Trace Mineral Nutrition of Cattle*

For cattle to have optimal performance in growth, reproduction and immunity they require adequate trace mineral status. Adequate trace mineral status meets the needs of the animal with a small reserve of storage to combat and deficiencies caused by antagonists (Puls, 1994). Trace minerals are required by cattle because of their roles in the production of hormones, enzyme activity, synthesis of tissues, energy production and collagen formation (Paterson and Engle, 2005). Clinical and subclinical deficiencies in trace minerals can decrease animal performance because of their requirement for these physiological activities (Paterson and Engle, 2005). Subclinical deficiencies can causes decreased immune response, growth and reproductive performance and are more common than clinical deficiencies (Wikse, 1992). Clinical deficiencies are less common and can be seen in animals depleted of trace minerals demonstrating pathological or visual signs of a deficiency (Puls, 1994).

Trace minerals that are typically supplemented to cattle are Cu, Co, I, Mn, Se and Zn (Paterson and Engle, 2005). This review will focus on Cu, Mn, Se and Zn as those are the trace minerals that were under investigation in this thesis research.

Requirements of some trace minerals change for animals being used for breeding purposes. Specifically, the requirement of Mn differs between growing and reproducing animals as reproducing animals have a higher Mn requirement (NRC, 2000; Table 1).
Also Zn is thought to be required at greater amounts during gestation, but exact requirements have not been defined (NRC, 2000; Table 1).

**Role of Cu, Mn, Se and Zn in reproduction, growth and immunity**

**Copper**

Copper requirements of cattle are based on their needs for growth and reproduction. In the body, Cu plays important roles in the formation of hemoglobin, bone, melanin and keratin (NRC, 2000). Copper is also an essential component of enzymes that have activities in cellular respiration, cross-linking of connective tissue, central nervous system formation, reproduction and immunity (NRC, 2000). Because of Cu’s role in these events, deficiencies of Cu have been linked to decreased performance in growth, reproduction and weak immunity.

In the reproductive cycle, Cu plays an important role in the function of many enzymes. A clinical deficiency in copper can lead to decreased conception rates, infertility, anestrus, and fetal resorption in murine, porcine and bovine (Hostetler et al., 2003). In rats, supplementation of Cu has been found to influence ceruloplasmin and superoxide dismutase activity, which in turn has impacts on fetal development (Hawk et al., 1998; Beguin et al., 1985). Female rats fed diets containing either 1.3 or 11.1 ppm of Cu, on d 11 of gestation, had similar implantation sites and number of fetuses; however by d 13, females consuming 1.3 ppm Cu, had fetuses that were necrotic and reabsorbed (Beguin et al., 1985). Many studies have looked at the effect of feeding high levels of Cu to sows during lactation and have observed an improvement in weaning weights of piglets and piglet survival (Roos & Easter, 1986; Thacker, 1991; Wallace et al., 1966).
When sows were fed high levels of Cu (250 ppm) for an extended period of time, litter size was increased (Cromwell et al., 1993). However, there have been conflicting reports of Cu status and its subsequent effects on reproduction in cattle. Dairy cattle with greater serum Cu values had significantly lower days to first service, services per conception and days to conception compared to cows with low serum Cu (Kappel et al., 1984). In 1993, Abdelrahman and Kincaid (1993) measured the concentration of Cu of bovine fetal tissue at different stages of gestation and discovered that the stage of gestation did not affect Cu concentrations in fetal liver or kidney meaning that increasing supplementation late in gestation will not increase the status of the calf at birth. It was also reported by Gooneratne and Christensen (1989) that bovine fetuses had higher liver Cu concentrations than their dams. This is important because Cu is not adequately transported via milk and the calf will not start consuming forages or supplement which contain Cu until approximately 45 days old meaning that its Cu stores at birth must meet its requirements for 45 days. An increase in fetal liver Cu concentration was also seen in porcine fetuses (Hostetler et al., 2000). These studies show there may be an increased demand of Cu by the fetus. To meet that fetal requirement, the dam may be transferring extra Cu to the embryo and fetus (Hostetler et al., 2003). A common disorder demonstrating the relationship between dam and fetus and Cu deficiency is enzootic neonatal ataxia (swayback) in lambs. This disorder results from pregnant ewes grazing Cu deficient pastures; when lambs from these Cu deficient ewes (liver concentration; 12.4 ± 1.1 mg Cu /kg DM) are born, they are unable to coordinate their legs and some may be born dead (Bennetts and Chapman, 1937). These symptoms are caused by low brain Cu concentrations causing a deficiency in cytochrome oxidase in their motor
neurons and results in aplasia of the myelin surrounding the neurons (Fell et al., 1965; Keen et al., 1998; Mills & Williams, 1962). This demonstrates the importance of Cu supplementation to animals during their reproductive cycle because of its importance in development of the fetus.

There is also evidence that cattle exhibiting a Cu deficiency have decreased growth and a weak immune response. Cattle were fed a diet containing 10 mg/kg of Cu with either 5 mg/kg of Mo or 500 mg/kg of Fe to induce a Cu deficiency (Boyne and Arthur, 1986). Cattle fed the diets with Fe or Mo had depressed ability of neutrophils to ingest and kill C. Albicans (Boyne and Arthur, 1986). Feeding heifers low amounts of Cu (1.54 mg/kg DM) with the antagonist sodium molybdate added to the diet to achieve a Cu:Mo ratio of 1:1.5 for 60 d did not affect the neutrophil bactericidal capacity relative to Cu-adequate control heifers (Arthington et al., 1995). When heifers were fed Cu deficient diets the calves produced were severely Cu deficient prior to weaning and had reduced average daily gain (ADG) (Gengelbach et al., 1994). This decrease in growth caused by a Cu deficiency from excess Mo has also been seen in other studies investigating trace mineral supplementation to growing calves (Humphries et al., 1983; Phillippo et al., 1987). This decrease in growth seen in young cattle could be due to the fact that Cu is required for cross-linking of connective tissue and formation of the central nervous system. When young cattle are Cu deficient it decreases their bodies ability to perform these functions and decreases growth.
Manganese

The Mn requirement of growing and finishing cattle is 20 mg/kg whereas the requirement for breeding cattle is higher at 40 mg/kg (NRC, 2000). This higher requirement for breeding cattle was demonstrated in 1965 by Rojas et al. where cows fed a diet containing 15.8 mg Mn/kg exhibited lower conception rates, requiring four services to conceive compared to control animals that required two services to conceive that were fed 25 mg Mn/kg. In another study performed by Bentley and Phillips in 1951 heifers fed Mn at 10 mg/kg had impaired reproduction seen as a delay in cyclicity and reduced conception rates but exhibited similar growth rates to that of control group that was fed 30 mg Mn/kg.

Manganese’s importance in steroid hormone synthesis may be its most important role in reproduction. It is suggested that Mn plays an important role within the ovary in hormone synthesis where it stimulates cholesterol synthesis which indirectly influences steroid hormone synthesis (Di Costanzo et al., 1986). Manganese role in hormone synthesis is as a cofactor for an enzyme that converts mevalonic acid to squalene, where squalene stimulates the synthesis of cholesterol (Olson, 1965). Estradiol which is secreted from the conceptus as the signal for pregnancy recognition in swine may be affected by Mn because of squalene’s role as a precursor in steroid hormone production (Hostetler, 2003).

It has also been hypothesized that Mn could also play a role in the secretion of progesterone. This is because in ewes, Mn concentrations of the corpus luteum (CL) were increased on d 11, compared to d 4, when progesterone secretion would be the
highest (Hidiroglou & Shearer, 1976). Progesterone plays an important role in early embryonic life and sub-optimal concentrations of progesterone can lead to early embryonic loss (Wilmut et al., 1986; Wright et al., 1982).

It was also proposed by Hignett (1950) that adequate levels of Mn are required for high conception rates and calcium and phosphorus can negatively affect Mn concentrations. This was seen in dairy cows that were fed low levels of Mn (< 40 ppm) with either adequate or antagonistic levels of Ca and Phosphorus, those cattle receiving high calcium and phosphorus, which are Mn antagonists, had lower fertility (King, 1971).

It is hypothesized that Mn may play a significant role in the activity of certain endocrine organs. It has been observed that the pituitary and ovary are relatively rich in manganese (5.9 and 5.2 ug/g) (Hostetler et al., 2003). Ovarian follicles and CL of ewes absorbed a larger amount of radioactively labeled manganese than ovarian or extraovarian reproductive tissues, suggesting the accumulation of Mn within the CL and follicles for the synthesis of hormones (Hidiroglou et al., 1976). These studies demonstrate the role and importance of adequate Mn nutrition in cattle during their reproductive cycle.

It is also hypothesized that Mn may play an important role in immune function because of its role in removing superoxide radicals produced by active immune cells (Tomlinson et al., 2008). This is seen in the activity of superoxide dismutase (SOD) a Mn dependent enzyme whose activity is to protect mitochondrial membranes from attack by free radicals, however evidence of Mn supplementation improving the bovine immune systems response to an immune challenge has been limited (Tomlinson et al., 2008).
Manganese supplementation effect on cattle growth has not shown significant results. In a study designed to test dietary levels of Mn on heifer growth and reproductive performance, supplemental levels (0, 10, 30, 50 mg/ Mn kg DM) added to a diet containing 15.8 mg of Mn/kg of diet DM did not affect ADG, dry matter intake or feed efficiency of heifers (Hansen et al., 2006). It is hypothesized by the author that the dietary level 15.8 mg of Mn/kg of DM was sufficient to meet the heifer’s needs for growth and could explain why responses were not observed (Hansen et al., 2006). Heifers supplemented the additional 50 mg Mn/kg DM saw numerical increases in the number of heifers conceiving to AI in response to estrus, however there was no statistical significance observed (Hansen et al., 2006). It is thought that statistical significance was not seen because of the low number of heifers (n =20) used in each treatment group. No effect on growth has also been seen in other trials investigating dietary levels of Mn (Legleiter et al., 2005; Bentley and Phillips, 1951). From these studies it is concluded that Mn plays important roles in the body and has been shown to be required at greater levels for cattle during reproduction.

**Selenium**

Selenium is an important trace mineral in livestock. Selenium is required for function of many enzymes and proteins specifically glutathione peroxidase, deiodinases, and thioredoxin reductases (Allan et al., 1999). These enzymes play an important role in cell integrity because of their involvement in the catabolism of peroxides that are synthesized during lipid oxidation (Burk, 1991). Because of selenium’s role in the
function of these enzymes it is hypothesized that Se plays an important role in immunity and reproduction. A clinical deficiency in Se can lead to many reproductive disorders such as retained placentas, infertility, cystic ovaries, metritis, delayed conception, and erratic, weak or silent heat periods leading to poor fertilization (Corah and Ives, 1991).

During early fetal growth, Se functions as a reductase to help protect against mortality from oxidative damage (Hostetler, 2003). When injected with supplemental Se (15 mg Se/kg as selenate) and vitamin E (680 international units; IU), beef cows produced more fertilized ova when super ovulated than control animals that were not supplemented (Kappel et al., 1984). However, when cattle were Se adequate (210 – 1200 ng Se/mL in blood) and given supplemental Se (50 mg of Se as sodium selenite) and Vitamin E (680 IU) via an injection there was no enhancement in reproductive performance (Kappel, 1984 and Hidiroglou, 1987). Historically, the relationship of Se and Vit E was based on the assumption that deficiencies in Se can be solved by supplementation of Vit E (Corah and Ives, 1991). Selenium acts through the activity of glutathione peroxidases in the cytosol to reduce hydrogen peroxide and other organic peroxides into less damaging products (Corah and Ives, 1991). This is similar to the role of Vit E as it works in the cellular membrane to reduce peroxides into less harmful products (Underwood, 1981). As both Se and Vit E function as antioxidants, it would lead to the conclusion that Se adequate herds may not have improved reproductive performance, but Se deficient herd’s supplementation with Se, Vit E or both may improve reproduction.

An Australian study investigated the effects of supplementation of Se to ewes grazing Se deficient (0.05±0.005 mg Se/kg DM) pasture (Godwin et al., 1970). Four to
eight weeks before the breeding season Se was supplemented via an oral dose of sodium selenite, or from an intraruminal selenium pellet containing elemental selenium and iron (Godwin et al., 1970). Ewes that received Se supplementation had improved conception rates (76%) compared to those that did not receive Se supplementation (49%) (Godwin et al., 1970). However, in other studies there was no effect of supplementing Se to ewes grazing Se deficient pastures on conception rates (Maxwell, 1972; Gardiner et al., 1962; Davies, 1966).

Selenium supplementation of Se deficient dairy cows has shown promising results in increased reproductive performance. Cows that were Se deficient (< 25 ug Se/L in plasma) were either injected intramuscularly with 50 mg of Se as selenite 21 d pre-partum or fed 0.92 mg/kg of Se as selenite in a ration 60 d pre-partum, both forms of supplementation were successful in reducing incidences of retained placentas from 38% to 0% (Julien and Conrad, 1976). These studies demonstrate the importance of adequate Se supplementation to cattle to ensure success in reproduction.

Selenium supplementation to ensure adequate status improves growth and immune function of cattle. Selenium’s role in immunity centers on its activity in glutathione peroxidase which inactivates hydrogen peroxide preventing it from causing cellular damage (Tomlinson et al., 2008). Selenium deficient calves [9.82 u/g whole blood glutathione peroxidase activity (Hb GSH-Px)] produced lower anti-IBRV titers when challenged with infectious bovine rhinotracheitis virus than Se adequate calves (100 u/g Hb GSH-Px) (Reffett et al., 1988). Growth response to Se supplementation was reported in beef calves. Calves receiving an injection containing Vit E and Se (30 mg Se as sodium selenite and 408 IU Vit E) at birth and nursing dams that had received the
same injection 120-150 d pre partum had slightly increased adjusted weaning weights than control calves nursing control dams, cows in both treatment groups had adequate GSH-Px activity throughout the study (> 19 u/g Hb) (Spears et al., 1986). In a three year producer study cows were not supplemented Se in the first year and observed high morbidity and low weaning weights in the calf crop (Koller et al., 1983). The following two years cows were supplemented 90 mg Se/kg via a salt, mineral mix, and the calves of these cows had significantly increased weaning weights and indications of decreased incidence of infectious diseases (Koller et al., 1983). These studies prove the importance of Se supplementation to meet the requirements of cattle to insure performance in growth, reproduction and immunity.

**Zinc**

Zinc is another essential trace mineral in beef cattle production and should be supplemented because of its role in the function of over 300 enzyme systems (Cousins and King, 2004). These enzymes play roles in the metabolism of carbohydrates and energy, protein synthesis, nucleic acid metabolism, epithelial tissue integrity, and the division and repair of cells (Cousins and King, 2004). Specific requirements of Zn during reproduction are not defined, but it is hypothesized that its requirements are higher during this time than any other in a cow’s life (NRC, 1996).

Zinc deficient cows appear to exhibit abnormal estrus and a decrease in fertility, but all phases of the reproductive process may be affected (Underwood, 1981). Adequate Zn is important during early pregnancy because of its importance in the configuration of RNA and DNA (Chesters, 1978), mostly in the formation of zinc fingers which are
essential for binding a steroid receptor complex to DNA (Freedman, 1992). These steroid receptors turn on genes that are active in protein synthesis which is important in early pregnancy (Hostetler, 2003).

Zinc influences pregnancy through insulin-like growth factors (IGFs) (Hostetler, 2003). During early pregnancy IGFs exist at high levels within the uterus of several livestock species including cattle (Geisert et al., 1991; Ko et al., 1991; Simmen et al., 1992). These IGFs are powerful stimulators of tissue differentiation and cell proliferation (Zapf and Froesch, 1986). Specifically during this time of uterine renewal growth factors are active in fetal development (IGF-II), embryonic implantation (IGF-I) and conceptus growth (Hostetler, 2003). During a Zn deficiency IGF-I levels are decreased, therefore lowering their activity in conceptus development (MacDonald, 2000). These results show that the presence of Zn at adequate levels within the animal has an impact on the animal’s reproductive performance specifically during early pregnancy and the maintenance of pregnancy.

The enzymes that require Zn also play important roles in growth and immune function. In regards to innate immunity, Zn has critical roles in maintenance and integrity of skin because of its role in cellular repair which can help with wound healing (Moynahan, 1981). Similar to Cu and Mn, Zn is required for the activity of SOD which helps to stabilize the cellular membrane against oxidative damage caused by superoxide radicals (Moynahan, 1981). Zinc is also required for the production of antibodies (Tomlinson et al., 2008). Increasing the level of supplemental Zn from 30 to 100 mg/kg diet slightly reduced morbidity from respiratory diseases in newly weaned calves after transportation (Galyean et al., 1995). In terms of growth the roles of Zn in protein
synthesis and carbohydrate and energy metabolism come into play. When calves that were known to be Zn deficient, determined by calves having decreased concentrations of serum alkaline phosphatase (2.03 vs 4.48 u/mL, deficient diet vs adequate diet, respectively), or Zn adequate were fed at the same intake, Zn deficient calves grew at a slower rate than adequate calves (Miller et al., 1965). This decrease in growth could probably be explained by decreased efficiency of deficient animals.

The trace minerals discussed here are commonly present in forage consumed by cattle. However, forages commonly do not provide adequate amounts of Cu, Se and Zn to meet requirements of beef cattle.

**Trace Mineral Deficiencies of Forage Based Production**

Trace minerals that are commonly supplemented to beef cattle are Cu, Se and Zn, as forage produced in the US are commonly deficient in these trace minerals (Mortimer et al., Table 2 and 3). However, throughout the US Mn is typically adequate in forages (Table 2 and 3). More specifically, in the Northwest United States, Cu and Zn were seen to be deficient in forages year round, while Mn was seasonally deficient and differed by year (Ganskopp and Bohnert, 2003). Selenium concentration of forages in the Northwest US is extremely variable with deficiencies and toxicities seen in the same region (Carter, 1968). Many factors affect the concentration of trace minerals in forage. Factors that can affect the trace mineral concentrations within forage are climatic conditions, stage of plant growth, plant species, soil characteristics and in some cases application of fertilizers (Greene, 2000).
Climatic conditions control the type of forage grown, as well as the growing season. Environmental aspects, such as rainfall and temperature, will affect plant growth and in turn affect the capability of a plant to absorb minerals available in the soil into plant tissue, causing a variation in the plants mineral quantity (Greene, 2000). In early plant growth, uptake of minerals from the soil is quick, but mineral content will decline as the plant matures because minerals are concentrated in the roots and become diluted throughout the plant (Greene, 2000). Further, as the plant grows the production of dry matter material takes priority over mineral uptake creating a decline in mineral concentration (Underwood, 1981; McDowell and Valle, 2000). As plants mature there is a decrease in the accumulation of the trace minerals Cu, Zn, Fe, Co, and Mo (Underwood, 1981). Mineral concentrations between species of plants differ greatly and do not always reflect the soils concentration of mineral, especially in range and pasture plants. When plants grow in soil with inadequate mineral concentrations, the plant may experience a decrease in growth as a result of the deficiency or may reduce the concentration of the deficient mineral within the plant’s material (Greene, 2000). The foliar application of some trace minerals can increase trace mineral consumption of cattle (McDowell, 1996). But, the application of mineral fertilizer should only be used to increase production of the plant; fertilization to increase animal consumption is costly and can be achieved by other methods that will be described later is this chapter (Olson, 2007).

