THE EFFECT OF A SUPPLEMENTAL TRACE MINERAL INJECTION ON DEVELOPING BEEF BULL AND HEIFER REPRODUCTION

by

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Abstract

Trace mineral supplementation is necessary for proper reproductive success. Little research has evaluated the effect of an injectable trace mineral product, in conjunction with a dietary mineral supplementation program, on reproduction. This thesis includes two separate studies evaluating the use of an injectable trace mineral product, in addition to a dietary mineral program, on the reproductive success of yearling bulls and heifers. In the first study, we hypothesized that when dietary trace mineral needs are met, administration of an injectable trace mineral product to developing beef bulls would cause a short-term increase in circulating trace mineral concentrations, but not alter semen quality nor ability to pass a breeding soundness examination. Trace mineral treatment did not affect scrotal circumferences and BW of bulls throughout the trial ($P \geq 0.20$). Trace Mineral bulls had greater ($P \leq 0.0001$) trace mineral concentrations at 8 h post-treatment than Control bulls. Semen trace mineral concentrations on d 42 and 91 were similar ($P \geq 0.52$) between treatments. Sperm parameters improved ($P \leq 0.003$) from d 42 to 91, but did not differ ($P \geq 0.06$) between treatments. A similar ($P = 0.94$) percentage of Trace Mineral (67%) and Control (68%) bulls passed a BSE 91 d post-treatment. In the present study, supplemental trace mineral injection was successful at raising circulating trace mineral levels, but did not alter semen trace mineral levels nor improve semen quality. In the second study we hypothesized that when dietary trace mineral needs are met, the use of an injectable trace mineral product in developing heifers would not affect pregnancy rates at single service fixed-time artificial insemination (FTAI). Trace Mineral heifers had greater ($P = 0.02$) pregnancy rates (51.28%) than Control heifers (25.58%). The percentage of Trace Mineral (30.77%) and Control heifers (47.50%) that displayed estrous behavior prior to FTAI as indicated by a red estrous detection patch was not different ($P = 0.13$) between treatments. In the

present study, despite dietary trace mineral requirements being met, use of an injectable trace mineral injection improved pregnancy rates following FTAI, but did not affect estrous behavior.

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Chapter 1 - Literature Review

Role of trace minerals in beef cattle diets

Trace minerals are dietary elements required by the body in minute amounts, ranging from 0.10 to 50.0 mg/kg dry matter in beef cattle diets (NRC, 2000). Care must be taken to meet, but not to overly exceed, requirements to prevent deficiency and toxicity. Of the 15 total trace minerals, 8 are considered when evaluating a large ruminant diet. To ensure that an animal is meeting its trace mineral requirements, one must understand the two ways by which deficiency can occur. The first type of deficiency, a dietary deficiency, occurs when the animal is not consuming adequate levels of trace mineral to meet requirements. The second type, deficiency due to dietary interaction, involves proper intake but reduced mineral bioavailability. Common trace minerals potentially deficient in grazing beef cattle diets include cobalt (Co), copper (Cu), iron (Fe), iodine (I), manganese (Mn), selenium (Se), and zinc (Zn; Corah and Ives, 1991).

These trace minerals are all necessary for the biochemical processes of the body that support proper growth and maintenance. For example, Cu is necessary for the function of superoxide dismutase and its removal of toxic byproducts from metabolic pathways (Underwood, 1981). The removal of these toxic byproducts allows for metabolism to proceed efficiently, uninhibited by damaging oxygen free radicals. Zinc, an important trace mineral for enzyme function, aides in the regulation of nucleic acid production, carbohydrate metabolism, and protein synthesis thus providing a stable framework for development (Smart et al., 1981).

Trace minerals also play an important role in regulating reproduction, specifically fertility and gonadal development. In females, Mn functions to properly regulate synthesis of ovarian hormones (Hidrioglou, 1979). Copper and Zn are required to prevent anestrus, and adequate Se levels can reduce the risk of a retained placenta (Julien and Conrad, 1976; Hidiroglou, 1979). In

males, a Zn deficiency can lead to delayed puberty and under-developed testes (Martin et al., 1994). Although the quantity of trace minerals consumed in the diet is very small, the impact trace minerals have on bodily functions cannot be overlooked. Not only are trace minerals important for the biochemical process of the body, but they are also important for the efficiency and productivity of the animal as a whole. Without proper trace mineral consumption, reproductive, developmental and immunological functions can suffer, leading to economic losses.

Supplementation

Forage-based grazing diets typically require mineral supplementation to avoid deficiency. In areas were the available trace mineral content from the forage is under the recommended requirement, supplementation will be necessary. Forage deficiency of trace minerals typically follows a regional pattern due to soil chemistry and characteristics (Fig. 1.1; Reid and Horvath, 1980). Forages from states in the Pacific Northwest and the Atlantic regions are typically deficient in Se and Cu, whereas northeastern states are often deficient in Zn. States in the Midwest commonly experience a combination of Cu and Zn deficiencies (Kubota et al., 1987). In a survey compiled by the National Animal Health Monitoring System in 1996 to evaluate the trace mineral status of various forages across the United States, researchers reported that in 352 forage samples from 18 states, 14.2% of samples were below the NRC requirements for Cu content and 49.7% of samples met Cu content requirements, but did not exceed them. Copper inadequacies in this study can be attributed to various interactions with high levels of forage Fe and Mo, both of which can decrease availability of Cu in the diet. Even more concerning, only 2.5% of forage samples tested adequate in Zn content (Corah et al., 1996).

Various forms of trace mineral supplementation are commercially available to the beef cattle producer. Options include: *ad libitum* salt-based free-choice blocks or loose mineral, trace mineral pre-mixes added to rations, oral boluses, and more recently, injectable trace mineral boluses. In a grazing situation, the most commonly used form is a free-choice, salt-based granular supplement (Greene, 2000). Salt-based trace mineral blocks are hard pressed blocks containing sodium chloride and various minerals. These trace mineral blocks offer both convenience and ease of use. The product is placed in a pasture and the cattle consume trace mineral *ad libitum*. Trace mineral blocks work well with grazing cattle, but it is difficult to ensure adequate consumption. Although cattle are unable to balance their mineral requirements while being supplemented in an *ad libitum* form, there are few other practical supplementation programs for grazing situations (McDowell, 1985).

Grazing cattle operations also have the option of free-choice, loose mineral supplementation. Loose mineral is typically easier for the animal to consume because it is not in a hard, block form, but weather loss can occur. Similar to trace mineral blocks, monitoring consumption of loose mineral on a per animal basis is very difficult. For cattle fed at a bunk, a pre-mix, including trace minerals and other elements formulated to meet the animal's requirements, is added to the ration. As with pasture supplementation, individual animal consumption is still difficult to assess. In a study conducted to determine variability of intake in cattle provided an *ad libitum* trace mineral supplement in a dry lot setting, an intake variation of 81% was illustrated on a day to day basis as compared to a hand-fed forage cube supplementation program where daily variation in intake did not occur. Variation did not occur in the hand-fed cattle because all animals consumed all cubes offered (Ominski et al., 2006).

In addition to the consumption challenges associated with *ad libitum* mineral products, dietary interactions among trace minerals are also of concern. In cases of dietary interaction, trace mineral intake meets requirements, but reduction in mineral bioavailability causes a deficiency. The most common minerals that form dietary interactions are: Cu, Mo, and S, all trace minerals found in a typical grazing diet. This dietary interaction frequently results in a secondary Cu deficiency, the most frequently reported trace mineral deficiency in grazing cattle (Baldwin et al., 1981). Molybdenum and S, when fed in excess, form thiomolybdate complexes with Cu in both intestines and tissue, rendering Cu unavailable to the animal (Allen and Gawthorne, 1987; Gooneratne et al., 1989). In addition to forming thiomolybdate complexes, sulfates, which are reduced to sulfides in the rumen, can react directly with Cu, reducing its availability. Copper availability and absorption can also be compromised by dietary phytates, Cd, Zn, Ag, Hg, and Fe (Smart et al., 1981). Other trace minerals, such as Se, can have compromised ruminal availability when fed with high levels of heavy metal in the diet such as mercury and cadmium (Whanger, 1985). Sulfur and sulfates can also reduce Se absorption (Puls, 1988). Somewhat similar to the interactions seen by Mo and Cu, availability of Zn can be compromised when consumed in conjunction with a high Mo diet. If Zn deficiencies exist, high levels of Ca and P can interact to form an even greater Zn deficiency (Stake, 1977). An option that eliminates consumption variation and dietary interaction is injectable bolus trace mineral supplementation.

Injectable Trace Mineral Supplementation

To combat the problem of varying consumption rates and dietary interactions, an injectable trace mineral bolus has been developed. Designed to be administered in conjunction with a pre-existing dietary mineral program, injectable bolus supplementation can ensure a

consistent rate of trace mineral supplementation on a per animal basis. Bolus trace mineral injections can also be utilized to treat animals with a suspected mineral deficiency. By injecting the bolus, dietary mineral interactions in the rumen are avoided, thus enhancing the bioavailability of the individual trace minerals. In addition to enhancing the bioavailability of various trace minerals, an injectable trace mineral product can be used to elevate trace mineral status prior to key management practices. Various times when the animal may benefit from additional trace mineral supplementation include but are not limited to: transportation of young weaning calves, prior to a bull breeding soundness exam, and before synchronizing cows and heifers to fixed time artificial insemination.

In addition to several advantages, the use of an injectable trace mineral product also has several drawbacks. One of these drawbacks is frequency of administration. MULTIMIN 90 recommends that bulls are treated 3 times/year, heifers 4 times/year, and calves and cows 2 times/year (MULTIMIN USA, Fort Collins, Co). At a cost of approximately $0.43/mL, an average bull dose would cost $3.44/head, or $10.32/head/year. If a producer were to treat a herd of 60 pairs, 2 bulls, and 8 developing heifers, they would spend approximately $488.48/year in injectable trace mineral alone. In addition, an injectable trace mineral product is not intended to replace a pre-existing dietary mineral program.

A bolus injectable trace mineral supplement (MULTIMIN 90, MULTIMIN USA, Fort Collins, CO) containing 60 mg Zn/mL (as Zn disodium EDTA), 10 mg Mn/mL (as Mn disodium EDTA), 5 mg Se/mL (as sodium selenite), and 15 mg Cu/mL (as Cu disodium EDTA) is currently being marketed for use both in dairy and beef cattle. Use of this product has been shown to improve production of neutralizing antibodies in calves receiving viral vaccinations, as well as improve feed efficiency in finishing steers (Clark et al., 2006; Arthington and Havenga,

2012). In a study evaluating the effects of a trace mineral injection at initial processing on newly received heifers, heifers receiving an injectable trace mineral product had less antibiotic treatment costs, reduced morbidity to bovine respiratory disease, and improved feed conversion as compared with untreated heifers (Richeson et al., 2006).

The unique advantages of an injectable trace mineral product can partially be attributed to how the trace minerals circulate and are stored in the body. Because the trace minerals are able to bypass the rumen and directly enter the blood stream, they are more available for storage and use throughout the varying body systems. In a study done by Pogge et al. (2012) to evaluate plasma mineral concentrations and liver mineral stores, steers that received a trace mineral injection (MULTIMIN 90) had greater plasma concentrations of Zn, Cu, Se, and Mn at 8 to 12 h following treatment compared with untreated steers. The difference in plasma trace mineral concentrations was no longer apparent 24 h post injection. In the same study, liver concentrations of Se, Cu, Mn, and Zn peaked one day post-injection. Compared with untreated steers. Selenium and Cu liver concentrations remained elevated through d 15 of the trial (Pogge et al., 2012). In a study evaluating the use of an injectable trace mineral product (MULTIMIN 90) combined with injectable vitamin E in pre-calving and pre-breeding beef cows grazing native range, liver Cu concentrations in treated cows remained elevated for 96 days following treatment as compared with control cows (Daugherty et al., 2002). These results lead one to believe that following a trace mineral injection, trace minerals circulate and are stored for long periods of time. This prolonged storage may provide a reservoir for the animal should a potential stressor arise and dietary trace mineral consumption falls below requirements.

Bull Reproductive Management

Accounting for half of a herd's potential reproductive success, the bull plays a very important role in overall productivity. To ensure reproductive success, bulls should be subject to several different management strategies over the course of their life. Ranging from proper nutrition during growth and development to breeding soundness exams prior to a breeding season, each factor in bull reproductive management is significant.

Nutrition

Nutrition during the growth and development stage is the first critical component to bull reproductive success. Because many producers utilize yearling bulls, it is important that the nutritional plane of the animal allow for the attainment of puberty by 12 to 14 months of age. Puberty in the beef bull is defined as the ability to produce an ejaculate containing 50 million spermatozoa with 10% progressive motility (Wolf et al., 1965).

To achieve this physiological goal, bulls are typically fed a high energy ration intended to attain 0.68 – 0.91 kg ADG from weaning till about a year of age (Corah, 1987). This high energy ration goes beyond energy levels required for maintenance and allows the body to utilize additional energy for growth and reproduction.

Following 140 d post weaning, the concentrate component of the ration is reduced. Because bulls are typically managed in a matter to push gain and achieve physical characteristics appealing to buyers at a sale, they have the potential to be over conditioned upon purchase. This period when the bull is gradually adapted to a lower energy feed, allows the bull to shed excessive back fat and reach an optimum body condition for the breeding season. Mature bulls also have specific nutrient requirements to maintain optimum reproductive performance. Over-

conditioned or under-conditioned bulls should be evaluated and reconditioned 1 to 2 months prior to the breeding season (Corah, 1987).