In 1993 (Corah and Dargatz, 1996) and 1997 (Mortimer et al., 1999), cow/calf producers from the US were selected to participate in a survey of management procedures and animal health. To be selected, producers must have at least 5 beef cows
or heifers with 50% of their calf crop being born between January and June of 1992 (Corah and Dargatz, 1996) or 1996 (Mortimer et al., 1999). Producers that participated in the USDA’s National Animal Health Monitoring System (NAHMS) Cow/Calf Health and Productivity Audit (CHAPA) were given the opportunity to have a forage sample collected and analyzed. In 1993, samples were collected from 18 states and, in 1997, samples were collected from 23 states for a total of 352 samples submitted in 1993 and 709 samples in 1997. For analysis purposes, forages were categorized into 10 categories in 1993 and 11 categories in 1997. In 1993 and 1997 the categories were alfalfa/alfalfa mix (n = 69 and 196), brome / brome mix (n = 8 and 20), bermuda/ bermuda mix (n = 37 and 112), fescue/fescue mix (n = 22 and 73), sudan/sudan × sorghum (n = 27 and 61), cereal forages (n = 17 and 46), native (n = 30 and 38), grass (n = 109 and 70), silage/silage mix (n = 9 and 31), and “other” which included samples that did not fit into these categories (n = 26 and 28) (1993 and 1997, respectively). In 1997, the category orchard grass (n = 15) was added. Samples were classified by their trace mineral concentrations for Cu, Mn, Se and Zn as adequate, marginally deficient, or deficient (Table 2). In 1997, the ranges of marginal and adequate status were changed slightly for Cu, Se, and Zn, as well as antagonistic levels of minerals were redefined (Table 2).

Copper

Cattle require 10 mg/kg DM of Cu to meet their requirement for growth and reproduction (NRC, 2000). However, forage concentrations of Cu are most often marginal, out of 709 forage samples collected from 23 states 66% of those were determined as having a marginal (4 – 10 mg/kg DM) Cu status and 33.3% determined as adequate (> 10 mg/kg DM) in Cu (Mortimer et al., 1999). Due to its antagonistic
relationship with Mo, copper is often recorded as a ratio with Mo (Corah and Dargatz, 1996). An adequate Cu:Mo ratio is 4.5-5:1, as Mo can bind Cu, making it unavailable to the animal (Corah and Dargatz, 1996). A primary deficiency occurs when dietary levels are below the animal’s requirement (Wikse, 1992). When Mo concentrations in the diet are high, a secondary Cu deficiency may occur, due to this antagonism (Corah, 1996). A secondary deficiency occurs when there are high levels of an antagonistic mineral, making the mineral unavailable to the animal and creating a deficiency, even when dietary intake may be adequate (Wikse, 1992).

Iron and sulfur are also antagonistic minerals to Cu (Spears, 2003). Though Cu may be of adequate or marginal concentration in the diet, ruminal interactions of Cu with S, Mo, and Fe may cause a Cu deficiency as the interactions between these minerals create thiomolybdates, which are inabsorbable by the animal (Spears, 2003). Antagonistic levels of both Fe and Mo were observed in forages in the US. Antagonistic levels of Fe were determined in 28.7% of forages as high (> 400 mg/kg DM) or marginally high (> 200 – 400 mg/kg DM) and 57.8% were marginally high (1 -3 mg/kg DM) or high (> 3 mg/kg DM) in Mo (Corah and Dargatz, 1996, Table 3). This would indicate that both Fe and Mo are present at levels that could cause a reduction in Cu availability in cattle consuming forages produced in the US (Corah et al., 1996, Table 3). The same can be said for S, where 46.4% of 709 samples were determined to be high (> 0.30 % DM) or marginally high (> 0.20 – 0.30 % DM) (Mortimer et al., 1999). This demonstrates that although forage in the US is typically marginal or adequate in Cu, the presence of Fe, Mo, and S at antagonistic levels may be of concern and so supplementation of Cu is recommended.
Research has also looked into the differences primary and secondary deficiencies may have on reproductive performance of cattle. Conception rates, attainment of puberty and ovulation rates were measured in heifers consuming diets deficient in Cu (4 mg Cu/kg) with the antagonist Mo (5 mg Mo/kg) and Fe (800 mg Fe/kg) or both Mo and Fe during a 32 week study starting when heifers were 5 months old until they were bred (Phillippo et al., 1987). Results showed that heifers consuming the marginal Cu diet + antagonistic levels of Mo had reduced liver Cu concentrations (112 mg Cu/kg DM) and exhibited delayed puberty, lower rates of ovulation, and lower conception rates, while heifers consuming the Cu deficient diet without antagonists did not show this decrease in performance and had higher liver Cu concentrations (380 mg Cu/kg DM) (Phillippo et al., 1987). It is hypothesized by Wikse (1992) that it is not necessarily the secondary Cu deficiency, but possibly the formation of thiomolybdates reducing performance. Supplementation of Cu should be performed with the consideration of Cu antagonists and their effects on overall performance.

Manganese

The trace mineral of least concern in terms of forage concentration of those I am discussing is Mn. Beef cattle requirements for Mn are 20 mg/kg DM for growing and finishing cattle, but are increased to 40 mg/kg DM for breeding cattle (NRC, 1996). In the 1996 and 1999 studies, 76% and 85% of the forage samples were of adequate (> 40 mg/kg DM) Mn concentration (Corah et al., 1996; Mortimer et al., 1999; Table 3). More specifically, in the North West United States, Mn concentration was determined as adequate at 38.6 ± 1.3 mg/kg in seven grass species (Ganskopp & Bohnert, 2003).
Manganese bioavailability can be impacted by high levels of Fe (> 400 mg/kg DM) fed to cattle (Hansen et al., 2010). Iron and Mn are transported by divalent metal transporter 1 (DMT1) (Garrick et al., 2006). When there were high levels of Fe supplemented (750 mg Fe/kg DM) Fe concentrations in the duodenum were not affected, but DMT1 protein levels decrease, in turn decreasing the transportation of Mn for utilization by the animal (Hansen et al., 2010). Hansen et al. (2010) suggest that this is caused by the competition between Fe and Mn for transport via DMT1.

*Selenium*

Concentrations of selenium in forages are extremely variable among different locations within the US. In Mortimer et al. (1999), Se was deficient (< 0.1 mg/kg DM) in 43% and reached the maximum tolerable level (2.0 mg/kg DM) in 0.3% of samples. The maximum tolerable level is defined as “the dietary level, when fed for a limited period of time, that will not impair animal performance and should not produce unsafe residues in human food derived from the animal” (Mortimer et al., 1999). This study suggests that Se concentrations of forage are extremely variable in the US. Even within a region, forage concentrations can differ drastically. When Se concentrations of 361 forage samples taken in the Pacific Northwest were analyzed, two-thirds contained less than 0.10 mg Se/kg DM, which is the level determined by the NRC (1996) for requirement of Se in beef cattle demonstrating supplementation should be provided (Carter et al., 1968).

Spears (2003) suggest that there may be antagonistic interactions between Se and sulfur. It is thought that because Se and sulfur have similar physical and chemical properties that they likely interact competitively for the same transporters (Gutzwiller, 1993). In sheep, concentrations of Se in the liver were reduced when dietary sulfur was
increased from 2.2 to 4 g/kg of the diet (Gutzwiller, 1993). Also Se bioavailability can be impacted by cyanogenetic glycosides which are antagonists of Se found in certain legumes and can be metabolized to cyanide in the rumen (Spears, 2003). Ewes fed white clover high in cyanogenetic glycosides had lower Se status than ewes fed white clover with low concentrations of cyanogenetic glycosides (Spears, 2003).

**Zinc**

Zinc is likely to be deficient in forage based cattle diets (Corah and Dargatz, 1996 and Mortimer et al., 1999). Cattle require 30 mg/kg of Zn for optimum performance in growth and reproduction (NRC, 2000). In forage samples from cow/calf producers analyzed for Zn, 63% of those were found to have less than 20 mg Zn/kg DM (Corah and Dargartz, 1996, Table 3). In another study of forage samples from the US, 33% of samples had less than 20 mg Zn/kg DM and 44% were marginal (20 – 30 mg/kg DM) (Mortimer et al., 1999). In the Northwest the mean Zn concentration of 7 species of grass was 12.1 ppm, which is less than half of that required by beef cattle (30 ppm) as determined by the NRC (2000). Similar to Mn and Fe, Zn is also transported by DMT1 (Garrick et al., 2006). At high dietary levels of Fe, DMT1 concentrations are decreased and transport of Zn is reduced, reducing Zn bioavailability to the animal (Patterson et al., 1999).

As trace mineral concentrations of forages can be erratic, supplementation can increase the likelihood that cattle are receiving trace mineral concentrations to meet their requirements. The formation of trace mineral supplements to meet the needs of beef cattle can be difficult to achieve due to the interaction between specific minerals within
the rumen and intestine and the limited knowledge of minerals provided through the forage. Even when cattle are being provided supplemental trace minerals, supplementation may not always be adequate to overcome mineral antagonists present in the forage and/or diet, therefore, assessment of trace mineral status is needed.

**Trace Mineral Status Indices**

Adequacy is defined by Kincaid (1994) as “levels that are sufficient for optimum functioning of all body mechanisms with a small margin of reserve to counteract commonly encountered antagonistic conditions.”

**Copper**

Adequate ranges of Cu in bovine blood are 0.7 to 0.9 mg/L for plasma and 0.32 - 1.20 mg/L for serum (Kincaid, 2000 and Puls, 1994). However, serum Cu can be influenced by viral and bacterial infections as well as stress, which will stimulate the liver to release high amounts of Cu, changing serum Cu concentration; therefore, plasma and serum concentrations are poor indicators of Cu status (Puls, 1994). Signifying that elevated serum and plasma Cu concentrations could indicate stress when coupled with a low liver Cu concentration. Concentrations of Cu within the liver are a reflection of the bioavailable Cu within a ruminant’s diet (McDowell, 1996), and liver concentrations of Cu can be affected by the needs of the animal, such as whether they are in a growing vs gestating/lactating phase of production (Kincaid, 2000). It is thought that in late gestation cows have higher requirements for Cu as Cu will be transferred to the fetus, depleting the dam’s stores, though exact requirements are not yet defined (Kincaid,
Kincaid suggests that 125 mg/kg DM is the concentration in the liver in which cattle have an adequate Cu status.

**Manganese**

Adequacy of Mn in serum is between 6 to 70 ug/L (Kincaid, 2000). However, this is not a good indication of Mn status as the liver removes Mn from blood to maintain homeostasis, where it is then excreted via the bile (Kincaid, 2000). Adequate concentrations of Mn in liver are greater than 13 mg/kg DM (Kincaid, 2000). Liver Mn is also not a sufficient way to represent the body’s Mn status as storage of Mn in the liver is not a good reflection of dietary intake because the majority of Mn is excreted via bile on its first pass through the liver (Kincaid, 2000). This was reported by Underwood and Suttle (1999), where only a fourfold increase in liver Mn was observed when dietary Mn was increased 130- to 140- fold (Ivan and Hidiroglou, 1980; Watson et al., 1973). The only proven way to demonstrate dietary intake of Mn stored in the body of cattle is by activity of the Mn dependent enzyme superoxide dismutase (SOD), as it has been significantly correlated to Mn dietary intake (Masters et al., 1988).

**Selenium**

The level of adequacy as determined by Kincaid (2000) for bovine whole blood Se is 210 to 1200 ug/L. It is stated that whole blood Se concentration is approximately three times as high as that of serum; for this reason, whole blood is a better marker for Se determination. Any hemolysis of erythrocytes can cause serum to have falsely high level of Se (Kincaid, 2000). Adequacy of Se, determined by Puls (1994), is 80 to 300 ug/L of serum or plasma. Adequate liver status of mature cattle for Se is 1.25 to 2.5 mg/kg DM,
determined by Kincaid (2000). Between 4-9% of Se found in the body is in the liver, with the liver tending to have the highest Se concentrations of tissues, making it the most common measure of Se status (Kincaid, 2000). However, whole blood Se concentrations are highly responsive to Se intake (Levander, 1986). It can be stated that whole blood Se and liver Se would both be adequate measures of Se status of bovine.

**Zinc**

The level of adequacy for Zn in plasma and serum is 0.8 to 1.4 mg/L (Kincaid, 2000 and Puls, 1994). Liver Zn concentrations must fall within 40 to 200 mg/kg DM to be considered adequate (Kincaid, 2000). However, it is difficult to get a true measure of Zn status due to its role in over 300 metalloenzymes (McCall et al., 2000). Because of this storage of zinc in the liver is inconsistent as it is stored in labile pools before being stored in the liver.

**Method of Mineral Supplementation**

In cattle being fed in confinement or a dry lot, supplementation of trace mineral through a total mixed ration can be effective (Olson, 2007). However, total mixed rations are not typically fed to beef cows and calves, as these animals are typically grazing forages, so formulated rations are not provided. Mineral supplementation can be achieved in forage-based beef production systems in a variety of ways, some of which are free choice supplementation, ruminal boluses, and by injection.

**Free Choice Mineral**
The most common form of mineral supplementation to cattle in a grazing system is free choice mineral; these are salt based products that are designed for self-feeding. However, with free choice mineral, cattle do not regulate their mineral consumption based on their body's needs for minerals, but rather on their taste for salt (Mundell et al., 2012). Therefore, supplementation via free choice minerals can lead to deficiencies or toxicities within a herd, as some cattle may not consume an adequate amount of mineral and some may overconsume (McDowell, 1996).

Source of the mineral in free-choice mineral plays a large role in their bioavailability. The bioavailability of essential trace minerals was defined by Levander in (1983) as “A quantitative measure of the utilization of a nutrient in a food, meal, or diet under specific conditions to support normal structural and physiological processes occurring within the body”. There are many interactions within the digestive tract that can affect absorption, availability of a trace mineral, and utilization of essential trace minerals, as well as the method by which supplementation is provided can affect how it is utilized by the animal (Spears, 1996).

Historically, trace mineral supplements have been provided to livestock as inorganic salts, sulfates, oxides, and chlorides (Paterson et al., 1999). However, not all of these sources are created equally in terms of their bioavailability to the animal (Table 4). As trace minerals are consumed by cattle, they encounter biochemical reactions within the rumen decreasing their availability to be utilized by the animal (Spears, 1996). Some of the least bioavailable sources are inorganic salts and oxide sources because of their high reactivity in the rumen (Spears, 1996). In contrast, organic and chelated trace minerals are able to bypass the rumen with little degradation and there for can be utilized
by the animal at higher concentrations (Paterson et al., 1999). Chelated trace minerals are stable in the digestive tract because they are protected from forming complexes with other dietary components that inhibit absorption and in turn allow for greater absorption by the animal (Spears, 1996). In some cases ruminants supplemented organic or chelated trace minerals have seen improvements in growth, reproduction and health (Spears, 1996). Trace minerals are present in the body as organic complexes or chelates. When they are absorbed in this form, they are more available to be used by the animal (Spears, 1996). For the animal to use inorganic trace minerals, they must first convert them to their organic complexes, hence why they are less bioavailable to the animal (Spears, 1996). Also of note, is that naturally occurring trace minerals in feed exist primarily in organic or chelated forms which are more available to the animal (Spears, 1996). There have been multiple studies (Ahola et al., 2004; Stanton et al., 2000) looking at the differences in performance of cattle being supplemented with an organic trace mineral supplement vs. those being supplemented an inorganic supplement. These studies reported an increase in performance, seen as an increase in body weight (BW), artificial insemination (AI) pregnancy or overall pregnancy, believed to be due to increased bioavailability of organic trace mineral sources over that of inorganic sources; however these results have not been seen in all trials investigating organic vs inorganic trace minerals.

Stanton et al. (2000) conducted a 209 d trial using beef cows (n = 100) supplemented trace minerals via free choice supplement with high amounts of organic trace minerals (11 mg Cu/kg, 31 mg Zn/kg, 18 mg Mn/kg, and 1.1 mg Co/kg) high amounts of inorganic trace minerals (n = 100; 11 mg Cu sulfate/kg, 31 mg Zn sulfate/kg,
18 mg Manganous oxide/kg, 1.1 mg Co carbonate/kg) or low amounts of inorganic trace minerals (n = 99; 5 mg Cu sulfate/kg, 22 mg Zn sulfate/kg, 12 mg Manganous oxide/kg, 0.11 mg Co carbonate/kg). Cows were provided these supplements from March 12, 1997 (start of calving season) to September 29, 1997.

Cows receiving high levels of organic or inorganic trace minerals liver Cu was greater 60 d after the start of supplementation than those animals receiving low levels of inorganic trace minerals, but all animals were still in the deficient range (25 vs 11 mg/kg DM; high vs low, respectively). Liver trace mineral status was also sampled from calves. Calves nursing cows fed the inorganic low-level treatment (n = 12; 75 mg Cu/kg DM) demonstrated greater liver Cu concentrations on May 13, at the end of calving season, when compared to calves from cows fed organic high-levels of trace minerals (n = 15; 33 mg Cu/kg DM). Stanton et al. (2000) hypothesize that this difference may be a result of measurably lower trace mineral consumption of the organic high-level treatment group through calving (March 12- May 13). From the end of calving season (May 13) to the end of the trial (September 29) calf liver Cu concentrations decreased significantly, but did not differ between treatment groups at the end of the trial (Stanton et al., 2000). There were no other differences in trace mineral concentrations measured and cows were adequate in Zn (117 mg Zn/kg DM) and marginally deficient in Mn (8 mg Mn/kg DM) regardless of treatment.

Cows consuming the high levels of organic TM had greater pregnancy to AI (75/100; 75%) than the other two groups (Inorganic high level, 56/100; 56% and Inorganic low level, 60/99; 61%). However, this did not carry over to overall pregnancy (88, 81, 88% pregnant; inorganic low, inorganic high, organic high, respectively).
Stanton demonstrated in this study that when cows are supplemented organic trace minerals, it can increase calves ADG and cow’s pregnancy to AI. This study has demonstrated the importance of trace mineral supplementation specifically of Cu where organic supplementation increased liver concentrations of Cu and subsequent performance.

At the Eastern Colorado Research Center in Akron, CO, Ahola et al. (2004) designed a study to determine the effect that source and supplementation of Cu, Zn, and Mn have on trace mineral status, reproductive performance and growth of grazing beef cattle. Cows were stratified by age, expected calving date, BW, BCS, and initial liver mineral status and randomly assigned to one of three treatments. A control group received no supplemental Cu, Zn or Mn; the organic supplemented group was supplemented with 50% organic and 50% inorganic Cu, Zn, and Mn; and the inorganic supplemented group was supplemented with 100% inorganic Cu, Zn and Mn. Inorganic trace minerals were supplemented as CuSO$_4$, ZnSO$_4$, and MnSO$_4$ whereas organic trace minerals were provided from a commercially available mineral proteinate source. Mineral treatments were offered as a free choice mineral 81-82 d before the start of the calving season to 110 (Year 1) and 135 (Year 2) d after the average calving date. During the 160 d between discontinuation of treatment post-calving to 81 d pre-calving, all cows were offered the control mineral supplement, which contained no supplemental Cu, Zn or Mn. Calves were offered the same trace mineral treatment as their dam with access via creep feeders starting at 90 (Year 1) and 99 d (Year 2) of age and continued their treatments until weaning at 185 (Year 1) and 164 (Year 2) d of age. Based on mineral disappearance, it was indicated that organic and inorganic supplemented cattle were
consuming enough Cu, Mn and Zn to meet the NRC recommendations from the free choice supplement.

Liver trace mineral concentrations for Cu and Mn were affected by supplementation and year. Liver Cu concentrations were greater in supplemented vs. control cows in both years, at the end of year two, control cows were deficient and had the lowest liver Cu concentration (43.7 mg/kg DM). Concentrations of liver Cu were not different between the supplemented groups and they were both adequate in liver Cu [Inorganic supplemented cows (141.8 mg/kg DM) and organic supplemented cows (156.1 mg/kg DM)].

Liver Mn concentrations as a result of trace mineral supplementation were less clear. Liver Mn concentrations were greater ($P < 0.01$) at the end of year 1 in supplemented cows (8.9 and 9.2 mg/kg DM, organic and inorganic, respectively) vs. control (8.0 mg/kg DM). At the end of year 2 the opposite was observed with control cows having a higher liver Mn concentration (8.9, 8.8, and 9.5 mg/kg DM, organic, inorganic, and control; respectively). Generally in ruminants, concentration of Mn in the liver concentration does not respond to Mn supplementation and at all-time points measured the cows were marginally deficient in Mn.

Liver Zn concentration was also effected by supplementation, with supplemented cows (106.3 and 105.3 mg/kg DM, organic and inorganic; respectively) having higher ($P < 0.05$) liver Zn at the end of year 1 than control cows (89.1 mg/kg DM), however in year 2 liver Zn concentrations did not differ ($P > 0.56$) between supplemented (85.0 and 93.9
mg Zn/kg DM, organic and inorganic; respectively) and control cows (91.5 mg Zn/kg DM) and at all-time points cows had adequate concentrations of liver Zn.

Throughout the two-year experiment, mean BW and BCS were not affected by trace mineral supplementation. Supplementation of trace minerals also did not affect the rate of estrous cyclicity, which was determined by blood samples taken 10 d apart. Cows were determined as cycling if at least one blood sample had a serum progesterone concentration greater than 1.0 ng/mL, indicating the presence of a functional corpus luteum. In the first year of the study, pregnancy to AI was not different between control (34/52, 65%) and supplemented cows but cows receiving organic supplementation of trace minerals tended ($P < 0.08$) to have a higher pregnancy rate to AI (36/54, 67%) than inorganic supplemented cows (29/56, 52%). However, in year two of the study, supplementation of trace minerals increased ($P < 0.02$) pregnancy rate to AI in supplemented cows (26/46, 57% and 25/43, 58%; organic and inorganic, respectively) over the control group (15/44, 34%). This increase in AI pregnancy rate of supplemented cows in year two, but not year one, could have been a result of control cows not being supplemented Cu, Zn, and Mn for over one year; therefore, depleting their body’s storage of these minerals. It was also reported that overall pregnancy rate after a 60 d breeding season in both years tended ($P < 0.10$) to be increased by free choice supplementation of trace minerals from organic or inorganic sources [(85/96) 89%, (94/101) 93%, and (98/103) 95%; control, organic and inorganic; respectively].

Calves in all groups consumed more free choice mineral from creep feeders in year two than in year one. Interestingly, in both year one and two, there was more ($P <
0.02) kilograms of calf weaned per cow exposed in the control treatment (200.3 kg) than in the supplemented treatments (184.7 and 198.8 kg, organic and inorganic; respectively).

Use of organic or chelated trace minerals should be considered when antagonists are present in the forage or in cases where forages are known to be deficient in essential trace minerals. The bioavailability of these trace minerals are greater than the inorganic sources and so performance can be increased in some cases where status of the animals are low. However, the most appropriate way to supplement these more available sources is in addition to cheaper sulfate sources which also have high bioavailability but the combination of both can decrease the cost but also provide good trace mineral supplementation.

Trace Mineral Boluses

In situations where trace mineral supplementation via a free choice mineral is problematic due to terrain or antagonists in the forage, long acting reticulorumen trace mineral boluses have been utilized to supplement trace minerals to cattle (Sprinkle et al., 2006). Boluses are beneficial because they provide trace minerals over an extended period of time and ensure each animal receives supplementation. A study to determine the effectiveness of these boluses was performed in central New Mexico where there is a reported deficiency in Se (Kubota et al., 1967) as well as Cu during some time periods (Sprinkle et al., 2006). Cows were blocked by breed type, age, and weight, and randomly assigned to receive a bolus or no treatment (control); cows remained in their treatment group for the three year study. Bolus cows received boluses in January of each year;
bolus cows were orally dosed with two 100-g Cosecure boluses consisting of 0.30% (wt/wt) Se as sodium selenate, 13.4% (wt/wt) Cu, and 0.5% (wt/wt) Co. Boluses dissolve over the course of 175 d (6 mo) releasing 156 mg of Cu, 5.9 mg of Co, and 3.4 mg of Se per day (validated by ruminally fistulated cattle). Cows remained in the same treatment without any trace mineral supplements over the three-year period with the exception of free-choice access to white, iodized salt blocks. Most cows (67%) calved by May 1 in all years with the breeding season starting by mid-May in the first two years and late June in the last year. Liver biopsy, BW and BCS were collected in January and May of each year, with the exception of the first year of the trial where liver biopsy were not taken in January.

Concentration of Cu in the forage varied by year. In year one Cu (9.2 ± 0.46 mg/kg DM) concentration in the forage was close to meeting the dietary requirement of cows, year two the forage was very low (3.9 ± 0.46 mg/kg DM) and in year three Cu was low (4.9 ± 0.45 mg/kg DM). Selenium and Zn concentrations of the forage were deficient (either marginally or severely) at all times during the study.

Liver Cu concentration was deficient (<125 mg/kg DM) in control cows and adequate (> 125 mg/kg DM) in bolused cows over the three year study. Interestingly, even though Zn concentrations of forage were deficient at all times during the three year study, concentrations of liver Zn never fell below adequacy (40 mg Zn/kg DM). There were also no observed differences in Zn liver concentrations between control and bolused cows.
Calves of biopsy cows were sampled in May (birth) and September (184 DOA) for serum Cu and Zn. These measurements demonstrated no difference in serum Cu (0.67 ± 0.019 ug/mL vs 0.65 ± 0.019 ug/mL, control vs bolused, respectively) or Zn (1.05 ± 0.030 ug/mL vs 1.05 ± 0.036 ug/mL, control vs bolused, respectively) concentrations between bolused and control cows calves. Though serum Cu concentrations fell below what is considered adequate (0.70 ug/mL; Kincaid, 2000) in the first year of the study suggesting that there was little transfer in the milk.

Whole blood Se was also collected from biopsy cows and their calves in May and September of each year. There was no difference in whole blood Se between treatment groups in January when boluses were given, but five months after administration, in May, bolused cows had greater whole blood Se concentrations. This was also seen in the calves of bolused cows, having greater whole blood Se concentrations than control calves. Suggesting that Se supplemented via the dams bolus, was transferred to the nursing calves via milk, however milk Se concentrations were not measured. Whole blood Se levels of bolused cows also depended on age of the cow with mature cows (5-10 yrs of age) having higher whole blood Se levels than younger (< 5 yrs of age) and older cows (> 10 yrs of age) within their herd. This is interesting to consider that older cows and younger cows may have a higher trace mineral requirement than mature cows and so may need added supplementation to achieve adequate status.

Over the course of the three year study, cows that received boluses tended (P = 0.07) to have greater BCS in January than control cows (Sprinkle et al., 2006). However this did not follow through to May (calving) and September (weaning) where BCS was not affected by bolus. It was reported that cows treated with boluses lost more weight
from January to May. The author gave two explanations for why cows receiving boluses lost more weight during late gestation and early lactation (January to May). One hypothesis was that these cows had greater early lactation production than the control cows. Lacetera et al. (1996) reported that dairy cows provided supplemental Se tended to have greater milk production ($P = 0.06$) and did have greater total milk solids than a control group. The other explanation was that the increased supplementation of Cu could have had an antagonistic effect on cow weight by interacting with other trace minerals in the forage (Spears, 1991) or by decreasing the forage digestibility (Arthington et al., 2003).

These differences in BW and BCS of cows did not, however, relay onto the calf crop, where there was no observed difference in the adjusted weaning weights or weight per day of age (WDA). It is thought that weight gain of calves nursing cows supplemented with trace minerals are dependent on numerous factors, such as dietary trace mineral concentrations and the presence of antagonistic trace minerals in the diet which can decrease the dams status and the transfer of trace minerals via milk (Sprinkle et al., 2006).

Supplementation with a long acting bolus releasing trace minerals Cu and Se over a six month period seemed to affect body stores of said minerals, as well as that of the calf. The bolus was successful in increasing liver Cu of cows and blood Se in cows and calves. Use of the bolus had variable results on BW change from January to May. The benefit of these trace mineral boluses is the release of trace minerals over an extended period of time. In this study it was during the calving and breeding season when trace mineral requirements of late gestation and early lactation cows were higher.
Supplementation of trace minerals at this time can also be achieved with trace mineral injections which can bypass antagonisms in the rumen that boluses cannot avoid. Therefore injectable trace minerals may be a more advantageous form of trace mineral supplementation.

**Trace Mineral Injection**

Copper, Se, Mn, and Zn are the trace minerals that can be supplemented through supplementation by injection. Many studies have investigated the use of trace mineral injections in beef cattle, and the transfer and storage of Cu, Mn, Se and Zn from the injection throughout the body. The benefit of injectable trace minerals is their rapid availability and transport in the blood. The initial spike caused by a trace mineral injection in plasma and serum levels are only elevated for a short period of time (24 hours), and then decrease slowly over the following days (14 – 15 d) as they are stored in the body (Bohman et al., 1984). That is why it is more important to consider liver tissue as a marker for overall trace mineral status.

In a recent study by Pogge et al. (2012), beef steers (n = 20, 10 injected and 10 control) were used to determine the effectiveness of an injectable trace mineral solution [Multimin 90; 15 mg Cu/mL (as Cu disodium EDTA), 60 mg Zn/mL (as Zn disodium EDTA), 10 mg Mn/mL (as Mn disodium EDTA) and 5 mg Se/mL (as sodium selenite)] on increasing liver and plasma concentrations of Cu, Mn, Se and Zn. Jugular blood was collected prior to receiving injections and 8, 10 and 24 h post injection and 8 and 15 days
post injection. Liver biopsies were collected 3 days prior to receiving injections and 1, 8 and 15 d post injection.

At 8 and 10 h post injection there were significant increases in plasma concentrations of Se, Mn and Zn but not Cu in the injected cattle. However, by 24 hours post injection only plasma Se concentrations were still elevated in the injected group over the control group and by 8 d post injection there were no increases observed in plasma concentrations. Liver concentrations of Cu (113.5 vs 177.6 mg/kg DM, control vs injected, respectively), Se (1.7 vs 6.2 mg/kg DM, control vs injected, respectively) and Zn (77.8 vs 88.3 mg/kg DM, control vs injected, respectively) were increased over the 15 d period and there was a tendency ($P = 0.06$) for Mn (6.2 vs 6.8 mg/kg DM, control vs injected, respectively) to be increase in injected cattle.

In another study, beef calves were fed a diet deficient in Cu (4.1 mg/kg DM), Mn (25.7 mg/kg DM), and Zn (33.9 mg/kg DM) that also contained supplemental antagonists Fe (300 mg iron sulfate / kg diet) and Mo (5 mg sodium molybdate / kg diet), after a 20 h shipping stress, calves from both groups were administered a TMI or sterilized saline (Genther and Hansen, 2014). Blood was collected on d 0 prior to injections and d 1. Blood and liver biopsies were collected on d 8, 15, 29, 57 and 85 days post injection.

Plasma Se, Zn and Mn concentrations were increased on d 1. Selenium plasma concentrations continued to be increased until d15 post injection but were similar to controls by d 29. There was no effect of the trace mineral injection on plasma concentrations of Cu; and Se and Zn plasma concentrations were similar to controls by 8 d post injection (Genther and Hansen, 2014).
Steers that received trace mineral injections had increased liver concentrations of Cu and Se until 29 d post injection. The amount of increase in liver Cu in response to injection was greater in animals fed a trace mineral adequate diet over those fed the trace mineral deficient diet with antagonists. It is assumed that animals fed the adequate diet were able to store more Cu in their liver whereas Cu deficient animals utilized more from the injection resulting in less Cu storage in the liver after 29 days. Differences in liver Cu and Se concentrations as a result of the injection were not seen past d 29. Use of the trace mineral injection did not increase liver concentrations of Mn (7.81 vs 7.58 ± 0.207 mg/kg DM, control vs injected, respectively) or Zn (73.2 vs 74.7 ± 2.01mg/kg DM, control vs injected, respectively) at any of the time points measured. The lack of an increase in Mn was not unexpected because liver is not a good indicator of dietary intake of Mn suggesting that cattle are dependent on constant intake and do not store Mn.

In a study performed by Arthington and Havenga (2012), beef steer calves receiving a TMI had increased serum Cu, Se and Zn 14 days after injection when compared to control animals however increases in Mn were not observed on d 14. Richeson and Kegley (2011) performed a similar experiment where highly stressed crossbred beef heifers were injected with one of two TMI treatments containing different concentrations of Zn, Mn, Cu, and Se, blood was collected from these heifers prior to receiving injections and 28 d post injection. There was no increases observed in serum trace mineral concentration as a result of receiving injections in there heifers.

These data suggest that the use of a TMI is an effective way to immediately influence the concentrations of Cu, Se, Mn and Zn in the blood. Blood plasma concentrations of Cu, Se, Mn, and Zn have consistently been elevated for up to 24 h post
injection when compared to control animals (Pogge et al., 2012, Genther and Hansen, 2014). This increase in blood plasma has also been observed as far out as 14 d in Cu, Se and Zn (Arthington and Havenga, 2012). As trace minerals travel through the blood they are excreted or stored in the liver, these studies have demonstrated that trace mineral supplementation via an injection is an effective way to enhance the storage of Cu, Mn, Se and Zn in the liver. Concentrations of these minerals in the liver were greater for up to 14 d in animals receiving a trace mineral injection over that of control animals (Pogge et al., 2012). Trace mineral injections are proven to be advantageous for a short term boost in Mn and Zn status and have long term storage of Cu and Se in the liver.

Though these studies have shown that the trace mineral injections are a good way to increase trace mineral status of cattle the question remains on if this increase of status can increase cattle performance. Little work has investigated the use of injectable trace minerals in a beef cow/calf system. The ability to inject trace minerals to beef cows and calves is possibly beneficial to the cow/calf production system because cows and their calves are typically produced in grazing systems, where trace minerals are provided free choice. Free choice trace mineral supplementation results in variable intake by individual animals leaving some of the cows and their calves’ deficient. Deficiencies are greater in situations where the forage consumed by the herd is also deficient in minerals. For this reason, use of injectable trace minerals in the beef cow calf system may insure all animals are receiving adequate trace minerals. Potential benefits of a trace mineral injection are that animals receive this boost in trace mineral status prior to times of a higher requirement, such as pre-calving and pre-breeding. This could potentially improve the performance of deficient and marginally deficient animals.
In 2012, a study conducted by Mundell et al. looked at the use of a TMI containing Cu, Se, Mn and Zn in beef cows and their calves grazing native range with access to free choice mineral. Injections were administered to cows 105 d before the first projected calving date and again 30 d before fixed timed AI. At birth, calves received that same treatment (TMI vs saline injection) as their dam and again at 71 d of age. At the initiation of the study serum concentration of Cu, Se, Mn and Zn were determined. Cows in the herd were below the adequate range for Mn (3.5±1.84 ug/kg vs 2.2±0.80 ug/kg, TMI injected vs saline injected) with adequate concentrations being 6.0 – 7.0 ug Mn/kg. When looking at the standard deviation of trace mineral concentrations of the herd a significant portion of the herd had marginal status of Cu, Se and Zn. The change in BCS and body weight of cows from time of the pre-partum injection to calving was not different between treatment groups. However, cows that received trace mineral injections had greater ($P = 0.04$) BCS increase from calving to AI. Before timed AI similar proportions of cows in each group were demonstrating estrus however conception rate to fixed-time AI was greater ($P = 0.05$) for cows receiving a TMI injection (60.2%) than those who received saline (51.2%). Overall pregnancy rates of the treatment groups did not differ ($P = 0.24$). The next spring, however, the calving distribution of those cows that had received a TMI injection was more favorable as they calved earlier in the spring.

Beef cow response to trace mineral supplementation is variable and can be influenced by a number of factors. In this scenario, when cows were grazing native range the use of a TMI tended to increase BCS from calving to AI, fixed-time AI conception rate and calving distribution which are all advantageous responses. Also, in research by
Daughtery et al. (2002), cows that received TMI saw improvements in trace mineral status. However, when looking at the effect the trace mineral supplement to the cow might have on the calf, there were no effects on the passive immune status or survival rates of the calves (Daughtery et al., 2002).

Calf response to trace mineral supplementation has been even more variable than in cows, possibly because of the transfer of trace minerals from their dam during gestation and transfer of trace mineral through the milk. Therefore, the use of trace mineral injection to the calf to ensure each calf is receiving trace minerals may be more beneficial to calves. However, results from Mundell et al. (2012) showed no benefit from administering trace minerals to calves via an injection at birth and 71 DOA. Serum mineral concentrations collected prior to injections were administered indicated that calves were adequate in Zn (1.0 ± 0.37 mg/kg) and marginally deficient in Cu (0.6 ± 0.17 mg/kg), Mn (2.6 ± 1.13 ug/kg), and Se (66 ± 10.35 ug/kg). Calf BW at birth (\( P > 0.91 \)), ADG (\( P \geq 0.36 \)) and 205-d adjusted weaning weight (\( P = 0.48 \)) were not different between those calves that received TMI and those that received saline injections. It is unknown why the TMI to calves is not as effective as it is in cows, so further research to understand this is needed. Research on TMI use at post weaning, however, has shown significant improvements in feed intake during receiving. Improvements in feed efficiency and greater ADG was observed in highly stressed beef heifers that were marginally deficient in Zn and adequate in Cu at time of injection (Berry et al. 2000, Richeson and Kegley, 2011, and Genther and Hansen, 2014).