In addition to meeting the energy requirements of the developing beef bull, trace mineral requirements are also important. In developing bull rations, 3-10 mg Cu/kg diet is required. Copper supplementation requirements are lower for developing bulls compared to grazing cattle due to the availability of Cu in high concentrate diets (NRC, 2000). Meeting the Cu requirements of the developing bull is necessary to ensure proper bone development, cardiac function, and spermatogenesis. Adequate supplementation of Mn is also necessary. Recommended at approximately 40 mg Mn/kg diet, Mn is a key trace mineral for reproductive efficiency (NRC, 2000). Breeding cattle fed significantly under the NRC requirements for Mn experienced delayed cycling and reduced conception rates while growth was unaffected (Bentley and Phillips, 1951). Selenium, although very important, is required at the lowest level of all the trace minerals. Recommended at 0.1 mg Se/kg diet, Se is necessary for proper function of the immune and musculoskeletal systems. Glutathione peroxidase, an enzymatic product of Se metabolism, is crucial to avoid states of Heniz body anemias and white muscle disease, two disease states characteristic of oxidative damage (NRC, 2000). Zinc, required at 30 mg Zn/kg diet, is necessary for effective feed conversion, foot soundness, and testicular growth (Miller and Miller, 1962).

Breeding Soundness Exam

After proper nutritional requirements have been met, it is important to determine if a bull is reproductively sound. To evaluate this, several assessments of potential fertility and libido can be performed. Most commonly referred to as a breeding soundness exam (BSE), this common fertility assessment evaluates the motility and morphology of the sperm, as well as testicular and

scrotal development, and the physical ability to breed. Commonly performed prior to a sale or 60 to 90 d prior to the breeding season, a BSE allows for proper reproductive management of the herd. The breeding soundness exam does not account for bull libido. Although rarely performed in the United States, a separate observational examination can be done to determine if a bull has the correct mating behavior to successfully breed. There is not a uniform assessment of libido, but all tests include the following: 1) at least one service within 20 min of observation (Bos Taurus); 2) normal penis and musculo-skeletal function; and 3) no other factor that might limit service capability (Entwistle and Fordyce, 2003).

Breeding Soundness Exam: Physical Examination

The physical examination portion of the breeding soundness exam includes palpation of the penis, as well as the sigmoid flexure, prepuce, accessory sex glands, the scrotum and its contents, a measure of scrotal circumference, and a collection of semen. These results are then combined to determine if each bull is a "satisfactory," "questionable," or "unsatisfactory" potential breeder (Hopkins and Spitzer, 1997). Scrotal size is included in the exam because of its ability to predict sperm output and inverse relationship to age at puberty of both male and female offspring (Almquist et al., 1976; Brinks et al., 1978; Coulter and Keller, 1982; Coulter and Kozub, 1984; Gipson et al., 1987; Smith et al., 1989; Graser and Raznozik, 1992; Brinks, 1994; Moser et al., 1996).

Bulls that have reached puberty, or the ability to produce an ejaculate containing 50 million spermatozoa with 10% progressive motility, typically have a minimum scrotal circumference between 28 and 30 cm depending on breed (Wolf et al., 1965; Spitzer and Hopkins, 1997). Because of the relationship between puberty and scrotal size, the Society for Theriogenology recommends a minimum scrotal circumference of 30 cm in all yearling bulls used for breeding.

In the past twenty years there has been discussion to increase the minimum scrotal circumference required for breeding bulls from 30 cm to 32 - 33 cm, but no such changes have been officially made (Kasari et al., 1996). Although no changes have been made, research has indicated that young bulls with larger scrotal circumferences are more likely to produce greater volumes of high quality sperm than their peers with smaller scrotal circumferences (Hahn et al., 1969). Following puberty, scrotal circumference continues to increase. Bulls older than 2 yrs of age should exhibit a minimum scrotal circumferences of 33 cm. Although the minimum requirement for scrotal size is 33 cm, Guerra et al. (2013) reported average scrotal circumference of 2 year old Angus bulls as 38.55 cm. Hereford and Simmental 2 year old bulls average a scrotal circumference of 39.15 cm and 39.19 cm respectively (Guerra et al., 2013). Any changes in scrotal size after 2 yrs of age are typically related to further growth, breed variations, or are nutritionally dependent (Coulter, 1991; Chenoweth et al., 1997; Hopkins and Spitzer, 1997).

The penis and sheath are also observed to determine if any deformities or irregularities exist. In yearling bulls it is common to find frenulums that must be detached to ensure proper erectile function. Other common deformities are spiral deviations and hematoma of the penis, all of which can be surgically corrected if observed prior to the breeding season. The accessory glands are also rectally palpated to check for any signs of inflammation or abnormality. During the physical examination, care is taken to evaluate the structure of the feet and legs, as any structural problems may leave a bull unwilling or unable to breed.

Breeding Soundness Exam: Semen Evaluation

To pass the semen quality portion of a breeding soundness exam, the Society for Theriogenology recommends that a bull must have 70% or greater normal sperm morphology and 30% or greater sperm motility (Hopkins and Spitzer, 1997). Sperm must travel in a forward,

progressive manner. Bulls that do not meet these requirements should not be immediately culled, but rather reevaluated in 6 to 8 wks, the minimum time period required for development of new sperm. Various sperm abnormalities may exist in bulls that fail to pass a breeding soundness exam. These abnormalities may include defects in the sperm head, midpiece, and tail. Abnormalities that may cause a bull to fail a breeding soundness examination include, but are not limited to: a high percentage of distal midpiece reflexes, nuclear vacuoles, and a high percentage proximal droplets (Barth, 2007).

Sperm abnormalities can be classified into two groups: compensable and uncompensable defects (Barth, 2007; Parkinson, 2004). Compensable defects are defects that can be overcome by increasing the sperm dose. With an increase in sperm dose, competent sperm are able to fertilize the egg (Saacke, 2013). Uncompensable defects are those defects that result in subfertility regardless of sperm dose (Barth, 2007). Sperm with uncompensable defects can penetrate the zona, but then are unable to cause cleavage, resulting in unviable embryos. Bulls with uncompensable sperm defects should not be used for breeding (Saacke, 2013).

Libido and Service Capacity Testing

Although not commonly assessed, and difficult to evaluate chute-side, a libido assessment can be performed by the producer in the field. It is important to note that although libido is a critical component in the mating process, its correlation with actual fertility is not well understood. This is apparent in a trial where bulls with a moderate number of mounts exhibited higher fertility than bulls with either low or high numbers of mounts. Libido and service testing also allows the observer to see any issues that a bull may have with penile extension and gait (Mossman, 1983; Bertram et al., 2002). Because of the shortcomings of both the breeding soundness exam and libido tests, the results should be analyzed in conjunction. It is not unusual

to find bulls that have high libido do not necessarily have high quality semen and vice versa (Makarechian et al., 1983; Crichton et al., 1987; Boyd et al., 1989). Again, young bulls that exhibit low libido in a field trial should not be immediately culled. A Kansas study determined that libido is a learned trait and additional experience may increase libido over time (Boyd and Corah, 1987).

Specific Trace Minerals and Male Reproduction

The potential to maximize a bull's reproductive success can be found in a combination of adequate nutrition and proper reproductive management. If a reproductive problem does arise, it is often difficult to pinpoint where exactly the issue stems. Deficiency in trace elements commonly takes the blame. With variability in animal intake and the potential for dietary mineral interactions to occur, providing *ad libitum* access to supplementation or incorporating supplementation into a ration poses a risk to reproductive management. In an effort to eliminate risk, use of an injectable trace mineral supplement could ensure proper supplementation levels on a per animal basis and avoid dietary interactions in the rumen. An injectable product (MULTIMIN 90) currently marketed for use in conjunction with a pre-existing mineral program, provides additional supplementation of Zn, Se, Cu, and Mn to ensure that all animals have met their trace mineral needs. These 4 trace minerals combined with an existing supplementation program, high quality feedstuffs and proper management, work within the body to ensure proper gonadal, endocrine, and hormonal function, all necessary for reproduction.

Injectable Trace Minerals and Male Reproduction

Although there is little data on the reproductive benefits of a bolus injectable trace mineral product in bulls, the improvements demonstrated in female reproduction may likely be reproducible in males. In a study evaluating the use of injectable, radiolabeled Se in bulls,

elevated Se concentrations were observed in semen 40 d following treatment. This demonstrates the likely uptake of injected Se into the reproductive accessory glands (Smith et al., 1979). Wu et al. (1973) concluded that certain trace mineral retention in reproductive tissue may be important to semen quality, sperm maturation, and structural integrity.

Male reproductive improvements, including improved motility, morphology, and sperm concentration, seem likely following a bolus trace mineral injection. Important stipulations to achieve improved reproductive parameters include: a prolonged increase in circulating trace mineral status, testicular trace mineral uptake, and testicular trace mineral storage following prolonged liver storage.

Zinc

Required by the bull at 30 mg/kg diet, Zn has been found to be a very important component of bull reproduction (NRC, 2000). Circulating Zn concentrations in bovine plasma should be within 0.6 – 1.9 µg/mL serum to ensure that a Zn deficiency does not occur (Herdt and Hoff, 2011; Table 1.1). Adequate Zn stores in the bovine liver typically range from 90-400µg/g liver biopsy (Herdt and Hoff, 2011). Zn is typically found in bovine ejaculate at a concentration of 0.3 to 2.5 µg/mL (Aguiar et al., 2012; Table 1.2).

Particularly abundant in the testes and prostate, Zn acts to facilitate the maturation of testis germinal cells into spermatozoa (Bedwal and Bahuguna, 1994). During times of Zn deficiency, there is a reduction of Zn in the testis, epididymis, and prostate and testicular growth is inhibited (Miller et al., 1958; Miller and Miller 1962). Although testicular growth is impaired during a Zn-deficient state, if Zn status returns to normal, testicular growth will resume with no apparent lasting effects on reproductive efficiency (Pitts et al., 1966).

Zinc is also necessary for its catalytic capacities necessary for enzymatic function throughout the body, including the reproductive tract. Metalloenzymes, enzymes with a metal cofactor, participate in actives ranging from free radical removal to protein maturation (Byar, 1974; Hurley and Doane, 1989). These activities are necessary for proper maturation of germinal cells in the testes. Research has also indicated that Zn is an important cofactor for DNA and RNA polymerase activities. Although DNA and RNA polymerase activity occurs at the cellular level, it is still important to reproduction as sperm with reduced DNA content have been found sub-fertile (Roote et al., 1979).

Proper Zn concentrations are also necessary for the production and secretion of testosterone (Martin et al., 1994). Testosterone, a necessary androgen in male reproduction, promotes growth, spermatogenesis, and accessory gland functions. In a trial evaluating dietary Zn supplementation beyond required levels in Cashmere goats, supplemented goats had increased levels of plasma testosterone (Liu et al., 2015). Similar studies conducted in Zn deficient rats demonstrated reduced pituitary gonadotrophin and androgen output (Miller et al., 1958; Kellokumpu and Rajaniemi, 1981). The reduction in androgen and gonadotrophin output could be due to failure of spermatogenesis at the testicular level or may be due to Zn acting directly on the pituitary gland.

In addition to testosterone production, Zn maintains many of the mechanical properties of sperm. Found in bull spermatozoa at a concentration of 30.6 ± 6.6 nmol Zn/108 cells, Zn functions to maintain the mechanics of accessory fibers, tail morphology, and membrane stability (Underwood and Somers, 1969; Boursnell and Roberts, 1974; Swarup and Sekhon, 1976; Chesters, 1978; Apgar, 1985). Overall sperm motility is also regulated by Zn, as Zn is closely

involved with the mechanism that provides energy for progressive sperm movement (Hidiroglou and Knipfel, 1984).

Zinc supplementation can cause many favorable reproductive responses. In a study performed on crossbred 2 yr old bulls, Zn supplementation above the animal's daily requirement increased ejaculate volume, sperm concentration, and number of sperm per ejaculate. Sperm of supplemented bulls had increased motility, a greater percentage of intact acrosomes, and a greater percentage of live sperm as compared with control bulls (Kumar et al., 2006).

Selenium

Selenium is required by the bull at a level of 0.1 mg/kg diet (NRC, 2000). Because Se is required at such a small level, care must be taken to avoid over-supplementation. Adequate circulating Se in bovine serum ranges from 65-140 ng/mL serum (Herdt and Hoff, 2011; Table 1.1). Bovine liver Se concentrations range from 0.7-2.5 μg/g liver biopsy (Herdt and Hoff, 2011). Bovine seminal concentrations range from 100.0 to 1,100.0 ng/mL (Aguiar, et al., 2012; Table 1.2). Concentrations above these values put the bull at risk for Se toxicity.

In male reproduction, the role of Se is somewhat poorly defined. Although the role of Se in male reproduction, beyond its removal of free radicals, is somewhat uncertain, the testes can accumulate a large portion of an intravenous dose of Se-selenite (Brown and Burk, 1972). Current research indicates that Se typically can be found reaching levels of 0.8 ± .01 mg/L in the ejaculate (Aguiar et al, 2012). This may indicate that Se plays a more direct role in reproduction than previously thought.

In addition to its accumulation in the testes, Se has distinct antioxidant functions that protect the body from harmful free radicals and resulting oxidative degeneration (Diplock, 1981; Freeman and Crapo, 1982; Burton and Ingold, 1984). Selenium is a key component in

glutathione peroxidase activity and the subsequent reduction of cytosolic peroxides (Diplock, 1981; Freeman and Carop, 1982). Glutathione peroxidase, which functions in the reduction of harmful peroxides, is active in the semen of the bull (Brown et al., 1977). In the bull, glutathione peroxidase is associated with protecting the sperm cell membranes within the seminal plasma from oxidative damage (Mills and Randall, 1958; Cohen and Hochstein, 1963; Combs et al., 1975; Brown et al., 1977; Brown and Senger, 1977). In Se-deficient bulls, an inhibition of spermatogenesis can be seen (Smith et al., 1979). Aside from antioxidant functions, Se supplementation can increase testosterone secretion in goats and increase both scrotal length and circumference (Marai et al., 2006; El-Sisy et al., 2008).