**Conclusion**
Trace mineral supplementation is vital to optimize production of beef cattle because of the roles trace minerals play in the body. In development of replacement heifers, trace minerals Cu, Se, Mn and Zn play roles in bodily functions that support reproduction. In the cow/calf system, multiple studies have shown the importance of trace mineral supplementation. Supplementation of trace minerals improve conception rates beef cows in different situations, factors that affect the impact trace mineral supplementation may have on performance is the trace mineral status of the animal prior to receiving supplementation. Cattle that have adequate trace mineral status and are not demonstrating decreased performance because of a trace mineral deficiency will not benefit from additional supplementation.
Literature Cited


Table 1. Trace Mineral Requirements for Growth and Reproduction of Beef Cattle [Adapted from NRC (2000)]

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Requirement (mg/kg, DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
</tr>
<tr>
<td>Copper</td>
<td>10</td>
</tr>
<tr>
<td>Manganese</td>
<td>20</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.1</td>
</tr>
<tr>
<td>Zinc</td>
<td>30</td>
</tr>
</tbody>
</table>
Table 2. Forage trace mineral concentrations relative to their ability to meet dietary requirements of beef cattle or cause antagonistic problems [Adapted from Corah and Dargatz (1996) and Mortimer et al., (1999)]

<table>
<thead>
<tr>
<th></th>
<th>Cu (mg/kg)</th>
<th>Mn (mg/kg)</th>
<th>Se (mg/kg)</th>
<th>Zn (mg/kg)</th>
<th>Fe (mg/kg)</th>
<th>Mo (mg/kg)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deficient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corah &amp; Dargatz</td>
<td>&lt; 4</td>
<td>&lt; 20</td>
<td>&lt; 0.1</td>
<td>&lt; 20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al.</td>
<td>&lt; 4</td>
<td>&lt; 20</td>
<td>&lt; 0.1</td>
<td>&lt; 20</td>
<td>&lt; 50</td>
<td>-</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td><strong>Marginal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corah &amp; Dargatz</td>
<td>4 – 7</td>
<td>20 - 40</td>
<td>0.1 - 0.15</td>
<td>20 - 40</td>
<td>50 - 200</td>
<td>&lt; 1</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al.</td>
<td>4 – 10</td>
<td>20 - 40</td>
<td>0.1 - 0.2</td>
<td>20 - 30</td>
<td>200 - 400</td>
<td>1 - 3</td>
<td>&gt; 0.2</td>
</tr>
<tr>
<td><strong>Adequate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corah &amp; Dargatz</td>
<td>&gt; 7</td>
<td>&gt; 40</td>
<td>0.15 - 0.3</td>
<td>&gt; 40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al.</td>
<td>&gt; 10</td>
<td>&gt; 40</td>
<td>0.2</td>
<td>&gt; 30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corah &amp; Dargatz</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt; 400</td>
<td>&gt; 3</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt; 400</td>
<td>&gt; 3</td>
<td>&gt; 0.3</td>
</tr>
<tr>
<td><strong>MTC(^1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corah &amp; Dargatz</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al.</td>
<td>100</td>
<td>1000</td>
<td>2</td>
<td>500</td>
<td>1000</td>
<td>5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

\(^1\) Maximum tolerable concentration (MTC)
Table 3. Trace mineral classification of forage samples in the US collected from Cow/Calf Herds in two surveys [Adapted from Corah and Dargatz (1996) and Mortimer et al. (1999)]

<table>
<thead>
<tr>
<th>Antagonist Levels</th>
<th>Cu</th>
<th>Mn</th>
<th>Se</th>
<th>Zn</th>
<th>Fe</th>
<th>Mo</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corah &amp; Dargatz, %</td>
<td>14.2</td>
<td>4.7</td>
<td>44.3</td>
<td>63.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al., %</td>
<td>0.71</td>
<td>0.56</td>
<td>43.4</td>
<td>33.29</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Marginal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corah &amp; Dargatz, %</td>
<td>49.7</td>
<td>19.3</td>
<td>19.3</td>
<td>34.1</td>
<td>17</td>
<td>48.6</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al., %</td>
<td>66</td>
<td>14.1</td>
<td>26.1</td>
<td>43.72</td>
<td>18.6</td>
<td>40.3</td>
<td>33.6</td>
</tr>
<tr>
<td>Adequate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corah &amp; Dargatz, %</td>
<td>36</td>
<td>76</td>
<td>19.7</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al., %</td>
<td>33.3</td>
<td>85.3</td>
<td>30.2</td>
<td>22.99</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Very High</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corah &amp; Dargatz, %</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.7</td>
<td>9.2</td>
<td>-</td>
</tr>
<tr>
<td>Mortimer et al., %</td>
<td>-</td>
<td>-</td>
<td>0.281</td>
<td>-</td>
<td>8.04</td>
<td>8.18</td>
<td>12.8</td>
</tr>
</tbody>
</table>

1Maximum tolerable level
Table 4. Bioavailability of trace minerals from different sources (Adapted from Paterson et al., 1999)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Organic</th>
<th>Sulfate</th>
<th>Oxide</th>
<th>Chlorides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>130</td>
<td>100</td>
<td>0</td>
<td>105</td>
</tr>
<tr>
<td>Manganese</td>
<td>176</td>
<td>100</td>
<td>58</td>
<td>-</td>
</tr>
<tr>
<td>Selenium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zinc</td>
<td>159 - 206</td>
<td>100</td>
<td>-</td>
<td>40</td>
</tr>
</tbody>
</table>
Chapter III

Effect of a trace mineral injection on performance and trace mineral status of beef cows and calves grazing irrigated pasture

C. J. Brasche,* J.B. Hall, † PAS, M.E. Drewnoski,*

*Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908;
†Department of Animal and Veterinary Science, University of Idaho, Moscow, ID 83844
Abstract

Beef cows (n = 200) were blocked by age and expected calving date and randomly assigned to treatment to receive either an injectable trace mineral (TMI) containing copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) or no injection (CON) prior to calving and breeding over two consecutive years. Calves born to cows receiving TMI also received TMI at birth (both years) and at spring branding (49 ± 1.3 days of age; in year one). Cows were synchronized using CIDR based protocols and exposed to fertile bulls. Cows and calves were grazing irrigated pasture and had access to free choice mineral containing Cu, Se and Zn. Treatment did not affect the change in BCS (P = 0.18) of cows from pre-calving to pre-breeding. Body condition score of CON (5.6 ± 0.07) and TMI cows (5.5 ± 0.07) at pre-breeding did not differ (P = 0.49) in year 1 but was lower (P = 0.04) for TMI (5.5 ± 0.10) than CON (5.8 ± 0.10) in year 2. In year 1, TMI increased cow liver concentrations of Se (1.2 vs 1.8 ± 0.15 mg/kg DM, CON vs TMI, respectively; P < 0.01), tended to increase Cu (185 vs 229 ± 12 mg/kg DM, CON vs TMI, respectively; P = 0.12), had no effect on Zn (93 vs 97 ± 2.7 mg/kg DM, CON vs TMI, respectively; P = 0.25) and decreased Mn (8.9 vs 8.0 ± 0.35mg/kg DM, CON vs TMI, respectively; P = 0.05) at breeding 15 d post injection. In year 2, TMI increased liver concentrations of Se (0.91 vs 1.6 ± 0.07 mg/kg DM, CON vs TMI, respectively; P < 0.01) and Zn (101 vs 124 ± 5.2 mg/kg DM, CON vs TMI, respectively; P < 0.01), tended to increase Cu (178 vs 213 ± 15.4 mg/kg DM, CON vs TMI, respectively; P = 0.12) and had no effect on Mn (13.6 vs 13.3 ± 0.74 mg/kg DM, CON vs TMI, respectively; P = 0.20) at breeding 18 d post injection. Pregnancy to AI did not differ (P ≥ 0.41) between CON (48 and 38 %, year 1 and year 2, respectively) and TMI (44 and 39%, year 1 and
year 2, respectively). Overall pregnancy rate after a 45-49 d breeding season did not differ \((P \geq 0.19)\) between CON (93% year 1 and 2) and TMI cows (93 and 90%, year 1 and year 2, respectively). Use of a TMI in calves increased plasma concentrations of Mn \((P = 0.05)\), Se \((P = 0.03)\), and Zn \((P = 0.05)\) but not Cu \((P = 0.54)\) at branding. Calves receiving TMI had greater liver concentration of Cu \((P = 0.02)\) and Se \((P = 0.01)\) but not Mn \((P = 0.48)\) and Zn \((P = 0.24)\) at weaning. There was no effect of TMI \((P \geq 0.38)\) on ADG, actual weaning weight or 205 d adjusted weaning weight (284 vs 286 ± 2.5 kg, CON vs TMI, respectively). These data suggest that TMI does not improve performance of beef cows and calves with marginal to adequate trace mineral status grazing irrigated pasture in Idaho.

Key words: beef cow/calf, conception, growth, injectable trace mineral
Introduction

Forage is the primary component of beef cow diets, however, forage often does not provide adequate amounts of mineral, therefore, mineral supplementation is often required (Greene, 1997). The most common form of mineral supplementation in grazing systems is free choice mineral. Free choice mineral is salt based and designed for self-feeding (Greene, 2000). A cow’s appetite for a free choice mineral is not related to her mineral requirement but rather on the animal’s taste for salt, thus intake of free choice mineral and subsequently the mineral status of cows within a herd can be variable (Arthington and Swenson, 2004). Trace mineral status of cows at calving can affect the calf mineral status at birth and health in early life (Hostetler et al., 2003). Additionally, a cow’s reproductive performance can be impacted by her trace mineral status (Corah and Ives, 1991). Specifically the trace minerals Cu, Mn, Se and Zn play important roles in reproduction and health (Hostetler et al., 2003).

Injectable trace minerals have been proven to be an effective method of supplementation in which known amounts of trace minerals are delivered to each animal (Genther and Hansen, 2014). However, little research has been conducted to evaluate the effects of supplementation of trace minerals through an injection in cow-calf production systems. Mundell et al. (2012) reported improved conception to fixed time AI in beef cows given trace mineral injections 105 d before calving and again 30 days before breeding when grazing native range with access to free choice mineral. However, calves that were nursing the cows receiving trace mineral injections and receiving trace mineral injections at birth and at approximately 71 d of age did not have greater ADG or 205 d weaning weight than controls.
The objective of this trial was to determine the effects of a trace mineral injection on trace mineral status of cows and calves grazing irrigated pasture in Idaho and the resulting impacts on reproductive performance of the cows and growth and health of their nursing calves.

Methods

Crossbred beef cows (Angus, Hereford and Angus × Hereford cross) and their calves located at the Nancy M. Cummings Research Extension and Education Center in Carmen, ID were used in this study. The summer prior to the initiation of the study, approximately half of the 200 cows used in this study grazed native range, while the remainder were maintained on irrigated pasture; cows were stratified by their summer grazing regimen when assigned to a treatment. Cows were also stratified by age and expected calving date and assigned to receive trace mineral injections (TMI) of Multimin 90 (Multimin, USA; Fort Collins, CO) containing 15 mg/ mL Cu, 10 mg/mL Mn, 5 mg/mL Se and 60 mg/mL Zn at pre-calving and pre-breeding or remain as untreated controls (CON). Cows remained on the same treatment over the two year trial.

In year 1, pregnancy data was collected on 86 CON and 88 TMI cows. Cows (n = 14 CON and n = 12 TMI) were removed from the study during year 1 due to being culled from the herd for loss of calf (n = 1 CON and n = 2 TMI) or other management reasons (behavior or conformation). Additionally, cows were culled at pregnancy check in October for failing to conceive. Bred heifers which had received a TMI (n = 11) or not (n = 10) prior to breeding as heifers in year 1 (but were not a part of this study in year 1) were added to the trial in year 2 starting at time of pre-calving injection (d 0) to replace
cows removed from the study. Cows that were less than 30 days post-calving at the start of the breeding (n = 5 CON and n = 7 TMI) were not synchronized and were removed from the pregnancy analysis in year 2. Cows that lost calves between calving and breeding were culled from the herd (n = 3 CON and n = 2 TMI). After the breeding season began any cow culled from the herd for health issues (udder and conformation issues) was removed from data. In year 2, pregnancy data was analyzed on 94 CON and 84 TMI cows.

Calves were sired by Angus, Hereford or Simmental bulls, with calves from TMI cows receiving a 1 ml injection of Multimin90 between birth and 24 h of age in both years. In year 1, TMI calves also received an injection at branding (1 mL/45 kg BW, 49 ± 1.3 days of age; DOA). In year 1, jugular blood was collected from calves (n = 40, 20 per treatment) at branding to determine plasma trace mineral concentrations. Weight of calves was recorded at birth, branding (49 ± 1.3 DOA, year 1 only), summer pregnancy check (128 ± 1.3 DOA, year 1; 128 ± 1.2 DOA, year 2), and at weaning (197 ± 1.3 DOA, year 1; 193 ± 1.2 DOA, year 2). Two-hundred and five day adjusted weaning weights were calculated using the Beef Improvement Federation (BIF) guidelines (BIF Guidelines, 2010). Calves were removed from analysis if they died (n = 5 CON, 6 TMI, 2 CON, and 3 TMI in year 1 and year 2, respectively) or were born as twins (4 and 3 sets of twin for CON and TMI, respectively in year 1). Thus, 91 CON and 91 TMI calves were used to evaluate the effects of TMI in year 1 and 98 CON and 97 TMI calves were used in year 2. Calves requiring treatment for pink eye, foot rot, or bovine respiratory disease (BRD) prior to weaning were recorded by the farm crew.
Liver biopsies were collected from randomly selected cows that were stratified across age (n = 40; 20 CON, 20 TMI) at pre-calving, pre-breeding, post pre-breeding TMI (breeding), and at weaning to determine liver trace mineral concentrations in both years. Biopsies were collected from the same cows at each sampling date. Liver biopsies were also collected from the calves (n = 40, 20 CON, 20 TMI) of sampler cows at weaning. Liver biopsies were collected using the method of Engle and Spears (2000). Liver biopsy samples were placed in a plastic culture tube, transported on ice to the laboratory and frozen at -20°C. Liver samples were then dried in a forced-air oven (60°C). Samples were analyzed for trace mineral concentration by the Diagnostic Center for Population and Animal Health (Lansing, MI). Once at the laboratory tissues were dried overnight in a 75°C oven and then digested overnight in nitric acid. Elemental analysis followed the methods of Wahlen et al. (2005) using an Agilent 7500ce Inductively Coupled Plasma – Mass Spectrometer (Agilent Technologies Inc., Santa Clara CA, 95051). Elemental concentrations were calibrated using a 4-point liver curve of the analyte-internal standard response ratio. The lowest concentrations points were 0.1 ug/mL for Cu and Zn, 0.5 ng/mL for Mn, and 0.1 ng/mL for Se. Standards were from GFS (GFS Chemicals, Powell, OH, 46065). A National Institute of Standards and Technology (NIST, Gaithersburg, MD, 20899) bovine liver standard was used as a control.

Forage was sampled by hand clipping forage at a height of 6 cm from randomly selected areas (0.093 meter$^2$, n = 10 per pasture) in July (58 d post AI) of year 2 in three irrigated orchard grass based (Dactylis glomerata) pastures (8 – 40 ha) located in different regions of the 4,451 ha ranch. During the winter (December – May) cows were
being fed alfalfa hay and wheat straw to meet energy and protein requirements during late gestation and early lactation. Trace mineral concentrations of the forage were determined by a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD). Analysis was conducted by instructions described for Metals and Other Elements in Plants (Association of Official Analytical Chemists, 2000). Modifications to the protocol included ashing 0.35 g sample for 1 hr at 535°C and digesting in open crucibles for 20 minutes in 15% nitric acid on hotplates. Samples were then diluted to 50 ml and analyzed on Inductively Coupled Plasma. Trace mineral concentrations of forage (Table 1), alfalfa and straw (Table 2) are reported.

A custom made free choice mineral supplement (Table 3) was offered throughout the entirety of the trial with the exception of a 53 d period in year 1 (32 – 85 d post AI) when they were provided with a commercial free choice mineral supplement (Purina Wind & Rain Storm All Season 7.5 Complete).

All cows were evaluated for body condition score (BCS; 1- emaciated, 9 - obese), described by Selk (2004) before the start of calving (December; d 0), one month prior to the start of breeding (April; d 117 – 114) and at preg check (July; d 195 - 202). Each year, there were different estrus synchronization and AI practices performed; therefore, methods will be described for each year separately.

**Year 1**

In December of 2012, (50 days prior to the start of calving season) cows in the TMI group were injected with 6 mL of TMI (0.67 mL/68 kg of body weight). Twenty-three days prior to AI (117 d post pre-calving injection), cows in the TMI group were
again given 6 mL of TMI (0.69 mL/68 kg of BW). All cows were estrus synchronized using a 5 day Co-Synch plus CIDR protocol in which cows were given an injection of gonadotropin releasing hormone (GnRH, 43 µg/mL; Fertagyl, Merck Animal Health) and received an insert of a controlled internal drug release device (CIDR vaginal insert containing 1.38 g of progesterone; Eazi-Breed CIDR, Zoetis Animal Health, New York, NY) 8 days prior to AI (d 132). The CIDR was removed 5 days later (d 137) and an injection of prostaglandinF\(_2\alpha\) (PG; 25 mg; Lutalyse, Zoetis Animal Health) followed by a second PG injection 5.6 h later was administered. Cows were administered an injection of GnRH and inseminated either 72 or 80 h after CIDR removal (d 140) with sexed semen. Cows were stratified across injection treatment to one of the two insemination times (72 or 80 h). Cows were randomly inseminated by one of two technicians then exposed to fertile bulls for natural-service breeding 17 d after AI (d 158) and remained with the bulls for 45 days. Pregnancy was determined using ultrasonography at 55 d post AI (d 195) and by rectal palpation at 105 d post AI (d 245). Artificial insemination pregnancy rate was calculated using \([n \text{ pregnant to AI} / \text{total n synchronized}] \times 100\).

**Year 2**

In December of 2013, (36 d prior to the start of calving season; d 0) cows in the TMI group were injected with Multimin90 (0.75 ± 0.014 mL/68 kg of BW). Twenty-eight days prior to AI (d 114), cows in the TMI group were again given a dose of Multimin90 (0.75 ± 0.020 mL/68 kg of BW). Cows were estrus synchronized using a 5 day Co-Synch plus CIDR protocol in which cows received an injection of GnRH and CIDR insert (d 135 or d 136). The CIDR was removed 5 days later (d 140 or 141) and an injection of PG was administered. Cows were then inseminated with non-sexed (n = 72
CON and 65 TMI) or sexed semen (n = 22 CON and 19 TMI) by one of three technicians (d 142 through 144) to synchronization response, determined by a full or partial rub on a heat patch. Cows that showed no response to synchronization by 72 h post CIDR removal were inseminated at 96 h post CIDR removal. Cows were exposed to fertile bulls on d 153 and co-mingled for 49 days. Pregnancy was determined using ultrasonography at 56 d post AI (d 202) and by rectal palpation at 150 d post AI (d 294). The change in calving date of cows from year 1 to year 2 was determined by the equation (Date of calving in year 1 – Date of calving in year 2 = change in calving day). The days post-partum at time of AI was calculated using the equation (Day of calving – Day of AI = Days post-partum at time of AI).

Statistical Analysis

Significance was declared at $P \leq 0.05$, tendencies are discussed when $P > 0.05 \leq 0.15$ and covariates were used when $P \leq 0.20$. Cow BCS at pre-calving, pre-breeding and changes in BCS from pre-calving to pre-breeding were analyzed using a mixed-effects model (PROC MIXED; SAS Inst., Inc., Cary, NC). The covariate of dam age (using BIF age groupings for 205 d adjusted weight calculations; Table 4) was tested for both years. Dam age was not a significant covariate ($P > 0.90$) for pre-calving BCS in both years as well as change in BCS or BW in year 1 and was removed from the model. In year 1 the model included the fixed effect of previous grazing (range or irrigated pasture) during the previous summer. Previous grazing $\times$ TMI was not significant ($P > 0.24$) for any models and was removed from the model.
Pregnancy rates were analyzed using logistic regression (PROC GENMOD; SAS Inst., Inc., Cary, NC). For year 1, the model used to assess differences in AI pregnancy rate tested the effects of TMI, previous grazing, days post-partum (grouped as 29-60 d post-partum, 61 – 80 d post-partum, or >81 d post-partum), dam age, and AI timing treatment (72 vs 80 h) and their interactions. There were no significant interactions ($P > 0.54$) and therefore they were removed from the model. The fixed effects of previous grazing, day’s post-partum, dam age and AI timing treatment were not significant ($P > 0.37$) and were removed from the model. The covariates of AI sire and AI technician were tested. The covariate of AI technician was not significant ($P = 0.19$) and removed from model. For year 1, the model to assess differences in overall pregnancy rate included TMI and previous grazing and their interactions. The interaction between TMI and previous grazing was not significant ($P = 0.43$) and so it was removed from the model.

For year 2, the model used to assess differences in AI pregnancy rate included TMI, semen type (sexed vs non-sexed), dam age, days post-partum, and timing of AI [heat response (72 h) vs delayed 96 h after CIDR removal] and their interactions. There were no significant interactions ($P > 0.42$) and all interactions were removed from the model. The fixed effects semen type, dam age, days post-partum, and timing of AI were not significant ($P \geq 0.31$) and removed from the model. The covariates of AI sire and AI technician were tested. The covariate of AI sire was not significant ($P = 0.47$) and was removed from the model. In year 2, the model used to assess differences in overall pregnancy rate and change in calving date included TMI, dam age and their interaction. The interaction was not significant ($P \geq 0.21$) and was removed from the model.
Liver mineral concentrations of Cu, Mn, Se, and Zn at each time point were analyzed using PROC MIXED with the fixed effect of TMI. The covariates of initial pre-treatment liver concentrations (d 0 in year 1) and dam age were tested and were used for all models for that mineral when the majority of time points had a $P$-value that was less than or equal to 0.20. Therefore pre-treatment liver concentrations of Cu and Mn were used as a covariate in models testing the effects on Cu and Mn, respectively and dam age was also used for Cu.