The use of an injectable trace mineral product containing Se could be beneficial in raising the amount of seminal Se. This was demonstrated by Smith (1979) where bovine seminal Se concentrations were elevated following treatment with an intramuscular injection of a radiolabeled Se and remained elevated 40 days after. This increase in seminal Se concentration may allow for additional free radical removal within the seminal plasma and provide a better environment for spermatogenesis.

Copper

Like other various trace elements, Cu is required in the bull at a level of 3-10 mg/kg diet and is necessary for many copper containing proteins with various enzymatic functions (Bremner, 1980; Cass and Hill, 1980; Mills, 1980; NRC, 2000). Adequate Cu supplementation is seen when circulating bovine Cu concentrations are between 0.6 - 1.1 µg/mL serum and bovine liver Cu concentrations fall between 50 - 600 µg/g liver biopsy (Herdt and Hoff, 2011; Table 1.1). Typical bovine seminal Cu concentrations range from 0.02-0.15 µg/mL (Aguiar et al., 2012; Table 1.2). Copper is known to be a component of the antioxidant superoxide dismutase

(SOD) which aides in the removal of harmful oxidative byproducts produced by several of the metabolic pathways (Bull, 1980; Smart et al., 1981; Underwood, 1981; Gay and Madson, 1986; Maas, 1987).

In the bull, the effects of Cu deficiency are not well understood, as most symptoms of either clinical or subclinical Cu deficiency exist in the female. Most of the clinical signs of Cu deficiency in bulls are due to high Mo in the diet, or an improper Cu:Mo ratio. Excess Mo in the diet impairs Cu metabolism and prevents the efficient utilization and mobilization of Cu from various tissues, in turn elevating the animal's dietary Cu requirement (Mills, 1980). Because excess Mo in the diet raises the animal's requirement for Cu, bulls that experience this sort of nutritional compromise can have diminished libido, as well as various stages of testicular degeneration, particularly in the seminiferous tubules and the interstitial tissue. Ejaculate samples may also be completely devoid of sperm (Thomas and Moss, 1951).

Manganese

Manganese is required by the bull at a level of 40 mg/kg diet and is a necessary cofactor for several metalloenzymes and various redox reactions (Georgievskii, 1981; NRC, 2000). Adequate bovine circulating Mn concentrations fall between 0.9 - 6.0 ng/mL serum and adequate bovine liver concentrations typically range from 5 - 15 µg/g liver biopsy (Herdt and Hoff, 2011; Table 1.1). Seminal concentrations typically range from 9.5 - 64.4 ng/mL (Aguiar et al., 2012; Table 1.2). In the rare occasion that deficiency occurs in ruminants, male reproductive capacity can be compromised (Doisy, 1974; Underwood, 1977). Manganese may also play an important role in the function of various endocrine organs, as Mn is critical for the synthesis of sterols, and resulting gonadal hormones (Benedict et al., 1965).

Heifer Reproductive Management

Properly managing heifers for reproductive success and efficiency allows for greater overall herd productivity. Not only does proper reproductive management increase the number of heifers that become pregnant early in their first breeding season, but various management practices also ensure that all heifers are capable of becoming pregnant well before the breeding season begins.

In many management systems, especially purebred operations, heifer reproductive success and efficiency is initially measured by conception rate to single service artificial insemination (A.I.). Calculated by number of conceptions per number of inseminations, conception rate to first service A.I. predicts heifers that are capable of producing calves that will be older, heavier, and ultimately more profitable at weaning.

To ensure that heifers are capable of conceiving at the first insemination, they must be managed properly prior to this point. Important management factors to consider when preparing heifers to achieve pregnancy include a high plane of nutrition, early attainment of puberty, and the ability to support a pregnancy.

Attainment of Puberty

To ensure productivity and first calving by 24 mo of age, heifers must be pubertal before their first breeding season. Puberty, as defined in beef heifers, is when a heifer is first able to express estrous behavior and simultaneously ovulate a fertile oocyte (Moran et al., 1989). Ideally, heifers should be pubertal by the ages of 11 to 13 mo to allow for 3 to 4 wks time of managed breeding before the start of the mature cow breeding season (Larson, 2007). This 3 wk window allows for the producer to focus on calving difficulties that are more frequent in heifers before the onset of calving of the cow herd. Because heifers typically take more time than cows

to return to cyclicity post-gestation, this additional time may help heifers regain cyclicity before the next breeding season begins (Short et al., 1990). Lesmeister et al. (1973) found that heifers that calved earlier in the calving season had higher lifetime calf production than their late-calving peers.

Nutrition

Puberty in the heifer is dependent on age, weight, and breed (Wiltbank et al., 1969; Nelsen et al., 1982; Oeydipe et al., 1982; Nelsen et al., 1985). Prior to breeding, heifers must reach a target weight of approximately 60% of their mature weight in order to achieve puberty and carrying a calf through gestation (Dziuk and Bellows, 1983; Wiltbank et al., 1985). Similar to bulls, heifers are typically fed a high concentrate diet. This high concentrate diet is intended to allow the heifer to achieve an ADG of 0.68 - 0.91 kg/d (Lancaster and Lamb, 2014). Developing heifers fed to meet this target ADG typically become pubertal earlier, experience improved udder development, and increased conception rates as compared with heifers fed to achieve less than the target ADG (Wiltbank et al., 1969; Bond and Wiltbank, 1970; Short and Bellows, 1971; Oeydipe et al., 1982; Patterson et al., 1989).

In addition to a high concentrate diet, trace mineral supplementation is necessary to ensure the heifer is capable of reproduction. In heifers fed a high concentrate diet, 3-10 mg Cu/kg diet is required. If the developing heifers are grazing, requirements increase to 7-14 mg Cu/kg diet. This increase is predominately due to Mo interaction commonly observed in forage based diets (NRC, 2000). In the female, Cu is necessary for bone development, cardiac function, and the proper expression of estrus (NRC, 2000; Underwood, 1981). Supplementation of Mn is also necessary. Recommended at 40 mg Mn/kg diet for breeding cattle, Mn is necessary for reproductive success. In studies evaluating dairy cattle diets deficient in Mn, conception rates

were reduced and estrous cycles were delayed in cows that received Mn deficient diets compared with cows that received diets with adequate Mn supplementation (Bentley and Phillips, 1951). Selenium, required by the developing heifer at 0.1 mg Se/kg diet, is necessary for the function of antioxidants, and immune, and skeletal systems (NRC, 2000). Zinc, although better known for its benefits in male reproduction, is required by the developing heifer at 30 mg Zn/kg diet. Proper Zn supplementation is necessary for proper gain, skin health, and proper immune function (Miller and Miller, 1962; Mayland et al, 1980; Perryman et al., 1989).

Artificial Insemination

In addition to proper nutrition and attainment of puberty, there are several other management practices that can help ensure heifer reproductive success. Artificial insemination, AI, is a management practice that is becoming more widely accepted in the beef industry. Approximately 12.8% of beef heifers are bred to AI. This percentage is significantly below the 66% of all dairy heifers bred to AI (NAHMS, 2009). Although the percentage of beef heifers bred to AI is low compared to the dairy industry, it is growing. Percentage of AI use in beef herds has increased by approximately 4% in a 12 year span (NAHMS, 1997; NAHMS, 2009).

Artificial insemination allows for many advantages in the beef herd. Artificial insemination use allows the producer to more quickly improve the genetic merit of the herd without the cost of purchasing a genetically superior bull. In addition to improving genetics, beef producers can utilized AI breeding to ensure that the heifer calving season begins prior to the cow calving season. By separating heifer from the cow calving seasons, the producer would be able to focus on calving problems more typical of heifers. To do this, the producer would AI breed heifers 3 - 4 weeks prior to turning the cows and heifers out with the bulls. In addition to improving the genetic merit of the herd and allowing for a heifer calving season, AI use has been

shown to have economic benefit as well. In a New Zealand study evaluating a management system using an AI/natural service combination compared with a management study using solely natural service, the AI/natural service combination netted $2,000 more than the natural service herd. This increase in value can be attributed to the reduction in grazing costs associated with a shorter natural service breeding season (Anon, 2001).

Although AI has many potential advantages, there are drawbacks that have prevented its widespread use in the beef industry. Time, labor, difficulty of implementation, and lack of facilities are most commonly cited as reasons for beef cattle producers to have not adopted AI use (NAHMS, 2009). In addition to concerns pertaining to management inputs, there are other concerns pertaining to pregnancy rates and AI use. The beef cattle industry has anecdotally assumed that natural service increases herd reproductive success. This can be attributed to beliefs that the bull is better at detecting estrus than humans. Several studies have dispelled this myth and have concluded that there is no differences in calving rates between herds that use AI as compared with herds that solely use natural service (Rupp et al., 1977; Healy et al., 1993).

To combat these concerns, several additional management practices can be added to AI to reduce the time and labor needed to attain satisfactory pregnancy rates. Among these various practices is estrous synchronization. One specific estrous synchronization protocol (Select-Synch + CIDR) employs the use of a CIDR followed by an injection of a prostaglandin F2α to synchronize the estrous cycle. CIDRs (EAZI-BREED CIDR, Zoetis, Florham Park, New Jersey) are intravaginal progesterone releasing devices that are a component of some estrous synchronization programs. Prior to CIDR insertion, GnRH is given to reset follicular waves. The progesterone in the CIDR suppresses the release of GnRH, subsequent gonadotropin (LH and FSH) release, and ovulation. After 7 d of use, the CIDR is removed and prostaglandin F2α is

administered. The prostaglandin administration causes the corpus luteum to regress and GnRH pulse frequency to increase in response to lower circulating progesterone. Two to 3 days following the prostaglandin administration, synchronized cows will display estrus. Following heat detection, cows will be inseminated.

Although CIDRs and prostaglandins can be used independently to synchronize estrus, CIDR/prostaglandin combinations have been found more effective. Sole CIDR usage (or any other sole progestin) is not recommended mainly due to the formation of persistent follicles with long-term progestin treatment, whereas sole prostaglandin use is only effective in cycling females from day 7 to 17 of the estrous cycle (Lauderdale et al., 1974; Kinder et al., 1996).

Following estrous synchronization, the utilization of ovulation synchronization and fixed-time artificial insemination are very beneficial in reducing labor and time costs associated with visually detected heats. Defined as artificial insemination following ovulation synchronization, fixed-time AI allows for satisfactory conception rates without the need to physically detect estrus.

Ovulation is synchronized prior to AI by a protocol including treatment with GnRH and a prostaglandin. This treatment allows for ovulation to be synchronized within an 8 hour window. GnRH, the first treatment given in the protocol, is intended to synchronize the follicular waves of heifers. GnRH is then followed by 7 days of a progestin, typically a CIDR. Following CIDR removal at day 7, a prostaglandin is given to induce luteolysis. The third and final treatment, GnRH, is given 48-60 hours following the prostaglandin (7-day CO-Synch + CIDR). This final GnRH injection induces a preovulatory LH surge and thus ovulation and is followed by artificial insemination.

Typical pregnancy rates for fixed-timed AI protocols including CIDRs range from 43-62% (Martinez et al., 2002; Busch et al., 2007). In addition to financial benefits and satisfactory pregnancy rates associated with AI, calves born to heifers that conceived following first service AI are older and have heavier weights at weaning, resulting in more profitable calves than calves conceived from natural service (Schafer et al., 1990).

Specific Trace Minerals and Female Reproduction

Similar to bulls, maximization of a heifer's reproductive success can be found in a combination of adequate nutrition and proper reproductive management. Like their male counterparts, it is difficult to determine the source of various reproductive shortcomings. If no other explanation exists, trace mineral analysis may occur to rule out any potential deficiencies. Because reproductive concerns are a common sub-clinical manifestation of trace mineral deficiency, it is important to ensure that all trace mineral requirements are being met.

Injectable Trace Minerals and Female Reproduction

In addition to improved immune status and growth, supplementation with an injectable trace mineral product has been shown to improve reproductive status in cattle. When an injectable trace mineral product (MULTIMIN 90), in addition to an existing dietary mineral program, was administered to beef cows and heifers on native range, conception to first service AI improved (Mundell et al., 2012). Calving distribution was also found to be favorable with more cows calving at the beginning of the calving season as compared with the middle and end (Mundell et al., 2012). In heifers treated with an injectable trace mineral product (MULTIMIN 90) prior to synchronization for timed embryo transfer (ET), treated heifers experienced an increase in transfer rates (embryo survival) at 23 and 48 days following embryo transfer. Although treated heifers had improved embryo transfer rates as compared with control heifers,

treatment with an injectable trace mineral product did not increase the number of heifers successfully synchronized to receive an embryo (Sales et al., 2011).

In a two part study evaluating the use of an injectable trace mineral product in developing beef heifers, Brasche et al. (2015) reported conflicting results. In experiment one, a trace mineral injection prior to breeding did not improve pregnancy rates after artificial insemination following a 14 d CIDR-PG or 5d Co-Synch plus CIDR protocol. Contrastingly, in experiment two, heifers that received a trace mineral injection prior to artificial insemination and a 7d Co-Synch Plus CIDR protocol had greater pregnancy rates than control heifers. Brasche et al (2015) speculated that the conflicting results stemmed from a forage trace mineral inadequacy in experiment two, allowing treated heifers an advantage over control heifers.