Calf birth BW, average daily gain (ADG), weaning weight and 205 d adjusted weaning weight was analyzed using a mixed-effects model (PROC MIXED; SAS Inst., Inc., Cary, NC) including the fixed effect of TMI, year, and breed type (determined by sire breed and at least half of the known dam breed) and their interactions. Calf gender and dam age were tested as covariates for calf birth BW, average daily gain (ADG) and weaning weight models. For birth BW, ADG, and weaning weight there were no significant interactions ($P > 0.33$) therefore interactions were removed from these models. For 205 d adjusted weaning weight there were no significant interactions between TMI $\times$ year $\times$ breed type ($P = 0.21$) and the covariate of dam age was not significant ($P = 0.31$) and both were removed from the model.

Liver mineral concentrations of Cu, Mn, Se, and Zn of calves were analyzed using the fixed effects of TMI and year and their interaction. Dam age and calf sex were tested as covariates. Interaction of TMI $\times$ year was removed from models for Cu, Zn, and Se ($P > 0.43$). Covariate of dam age was removed from the models for Mn and Zn ($P > 0.23$). Covariate of calf sex was removed from models for Mn, Zn and Se ($P > 0.27$).
Calf health was analyzed using logistic regression (PROC GENMOD; SAS Inst., Inc., Cary, NC). Incidence of foot rot, pinkeye and BRD were analyzed using the fixed effects of TMI, year and their interaction. There was no interaction \((P \geq 0.30)\) of TMI \(\times\) year on foot rot, pinkeye, or BRD, so the interaction was removed from these model and the two years of results were pooled.

Results & Discussion

Liver Mineral Status of Cows

Liver concentrations determined at the initiation of the trial (pre-calving in year 1) of Cu, Mn, and Zn did not differ \((P \geq 0.36)\) between CON and TMI groups. However, liver Se was greater \((P < 0.01)\) in TMI than CON cows at initiation of the trial (Figure 1).

At pre-breeding in year 1, 117 d post pre-calving and prior to pre-breeding injection, liver concentrations of Cu, Mn and Se did not differ \((P \geq 0.25)\) among treatments but Zn was greater \((P < 0.01)\) in TMI than CON. At breeding in year 1, 15 d post pre-breeding injection, liver concentrations of Cu and Se were greater \((P \leq 0.05)\) in TMI cows than CON. Although, still numerical greater in TMI, Zn \((P = 0.25)\) did not differ between treatment groups. In contrast, Mn liver concentrations were greater \((P = 0.05)\) in CON than TMI. At weaning, 176 days after pre-breeding injection, there was no difference \((P \geq 0.21)\) in liver concentrations of Cu, Mn and Se but Zn was greater \((P < 0.01)\) in TMI than CON cows (Figure 1).

At the start of year 2, prior to pre-calving TMI (247 d post pre-breeding injection) liver concentrations of Cu were not different \((P = 0.31)\) between treatment groups. However, liver concentrations of Se and Zn were greater \((P < 0.03)\) in TMI cows than
CON and Mn was greater \((P = 0.02)\) in CON than TMI. At pre-breeding, 114 d post pre-calving injection liver concentrations of Cu \((P = 0.48)\), Mn \((P = 0.95)\), Se \((P = 0.16)\) and Zn \((P = 0.77)\) were not different between treatment groups. At breeding, 18 d post pre-breeding injection, liver concentrations of Mn \((P = 0.79)\) were not different between treatment groups but Se and Zn were greater \((P \leq 0.01)\) and Cu tended \((P = 0.12)\) to be greater in TMI cows. Liver concentrations at weaning, 180 d post pre-breeding injection, of CON and TMI cows were not different for Cu \((P = 0.48)\), Se \((P = 0.17)\) and Mn \((P = 0.19)\) but Zn was greater \((P = 0.02)\) in TMI cows (Figure 1).

Concentrations of Cu in the forage of the predominately orchard grass pastures in this study were marginal \((8.7 \text{ mg/kg DM}; \text{ Table 1})\). Further, the Mo concentration of the forage \((2.2 \text{ mg/kg DM})\) was marginally antagonistic. Mortimer et al. (1999) defines marginally antagonistic levels of Mo in forage to be 1-3 mg/kg DM. The alfalfas hay provided to the cows during the winter contained adequate concentrations of Cu but the straw was low in Cu and both contained antagonistic concentrations of Mo (Table 2). However, the free choice mineral supplement provided contained 2000 mg/kg of Cu and appeared to overcome these issues as the Cu liver concentrations of both groups were considered to be adequate throughout the study \((\text{greater than } 125 \text{ mg Cu/kg DM as defined by Kincaid, 1999})\).

The Mn concentration of the pasture was adequate at 77 mg/kg (Table 1) but Mn concentration of alfalfa hay and wheat straw provided during the winter was marginal (Table 2). During the majority of the trial, both of the treatment groups had liver Mn concentrations below the level of adequacy \((< 13 \text{ mg Mn/kg DM as defined by Kincaid, 1999})\) suggesting that the Mn in the forage was not sufficiently available to the cows.
The free choice mineral supplement fed for the majority of this study did not provide supplemental Mn (Table 3).

The pasture, alfalfa hay and wheat straw were deficient (< 0.10 mg/kg DM, Mortimer et al., 1999) in Se (Table 1 and 2) and would have provided less than 40% of the cows' requirement. It is interesting that despite the fact all cows had access to a free choice supplement which contained 38 mg/kg DM as sodium selenite (Table 3) CON cows were marginally deficient during year 2.

Concentrations of Zn in the pasture, alfalfa hay and straw (Table 1 and 2) were marginal. However, Zn was supplemented in the free choice mineral supplied to the herd (Table 3). Therefore it is not unexpected that both the TMI and CON cows had adequacy Zn status (25 – 200 mg Zn/kg DM) during the trial.

At breeding, 15 to 18 d post pre-breeding injection, liver concentrations of Cu and Se were consistently elevated in the TMI cows. However, Zn and Mn were not increased. There have been variable results on a TMI’s influence on liver concentrations. Pogge et al. (2012) reported that the use of a TMI increased liver concentrations of Cu (113 vs 177 ± 5.3 mg/kg DM, control vs injected, respectively), Se (1.7 vs 6.2 ± 0.37 mg/kg DM, control vs injected, respectively), and Zn (77 vs 88 ± 3.0 mg/kg DM, control vs injected, respectively) and tended to increase liver Mn (6.2 vs 6.8 ± 0.19 mg/kg DM, control vs injected, respectively) concentrations 15 d post injection. However, at 29 d post TMI, Genther and Hansen (2014) found no effect of injection on liver Mn (7.8 vs 7.6 ± 0.21 mg/kg DM, control vs injected, respectively) or Zn (73 vs 75 ± 2.0 mg/kg DM, control vs injected, respectively) concentrations but did observe an increase in Cu (132 vs 157 ±
9.0 mg/kg DM, control vs injected, respectively) and Se (1.73 vs 2.09 ± 0.060 mg/kg DM, control vs injected, respectively).

Cow BCS

Initial measurements of BCS were recorded at time of pre-calving injection in year 1. Initial BCS of CON (5.4 ± 0.09) and TMI (5.2 ± 0.09) cows did not differ ($P = 0.27$) at the start of the trial (Table 5). The use of a TMI did not affect the change in BCS ($P \geq 0.60$) of cows from pre-calving to pre-breeding in either year (Table 5). However, at pre-calving in year two, CON cows tended ($P = 0.10$) to have a greater BCS (6.8 ± 0.08) than TMI cows (6.6 ± 0.08). At pre-breeding, 114 d later, there was a significant ($P = 0.04$) difference in BCS with BCS of CON (5.8 ± 0.10) cows being greater than that of TMI cows (5.5 ± 0.10). However, it should be noted that in the current study cows were in good condition throughout the study with a BCS of 5.0 or greater and thus effects on the cow’s reproduction would not have been expected due to differences in BCS.

In contrast to our results, Mundell et al. (2012) reported a positive effect of TMI on BCS, where cows receiving TMI had a greater increase ($P = 0.04$) in BCS from parturition to time of AI breeding. It was observed by Mundell et al. (2012) that all cows used in their study increased BCS during this time while they were grazing native range. In comparison with the cows used in our study who were losing condition from calving to pre-breeding in year 2 it could be hypothesized that the native range was of a higher nutritional value and could explain why there was an increase in BCS from the TMI. However, in other studies in which trace mineral supplementation via free choice
consumption has been evaluated, no effect of supplementation on BCS has been observed (Doyle et al., 1988; Gunter et al., 2003; Ahola et al., 2004).

Reproductive performance

Prior to pre-calving injection in year 1, cows were blocked by age (Table 3) and expected calving date. As designed, there was no difference ($P = 0.21$) between treatments in the number of days post-partum at breeding in year 1 (Table 6). In year 2, there was no ($P = 0.48$) effect of TMI on calving date (change in calving date for CON = $4 \pm 2.6$ d and TMI = $6 \pm 2.7$ d). Therefore, there was no difference ($P = 0.25$) among treatments in post-partum interval at time of AI in year 2. Trace mineral injection did not affect reproductive performance of cows in the current study as both pregnancy to AI ($P \geq 0.58$) and overall pregnancy rate did not differ ($P \geq 0.36$) due to TMI treatment in both years (Table 6).

In contrast, Mundell et al. (2012) observed that cows on native range receiving TMI had greater AI conception rates than those receiving a saline injection (60.2 vs 51.2 %, TMI vs saline, respectively). However, this did not follow through to overall pregnancy rate which did not differ ($P = 0.24$) between treatments. Due to the increase in AI conception, Mundell et al. (2012) reported that use of a TMI created a more favorable calving distribution with cows receiving a TMI generally calving earlier than cows receiving a saline injection.

Pre-treatment serum mineral concentrations collected from cows in the Mundell et al. (2012) study demonstrated that the cows in their trial were deficient in Mn and marginal in Cu, Se and Zn. This could explain why the cows that received a TMI in their
study had an increased conception to timed AI with the use of the injection. Both CON and TMI cows used in our study were adequate Cu, Se, and Zn at breeding in year 1 and adequate in Cu, Mn and Zn at breeding in year 2. This difference in trace mineral status could explain why there were differences in response between our study and that of Mundell et al. (2012).

Ahola et al. (2004) reported that after one year, free choice supplementation of Cu, Mn, and Zn resulted in no differences in conception to AI when compared to unsupplemented controls. However, after two consecutive years without supplementation, cows in the control group had lower AI conception rates (15/44, 34%) than those that received supplementation (26/46, 57% and 25/43, 58%; organic and inorganic, respectively). This increase in AI conception of supplemented cows in year 2, but not in year 1, could have been a result of control cows not being supplemented Cu, Mn and Zn for over one year thereby depleting their body’s storage of these minerals. It was reported that control cows did exhibit decreased concentrations of liver Cu but not Mn and Zn by the end of year 2. The control cows had 43.7 mg Cu/kg DM in their liver and would have been considered deficient.

**Trace Mineral Status of Calves**

Plasma Se concentrations were increased ($P = 0.03$) in TMI calves when compared to CON at branding in year 1 (Table 7). Calves in the CON group were below the level of adequacy (80 ug Se/L) suggested by Puls (1994). Unlike Se, concentrations of Cu, Mn and Zn in plasma and serum are not typically affected by status until the point of severe deficiency because of homeostatic regulation (Kincaid, 1999). Concentrations of Cu, Mn and Zn of control calves at branding would indicate that status of calves was
adequate as defined by Kincaid, 1999. No effect \((P = 0.54)\) of TMI on plasma Cu was observed at branding in year 1. However, both plasma Mn and Zn concentrations were increased \((P = 0.05)\) in TMI calves when compared to CON (Table 7). Many studies have investigated the use of a TMI on plasma or serum concentrations. A study investigating TMI use in feedlot steers demonstrated an initial increase in plasma of Mn, Se and Zn for up to 10 h post injection in TMI steers over a control group (Pogge et al., 2012). At 24 h post injection plasma Se was still elevated in TMI steers but Cu, Mn and Zn were not and by 8 days post injection plasma concentration of Cu, Mn, Se and Zn were similar to control animal concentrations (Pogge et al., 2012). In another study looking at plasma concentrations of trace minerals from an injection for up to 85 days there were no consistent trends seen in the concentration of plasma Cu and Zn (Genther and Hansen, 2014). However, concentrations of plasma Mn were increased for up to 8 days in TMI cattle and Se was increased in plasma for up to 15 days in TMI cattle but by 29 days post injection plasma concentrations of Cu, Se, Mn and Zn were similar to a control group (Genther and Hansen, 2014). This would indicate that trace minerals supplemented via an injection are not present in the plasma for a period longer than 15 days most likely because of blood homeostasis.

This suggests that the increase in plasma Mn, Se and Zn observed 49 days after injection in the current study may have been due to increased transfer in the milk from their dam which received injections prior to calving and breeding. Though, this cannot be confirmed as the trace mineral concentrations of milk were not determined in this study. Little information is available regarding the effects of Cu, Mn and Zn status of cows on the concentrations of these minerals in their milk. However, concentrations of Zn in dairy
cow’s milk are typically greater than the cows serum, concentrations of Mn in milk are comparable to serum and Cu concentration in cow’s milk is rather low with serum concentration being several-fold greater suggesting that milk may provide some supplemental Zn and Mn but little Cu (Pechova et al., 2008). Concentration of Zn in cow’s milk is increased with increasing dietary Zn concentrations (Miller, 1970). It has also been found that supplementation of Se as selenomethionine will increase Se in milk (Pechova et al. 2008). Further, it has previously been observed that calves, nursing cows supplemented with Se and Cu via a rumen indwelling bolus, had greater blood Se concentrations than control calves, suggesting that Se was increased in the dam’s milk. Unlike Se, the serum Cu of these calves was not increased (Sprinkle et al., 2006).

Liver Cu was increased ($P = 0.02$) in TMI calves compared to CON at weaning (Table 8). Calves in both groups had adequate concentrations of liver Cu as they were above 125 mg Cu/kg DM. Liver Se of TMI calves at weaning tended ($P = 0.10$) to be increased compared to CON suggesting that the TMI of the cows and calves had a long term effect on Se status of the calves. Adequate liver concentrations of Se are suggested be a minimum of 1.25 mg/kg DM (Kincaid, 1999). Using this threshold both the TMI and CON calves would be considered marginally deficient in Se at weaning.

There was a tendency for an interaction of TMI × year ($P = 0.15$) on liver Mn concentration at weaning. In year 1, CON calves liver Mn concentration was not different ($P = 0.59$) from TMI calves (8.8 ± 1.19 vs 9.76 ± 1.21 mg/kg DM; CON vs TMI respectively). However, in year 2 CON (15.6 ± 1.25 mg/kg DM) calves concentrations tended to be greater ($P = 0.13$) than TMI (12.9 ± 1.21 mg/kg DM). There was no significant difference ($P = 0.24$) in liver concentrations of Zn when sampled at weaning.
Calf Performance

There was no interaction of TMI and year on ADG ($P = 0.62$), birth BW ($P = 0.33$), weaning weight ($P = 0.49$) or disease incidence ($P \geq 0.25$) so data was pooled across years. There was no effect ($P = 0.19$) of TMI on birth BW of calves (Table 9). Use of a TMI had no effect ($P \geq 0.33$) on average daily gain (ADG) from birth to weaning, birth to pregnancy check, or from pregnancy check to weaning. There was no effect of treatment on actual weaning weight ($P = 0.82$).

There was a tendency for an interaction between TMI × year ($P = 0.13$) on 205 d adjusted weaning weight. In year 1 there was a tendency ($P = 0.14$) for calves receiving a TMI at birth and at branding (49 ± 1.3 DOA) to have a higher 205 d adjusted weaning weight (275 vs 282 ± 3.6 kg). However, this was not seen in year 2 when TMI calves (288 ± 3.4 kg) receiving injections at birth did not have different ($P = 0.51$) 205 d adjusted weaning weights from CON calves (291 ± 3.4 kg). Similar to the results observed in this study, Mundell et al. (2012) reported no difference in ADG from birth to weaning or 205 d adjusted weaning weights between control and TMI calves when calves nursed dams who received a TMI pre and post – partum and received a TMI at birth and at 71 ± 21 days of age.

There was no difference in the incidence of foot rot ($P = 0.35$) or pinkeye ($P = 0.85$) between the treatments groups (Table 10). However, there was a tendency ($P = 0.11$) for more calves that received TMI to exhibit symptoms of BRD. In contrast, in a trial comparing the effect of trace mineral injection on the immunity of highly stressed weaned calves coming into a feedlot, calves administered a TMI containing Zn (20
mg/mL), Mn (20 mg/mL), Cu (10 mg/mL) and Se (5 mg/mL) at receiving had less BRD morbidity than control calves (Richeson and Kegley, 2011).

**Implications**

These data suggest that the use of a trace mineral injection containing Cu, Mn, Se and Zn does not improve reproductive performance of cows or growth rate of calves with a good trace mineral status grazing irrigated pasture in Idaho. However, the use of a trace mineral injection was effective in enhancing the liver concentration of Cu and Se in cows prior to breeding. In calves, the use of the trace mineral injection enhanced plasma concentrations of Mn, Se and Zn at branding and increased liver concentrations of Cu and Se at weaning.
Literature Cited


Tables and Figures

Table 1. Trace mineral concentrations of irrigated forage\textsuperscript{1}, primarily orchard grass, grazed by cattle in summer of year 2

<table>
<thead>
<tr>
<th>Trace Mineral</th>
<th>Mean (mg/kg DM)</th>
<th>SD\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>8.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Iron</td>
<td>130</td>
<td>9.5</td>
</tr>
<tr>
<td>Manganese</td>
<td>77</td>
<td>44</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.037</td>
<td>0.012</td>
</tr>
<tr>
<td>Zinc</td>
<td>25</td>
<td>3.6</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Analysis conducted by Cumberland Valley Analytical Services, Hagerstown, MD.

\textsuperscript{2} Standard deviation
Table 2. Trace mineral concentrations\(^1\) of alfalfa hay and wheat straw fed to cows from winter to early spring in year 2.

<table>
<thead>
<tr>
<th>Trace Mineral</th>
<th>Alfalfa (mg/kg)</th>
<th>Wheat Straw (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Iron</td>
<td>143</td>
<td>137</td>
</tr>
<tr>
<td>Manganese</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>1.57</td>
<td>1.42</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Zinc</td>
<td>22</td>
<td>19</td>
</tr>
</tbody>
</table>

\(^1\) Analysis conducted by Cumberland Valley Analytical Services, Hagerstown, MD.
Table 3. Trace mineral concentrations of free choice supplement\textsuperscript{1,2} provided during the study

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Concentration mg/kg</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>2000 mg/kg</td>
<td>25% Cu Proteinate, 75% Cu Sulfate</td>
</tr>
<tr>
<td>I</td>
<td>125 mg/kg</td>
<td>Ethylenediamine Dihydriodide</td>
</tr>
<tr>
<td>Mn</td>
<td>0 mg/kg</td>
<td>-</td>
</tr>
<tr>
<td>Se</td>
<td>38 mg/kg</td>
<td>Sodium Selenite</td>
</tr>
<tr>
<td>Zn</td>
<td>2000 mg/kg</td>
<td>25% Zn Proteinate; 75% Zn Sulfate</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Provided for the entirety of the trial with the exception of a 53 day period (32-85 d post AI) in year 1 during which a free choice mineral containing 1200 mg Cu/kg from Basic Copper Chloride, 3600 mg Mn/kg from Manganese Sulfate, 27 mg Se/kg from Sodium Selenite and 3600 mg Zn/kg from Zinc Sulfate was provided.