In the dairy industry, Mitchell (2013) found that dairy cows treated with an injectable trace mineral product had significantly greater conception rates (defined as cows pregnant as a percentage of cows bred within a specific time frame) and pregnancy rates (defined as heat detection rate × conception rate) than control cows. He also noted a reduction in clinical mastitis in the treated cows. In this study, it is possible that because of the incidence of clinical mastitis, immune function of the cows was compromised. The addition of an injectable trace mineral product could have strengthened immune function, resulting in better reproductive success.

Conversely, Vanegas et al., (2004) reported that in dairy cows receiving a single dose of an injectable trace mineral product prior to calving and then again prior to breeding, treated cows had lower conception rates at first service than control cows. In this intensively managed dairy, all cows were fed a ration that met or exceeded NRC requirements for trace minerals. Similar findings were reported in beef cows grazing native range supplemented with dietary trace

minerals. Pregnancy rates to AI did not differ between cows treated with an injectable trace mineral (MULTIMIN 90) and control cows (Whitworth et al., 2014).

It is possible, that when all dietary trace mineral needs are met, adding additional trace minerals is not of direct reproductive benefit. In situations where dietary interactions are more likely to occur, such as a grazing environment, or in situations where additional stressors are put on the animal, the use of an injectable trace mineral product may help overcome dietary interactions and meet additional trace mineral needs.

Zinc

Similar to the male, a heifer requires 30 mg Zn/kg diet for various catalytic and enzymatic roles (NRC, 2000). Many of these enzymatic functions are present in reproductive tissues (Apagar, 1985). Although much is known about the importance of Zn in males, very little research has been completed regarding the role of Zn in female reproduction. What work is available illustrates that heifers supplemented with Zn had increased calving rates (93%) as compared to un-supplemented heifers (62%; Piper and Spears, 1982). Cows supplemented with Zn had increased conception rates and higher ovarian Zn content than un-supplemented cows. When supplementation was removed, fertility was compromised (Nedyjlkov and Krustev, 1969). Similar findings were reported in ewes supplemented with Zn over and beyond dietary requirements 1 month prior to mating through lambing. Serum Zn was found to be increased in supplemented ewes during late pregnancy (Ali et al., 1998). Supplemented ewes had greater feed consumption, greater fertility rates, and produced heavier lambs at both birth and weaning.

Decreased fertility and abnormal estrous behavior are typical symptoms for Zn deficiency. It is suspected that when a cow is Zn deficient, all stages of the reproductive process are compromised, beginning with estrus and ending with lactation (Underwood, 1981). As

mentioned above, once treatment for deficiency begins, fertility seems to return to normal (Ali et al., 1998).

Selenium

Selenium is required by the heifer at a level of 0.1 mg Se/kg diet (NRC, 2000). When supplementing Se, it is very important to be cautious of toxic levels of Se. The maximum tolerable concentration of Se in beef cattle is reported to be 2.00 mg Se/kg diet (NRC, 2000). Any concentrations above this can cause consequences ranging from reproductive failure to acute death.

The majority of work with Se in females has been completed in ewes. Although most small ruminant Se research focuses on Se toxicity, a major concern for small ruminant producers, there is some available research regarding Se deficiency. Because both Se toxicity and deficiency can have similar reproductive consequences, it is important to determine if a deficiency or toxicity is the underlying problem.

Selenium deficient ewes commonly experience embryonic and fetal loss. Ewes treated with oral Se supplementation one month before the breeding season through parturition exhibited increased lambing percentages and reduced fetal mortality as compared with un-supplemented ewes (Hartley et al., 1960; Hartley and Grant, 1961; Hartley, 1963).

What little cattle research exists investigates Se supplementation as a preventative measure against retained placentas. Cows fed a Se deficient diet and then supplemented with Se had lower incidence of retained placenta. In cows supplemented and fed a Se adequate diet, no difference in percentage retained placentas was noted (Julien and Conrad, 1976). Although Ugandan dairy producers attribute low fertility to regional grass Se deficiency, research concludes that Se supplementation does not improve pregnancy rates in herds battling low

fertility (Southcott et al., 1972; Long and Marshall, 1973; Scales, 1976; Kappel, 1984; Hidroglou et al., 1987)

It is well understood that Se and vitamin E interact closely. Hidiroglou (1979) speculates that some clinical signs of Se deficiency may actually be attributed to fluctuation in vitamin E concentrations, or a combined deficiency of Se and vitamin E. Studies looking at the combination of vitamin E and Se on fertilization rates in cattle with either a low or adequate plane of nutrition indicate that when inadequate diets are not supplemented with Vitamin E and Se, fertilization of the ova is compromised and rates of fertilization are reduced (Segerson et al., 1977).

Copper

Copper is required by the heifer at 3 - 14 mg Cu/kg diet and is a key factor in many Cu containing proteins with various enzymatic functions (Bremmer, 1980; Cass and Hill, 1980; Mills, 1980; NRC, 2000). Copper is also very important for the regulation of several hormones with important reproductive roles. Copper modulates the activity of prostaglandin E2 and it is speculated that the extracellular Cu released from axonal terminals may regulated PGE2's action on luteinizing hormone (Barnea et al., 1985).

Copper deficiency is commonly associated with early embryonic death, as Cu has been found to be required in greater amounts during fetal development (Hidiroglou, 1979; Hurley et al., 1980). In a study where pregnant goats were fed a semi-purified diet low in Cu, 50% of all goats aborted. Abortions occurred between the 2nd and 5th month of pregnancy. In cows grazing Cu deficient range forage, delayed estrus was observed (Allcroft and Parker, 1949; Annenkov, 1981). Other symptoms of deficiency include depressed ovarian activity, delayed estrus, reduced

conception rates, increased incidence of retained placenta, calving difficulty, and congenital rickets (Hidiroglou, 1979).

Although the symptoms of Cu deficiency can be severe, supplementation seems to reverse many of the negative effects Cu deficiency may cause. Copper deficient heifers with poor fertility were able to regain normal breeding patterns with Cu sulfate supplementation (Blakemore and Venn, 1950). Cows with slightly low blood Cu levels had increased conception rates when supplemented with Cu (Hunter, 1977). In a study that evaluated Cu supplementation in marginally deficient New Zealand dairy herds, administration of 400 mg Cu glycinate improved conception. Supplemented cows experienced conception rates of 72% as compared with 53% for untreated cows (Mahadevan and Zubairy, 1969).

As with many other trace minerals, Cu is subject to dietary interactions with Mo, S, Fe, Ca, Co, and Zn. Whereas Mo reduces the utilization of Cu, Co tends to maximize utilization (Blakemore and Venn, 1950). Delayed puberty and anestrus have been reported in cows grazing pastures high in Mo and low in Cu (Roberts, 1971). Failure to exhibit puberty and estrus is most likely due to an improper Cu:Mo ratio (Peterson and Waldern, 1977). It is unclear whether Mo alone impacts reproduction (Hurley and Doan, 1989). Molybdenum does seem to interact with many steroid hormone receptors important for reproduction. In vivo, excess Mo alters the release of luteinizing hormone (Phillippo et al., 1987). In vitro, Mo works to stabilize receptors in a non-steroid bound form (Dahmer et al., 1987). In some mammals, particularly humans and rats, interactions exist between estrogen and Cu. Upon treatment with diethylstilbestrol, Cu seemed to rise in proportion to the dose of estrogen received (Briggs et al., 1970).

Manganese

Manganese is required by the heifer at a level of 40 mg Mn/kg (NRC, 2000). Necessary for the function of various metalloenzymes, various endocrine organ function, and reproduction as a whole, Mn deficiency in ruminants is rarely observed (Georgievskii, 1981). If deficiency does occur, reproductive function can appear depressed or impaired (Rojas et al., 1965; Anke and Groppel, 1970; Underwood, 1977).

In ruminants, symptoms of Mn deficiency include anestrus, irregular return to estrus and extended periods of anestrus (Bourne, 1967; Wilson, 1966). A study looking at the effects of Mn deficient diets found that cows exhibited subnormal ovarian size, as well as delayed puberty, reduced conception rates, and a tendency towards abortion and weakened calves (Bentley and Phillips, 1951). Follicular development can also be compromised, resulting in poor or delayed ovulation and undetected estrus. In Mn deficient herds, conception rates of 35 to 40% are not abnormal (Wilson, 1966; Bourne, 1967). Similar to other minerals, the requirement for Mn is increased during gestation (Rojas et al., 1965).

Supplementation with Mn has proved to be effective in reversing adverse reproductive effects caused by deficiency. Dairy cattle supplemented with Mn experienced an increase in conception rates and overall fertility (Munro, 1957; Wilson, 1966). Ewes supplemented with Mn in South Australia also responded well. With supplementation, the numbers of lambs per year increased and overall reproductive performance improved (Egan, 1972). Overall responses to Mn supplementation across species include increased ovarian activity and improved conception rates (Di Costanzo et al., 1986).

High levels of Mn can be found in the pituitary gland and the ovary. Ovarian structures, such as large follicles and corpora lutea, are particularly responsive to high Mn levels (Hidiroglou, 1975). Manganese may also have a role in the regulation and synthesis of steroid

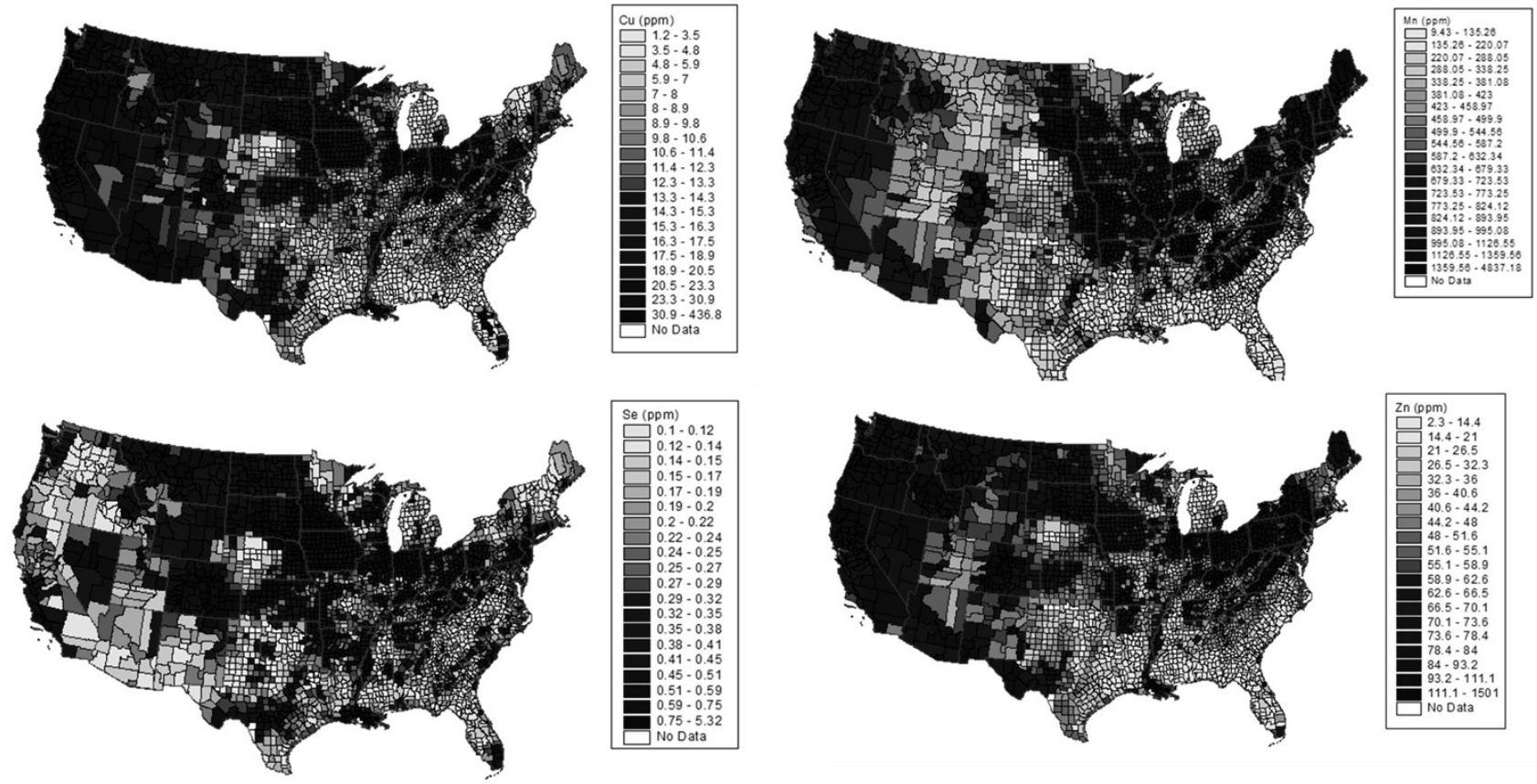
hormones. During times of deficiency, a lack of Mn prevents the formations of cholesterol precursors, in turn preventing the resulting steroid hormones (Doisy, 1974).

General Implications

With an adequate understanding of how individual trace minerals work to maintain reproductive function, one can better appreciate how an injectable trace mineral product could be of benefit in improving reproductive success. In addition to mitigating trace mineral consumption variation, as well as eliminating dietary interactions within the rumen, the use of an injectable trace mineral product can be used to manage cattle at various critical time points throughout the production year with the intent to improve overall reproductive success.

Figures

Figure 1.1 Maps of the United States depicting county soil concentrations of Cu, Mn, Se, and Zn as of 2008. Maps sourced from the US Department of the Interior, US Geological Survey and can be found at: http://mrdata.usgs.gov/geochem/doc/averages/countydata.htm.



Tables

Table 1.1 Reference values for serum trace mineral concentrations in beef cattle of various ages as reported by Herdt and Hoff, 2011

Herdt and Hoff, 2011	
	Serum Reference Ranges
Zinc µg/mL	0.6 - 1.9
Copper µg/mL	0.6 - 1.1
Manganese ng/mL	0.9 - 6.0
Selenium ng/mL	65.0 - 140.0

Table 1.2 Reference values for semen trace mineral concentrations in beef cattle of various ages as reported by Aguiar et al., 2012

Aguiar et al., 2012	
	Semen Reference Ranges
Zinc µg/mL	0.3 - 2.5
Copper µg/mL	0.02 - 0.15
Manganese ng/mL	9.5 - 64.4
Selenium ng/mL	100.0 - 1,100

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Chapter 2 - Effect of injectable trace mineral supplementation in yearling bulls on serum and semen trace mineral levels and reproductive parameters

Abstract

We hypothesized that administration of an injectable trace mineral product would cause a short-term increase in circulating trace mineral concentrations, but not alter semen quality nor ability to pass a breeding soundness exam (BSE). Sixteen Hereford, 52 Angus, and 22 Simmental bulls were blocked by breed and stratified by age (277±25 d) and weight (377±42 kg) and administered s.c. either a commercially-available trace mineral supplement (Trace Mineral; n=45; 15 mg Cu/mL, 60 mg Zn/mL, 10 mg Mn/mL, and 5 mg Se/mL; MULTIMIN 90, MULTIMIN USA, Inc., Ft. Collins, CO) or sterilized saline (Control; n = 45) at 1mL/45 kg BW. Bulls were maintained in a drylot and fed a grower ration that included trace minerals at NRC recommended levels. Blood was collected via jugular venipuncture immediately before treatment (h 0) and at 8 and 24 h. Body weight and scrotal circumferences were measured on the day of treatment (d 0) and d 20, 42, 59, and 91. Semen was collected via electroejaculation on d 42 and 91 and a BSE conducted on d 91. Serum samples from h 0, 8, and 24 and semen samples d 42, and 91 on a subset of 26 bulls per group were analyzed using inductively coupled plasma mass spectroscopy for concentration of Cu, Zn, Mn and Se. Data were analyzed using PROC MIXED, PROC GLIMMIX, and PROC FREQ in SAS with fixed effects of treatment and time of sampling and interactions with bull as experimental unit. Breed served as the random variable. Trace Mineral and Control bulls had similar ($P \geq 0.20$) scrotal circumferences and BW throughout the trial. Trace Mineral bulls had greater ($P \leq 0.0001$) trace mineral concentrations at 8 h post-treatment than Control bulls. Semen trace mineral concentrations on d 42 and 91 were

similar ($P \geq 0.52$) between Trace Mineral and Control bulls. Sperm motility, percent normal morphology, and concentration increased and percentage proximal droplets decreased ($P \leq 0.02$) from d 42 to 91, but did not differ ($P \geq 0.06$) between Trace Mineral and Control bulls. A similar ($P = 0.94$) percentage of Trace Mineral (67%) and Control (68%) bulls passed their BSE 91 d post-treatment. In the present study, a supplemental trace mineral injection increased circulating trace mineral levels, but did not alter semen trace mineral levels nor improve semen quality.

Introduction

Proper trace mineral supplementation is necessary for the various biochemical processes of the ruminant, including but not limited to enzymatic function and gonadal development. When developing beef bulls, it is necessary to meet trace mineral requirements to ensure reproductive success.

Previous research has indicated that supplementation with dietary Zn and Se improved overall sperm quality in bulls and rams (Arthington et al., 2002; Shi et al., 2010). Similar findings were demonstrated in a Kendall et al. (2000) trial evaluating supplementation with an oral bolus of Zn, Co, and Se in rams. Supplemented rams had increases in sperm motility and percentage of live sperm as compared to control rams. Supplementation with Se has been demonstrated to improve overall sperm morphology through improved antioxidant status (Brown et al., 1977). This improvement can be attributed to increased function of glutathione peroxidase, an enzyme that has been demonstrated to remove harmful free radicals from the plasma membrane (Mills and Randall, 1958; Cohen and Hochstein, 1963; Combs et al., 1975).

Recent research evaluating the circulating and storage potential of a commercially available injectable trace mineral product containing 60 mg Zn/mL, 10 mg Mn/mL, 5 mg Se/mL 15 mg Cu/mL (MULTIMIN 90, MULTIMIN USA., Fort Collins, Co) in beef steers indicated

that when administered in addition to a dietary trace mineral program, circulating trace mineral concentrations were elevated 8 h post treatment and liver concentrations remained elevated 15 d post-treatment (Pogge et al., 2012).

The information available on the storage and circulation of trace minerals combined with research evaluating the effect of trace mineral supplementation on male reproductive parameters has led to questions pertaining to the use of an injectable trace mineral product to improve both sperm quality and percentage passing a yearling BSE in developing beef bulls. We hypothesized that when dietary trace mineral needs are met, administration of an injectable trace mineral product to developing beef bulls would cause a short-term increase in circulating trace mineral concentrations, but not alter semen quality nor ability to pass a BSE. The objectives of this study were to compare serum and semen trace mineral concentrations of treated and untreated bulls and to determine if a bolus trace mineral injection improves semen quality.

Materials and Methods

This study was conducted in accordance with the Kansas State University Institution Animal Care and Use Committee (IACUC) at the Kansas State University Purebred Beef Teaching Unit. Ninety bulls (Angus, n=52; Simmental, n=22; Hereford, n=16) were blocked by breed and stratified by age [277±25 d of age on d 0 (day 0 = time of treatment)] and weight (377±42 kg). Bulls were then randomly assigned to 1 of 2 treatments: 1) s.c. injection of a commercially-available bolus trace mineral supplement [Trace Mineral; n=45; MULTIMIN 90, MULTIMIN USA, Inc., Ft. Collins, CO containing 60 mg Zn/mL (as Zn disodium EDTA), 10 mg Mn/mL (as Mn disodium EDTA), 5 mg Se/mL (as sodium selenite), and 15 mg Cu/mL (as Cu disodium EDTA)]; or 2) s.c. injection of sterile saline (Control; n=45). Treatments were

administered subcutaneously at a dose of 1 mL/ 45kg BW on d 0, 90 days prior to a complete breeding soundness examination (BSE; Fig. 2.1).

Bulls were housed in one pen measuring 97.6 m × 128.1 m. Throughout the study, bulls received a grower ration containing 70% concentrate and 30% roughage (47.49% corn gluten feed, 28.84% wheat straw, 20.30% steam flaked corn, and 1.8% premix on a dry matter basis; Table 2.1). All bulls were offered city water. Diets were formulated to achieve a 1.2 kg ADG and included trace minerals at NRC recommendations (23.89 mg/kg Cu, 82.05 mg/kg Mn, 0.25 mg/kg Se, and 99.79 mg/kg Zn).

Blood samples (5 mL) from all bulls (n=90) were collected via jugular venipuncture immediately before treatment (h 0) to determine pre-treatment serum concentrations of Cu, Mn, Se, and Zn (Fig. 2.1). To assess post-treatment serum concentrations of Cu, Mn, Se, and Zn, blood samples (5 mL) were collected again at 8 and 24 h post-treatment. Blood samples were allowed to clot and stored at 5ºC until centrifuged 24 h post-collection at 1,000 × g for 15 min to harvest serum. Serum was stored at -20ºC until assayed.

Body weights and scrotal circumferences were measured on the day of treatment administration (d 0) and d 20, 42, 59, and 91 post-treatment (Fig. 2.1). Scrotal circumferences were measured using a standard scrotal measuring tape by manually pulling the testicles to the bottom of the scrotum and measuring at the point of largest circumference.

Semen was collected via electroejaculation by a veterinarian on d 42 and 91 post-treatment (Fig. 2.1). Semen samples were obtained using a SireMaster Professional electronic ejaculator (SireMaster, Manhattan, KS) with a 6.35 cm probe (SireMaster, Manhattan, KS). All semen collections and evaluations were performed by the Kansas Artificial Breeding Service Unit (KABSU) personnel. Sperm concentrations were assessed using a NucleoCounter SP-100

(ChemoMetec, Denmark). Sperm motility was analyzed under microscopy (Olympus CX41, Center Valley, PA) at 20x magnification. Morphology was analyzed at 400x magnification. Semen samples (5 mL) from both d 42 and 91 were retained for analysis of Cu, Mn, Se, and Zn concentrations and stored in liquid nitrogen at -196 ºC until assayed. On d 91 a complete BSE was performed when all bulls were 362 ± 25 d of age. Bulls were considered to have passed their BSE when their semen sample contained 70% normal morphology, 30% progressive motility, had a scrotal circumference of at least 32 cm, and appeared to be free from physical deformities.

Serum and semen samples from a subset of 26 bulls per treatment group were analyzed for trace mineral concentrations by the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH, Lansing, MI) using inductively coupled plasma mass spectrometry (Varian ICP/MS/MS, Varian, Santa Clara, CA) for concentrations of Cu, Mn, Se, and Zn.

All data and statistical analyses were conducted using procedures in SAS (version 9.2, SAS Inst. Inc., Cary, NC). Bull was the experimental unit. Age, BW, scrotal circumferences, semen characteristics, and serum and semen trace mineral concentrations were analyzed using the mixed model (MIXED) procedure with fixed effects of treatment, time of sampling, and time by treatment. Breeding soundness data was analyzed using the general linearized mixed model (GLIMMIX) with fixed effect of treatment. For both models, breed served as the random variable. A total of 5 observations were withheld from analyses of semen characteristics as bulls did not have values recorded for various parameters. A P - value of ≤ 0.05 was considered significant. P -values $> 0.05 < 0.10$ were considered as tendencies.

Results and Discussion

As animals aged, BW and scrotal circumferences increased ($P < 0.0001$). There was no time by treatment interaction for either BW ($P = 0.99$) or scrotal circumference ($P = 0.89$). Likewise, there was no effect of treatment on BW ($P = 0.20$) or scrotal circumference ($P = 0.38$; Fig 2.2). These findings are in agreement with Arthington et al. (2002) who reported that bulls supplemented with dietary organic Zn above and beyond dietary requirements, did not differ in scrotal circumference or BW when compared to bulls supplemented within the recommended dietary range.

Treatment groups did not differ in pre-treatment serum trace mineral concentrations ($P \geq 0.57$). Pre and post-treatment serum trace mineral concentrations were within the range recommended for developing beef bulls (Herdt and Hoff, 2011). Although mean Cu pre-treatment serum concentrations (0.65 ± 0.04 µg/mL serum) were considered to be within the recommended range for adults and growing calves, the mean concentration was on the low end of the recommended range (0.6 - 1.1 µg/mL serum; Herdt and Hoff, 2011).

A time by treatment interaction ($P \leq 0.003$) was observed for all serum trace mineral concentrations evaluated (Fig. 2.3-2.4). A treatment effect, as well as a peak in serum trace mineral concentrations, occurred 8 h post-treatment with treated bulls having greater ($P < 0.0001$) serum concentrations of Cu, Mn, Se, and Zn than Control bulls. At 24 h post-treatment, concentrations of Se and Mn were greater ($P < 0.0001$) in Trace Mineral bulls than Control bulls. Serum Cu and Zn concentrations were similar ($P \geq 0.52$) between Trace Mineral and Control bulls 24 h post treatment (Fig. 2.3 - 2.4). This short term increase in circulating trace mineral concentrations, followed by a peak at 8 h is consistent with findings from Pogge et al. (2012) who observed a similar short term increase in circulating trace minerals followed by a peak at 8 h in beef steers.

Semen trace mineral concentrations in the present study were within a typical range as determined by Aguiar et al. (2012; Fig. 2.5-2.6). No time by treatment interaction *(P* ≥ 0.22) nor treatment effect *(P* ≥ 0.32) was apparent in semen trace mineral concentrations. Semen trace mineral concentrations at d 42 were similar between treatment groups (Cu: *P* = 0.38; Mn: *P* = 0.85; Se: *P* = 0.32; Zn: *P* = 0.36). At d 91 semen trace mineral concentrations remained similar between treatments (*P* ≥ 0.25).

Sperm parameters increased *(P* ≤ 0.0027) from d 42 to 91 in percent progressive motility, percent normal morphology, and sperm concentration. Percent proximal droplets decreased (*P* ≤ 0.0001) from d 42 to 91, but did not differ between treatments. Sperm concentration tended *(P* = 0.06) to be greater at d 42 for bulls receiving trace mineral treatment, but was similar *(P* = 0.83) between treatments at d 91 (Table 2.2). These findings are inconsistent with Shi et al. (2010) and Kendall et al. (2000). Shi et al (2010) reported that following supplementation of dietary Se-enriched yeast beyond Se NRC requirements, goats experienced increased semen volume, motility, viability, and concentration. Goats supplemented at the basal requirements for Se did not experience these improvements. Kendall et al. (2000) reported that in rams supplemented with a glass bolus containing Zn, Co, and Se, treated rams had greater sperm motility and proportion of live sperm than untreated rams.

A yearling breeding soundness examination was conducted 91 d post-treatment when bulls averaged 362 ± 25 d of age. A similar *(P* = 0.94) percentage of Trace Mineral (68.18%) and control (67.39%) bulls passed their breeding soundness examination (Fig. 2.7). A total of 29 bulls failed to meet the motility (30% progressive motility) and morphology (70% normal morphology) standards required to pass a breeding soundness examination. Of those bulls failing to pass the 91 d BSE, 30% failed due to a high percentage of sperm with proximal droplets. It is

not uncommon to find a higher percentage of proximal droplets in semen of bulls that are not yet sexually mature. These defects typically resolve with age and a reduction in proximal droplet percentage would be expected at breeding soundness exams conducted closer to time of sexual maturity (Barth and Waldner, 2002).