\textsuperscript{2} Vitamins A (222,222 IU/kg), D (22,222 IU/kg) and E (222 IU/kg) were provided in the free choice supplement provided from December through May.
Table 4. Dam age of cows receiving a trace mineral injection (TMI) or no TMI (CON) at pre-calving and pre-breeding

<table>
<thead>
<tr>
<th>Dam Age¹</th>
<th>CON</th>
<th>TMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Year 1</td>
<td>Year 2</td>
</tr>
<tr>
<td>2 yr old</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>3 yr old</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>4 yr old</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>5 - 10 yr old</td>
<td>54</td>
<td>51</td>
</tr>
<tr>
<td>11 + yr old</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

¹ Dam age was determined at time of pre-calving injection (d 0) of each year
Table 5. Comparison of trace mineral injection (TMI) or no trace mineral injection (CON) on cow performance measured as body condition score (BCS) and change in BCS and body weight (BW) throughout a two year trial

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TMI</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCS at pre-calving (d 0)(^{1,2})</td>
<td>5.4</td>
<td>5.2</td>
<td>0.09</td>
<td>0.27</td>
</tr>
<tr>
<td>BCS at pre-breeding (d 117)(^{1,2,3})</td>
<td>5.6</td>
<td>5.5</td>
<td>0.07</td>
<td>0.49</td>
</tr>
<tr>
<td>Change in BCS (d 0 - 117)(^{1,3,4})</td>
<td>0.22</td>
<td>0.29</td>
<td>0.132</td>
<td>0.60</td>
</tr>
<tr>
<td>BCS at preg check (d 195)</td>
<td>5.5</td>
<td>5.3</td>
<td>0.08</td>
<td>0.18</td>
</tr>
<tr>
<td>Year 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCS at pre-calving (d 0)(^1)</td>
<td>6.8</td>
<td>6.6</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>BCS at pre-breeding (d 114)(^{1,2})</td>
<td>5.8</td>
<td>5.5</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Change in BCS (d 0 - 114)(^{1,2,4})</td>
<td>-1.0</td>
<td>-1.1</td>
<td>0.11</td>
<td>0.63</td>
</tr>
<tr>
<td>BCS at preg check (d 202)</td>
<td>5.8</td>
<td>5.7</td>
<td>0.08</td>
<td>0.21</td>
</tr>
</tbody>
</table>

1 Body Condition Score (BCS); (1- emaciated, 9 - obese), described by Selk (2004).
2 Covariate of dam age (\(P < 0.20\))
3 Covariate of days post calving (\(P < 0.20\))
4 Change in BCS = (Pre-breeding BCS – Pre-calving BCS)
Table 6. Comparison of trace mineral injection (TMI) or no trace mineral injection (CON) on reproductive performance of cows at 105 (Year 1) or 150 days (Year 2) post artificial insemination (AI)

<table>
<thead>
<tr>
<th>Item</th>
<th>CON % (no. 2)</th>
<th>TMI % (no. 2)</th>
<th>SE¹</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days post calving at AI³, d</td>
<td>77</td>
<td>75</td>
<td>1.7</td>
<td>0.21</td>
</tr>
<tr>
<td>AI³ pregnancy, %</td>
<td>48 (42/86)</td>
<td>44 (39/88)</td>
<td>5.3</td>
<td>0.58</td>
</tr>
<tr>
<td>Bred⁵, %</td>
<td>93 (80/86)</td>
<td>93 (82/88)</td>
<td>2.9</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Year 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days post calving at AI³, d</td>
<td>65</td>
<td>63</td>
<td>2.1</td>
<td>0.25</td>
</tr>
<tr>
<td>AI⁴ pregnancy, %</td>
<td>38 (36/94)</td>
<td>39 (33/84)</td>
<td>5.1</td>
<td>0.86</td>
</tr>
<tr>
<td>Bred⁵, %</td>
<td>93 (88/94)</td>
<td>90 (76/84)</td>
<td>2.9</td>
<td>0.36</td>
</tr>
</tbody>
</table>

¹ SE calculated using Standard Error of Proportion
² Number of animals observed/number of animals evaluated
³ (Day of calving – Day of AI = days post calving at AI)
⁴ Artificial Insemination (AI)
⁵ Pregnant after AI and exposure to a bull for 45 - 49 days
Table 7. Effect of trace mineral injection (TMI) or no TMI (CON) on calf plasma concentrations at branding (49 ± 1.3 days of age) in year 1

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TMI</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper, mg/L</td>
<td>0.86</td>
<td>0.90</td>
<td>0.039</td>
<td>0.54</td>
</tr>
<tr>
<td>Manganese, ug/L</td>
<td>12.6</td>
<td>23.1</td>
<td>3.46</td>
<td>0.05</td>
</tr>
<tr>
<td>Selenium ug/L</td>
<td>51.9</td>
<td>88.8</td>
<td>10.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Zinc, mg/L</td>
<td>0.99</td>
<td>1.16</td>
<td>0.054</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 8. Effect of trace mineral injection (TMI) on liver trace mineral concentrations\(^2\) of calves at weaning

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TMI</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper, mg/kg</td>
<td>150</td>
<td>187</td>
<td>11.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Manganese, mg/kg</td>
<td>12.1</td>
<td>11.4</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Selenium, mg/kg</td>
<td>0.88</td>
<td>0.98</td>
<td>0.043</td>
<td>0.10</td>
</tr>
<tr>
<td>Zinc, mg/kg</td>
<td>108</td>
<td>113</td>
<td>2.9</td>
<td>0.24</td>
</tr>
</tbody>
</table>

\(^1\)Interaction of TMI \(\times\) year for Cu \((P = 0.76)\), Mn \((P = 0.16)\), Se \((P = 0.43)\) and Zn \((P = 0.74)\)

\(^2\) Concentrations reported on a dry matter basis
Table 9. Effect of trace mineral injection (TMI) or no injection (CON) on calf performance

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TMI</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG(^1), kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth – preg check(^2)</td>
<td>1.03</td>
<td>1.05</td>
<td>0.016</td>
<td>0.33</td>
</tr>
<tr>
<td>Preg check – weaning(^2)</td>
<td>1.17</td>
<td>1.17</td>
<td>0.019</td>
<td>0.94</td>
</tr>
<tr>
<td>Birth – weaning(^2)</td>
<td>1.14</td>
<td>1.15</td>
<td>0.009</td>
<td>0.38</td>
</tr>
<tr>
<td>Calf BW(^3), kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth BW(^3)</td>
<td>39.9</td>
<td>40.7</td>
<td>0.44</td>
<td>0.19</td>
</tr>
<tr>
<td>Actual weaning weight</td>
<td>265</td>
<td>264</td>
<td>2.3</td>
<td>0.82</td>
</tr>
<tr>
<td>205 d adjusted weaning weight(^4)</td>
<td>284</td>
<td>286</td>
<td>2.5</td>
<td>0.54</td>
</tr>
</tbody>
</table>

\(^1\) Average daily gain (ADG)

\(^2\) Calf body weights were recorded at birth (0 days of age (DOA)), preg check of cows (128 ± 1.3 DOA) and at weaning (197 ± 1.3 and 193 ± 1.2 DOA, year 1 and year 2, respectively) weights were pooled across years.

\(^3\) Body weight (BW)

\(^4\) Weaning weights adjusted according to Beef Improvement Federation guidelines (BIF, 1990).
Table 10. Effect of trace mineral injection (TMI) or no TMI (CON) on calf health combined over two years $^1$

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TMI</th>
<th>SE$^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot rot, %</td>
<td>11.5 (22/191)</td>
<td>14.8 (28/188)</td>
<td>2.44</td>
<td>0.35</td>
</tr>
<tr>
<td>Pinkeye, %</td>
<td>10.4 (20/191)</td>
<td>13.2 (25/188)</td>
<td>2.34</td>
<td>0.85</td>
</tr>
<tr>
<td>BRD$^3$, %</td>
<td>3.1 (6/191)</td>
<td>7.4 (14/188)</td>
<td>1.58</td>
<td>0.11</td>
</tr>
</tbody>
</table>

$^1$ Interaction of TMI × year on foot rot ($P = 0.39$), bovine respiratory disease ($P = 0.96$) and pink eye ($P = 0.01$). Trace mineral injection effect on pink eye in year 1 ($P = 0.43$) and year 2 ($P = 0.22$) was not significant within years so data was pooled between years.

$^2$ Standard Error of Proportion

$^3$ Bovine Respiratory Disease (BRD)
Figure 1. Effect of trace mineral injection (TMI) or no injection (CON) on liver concentrations of Cu, Se, Mn, and Zn in beef cows at pre-calving, pre-breeding, 15-18 days after pre-breeding injection (breeding), and weaning in year 1 and year 2 of the trial. Initial trace mineral concentration was used as a covariate in the analysis of Mn and Cu and dam age was also included as a covariate in analysis of Cu. Significance ($P < 0.05$) signified as (*) and tendencies ($P \geq 0.05 \leq 0.15$) as (**). The black bars represent adequate trace mineral status of mature cattle defined by Kincaid (2000).
Chapter IV

Effect of a trace mineral injection on trace mineral liver concentrations, AI conception and pregnancy rate of virgin beef heifers

C. J. Brasche,* J.B. Hall, † PAS, M.E. Drewnoski,*

*Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908;
†Department of Animal and Veterinary Science, University of Idaho, Moscow, ID 83844;
Abstract

Two experiments were conducted to examine the effect of using a commercially available injectable trace mineral on the reproductive performance of heifers. Angus-crossbred heifers (n = 109 and 112 in experiments 1 and 2, respectively) were blocked by weight and randomly assigned to receive a trace mineral injection (TMI) containing copper, manganese, selenium, and zinc or not (CON) approximately 30 d before breeding, both experiments used a $2 \times 2$ factorial design. In experiment 1, heifers were synchronized using a 14 d CIDR-PG protocol or 5 d Co-Synch plus CIDR protocol and assigned as either TMI or CON. In experiment 2, heifers were synchronized using the 7 d Co-Synch plus CIDR protocol and artificially inseminated using conventional or sexed semen and assigned as TMI or CON. In experiment 2, liver trace mineral concentrations were measured 24 d post injection to determine storage of injected trace minerals. Use of a TMI did not affect liver concentrations of Mn ($P = 0.85$) or Zn ($P = 0.19$) but did increase Cu ($P = 0.02$) and Se ($P = 0.01$) in experiment 2. However, effect on reproduction was inconsistent. In experiment 1, TMI did not affect AI pregnancy rate ($P = 0.94$). However, TMI increased overall pregnancy ($P = 0.02$) in experiment 1. In experiment 2, neither AI pregnancy ($P = 0.85$) nor overall pregnancy was affected ($P = 0.95$) by TMI. In experiment 1 heifer body weight (BW) was influenced by TMI, heifers receiving injections tended ($P = 0.09$) to have greater BW 88 d after injection than CON heifers. However, in experiment 2, TMI did not affect BW ($P = 0.58$) 98 d post injection. These data suggest that use of a TMI has inconsistent effects on reproduction and heifer growth but is an adequate way to enhance liver Cu and Se concentrations.

Keyword: beef heifer, pregnancy, estrus synchronization, injection, trace mineral
Introduction

It is well known that trace minerals are important for health, growth and reproductive performance of cattle. Reduction in pregnancy rates can occur as a subclinical effect of marginal trace mineral status (Hostetler et al., 2003). Deficiencies in Cu, Se, Mn, and Zn have been linked to abnormal estrus cycles, impaired ovulation and decreased conception rates (Hostetler et al., 2003; Underwood, 1981; and Wilson, 1952). In cattle with marginal trace mineral status, supplementation of trace minerals via an injection has the potential to improve reproductive performance.

In research the use of injectable trace minerals to supplement beef cattle, has improved reproductive performance of both cows and heifers. In beef heifers that were provided a free choice supplement, those which received an injection had a greater conception rate to timed embryo transfer when compared to control heifers (Sales et al., 2011). In beef cows grazing native range with access to a free choice supplement, cows receiving a trace mineral injection pre and post-partum exhibited higher conception rates to fixed timed artificial insemination over a control group (Mundell et al., 2012).

The objective of this study was to determine the effects of a trace mineral injection on conception to AI and overall pregnancy rates of beef heifers when administered approximately 30 days prior to initiation of the breeding season.

Materials and Methods

Two experiments were conducted at the Nancy M Cummings Research Extension and Education Center in Carmen, ID. Crossbred (Angus, Hereford and Simmental) beef heifers were retained from the university’s research cow herd. The trace mineral status of...
this herd during the two years that this study was conducted was adequate in Cu and Zn and marginal in Mn and Se (Brasche et al., 2015). In both experiments average initial BW of the heifers (approximately 30 days prior to the start of the breeding season) was between 55-60% (334 -364 kg) of the mature BW of the cow herd (608 ± 55 kg at a 5.5 BCS).

Experiment 1

Beef heifers (n = 109) were used in a 2×2 factorial design with two synchronization protocols (5 d Co-Synch plus CIDR vs. 14 d CIDR-PG) and either a trace mineral injection (TMI) or no injection (CON). Heifers were assigned to one of four 25 head pens and TMI treatment was represented within each of the four pens. Heifers in two of the pens were synchronized with the 5 d Co-Synch plus CIDR and heifers in the remaining two pens were synchronized using the 14 d CIDR-PG. Heifers were fed an alfalfa hay and wheat middling based total mixed ration (Table 1) that contained a liquid mineral supplement for ad libitum intake. Body weights were recorded on d 0 of the trial when treatments were administered and at pregnancy check in June, 88 days after TMI.

Thirty-three days prior to AI, heifers in the TMI group (n = 53, BW 356 ± 2.7 kg) were given 0.86 mL / 68 kg of BW of Multimin 90 (Table 2). For those heifers receiving the 14 d CIDR-PG protocol (n = 27 CON and 28 TMI), a controlled internal drug release device (CIDR, containing 1.38 g of progesterone; Eazi-Breed CIDR, Zoetis Animal Health, New York, NY) was inserted 33 d prior to insemination and was removed 14 d later. Prostaglandin F$_{2\alpha}$ (PG; 25 mg; Lutalyse, Phizer Animal Health) was injected 16 d after CIDR removal and heifers were administered an injection of gonadotropin releasing
hormone (GnRH, 43 µg/mL; Fertagyl, Merck Animal Health) and artificially inseminated 73 h later. For heifers receiving the 5 d protocol (n = 27 CON and 27 TMI), a CIDR was inserted 7 d prior to AI and an injection of GnRH was given. Five d later the CIDR was removed and a PG injection was given. A second PG injection was given 5.6 h later and heifers were artificial inseminated 55 h after the last PG injection. All heifers received an injection of GnRH concurrently with AI. Heifers were inseminated randomly by one of two technicians using one of two AI sires. Pregnancy to AI was determined using the equation [(n pregnant to AI / total n synchronized) × 100].

Following AI breeding, heifers were managed as one group and grazed orchard grass pastures which has previously been observed (Brashe et al., 2015) to have marginal Cu (8.7 mg/kg) and high concentrations of the antagonist Mo (2.2 mg/kg), adequate concentrations of Mn (77 mg/kg), low Se (0.04 mg/kg) and marginal Zn (25 mg/kg). Heifers had access to a free choice mineral containing 2000 mg Cu/kg with 25% being from Cu proteinate and 75% Cu sulfate, 38 mg Se/kg from sodium selenite and 2000 mg Zn/kg with 25% being from Zn proteinate and 75% Zn sulfate. Heifers were exposed to 3 fertile bulls for natural-service breeding 9 d after AI for a 46 d period. Pregnancy to AI [(n pregnant to AI / n synchronized × 100] was determined on d 88 by ultrasonography and BW was recorded of all heifers. Those pregnant 55 days post AI [(17/27) 14d CIDR-PG + CON, (25/28) 14d CIDR-PG + TMI, (21/27) 5 d Co-Synch plus CIDR + CON, (20/27) 5 d Co-Synch plus CIDR + TMI, respectively] were separated from open heifers. Open heifers were left with bulls for 20 more d. Overall pregnancy rate was determined at 105 d post AI by palpation (Figure 1). One hundred and seventy days after TMI liver biopsies were collected from heifers to access liver trace mineral status (Figure 1). Liver
biopsies were collected using the method of Engle and Spears (2000). Method of liver biopsy tissues for trace mineral analysis follows same procedures described by Brasche et al. (2015).

**Experiment 2**

In experiment 2, consecutive day weights were collected 7 days prior to the start of the trial and heifers (n = 112) were stratified by BW and randomly assigned to treatment in a 2×2 factorial design where they were given either a trace mineral injection (n = 56; TMI) or no trace mineral injection (n = 56; CON) and inseminated using sexed semen (n = 55; CON = 29, TMI = 26) or conventional semen (n = 53; CON = 27, TMI = 26). Heifers were fed a total mixed ration ad libitum that contained a liquid mineral supplement (Table 1). Heifers were penned in three groups with treatments stratified across pens. On d 0 of the trial heifers (BW 356 ± 3.7 kg) were given 0.96 ml/68 kg of BW of TMI. Liver biopsies were taken from randomly selected heifers (n = 20; 10 TMI, 10 CON) 24 days after the day of injection to determine trace mineral status (Figure 2).

On d 30 of the trial a CIDR was inserted intra-vaginally into all heifers concurrently with an injection of GnRH. The CIDR was removed 7 days later (d 36) and a heat patch was placed onto the tail head of all heifers. Forty-eight hours later heifers (n = 32 CON, n = 34 TMI) were inseminated using either conventional semen or sexed semen and were randomly inseminated by one of two technicians. There was one conventional and one sexed semen sire used. Heifers that were not exhibiting estrus (no rub on their heat patch; n = 12 CON, n = 11 TMI) at this time were given an injection of GnRH and inseminated 20 h later. Three bulls were co-mingled with the herd 13 d later (d 51) and
left with the herd for 47 days. On d 64 (26 d post AI) heifers were moved to irrigated pastures where they remained for the rest of the trial. Pregnancy was determined using ultrasonography at 60 d post AI and BW of all heifers were recorded. Overall pregnancy was determined at 111 d post AI by rectal palpation.

Statistical Analysis

Significance was declared at $P \leq 0.05$, tendencies are discussed when $P > 0.05 \leq 0.15$ and covariates were used when $P \leq 0.20$. Interactions with $P > 0.20$ were removed from the model.

In experiment 1, BW and liver mineral concentrations were evaluated using PROC MIXED (SAS Inst., Inc., Cary, NC) and included the fixed effect of TMI and synchronization protocol. Reproductive response data was analyzed using logistic regression (PROC GENMOD; SAS Inst., Inc., Cary, NC). The model used to determine effects on AI pregnancy rate and overall pregnancy of heifers included the fixed effects of TMI, synchronization protocol and their interaction. Interaction of TMI $\times$ synchronization protocol was not significant ($P = 0.16$) and removed from the model. Covariate of AI technician was tested for analysis of AI pregnancy and removed ($P = 0.61$). Covariate of AI sire was used as a covariate for analysis of AI pregnancy ($P = 0.01$).

In experiment 2, BW and liver mineral concentrations were evaluated using PROC MIXED and included the fixed effect of TMI. Reproductive response data was analyzed using logistic regression. To determine differences in AI pregnancy and overall pregnancy the fixed effect of TMI, semen type, delayed breeding and their interactions
were used. The covariate of AI technician was tested for AI conception but was not significant and was removed from the model. For AI pregnancy there were no significant interactions \( P > 0.37 \) and all interactions were removed from the model. For overall pregnancy interactions between TMI × semen type × delayed breeding was not significant \( P = 0.64 \) and was removed from the model. Also for overall pregnancy interaction of TMI × semen type was not significant \( P > 0.30 \) and was removed from the model.