We hypothesized that use of a trace mineral injectable product would temporarily increase circulating trace mineral levels but would not improve semen trace mineral concentrations, sperm parameters, or ability to pass a breeding soundness exam. In the present study, the use of a bolus trace mineral injection was beneficial in raising circulating trace mineral concentrations at 8 h post-treatment, but was unsuccessful at maintaining those levels to 24 h. Although not evaluated in the present study, liver trace mineral concentrations would also be expected to increase. Pogge et al., (2012) reported that in steers managed in a dry lot setting and treated with a trace mineral injectable product (MULTIMIN 90) in conjunction with a dietary trace mineral program, liver concentrations of Cu, Zn, Se were greater for trace mineral treated steers than for control steers. Manganese liver status tended to be elevated in trace mineral steers as compared with control steers. Liver trace mineral concentrations remained elevated throughout the 15 d sampling period. Similar observations were reported by Arthington et al. (2014). Calves receiving an injectable trace mineral product (MULTIMIN 90) following transport had increased liver trace mineral status throughout the 13 day sampling period as compared with control calves. Assuming that liver trace mineral concentrations are elevated in the present study, one can conclude that trace mineral stores were not elevated to an extent capable of increasing seminal trace mineral concentrations and improving reproductive parameters.

It is important to note the tendency for increased sperm concentration at d 42 for Trace Mineral bulls. Although dietary requirements for trace minerals were met, Cu serum concentrations were on the low end (0.62 μg/mL) of the recommended Cu range (0.6-1.1 μg/mL; Herdt and Hoff, 2011) for bulls of both treatments. Of the 26 bulls per treatment that were analyzed for semen and serum trace mineral content, 13 Trace Mineral bulls and 14 Control bulls were below the Cu recommendations set forth by Herdt and Hoff (2011) prior to treatment administration (0h). By 24 h, 8 Trace Mineral bulls and 14 Control bulls remained below recommended Cu concentrations.

Early research indicates that bulls suffering from extreme Cu deficiency may experience ejaculates devoid of sperm (Thomas and Moss, 1951). In the present study, it is possible that bulls were experiencing a slight subclinical Cu deficiency and had compromised sperm concentrations as a result. If a subclinical deficiency were to exist and an injectable trace mineral product was administered, one would expect an increase in liver Cu stores followed by an increase in circulating and seminal Cu concentrations (Underwood, 1981). We did not find this in the present study as Trace Mineral and Control bulls had similar *(P* = 0.47) seminal Cu concentrations at d 42 and liver concentrations were not measured. Although seminal Cu status was not elevated, it is possible that bulls treated with injectable trace mineral product were able to overcome obstacles pertaining to sperm concentration. Collection time points earlier than 42 d post-treatment would have been beneficial in further explaining this particular result.

In an attempt to explain the low Cu status in the present study, it is necessary to look at the effects of byproduct feeding. In the present study, all bulls were fed corn gluten feed, a byproduct of the wet milling process of corn. Because sulfurous acid is added within the wet milling process, variable levels of sulfur within the feed can be of concern. High levels of sulfur

has been shown to interact with Cu and Mo within the diet rendering Cu unavailable (Mills, 1980). In the present study, it is possible that the low Cu status of bulls of both treatment could be a result of dietary interactions due in part to byproduct feeding.

Additional research is needed to further evaluate the effect of a trace mineral injectable product on reproductive function. Testicular biopsy at d 0, 42, and d 91 would provide information regarding trace mineral uptake into the testes. Brown and Burk (1972) reported that testicular Se content peaked 2 weeks following an intraperitoneal injection of Se. Information pertaining to teste mineral content could provide unique information pertaining to the storage and circulation of various trace minerals within reproductive tissues.

A similar study performed on older bulls would also be beneficial. This would allow for more frequent semen collection and would better illustrate how quickly trace minerals are taken up and cleared from semen. This adaptation is supported by a Smith et al. (1979) study in which elevated Se concentrations were found in the semen 23 days following an intravenous injection with Se-selenite. A study in which bulls were supplemented below NRC recommendations for trace minerals would also be interesting to evaluate, as it would better illustrate the impact of the trace mineral injection on reproductive parameters when deficiency exists.

The goal of this study was to demonstrate the effects of a trace mineral injection on circulating trace mineral concentrations as well as semen trace mineral concentrations, sperm parameters, and ability to pass a yearling breeding soundness exam. Our findings indicated that treatment with an injectable trace mineral product, in addition to a dietary mineral program, can be successful at raising short-term circulating trace mineral concentrations, but does not appear to be effective in improving sperm motility, morphology, or percentage proximal droplets.

Treatment with an injectable trace mineral product also does not appear to be of benefit in passing a breeding soundness exam 91 d post- treatment.

Implications

A supplemental trace mineral injection to developing beef bulls, administered in conjunction with a dietary mineral program, is successful at raising short term circulating trace mineral levels, but does not improve serum trace mineral concentration nor ability to pass a breeding soundness exam. Opportunities for additional research include more frequent blood and semen collections, testicular biopsy, and the use of deficient bulls. Although no treatment differences were observed in the present study, the use of an injectable trace mineral supplement may be warranted in cases where known deficiency exists, areas where traditional mineral supplementation is made difficult, and in animals displaying symptoms of a trace mineral deficiency.

Figures

Figure 2.1 Collection schedule detailing times of blood collection for serum trace mineral concentrations (Blood), days of semen collections for semen trace mineral concentrations (Ejaculate; Breeding Soundness Exam), days of scrotal circumference measurements (Scrotal), and days of body weight measurement (Weight).

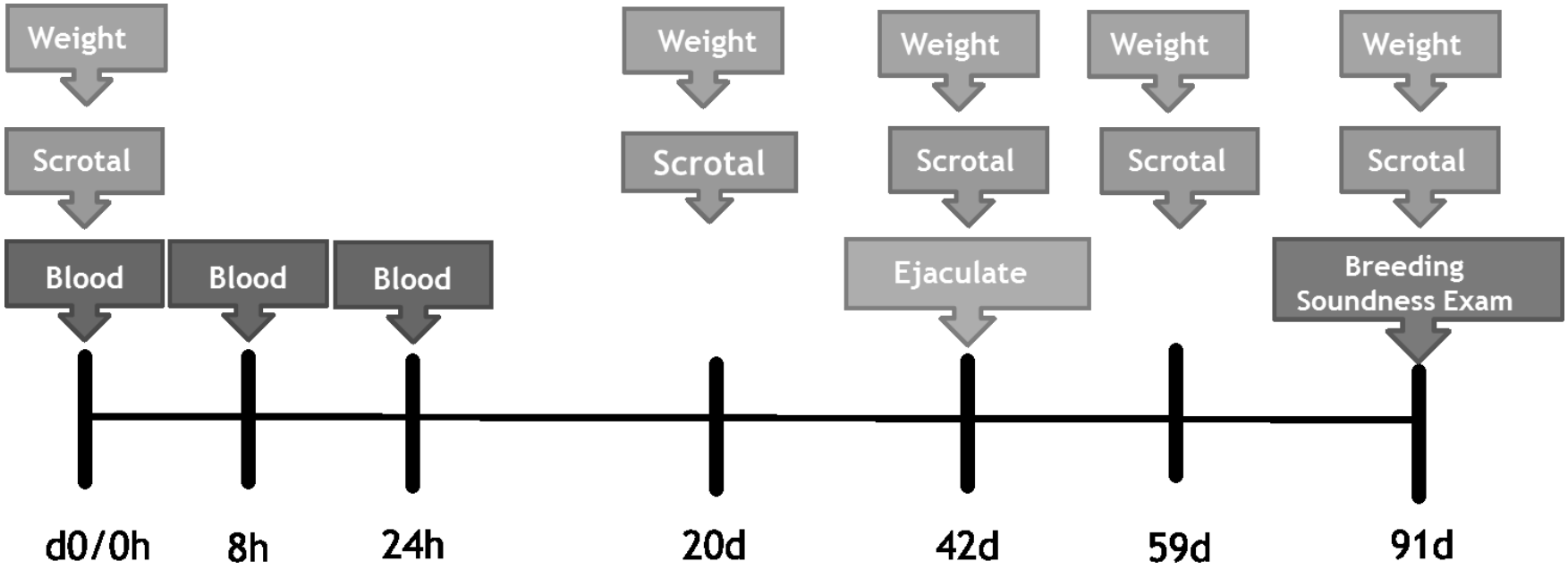


Figure 2.2 Weight and scrotal circumference of yearling Control and Trace Mineral bulls at d 0, 20, 42, 59, and 91 post-treatment with an injectable trace mineral product (Trace Mineral; n = 45) or saline (Control; n = 45); Weight: time × treatment ($P = 0.89$), time ($P \leq 0.0001$), treatment ($P = 0.20$); Scrotal Circumference: time × treatment ($P = 0.99$), time ($P \leq 0.0001$), treatment ($P = 0.38$).

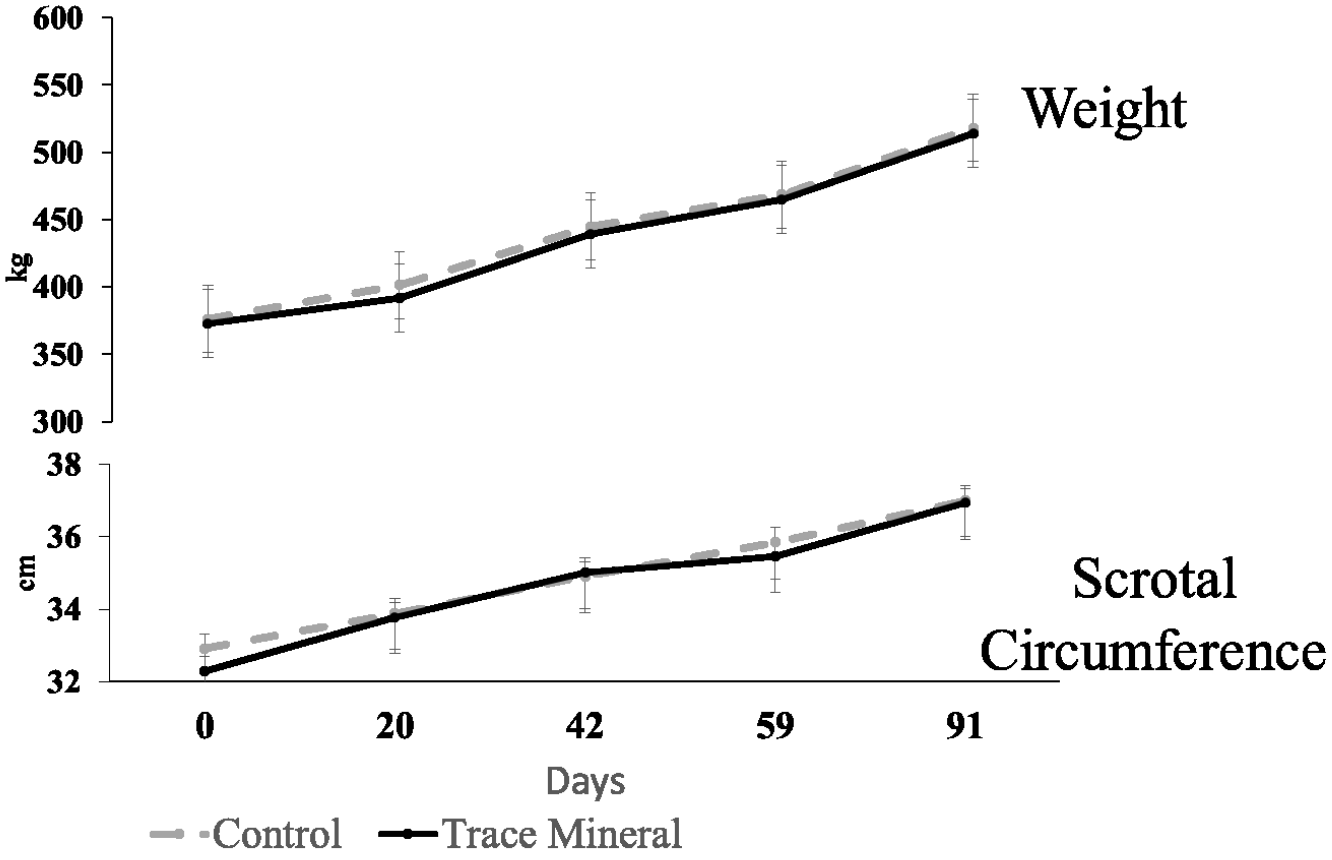


Figure 2.3 Serum Mn and Se concentrations of yearling Control and Trace Mineral bulls at 0, 8, and 24 hours post-treatment with an injectable trace mineral product (Trace Mineral; n = 26) or saline (Control; n = 26); time × treatment (*P* < 0.0001) and *treatment (*P* < 0.0001).

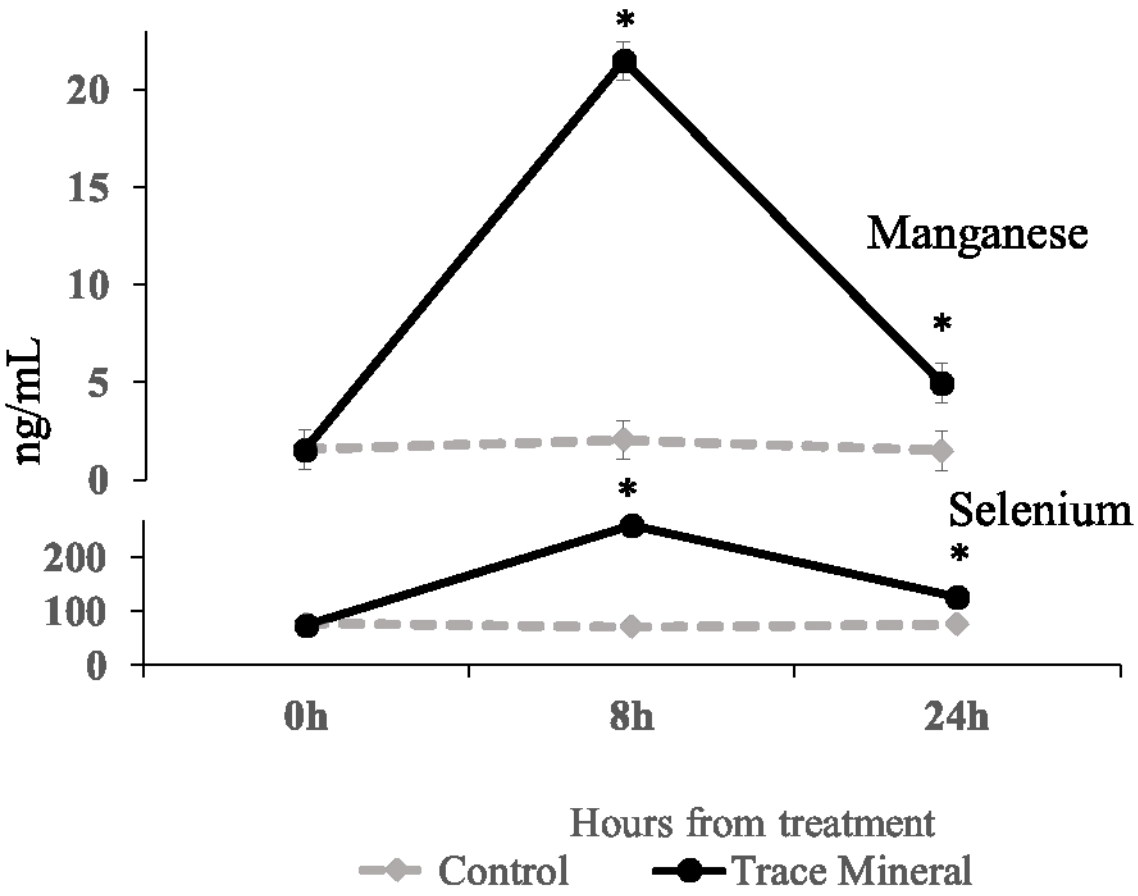


Figure 2.4 Serum Cu and Zn concentrations of Control and Trace Mineral bulls at 0, 8, and 24 hours post-treatment with an injectable trace mineral product (Trace Mineral; n = 26) or saline (Control; n = 26); time × treatment ($P \leq 0.003$), * treatment ($P < 0.0001$).

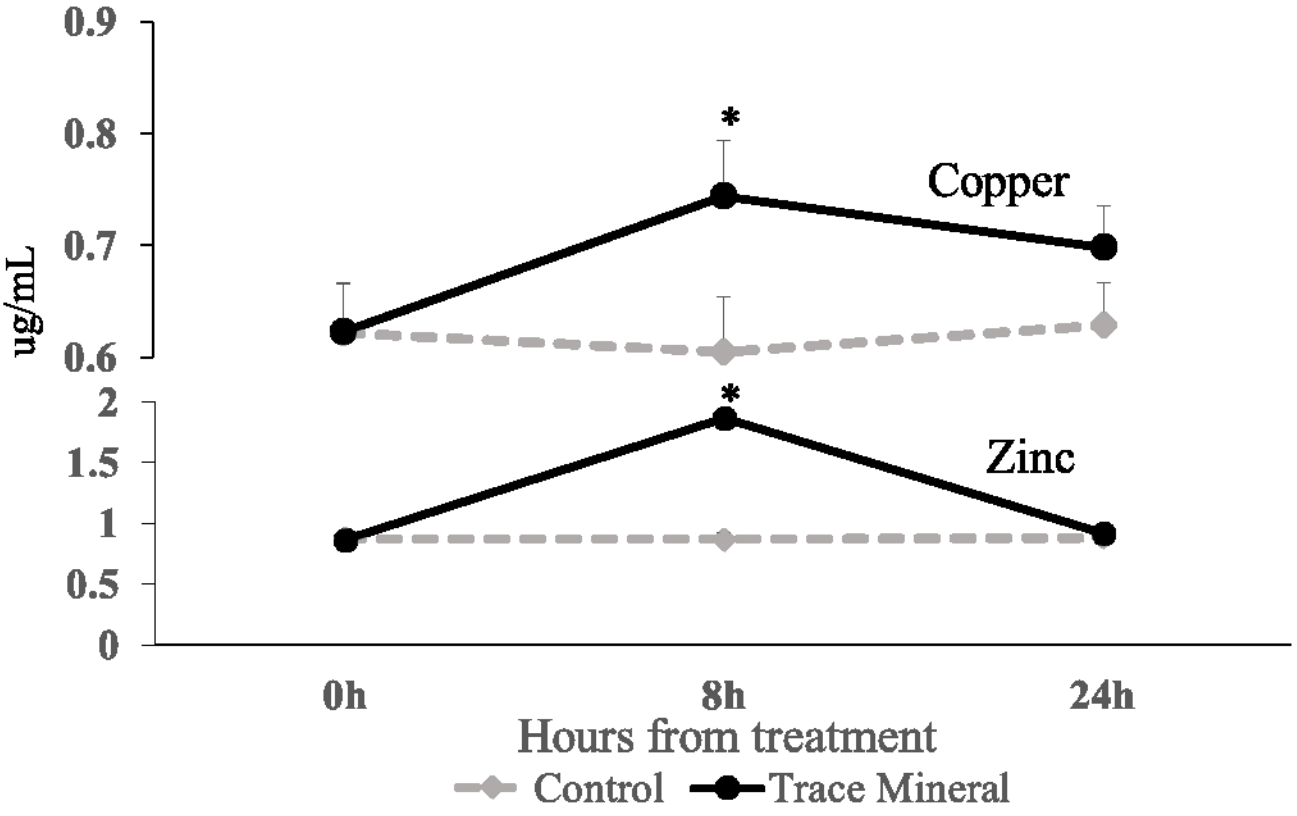


Figure 2.5 Semen Mn and Se concentrations of Control and Trace Mineral bulls at d 42 and 91 following administration with an injectable trace mineral product (Trace Mineral; n = 26) or saline (Control; n = 26); treatment ($P \geq 0.57$).

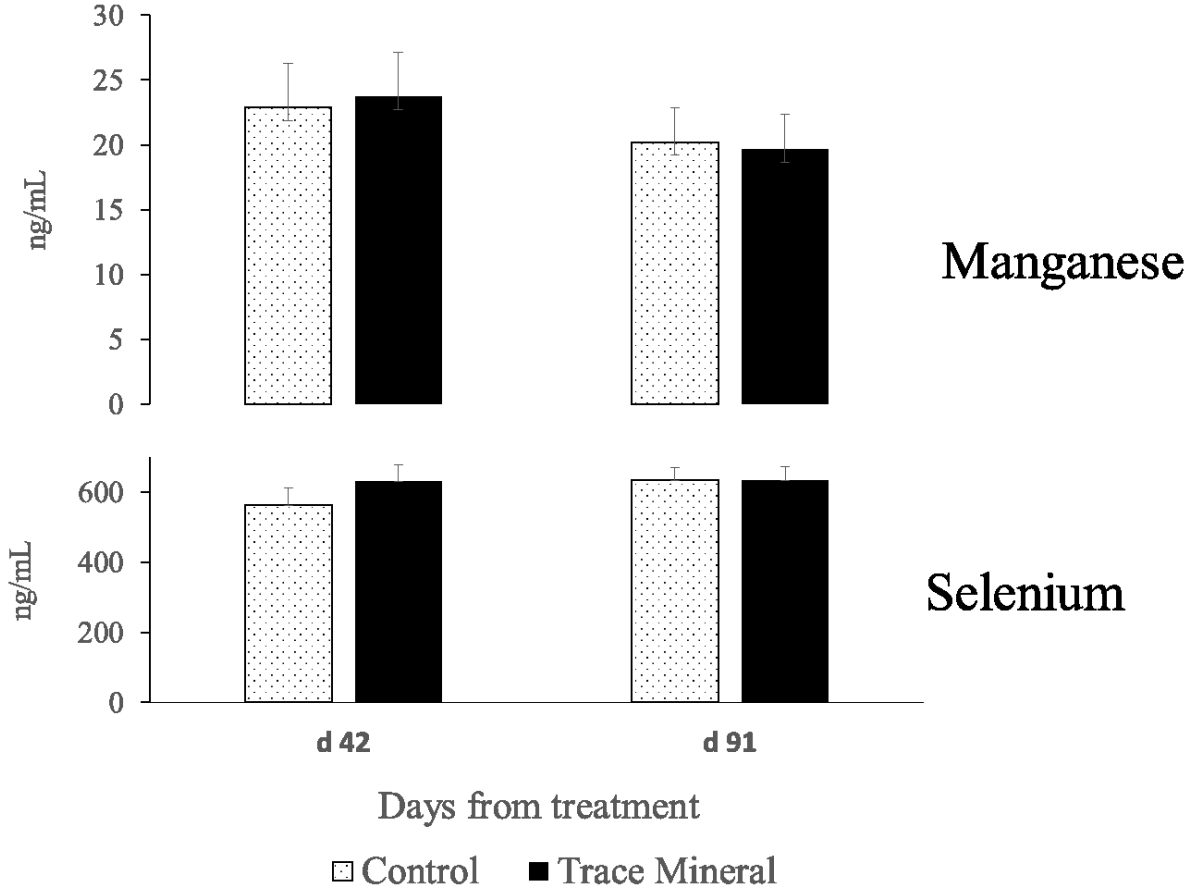


Figure 2.6 Semen Zn and Cu concentrations of Control and Trace Mineral bulls at d 42 and 91 following administration with an injectable trace mineral product (Trace Mineral; n = 26) or saline (Control; n = 26); treatment ($P \geq 0.79$).

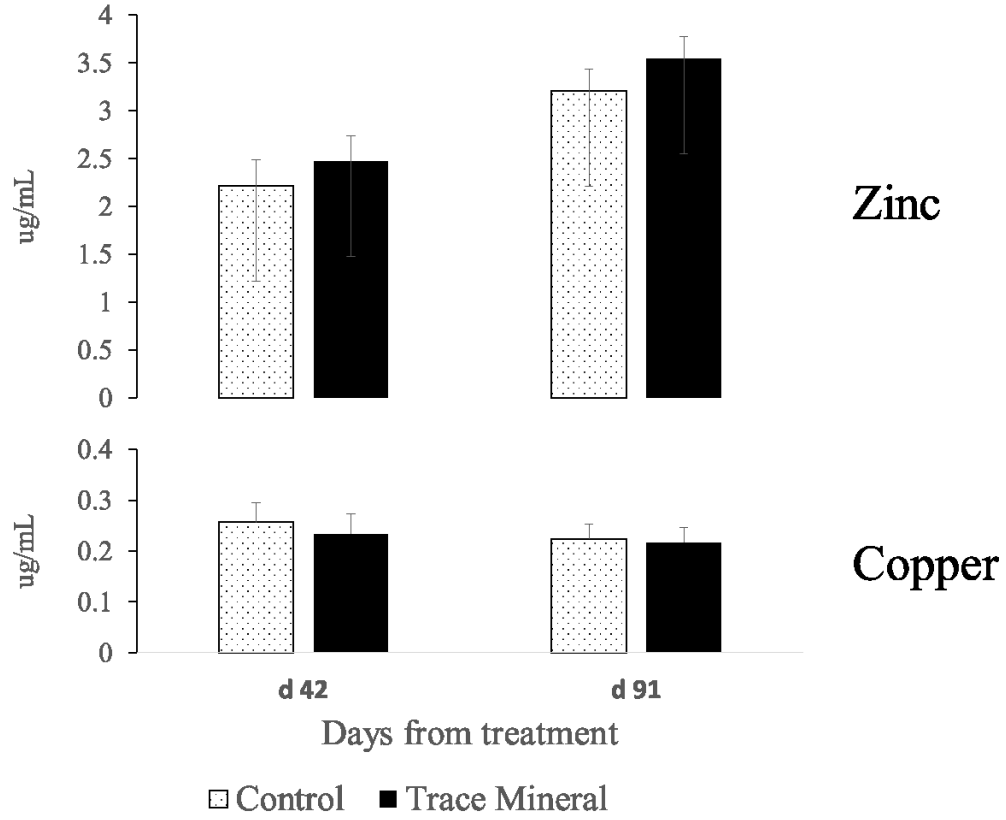
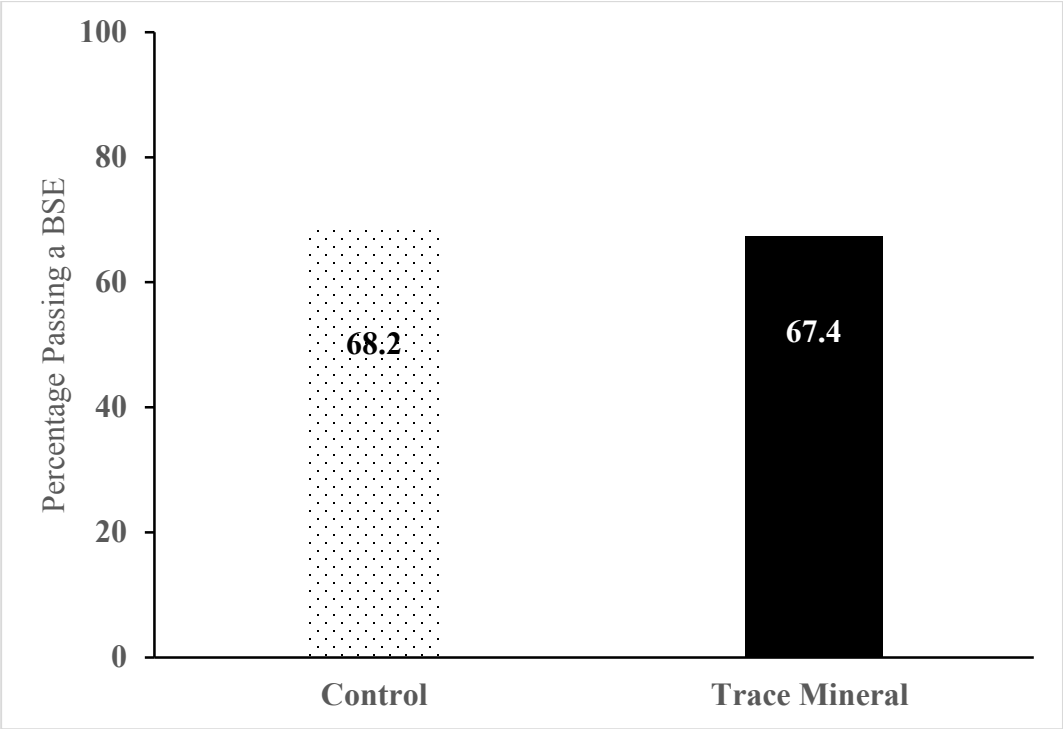