**Results and Discussion**

*Experiment 1*

When liver concentrations were determined 170 d after injection there was no difference \( P > 0.41 \) in Cu, Se or Zn among TMI and CON (Table 3). However, there was an interaction between synchronization protocol and TMI for liver concentrations of Se \( P = 0.03 \) and Zn \( P = 0.12 \). Within the group of heifers that were synchronized using the 5 d Co-Synch plus CIDR protocol there was a tendency \( P = 0.11 \) for CON heifers to have a greater liver concentration of Zn \( 98 \pm 4.0 \text{ mg/kg DM}, \text{CON vs TMI}, \text{respectively} \). The opposite was observed in liver concentrations of Se where TMI heifers synchronized with the 5 d Co-Synch plus CIDR tended \( P = 0.06 \) to have increased concentrations of Se \( 0.98 \pm 1.44 \pm 0.16 \text{ mg/kg DM}, \text{CON vs TMI}, \text{respectively} \). Also for those heifers in the TMI group there was a tendency \( P = 0.08 \) for heifers synchronized using the 5 d Co-Synch plus CIDR \( 1.44 \pm 0.164 \text{ mg/kg DM} \) to have a greater liver Se concentrations than 14 d synchronized heifers \( 1.00 \pm 0.164 \text{ mg/kg DM} \). Manganese liver concentration tended \( P = 0.07 \) to be greater in CON heifers than
TMI (Table 3). This trend was observed in other animals that were being utilized in similar trials at this location. In cows that were administered trace mineral injections twice a year; once prior to calving and again 30 days prior to the start of the breeding season, there was also a tendency for control cows to have a higher Mn liver concentration during the breeding season, 15 d post TMI (Brasche et al., 2015). It is important to note that the heifers in this trial as well as the cows in the previous experiment (Brasche et al., 2015) were not being supplemented Mn in their free choice mineral during grazing (d 42 -170). However, heifers in this experiment were being provided Mn in their ration during the AI breeding season while in the dry lot. The authors have speculated that the injection may have increased biliary excretion of Mn therefore lowering the Mn status of TMI animals. In trials investigating the use of TMI in feedlot steers consuming total mixed rations TMI increased liver Mn concentrations at 15 d post injection (Pogge et al., 2012) but not at 29 d post injection (Genther and Hansen, 2014).

As designed the initial BW of heifers was not different between synchronization protocols ($P = 0.46$; $396 \pm 4.4$ kg BW for 5 d Co-Synch plus CIDR protocol and $392 \pm 4.3$ kg BW for 14 d CIDR-PG protocol) or injection treatments ($P = 0.82$; Table 5). At pregnancy check (88 d post TMI) heifers that had received TMI tended ($P = 0.09$) to have a greater BW than CON heifers (Table 5). Use of a similar TMI in highly stressed, newly received beef heifers ($199 \pm 6.4$ kg) being fed a corn based diet in a feedlot have observed similar results (Richeson and Kegley, 2011). In a 55 d trial, heifers receiving injections containing Cu, Se, Mn and Zn had 10 to 12 kg greater final BW (Richeson and Kegley, 2011).
There was no interaction \((P = 0.16)\) between synchronization protocol and TMI for AI pregnancy rate. Also AI pregnancy rate was not affected \((P = 0.94)\) by TMI (Figure 3). There was no interaction \((P = 0.18)\) between synchronization protocol and TMI for overall pregnancy rate. However, there was a significant effect of TMI \((P = 0.02)\) on overall pregnancy rate with TMI heifers having a greater pregnancy rate than CON heifers after AI and exposure to clean-up bulls (Figure 4).

Experiment 2

There was no difference in liver concentrations of Mn \((P = 0.85)\) or Zn \((P = 0.19)\) between CON and TMI heifers when analyzed 24 days after injection (Table 5). However, TMI heifers did have significantly elevated liver concentrations of Se \((P < 0.01)\) and Cu \((P = 0.02)\) when compared to CON heifers. Both CON and TMI heifers were adequate in the trace minerals under investigation with the exception of Mn, in which both groups were marginally deficient (Table 4). Similar increases in liver concentrations of Cu and Se but not Mn and Zn have previously been reported when liver trace mineral concentrations were measured 29 d post-injection in steers \((BW = 309 \pm 14\ kg)\) receiving Multimin 90 at 1 mL / 68 kg BW (Genther and Hansen, 2014). Liver Cu and Se are good indicators of an animal’s status as the liver is a major storage organ for these minerals (Claypool et al., 1975; Kincaid, 2000). However, liver Zn is not a sensitive measure of status as it is active in over 300 metalloenzymes in the body and therefore status better measured in enzyme activity rather than liver concentration (McCall et al., 2000). As Mn is transported through the blood, the liver removes Mn and excretes excess via bile leaving little Mn stored in the liver (Kincaid, 2000).
As designed, BW of heifers was not different between AI semen type ($P = 0.99$) or TMI and CON at the initiation of experiment 2 ($P = 0.81$) or at pregnancy check ($P = 0.58$) 98 d post TMI (Table 6). When heifers were inseminated using sexed or conventional semen there was no interaction ($P = 0.37$) between semen type and TMI on pregnancy to AI. There was no interaction ($P = 0.39$) between delayed breeding and TMI on AI pregnancy. There was no difference ($P = 0.39$) between TMI and CON in AI pregnancy (Figure 5). For overall pregnancy there was a tendency ($P = 0.13$) for an interaction between TMI and delayed breeding. However, overall pregnancy was not significantly influenced ($P = 0.95$) by TMI (Figure 6).

The use of the TMI had variable results in these experiments. In experiment 1, TMI had no effect on AI pregnancy rate when heifers were synchronized using the 14 d CIDR-PG protocol or 5 d CIDR-PG protocol. In experiment 2, when a 7 d CIDR protocol was used TMI did not affect AI pregnancy. In experiment 1, overall pregnancy of heifers was increased by TMI but not in experiment 2.

Previously published research reported that when cows and heifers were grazing native range with access to free choice minerals and received a TMI at pre-calving and 30 d pre-breeding, they had a greater pregnancy to fixed timed AI than cows not receiving a TMI, however, no effect on overall pregnancy was observed (Mundell et al., 2012). It should be noted that prior to the initiation of Mundell et al. (2012) study pretreatment serum trace mineral concentrations for Cu, Se and Zn suggested that animals were likely marginally deficient in these minerals and were deficient in Mn. In experiment 1 and 2 heifers were marginally deficient in Mn.
Supplementation of high amounts of organic trace minerals (11 mg Cu/kg, 31 mg Zn/kg, 18 mg Mn/kg, and 1.1 mg Co/kg) to cows resulted in greater pregnancy to AI after 7 months of supplementation than cows supplemented high (11 mg Cu/kg, 31 mg Zn/kg, 18 mg Mn/kg, 1.1 mg Co/kg) or low (n = 99; 5 mg Cu/kg, 22 mg Zn/kg, 12 mg Mn/kg, 0.11 mg Co/kg) amounts of inorganic trace minerals (Stanton et al., 2000). Stanton et al. (2000) observed that 60 d after the initiation of supplementation cows receiving high levels of organic or inorganic trace minerals had greater liver Cu concentrations than those animals receiving low levels of inorganic trace minerals; however all of the animals sampled were deficient in Cu (highest mean reported was 25 mg/kg DM). These data suggest that effects on reproductive performance as a result of trace mineral supplementation is likely dependent on current trace mineral status and when cattle have adequate status supplementation is unlikely to be beneficial.

**Implications**

The use of a trace mineral injection containing Cu, Mn, Se, and Zn increased the liver Cu and Se concentrations 25 d post injection but did not affect Mn or Zn. However, increases in reproductive performance were inconsistent and are likely affected by initial trace mineral status. Use of a trace mineral injection should be considered under situations where cattle may suffer from trace mineral deficiencies caused by inadequate consumption or antagonisms in the forage as these deficiencies have been linked to reduced reproductive performance. However, no benefit is likely to occur in cattle with adequate status.
Literature Cited


### Table 1. Ingredient and chemical composition of diet fed in experiment 2 while heifers were in the drylot (prior to and during artificial insemination)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%, DM Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass / Alfalfa Hay Mix</td>
<td>69.2</td>
</tr>
<tr>
<td>Wheat Middlings</td>
<td>23.6</td>
</tr>
<tr>
<td>Liquid supplement(^1)</td>
<td>7.2</td>
</tr>
<tr>
<td>Analyzed composition(^2)</td>
<td></td>
</tr>
<tr>
<td>CP, %</td>
<td>16.2</td>
</tr>
<tr>
<td>NEm, Mcal/kg DM</td>
<td>1.28</td>
</tr>
<tr>
<td>NEg, Mcal/kg DM</td>
<td>0.71</td>
</tr>
<tr>
<td>Mn, mg/kg</td>
<td>117</td>
</tr>
<tr>
<td>Zn, mg/kg</td>
<td>111</td>
</tr>
<tr>
<td>Cu, mg/kg</td>
<td>28</td>
</tr>
<tr>
<td>Se, mg/kg</td>
<td>0.4</td>
</tr>
</tbody>
</table>

\(^1\) A liquid supplement containing rumensin (348.8 g / 909 kg) provided trace minerals via manganese sulfate (49 mg Mn /kg diet DM), zinc sulfate (60 mg Zn/kg diet DM), copper sulfate (18.4 mg Cu/kg diet DM), sodium selenite (0.36 mg Se/kg diet DM) and vitamins A (53,781 IU/kg), D (3,841 IU/kg), and E (82 IU/kg).

\(^2\) Analysis conducted by Cumberland Valley Analytical Services, Hagerstown, MD.
Table 2. Composition of injectable trace-mineral solution (Multimin® 90)

<table>
<thead>
<tr>
<th>Item</th>
<th>mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>15</td>
</tr>
<tr>
<td>Manganese</td>
<td>10</td>
</tr>
<tr>
<td>Selenium</td>
<td>5</td>
</tr>
<tr>
<td>Zinc</td>
<td>60</td>
</tr>
</tbody>
</table>

Multimin USA, Ft. Collins, CO.
Table 3. Effect of trace mineral injection (TMI) or no TMI (CON) on liver trace mineral concentration\(^1\) at 170 days after TMI in experiment 1

<table>
<thead>
<tr>
<th>Item</th>
<th>Adequate Level(^2)</th>
<th>CON</th>
<th>TMI</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper, mg/kg</td>
<td>125 - 600</td>
<td>240</td>
<td>227</td>
<td>32.4</td>
<td>0.42</td>
</tr>
<tr>
<td>Manganese, mg/kg</td>
<td>&gt; 13</td>
<td>11.5</td>
<td>10.5</td>
<td>0.38</td>
<td>0.07</td>
</tr>
<tr>
<td>Selenium(^3), mg/kg</td>
<td>1.25 – 2.5</td>
<td>1.2</td>
<td>1.2</td>
<td>0.12</td>
<td>0.67</td>
</tr>
<tr>
<td>Zinc(^3), mg/kg</td>
<td>40 - 200</td>
<td>94</td>
<td>91</td>
<td>2.8</td>
<td>0.48</td>
</tr>
</tbody>
</table>

1 Concentrations based on a dry matter basis  
2 Adequate defined by Kincaid (2000)  
3 Interaction of TMI \(\times\) synchronization protocol for Zn \((P = 0.12)\) and Se \((P = 0.03)\)
Table 4. Effect of trace mineral injection (TMI) or no TMI (CON) on liver trace mineral status\(^1\) 24 d after pre-breeding injection in experiment 2

<table>
<thead>
<tr>
<th>Item</th>
<th>Adequate Level(^2)</th>
<th>CON</th>
<th>TMI</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper, mg/kg</td>
<td>125- 600</td>
<td>292</td>
<td>371</td>
<td>22.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Manganese, mg/kg</td>
<td>&gt; 13</td>
<td>11.5</td>
<td>11.2</td>
<td>0.83</td>
<td>0.85</td>
</tr>
<tr>
<td>Selenium, mg/kg</td>
<td>1.25 – 2.5</td>
<td>1.5</td>
<td>2.3</td>
<td>0.09</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Zinc, mg/kg</td>
<td>40 - 200</td>
<td>95</td>
<td>101</td>
<td>3.3</td>
<td>0.19</td>
</tr>
</tbody>
</table>

\(^1\) Concentrations on a dry matter basis
\(^2\) Adequate defined by Kincaid (2000)
Table 5. Comparison of trace mineral injection (TMI) or no injection (CON) on heifer body weight

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TMI</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW(^1), kg (d 0)</td>
<td>357</td>
<td>358</td>
<td>3.7</td>
<td>0.83</td>
</tr>
<tr>
<td>June BW(^2), kg (d 88)</td>
<td>421</td>
<td>431</td>
<td>4.2</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW(^1), kg (d 0)</td>
<td>343</td>
<td>344</td>
<td>3.8</td>
<td>0.82</td>
</tr>
<tr>
<td>June BW(^3), kg (d 98)</td>
<td>436</td>
<td>439</td>
<td>4.1</td>
<td>0.58</td>
</tr>
</tbody>
</table>

\(^1\)Body weight at time of injection

\(^2\) Body weight was recorded 55 days after AI breeding

\(^3\) Body weight recorded 60 days after AI breeding
Figure 1. Timeline for experiment 1. Heifers received a trace mineral injection (TMI) or not (CON) on d 0, heifers were synchronized followed by artificial insemination (AI) on d 33. On d 42 heifers were exposed to fertile bulls, 46 d later pregnancy was determined and non-pregnant heifers remained with bulls for 20 more days. Overall pregnancy was determined on d 138 and on d 170 liver biopsies were performed.
Figure 2. Timeline of experiment 2. On d 0 heifers received a trace mineral injection (TMI) or no injection (CON). Liver biopsies were performed 23 d post injection and all heifers were synchronized beginning on d 29 with a 7 d CIDR protocol. Heifers were artificially inseminated (AI) on d 37 and 13 days later exposed to fertile bulls for 47 days. On d 97 pregnancy was determined using ultrasonography and overall pregnancy was determined on d 148 using rectal palpation.
Figure 3. Effect of trace mineral injection (TMI) or no injection (CON) on conception to artificial insemination (AI) in experiment 1.
Figure 4. Effect of trace mineral injection (TMI) or no injection (CON) on overall pregnancy after artificial insemination (AI) and exposure to bulls in experiment 1.
Figure 5. Effect of trace mineral injection (TMI) or no TMI (CON) on conception to artificial insemination (AI) when synchronized using a 7 d CIDR in experiment 2.
Figure 6. Effect of trace mineral injection (TMI) or no TMI (CON) on overall pregnancy when heifers were delay bred at artificial insemination (AI). There was a tendency for an interaction between delayed breeding × TMI ($P = 0.13$).
Chapter V

Effect of a trace mineral injection on pregnancy rate of Angus beef heifers when synchronized using the 14 day CIDR-PG protocol at a commercial feedlot

C. J. Brasche,* J.B. Hall, † PAS, S. Harrison, § M.E. Drewnoski,*

*Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908;
†Department of Animal and Veterinary Science, University of Idaho, Moscow, ID 83844;
§Riverbend Ranch, Idaho Falls, ID 83402
Abstract

Purebred Black Angus (PBA, n = 207, BW = 347 ± 3.0 kg) and commercial Black Angus heifers (CBA, n = 529, BW = 335 ± 2.3 kg) were used to examine the effect of an injectable trace mineral (TMI) providing Cu, Mn, Se and Zn on reproductive performance. Heifers were fed a ration supplemented with copper (10 mg/kg DM), manganese (15 mg/kg DM), selenium (0.17 mg/kg DM), and zinc (55 mg/kg DM). As heifers were processed 30 d prior to breeding alternating heifers through the chute received a 4 mL TMI (0.53/45 kg BW) or no injection (CON). Heifers were synchronized using a 14 d controlled internal drug-releasing (CIDR) plus prostaglandin F$_2$α protocol and timing of artificial insemination was determined using heat patches. Second service AI (SSA) was performed on CBA that were in heat after receiving GnRH and a 7 d CIDR. For PBA, there was no difference ($P = 0.67$) in overall AI conception between CON (52%) and TMI (52%). Conception of CBA to first service AI did not differ ($P = 0.52$) between the CON (55%) and TMI (50%) and there was no difference in the proportion of CBA (21 vs. 22% for CON and TMI, respectively). Overall pregnancy rate of CBA after AI and exposure to bulls for 27 days did not differ ($P = 0.53$) between the CON (87%) and TMI (85%). These data suggest that TMI has limited impact on reproductive performance of heifers being fed adequate concentrations of trace mineral in the diet.

Keyword: beef heifer, conception, estrus synchronization, trace mineral
Introduction

Trace minerals such as Cu, Mn, Se, and Zn play an important role in hormone synthesis, which is vital for reproductive performance (Paterson and Engle, 2005). A deficiency in copper can lead to decreased conception rates, infertility, anestrus, and fetal resorption (Hostetler et al., 2003). Zinc deficient cows appear to display abnormal estrus as well as experience a decrease in fertility (Underwood, 1981). Manganese deficiency will result in impaired ovulation (Wilson, 1952). A deficiency in Se can lead to cystic ovaries and erratic, weak or silent heat periods (Hostetler, 2003).

When mineral is supplied free choice, individual animal intake is highly variable (Olson, 2007). The use of an injectable trace mineral is a direct method of supplementation in which each individual animal receives a known amount of a mineral. In some studies a trace mineral injection (TMI) containing Cu, Mn, Se, and Zn has been seen to improve reproductive performance. Beef heifers being provided a free choice mineral supplement that received a TMI had a greater conception rate to timed embryo transfer when compared to control heifers (Sales et al., 2011). In a study performed by Mundell et al., (2012) beef cows receiving pre and post-partum TMI had improved conception to fixed time AI over a control group, when both groups were grazing native range and had access to free choice mineral. However, there has been little research to understand the effects that a TMI may have on conception to AI in beef heifers. The objective of this trial was to determine the effects of a trace mineral injection on reproduction of Black Angus heifers when administered approximately 30 days prior to initiation of the breeding season.
Materials and Methods

All procedures were approved by the University of Idaho Animal Care and Use Committee. This study was performed from March 2014 to August 2014 on a Black Angus Ranch (Riverbend Ranch) in Dillon, MT. One group of purebred Black Angus heifers (PBA, n = 207) as well as a second group of commercial Black Angus heifers (CBA, n = 529) were used in this study. Both groups were approximately 14 months of age at time of injection and were being housed in pens of approximately 100 head being fed a hay and barley silage-based total mixed ration (Table 1) that included a mineral supplement containing copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn).

Experiment 1. Purebred Heifers

The BCS (1 = emaciated, 9 = obese), BW, and reproductive tract score (RTS) of PBA heifers were determined 33 days prior to the start of AI. Reproductive tract scores were determined by the attending veterinarian using a scoring system he developed. Heifers were scored as: 1 – immature reproductive tract lacking tone of uterine horns and no palpable follicles, 2 – not currently cycling, but likely to reach puberty within 4 to 6 weeks, having slight tone of uterine horns and 8-10 mm follicles and 3 – mature/cycling reproductive tract presenting good tone in uterine horns and corpus luteum possible or present. Immediately after RTS heifers received an intra-vaginal controlled internal drug-releasing device (CIDR; 1.38 g of progesterone; Eazi-Breed CIDR, Zoestis Animal Health, New York, NY), and every other heifer that went through the chute was administered a 4 mL injection (n = 104, 0.52 ± 0.050 mL / 45 kg BW) of TMI containing 15 mg/mL Cu, 10 mg/mL Mn, 5 mg/mL Se and 60 mg/mL Zn (d 0). Heifers that did not
receive an injection of the trace minerals were considered controls (n = 103, CON). On d 14, the CIDR was removed from each heifer. Sixteen days later, (d 30) heat patches (Estrotec™, Rockway Inc., Spring Valley, WI) were placed according to manufacturer instructions onto the tail head of all heifers, and they received an injection of prostaglandin F₂α (PG; 25 mg; Lutelyse, Zoetis Animal Health, New York, NY). Seventy-two hours later (d 33), heifers with a fully activated heat patch (n =153) were determined to be in estrus and were deemed as responders; (n = 75 CON and n = 78 TMI) and sorted from heifers (n = 54) not presenting signs of estrus (partial activation or no activation of their heat patch) and deemed as non-responders (n = 28 CON and n = 26 TMI). Non-responders were injected with gonadotropin-releasing hormone (GnRH; 43 µg/mL; Fertagyl, Merck Animal Health, Omaha, NE) and penned together for 3 hours. Responders were inseminated between 73-76 hours after PG injection and received an injection of GnRH concurrently with AI. Non-responders were then inseminated 76 h after PG injection on d 33. Heifers were inseminated by the same certified AI technician to one of six AI sires (all sires were used in both treatment groups). Heifers were fed a total mixed ration (TMR) containing adequate trace minerals and 33 days later (d 66) conception was determined using ultrasonography. The protocol timeline for PBA heifers is illustrated in Figure 1.

Experiment 2. Commercial Heifers

On d 0 of the trial, the BW and BCS of CBA heifers were recorded and every other heifer that went through the chute was injected with 4 mL of TMI (n = 268, 0.54 ± 0.062 mL/45 kg BW). Heifers that did not receive an injection were considered the controls (n = 261, CON). All heifers received a CIDR at this time. On d 14, the CIDR
was removed from each heifer. Sixteen days later, (d 30) heat patches were placed onto the tail head of all heifers and they received an injection of PG. Seventy-two hours later, (d 33) heifers were separated into two groups of either non-responders or responders, according to their heat patch, as described in experiment one. Heifers designated as responders by 72 h post PG injection were inseminated between 73-76 hours after PG injection and received an injection of GnRH concurrently with AI (Group 1). The group of non-responding heifers was then sorted again at 76 h, as some of the heifers had come into estrus during the 4 h period. Heifers that had responded to synchronization between 72 and 76 hours after PG injection were inseminated at 82 hours post-PG injection and given an injection of GnRH concurrently with AI (Group 2). The remaining non-responding heifers were given an injection of GnRH at 76 h post-PG injection and inseminated 17 h later, at 93 hours post PG injection (Group 3). Heifers in Group 1 were randomly inseminated by one of two certified AI technicians, while heifers in Group 2 and 3 were inseminated by a third technician. All heifers were inseminated with the same AI sire.

All heifers received an injection of GnRH and insertion of a CIDR on d 45 to prepare for a second service of AI. Seven days later (d 52), CIDRs were removed. Estrus was detected via a heat patch from d 52 through 57 with any heifer coming into estrus (indicated by an activated heat patch) inseminated (n = 113; 54 CON and 59 TMI) with the same AI sire by the same technician. Heifers that received a second service of AI were transported approximately 160 kilometers to Kilgore, ID on d 57. At this time (d 57), 14 fertile bulls that had passed a breeding soundness exam were co-mingled with the heifers. The remaining heifers were transported to Kilgore, ID on d 61 and were co-
mingled with the other previously transported heifers and bulls. Heifers grazed native range and had access to free choice mineral (BioZyme® Incorporated VitaFerm - Beefmaker, St. Joseph, MO; Product # 56710) for the remainder of the trial. Bulls were removed from the herd 27 days later on d 86. Overall pregnancy was determined using ultrasonography on d 109 and d 110. The protocol timeline for the CBA heifers is illustrated in Figure 2.

Statistical Analysis

Purebred heifer initial measurements of BCS, BW, RTS and response to synchronization were evaluated using a mixed-effects model (PROC MIXED; SAS Inst., Inc., Cary, NC) including the fixed effect of TMI to determine if there was difference caused by the random assignment of heifers to treatment. Purebred heifer reproductive response data was analyzed using multinomial distribution (PROC GLIMMIX; SAS Inst., Inc., Cary, NC). The model for response to synchronization included the fixed effects of TMI, RTS and their interactions. The model used to determine differences in AI pregnancy rates of purebred heifers included the fixed effects of TMI, response to synchronization (responders at 72 h vs non-responders), RTS, AI sire and their interactions. Interactions with $P > 0.20$ were removed from the model. Commercial heifer initial measurements of BCS and BW were evaluated using a mixed-effects model including the fixed effect of TMI to determine if there was difference caused by the random assignment of calves to treatment. Commercial heifer reproductive responses were analyzed using logistic regression. The models for response to synchronization, (Group 1 vs. Group 2 and 3; Group 2 vs. Group 1 and 3; Group 3 vs. Group 1 and 2) included the fixed effects of TMI. The model used to determine effects on first service AI
conception contained the fixed effect of AI technician, TMI, and AI timing based on response to synchronization (Group 1, 2 or 3), and their interactions. The model for second service AI and overall pregnancy rate included the fixed effects of TMI. Interactions with $P > 0.20$ were removed. Treatment differences were discussed when $P \leq 0.05$ and tendencies were discussed when $P > 0.05$ and $\leq 0.15$.

**Results and Discussion**

At the start of the trial, the initial BW of the PBA heifers did not differ ($P = 0.58$) between treatments (347 ± 3.04 kg) but BCS of the CON (5.61 ± 0.037) tended ($P = 0.07$) to be greater than the TMI (5.52 ± 0.036). However, both groups were in good body condition for breeding. The initial BW (335 ± 2.3 kg) and BCS (5.5 ± 0.036) of the CBA heifers did not differ ($P > 0.20$) between treatment groups.

**Reproductive Tract Scoring**

There was no difference ($P > 0.20$) between treatments due to random assignment at the start of the trial in the proportion of PBA heifers with a reproductive tract score of 1, 2, or 3 (1 = 33 vs 34 %, 2 = 35 vs. 27 %, 3 = 32 vs. 39 % for CON and TMI, respectively). However, there was a tendency for an interaction ($P = 0.09$) between treatment and reproductive tract score on AI conception rate (Table 2). There was no difference ($P = 0.80$) in conception rate to AI for the heifers that were exhibiting signs of a mature reproductive tract (Score 3). However heifers determined to have an immature reproductive tract (Score 1) tended ($P = 0.15$) to have lower conception rate to AI when receiving a TMI than CON heifers (53 vs 34% for CON and TMI, respectively). Conversely when heifers were determined to be near maturity (Score 2) the use of a TMI
tended ($P = 0.10$) to increase conception rates 30 days later (47 vs 71% for CON and TMI, respectively). The ability of heifers to reach sexual maturity by the start of the breeding season is dependent on adequate concentration of hormones involved in development of the reproductive tract (Andersen et al., 1991; Day and Andersen, 1998). It has been stated that Cu, Zn, and Mn influence reproduction in mammals, possibly through hormone synthesis (Davis & Mertz, 1987; Hambidge et al., 1986; Hidiroglou, 1979; Hurley & Keen, 1987). In sexually immature heifers the use of a TMI tended to have negative effects on conception to AI, the cause of which is unknown. However, in heifers that were close to reaching sexually maturity it could be hypothesized that the supplemental trace minerals provided through the trace mineral injection could influence hormone synthesis and their ability to reach sexual maturity by the start of the breeding season 30 days later and conceive to AI.

Response to Synchronization

Purebred heifers synchronized with the 14 d CIDR exhibited no difference ($P = 0.41$) in the proportion of heifers that appeared to respond to synchronization by 72 hours after PG injection 73% (75/103) vs. 75% (78/104) % for CON and TMI, respectively. There was no interaction ($P = 0.67$) between RTS and TMI on response to synchronization as well. Commercial heifers were sorted into three groups based on their response to synchronization. There was a tendency for an effect ($P = 0.06$) of TMI treatment on response to synchronization. Control heifers tended to have a greater ($P = 0.06$) response to synchronization, seen as a higher proportion of CON heifers having an activated heat patch at 72 h post-PG injection (Group 1; 80% (208/261) vs. 73% (195/268) for CON and TMI, respectively). There was no difference ($P = 0.38$) in the
proportion of heifers [10% (25/261) and 12% (32/268), CON and TMI, respectively] that came into heat between 72-76 h post PG-injection (Group 2). However, there was a tendency ($P = 0.11$) for more TMI heifers to fail to respond to synchronization by 76 h post PG injection [15% (41/268) vs. 11% (28/261) for TMI vs. CON, respectively].

Estrus synchronization is a beneficial tool for cattle producers due to its ability to shorten the breeding season and create a more concise calving season (Larson et al. 2010). In the study presented purebred heifers exhibited no difference in their response to synchronization due to TMI. However, in commercial heifers the use of a TMI tended to decrease the response to synchronization at 72 h (Group 1) and resulted in more heifers failing to respond to synchronization by 76 h (Group 3). Heifers in both groups were being fed a diet containing trace mineral concentrations above NRC recommendations (Table 1).

**Conception**

Conception to AI for PBA heifers was determined at 33 d post AI. There was no interaction ($P = 0.57$) between response to synchronization and TMI in PBA heifers. There was no difference ($P = 0.67$) in AI conception for PBA heifers between CON 52.4% (54/103) and TMI 51.9% (54/104). When conception to first service AI was determined for CBA heifers there was no interaction ($P = 0.87$) between treatment and their response to synchronization (timing of AI). Conception to first service AI in CBA heifers did not differ ($P = 0.52$) between the CON and TMI (Table 3). However, there was a difference ($P < 0.01$) in pregnancy rates of the three groups based on their
synchronization response, Group 1 (63 ± 2.4 %) was greater ($P < 0.05$) than both Group 2 (26 ± 5.8 %) and Group 3 (12 ± 3.9 %) but Group 2 and 3 did not differ ($P = 0.36$).

There was no difference among treatments in the proportion of CBA heifers that received second service AI [21% (54/261) vs. 22% (59/268) for CON and TMI, respectively] but conception to second service AI was greater ($P = 0.05$) for TMI than CON (Table 3). Because bulls were introduced to the herd immediately following second service AI, the increase in conception of TMI heifers in questionable. Interestingly, the number of CBA heifers pregnant during the first 30 days of the breeding season (first and second service AI plus exposure to bulls for 3 to 7 days) did not differ ($P = 0.78$) among treatments. The increase in conception to SSA of TMI heifers as opposed to the higher conception rate of heifers in Group 1 (heifers responding at 72 h) which included a greater number of CON heifers could explain why the pregnancy rate in the first 30 days was not different between the treatment groups (Table 3). Overall pregnancy rate after AI and exposure to bulls for 27 days did not differ ($P = 0.53$) between the CON and TMI (Table 3).

Reproductive performances of cows and heifers as a result of supplementation of trace minerals have been inconsistent. Of consideration when determining the consequence of trace mineral supplementation on reproduction is the trace mineral status of the animal. Heifers being supplemented with free choice trace minerals meeting NRC requirements that received a TMI had a higher conception rate to timed embryo transfer than control heifers that did not receive TMI (Sales et al., 2011). Mundell et al. (2012) utilized 460 cows and heifers to determine the effects a TMI has on conception rates, animals receiving TMI had an increase in timed AI pregnancy rate over a control group.
The cows and heifers used in Mundell et al. (2012) were thought to be slightly deficient in Cu, Se and Zn determined by standard deviation of pretreatment serum and pretreatment serum Mn levels were below the normal range (Puls, 1994; Herdt and Hoff, 2011). Stanton et al. (2000) reported that cows (n = 100) supplemented with high amounts of organic trace minerals (11 mg Cu/kg, 31 mg Zn/kg, 18 mg Mn/kg, and 1.1 mg Co/kg) had greater pregnancy to AI than cows (n = 100) supplemented with high (11 mg Cu/kg, 31 mg Zn/kg, 18 mg Mn/kg, 1.1 mg Co/kg) or low (n = 99; 5 mg Cu/kg, 22 mg Zn/kg, 12 mg Mn/kg, 0.11 mg Co/kg) amounts of inorganic trace mineral supplements. In the study by Stanton et al. (2000) the cattle receiving high levels of organic or inorganic trace minerals, liver Cu was greater 60 d after the start of supplementation than those animals receiving low levels of inorganic trace minerals, but all animals were still in the deficient range (25 vs 11 mg/kg DM; high vs low, respectively). Ahola et al. (2004) reported that when cattle were grazing native pastures in eastern Colorado control cows receiving no supplemental Cu, Zn, and Mn for one year tended to have reduced (P = 0.10) pregnancy rates (89%; 85/96) after a 60 d breeding season compared to organic trace mineral supplemented (93%; 94/101) and inorganic trace mineral supplemented group (95%, 98/103). The supplemental trace minerals were provided through a free choice mineral. This demonstrates that cattle receiving inadequate amounts of trace minerals could benefit from supplementation.

However, it has also been seen that when cattle receive mineral supplementation above NRC requirements additional supplementation can have a negative impact on reproduction. Olson et al. (1999) found that when two-year old cows were supplemented Cu, Zn, Mn and Co in an inorganic or organic form at approximately twice their
requirement it reduced the cow’s reproductive performance. After a 70 d breeding season there were a greater number) of nonpregnant cows in the over supplemented organic and inorganic groups (n = 11/78, 11/78, and 0/80; inorganic, organic, and control, respectively; Olson et al., 1999). Olson et al. (1999) stated that the cows tended to show less estrual and breeding activity as a result of the over supplementation. When dairy cows and heifers, consuming a diet above NRC requirements for Cu, Mn, Zn, and Se (20, 47, 64, and 0.3 mg/kg of diet DM, respectively), were injected with a trace mineral supplement containing said minerals before calving and prior to breeding there was a decrease in first-service conception of the supplemented females (21.5% conception) compared to females that received no injection (31.5% conception; Vanegas et al., 2004). The results seen by Olson et al. (1999) and Vanegas et al. (2004) demonstrate that over supplementation of trace minerals may reduce the reproductive performance of cattle.

Thus trace mineral status of the animal can significantly affect the outcomes that trace mineral supplementation may have on reproductive performance. In comparison to free choice supplementation, supplementation of trace minerals via a TMR ensures that all animals are receiving the supplement and in the present trial the TMR fed to heifers contained trace minerals above NRC requirements in Se and Zn (Table 1). This may explain why added supplementation via TMI of these minerals did not appear to improve reproductive performance.

**Implications**

Use of a TMI before the start of the breeding season had minimal effect on AI conception of heifers. The trace mineral status of these heifers was not determined during
this trial. But the management practices of the ranch included mineral supplementation in a TMR during their time in dry lot (pre-breeding and during AI). The mineral supplementation of the diet exceeded dietary requirements for Se and Zn providing 55 mg Zn/kg and 0.17 mg Se/kg, provided the requirement for Cu (10 mg/kg) and provided 15 mg/kg of Mn. These data suggest that the use of a trace mineral injection may not improve reproductive performance of heifers being fed an adequate trace mineral supplement.
Literature Cited


Deutscher, D. C. Adams, D. J. Colburn, and A. B. Johnson. 1999. Effects of
supplementation of organic and inorganic combinations of copper, cobalt,
manganese, and zinc above nutrient requirement levels on postpartum two-year


Proc. Dep. of Anim. Sci. UT Ext. and Univ. Prof. Dev., Univ. of Tennessee,
Knoxville.

Clearbrook, BC, Canada.

copper, selenium, zinc and manganese on the pregnancy rate of crossbreds heifers
(Bos indicus x Bos Taurus) synchronized for timed embryo transfer. Livest. Sci.
142: 59-62.

Effects of trace mineral supplementation on cow-calf performance, reproduction,
and immune function. Prof. Anim. Sci. 16:121.

Bureaux.

supplement on first-service conception rate of dairy cows. J. Dairy Sci. 87:3665-
3671.

Wilson, J.G. 1952. Herd functional infertility, with reference to nutrition and mineral
intake. Veterinary Record. 64:621-623.
Tables and Figures

Table 1. Ingredient and analyzed composition of diet fed while heifers were in the dry lot (prior to and during artificial insemination)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%, DM basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley Silage</td>
<td>27.6</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>18.1</td>
</tr>
<tr>
<td>Grass Hay</td>
<td>15.8</td>
</tr>
<tr>
<td>Malt Sprout Pellets</td>
<td>13.4</td>
</tr>
<tr>
<td>Dry Rolled Corn</td>
<td>12.3</td>
</tr>
<tr>
<td>Potato Waste</td>
<td>7.0</td>
</tr>
<tr>
<td>Barley Straw</td>
<td>4.0</td>
</tr>
<tr>
<td>Supplement&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Analyzed composition<sup>2</sup>
- CP, %<sup>4</sup> 11.0
- NEm, Mcal/kg DM<sup>4</sup> 1.34
- NEg, Mcal/kg DM<sup>4</sup> 0.75

<sup>1</sup> A wheat middlings based pellet containing monensin sodium to provide 19.8 mg/kg of diet DM and supplemental Cu sulfate, Mn sulfate, sodium selenite and Zn sulfate to provide 10, 15, 0.17 and 55 mg/kg of Cu, Mn, Se and Zn to the diet DM. <sup>2</sup> Analysis conducted by Cumberland Valley Analytical Services, Hagerstown, MD.
Table 2. Conception to artificial insemination of purebred heifers receiving a trace mineral injection (TMI) or not (CON) with an immature reproductive tract (score 1), a tract that was near maturity (score 2) or a mature tract (score 3) 33 days prior to artificial insemination

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TMI</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 1</td>
<td>53 (18/34)</td>
<td>34 (12/35)</td>
<td>5.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Score 2</td>
<td>47 (17/36)</td>
<td>71 (20/28)</td>
<td>6.2</td>
<td>0.10</td>
</tr>
<tr>
<td>Score 3</td>
<td>58 (19/33)</td>
<td>54 (22/41)</td>
<td>5.8</td>
<td>0.80</td>
</tr>
</tbody>
</table>

1Trace mineral injection treatment × reproductive tract score interaction (P = 0.09).
2SE calculated using Standard Error of Proportion
3Number of animals observed/number of animals evaluated
Table 3. Effect of a trace mineral injection (TMI) or no trace mineral injection (CON) on reproductive performance of virgin black angus commercial heifers

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TMI</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First service AI conception</td>
<td>55 (143/261)</td>
<td>50 (134/268)</td>
<td>3.1</td>
<td>0.69</td>
</tr>
<tr>
<td>Second service AI conception</td>
<td>50 (27/54)</td>
<td>68 (40/59)</td>
<td>6.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Pregnant in first 30 days of breeding season</td>
<td>70 (184/261)</td>
<td>69 (186/268)</td>
<td>2.9</td>
<td>0.78</td>
</tr>
<tr>
<td>Overall pregnancy rate&lt;sup&gt;3&lt;/sup&gt;</td>
<td>87 (227/261)</td>
<td>85 (228/268)</td>
<td>3.3</td>
<td>0.53</td>
</tr>
</tbody>
</table>

<sup>1</sup> SE calculated using Standard Error of Proportion  
<sup>2</sup> Number of animals observed/number of animals evaluated  
<sup>3</sup> Pregnant after AI and exposure to a bull for 27 days
Figure 1. Experimental timeline of purebred heifers. Heifers were administered a trace mineral injection (TMI) or no injection and inserted with an intravaginal controlled internal drug-releasing device (CIDR) on d 0 which was removed on d 14. Heat patches (HP) were placed on the tail head and prostaglandin F$_{2\alpha}$ (PG) was injected on d 30. On d 33 gonadotropin releasing hormone (GnRH) was injected and heifers were artificially inseminated (AI). Pregnancy was determined by ultrasound 33 d after AI.
Figure 2. Commercial heifer’s experimental timeline. Heifers were administered a trace mineral injection (TMI) or no injection and inserted with an intravaginal controlled internal drug-releasing device (CIDR) on d 0. Heifers were synchronized using a 14 d CIDR plus prostaglandin F$_{2\alpha}$ protocol and timing of artificial insemination (AI) was determined using a heat patch (HP). Second service AI was performed on those heifers that were in heat after receiving GnRH and a 7 d CIDR. Heifers were then shipped to range pasture and co-mingled with fertile bulls for 27 days. Pregnancy was determined by ultrasonography 75 days after first service AI.