Figure 2.7 Percentage Control and Trace Mineral bulls (n=90) passing a yearling breeding soundness exam 91 days following treatment with an injectable trace mineral product (Trace mineral; n = 45) or saline (Control; n = 45); treatment *(P* = 0.93).



Tables

Table 2.1 Diet composition of both Trace Mineral and Control bulls.

Feedstuff	% Dry Matter	% As-fed
Corn Gluten Feed	27.83	47.49
Wheat Straw	25.65	28.82
Steam Flaked Corn	16.65	20.31
Limestone	1.16	1.16
Salt	0.22	0.22
Zinpro 4-Plex	0.11	0.11
Trace Mineral Premix	0.03	0.03
Sodium Selenite	0.02	0.02
Vitamin A, added	0.004	0.005
Vitamin E, added	0.003	0.003

Table 2.2 Sperm concentration, morphology, and motility of Control and Trace Mineral bulls at 42 and 91 days following treatment with an injectable trace mineral product (Trace Mineral; n = 42) or saline (Control; n = 43).

Parameter	Control		Trace Mineral		*P*-Value: Treatment		*P*-Value: Time
	d 42±SEM	d 91±SEM	d 42±SEM	d 91±SEM	d 42	d 91	
Sperm Concentration (10^6 sperm/mL)	90.49±39	219.04±29	151.51±39	226.66±29	0.06	0.83	<0.0001
Sperm Morphology (percent normal)	29.40±4.5	59.09±5.5	35.47±4.5	58.26±5.5	0.34	0.89	<0.0001
Sperm Motility (percent progressive)	38.33±2.6	44.43±2.6	39.19±2.6	42.33±2.6	0.82	0.56	0.01

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Chapter 3 - Effect of a supplemental trace mineral injection on pregnancy rates to first service artificial insemination in beef heifers

Abstract

We hypothesized that when dietary trace mineral needs are met, treatment with an injectable trace mineral product in developing beef heifers would not affect pregnancy rates at first service fixed-time artificial insemination. Fifteen Hereford, 47 Angus, and 20 Simmental heifers were blocked by breed and stratified by age (401.2 ± 9.7 d) and weight (315.0 ± 7.5 kg) and administered s.c. either a commercially-available trace mineral supplement (Trace Mineral; n = 39; 15 mg Cu/mL, 60 mg Zn/mL, 10 mg Mn/mL, and 5 mg Se/mL; MULTIMIN 90, MULTIMIN USA, Inc., Ft. Collins, CO) or sterilized saline (Control; n= 43) at 1mL/68 kg BW four weeks prior to breeding by fixed-time artificial insemination (FTAI). Day 0 is defined as day of treatment. Heifers were maintained in a drylot and fed a ration that included trace minerals at NRC recommended levels. Estrous cycles of heifers were synchronized using a modified 7-11 Co-Synch fixed time AI protocol. Heat detection patches were utilized to determine estrous behavior preceding and at time of FTAI. Pregnancy status was assessed via ultrasound at d 60 (32 days after FTAI). Data were analyzed using GLIMMIX in SAS with fixed effects of treatment, patch score, and treatment by patch score with heifer as the experimental unit. Breed served as a random variable. A greater *(P* = 0.02) percentage of Trace Mineral heifers (51.28%) became pregnant to FTAI than Control heifers (25.58 %). Patch score and treatment did not interact *(P* = 0.75) to affect pregnancy rate though a greater *(P* = 0.04) percentage of heifers with a red patch (48.39 %) at FTAI indicating estrous behavior became pregnant as compared with heifers with a grey patch (31.25 %) at FTAI. A similar *(P* = 0.13) percentage of Trace Mineral heifers (30.77%) and Control heifers (47.50%) displayed estrous

behavior as indicated by a red estrous detection patch at FTAI. In the present study, treatment of beef heifers with an injectable trace mineral 4 weeks before FTAI increased pregnancy rate to AI despite dietary trace mineral requirements being met.

Introduction

When managing developing heifers for a fixed-time artificial insemination protocol (FTAI), one must ensure that management factors promote conception at first service insemination. Nutrition, and, in particular, trace mineral nutrition, are important factors to consider prior to estrous synchronization.

Recent research has demonstrated that the use of a commercially available injectable trace mineral product containing 60 mg Zn/mL, 10 mg Mn/mL, 5 mg Se/mL 15 mg Cu/mL (MULTIMIN 90, MULTIMIN USA., Fort Collins, Co), administered in conjunction with a dietary trace mineral program, can be of benefit to improving reproductive success in cows and heifers. In a trial evaluating the use of an injectable trace mineral product (MULTIMIN 90) administered 30 days prior to fixed-time AI in grazing cows, conception rates of treated cows were greater than control cows (Mundell et al., 2012). Similar results were observed in heifers synchronized for timed embryo transfer (ET). Heifers treated with an injectable trace mineral product (MULTIMIN 90) 17 d prior to ET, had an increased percentage establishing pregnancy to embryo transfer as compared to control heifers (Sales et al., 2011).

Conversely, Vanegas et al., (2004) reported that for dairy cows receiving a single dose of an injectable trace mineral product or saline prior to calving and then again prior to breeding, treated cows had lower conception rates at first service than control cows. In this intensively managed dairy, all cows were fed a ration that met or exceeded NRC requirements for trace minerals.

The conflicting information regarding the use of an injectable trace mineral product in bovine females has led us to hypothesize that when dietary trace mineral needs are met, the use of a bolus injectable trace mineral product in developing beef heifers will not affect pregnancy rates at first service fixed-time artificial insemination (FTAI). The objectives of this study were to compare pregnancy rates of Control and Trace Mineral heifers at a 32 d ultrasound following FTAI and to compare estrous behavior immediately preceding FTAI as indicated by estrous detection patch color of Control and Trace Mineral heifers at time of FTAI.

Materials and Methods

This study was conducted in accordance with the Kansas State University Institution Animal Care and Use Committee (IACUC) at the Kansas State University Purebred Beef Teaching Unit. Eighty two heifers (Angus, n=47; Simmental, n=20; Hereford n=15) were blocked by breed and stratified by age (401.2 ± 9.7 days) and weight (315.0 ± 7.5 kg). Heifers were then randomly assigned to 1 of 2 treatments: 1) s.c. injection of a commercially available bolus trace mineral supplement [(Trace Mineral; n=39; MULTIMIN 90, MULTIMIN USA, Inc., Fort Collins, CO) containing 60 mg Zn/mL (as Zn disodium EDTA), 10 mg Mn/mL (as Mn disodium EDTA), 5 mg Se/mL (as sodium selenite), and 15 mg Cu/mL (as Cu disodium EDTA)]; or 2) s.c. injection of sterile saline (Control; n=43). Treatments were administered at a dose of 1 mL/68 kg BW four weeks prior to breeding by FTAI (Fig. 3.1). Day 0 was defined as day of treatment.

Heifers were housed in one pen measuring 97.6 m × 128.1m. Throughout the study heifer diets were formulated to achieve 1.2 kg ADG and supplemented at NRC recommendations for trace minerals on a DM basis (23.89 mg/kg Cu, 82.05 mg/kg Mn, 0.25 mg/kg Se, 99.79 mg/kg Zn).

Heifers were synchronized with a modified 7-11 Co-Synch protocol with a CIDR (Fig. 3.1). All heifers received a controlled internal drug release device releasing progesterone (CIDR; EAZI-BREED CIDR, Zoetis, Florham Park, New Jersey) on d 8 of the study. After a period of 7 days (d 15) the CIDRs were removed and an intramuscular prostaglandin F2α injection (Lutalyse, Zoetis Animal Health, Florham Park, NJ) was administered at a dose of 5mL/heifer. On d 19, the same CIDRs used for the previous 7 days were reinserted and an intramuscular GnRH injection (Cystorelin, Merial LTD, Duluth, GA) was given at a dose of 2 mL/heifer. CIDRs were then removed 7 days later (d 26) and a second prostaglandin F2α injection (Lutalyse, Zoetis Animal Health, Florham Park, NJ) was administered. At this time, estrous detection patches (ESTROTECT Heat Detectors, Rockway Inc., Spring Valley, WI) were placed midway between the hip bones and tail head of every heifer to further evaluate the effect of a trace mineral injection on estrous behavior. On d 28, 48 hours following removal of CIDRs, estrous detection patches were evaluated. Patches with 50% or more of the grey surface removed, exposing red underneath, were called red. Patches with less than 50% of the grey surface removed were called grey. Heifers with red patches were considered to have displayed estrous behavior (particularly mounting), while those with grey patches had not. Following patch scoring, all heifers received an injection of GnRH (Cystorelin, Merial LTD, Duluth, GA) at a dose of 2 mL/heifer, and were artificially inseminated following GnRH administration. Pregnancy status was assessed via transrectal ultrasound at d 60 (32 days after artificial insemination).

Statistical analyses were conducted using procedures in SAS (version 9.2, SAS Inst. Inc., Cary, NC). Heifer was the experimental unit. Pregnancy data and patch scores were analyzed using the general linearized mixed model (GLIMMIX) with fixed effects of treatment, patch

score, and treatment by patch score. Breed served as the random variable. A *P*- value of ≤ 0.05 was considered significant. Three observations were excluded from patch scores because heifers had failed to retain their patches at FTAI. P-values > 0.05 < 0.10 were considered as tendencies.

Results and Discussion

Treatment and patch score did not interact *(P* = 0.75) to affect pregnancy rates. A treatment effect *(P* = 0.02; Fig. 3.2) was observed at time of pregnancy assessment by ultrasound, with Trace Mineral heifers having greater pregnancy rates (51.28%; 20/39) than Control heifers (25.58%; 11/43). Although pregnancy rates differed among treatment groups, no such observation was noted for estrous detection patch scores. A similar percentage *(P* = 0.13; Fig. 3.3) of red patches were observed for Trace Mineral heifers (30.77%; 12/39) and Control heifers (47.50%; 19/40) at time of FTAI. A greater *(P* = 0.04) percentage of heifers that displayed estrous behavior (Red Patch at FTAI; 48.39%; 15/31) became pregnant as compared with heifers that did not display estrous behavior (Grey Patch at FTAI; 31.25%; 15/48; Fig. 3.4).

We hypothesized that use of a bolus injectable trace mineral product would not affect pregnancy rates at first service FTAI nor would it affect estrous behavior as measured by estrous detection patch scores. In the present study, the use of a bolus trace mineral injection improved pregnancy rates to first service AI, as measured by a 32 d pregnancy assessment, but did not affect estrous behavior. These findings are in agreement with Mundell et al. (2012) who reported increased pregnancy rates to FTAI in grazing cows and heifers receiving a trace mineral injection as compared with cows and heifers that did not receive a trace mineral injection. The findings of Mundell et al. (2012), although in agreement with the present study, may be attributed to cows grazing native range with various forage trace mineral inadequacies, variable trace mineral intake, and dietary trace mineral interactions. In the present trial, heifers were fed in a bunk

setting, with recommended trace mineral levels supplemented in the diet. Although variability in trace mineral intake and dietary trace mineral interactions could still occur, because heifers were fed in a bunk type setting, we speculate variability had a lesser effect in the present study as compared with other studies. The results of the present study are also in agreement with Brasche et al. (2015) who reported heifers that received a trace mineral injection prior to artificial insemination and a 7d Co-Synch Plus CIDR protocol had greater pregnancy rates than control heifers. Brasche et al. (2015) speculated that although heifers were fed in a bunk type setting, forage included in the mixed ration was inadequate in various trace minerals allowing those heifers treated with a trace mineral product an advantage over untreated heifers.

Conversely, the present study is in disagreement with data reported by Vanegas et al. (2004) who reported that dairy cows receiving a trace mineral injection prior to FTAI experienced decreased pregnancy rates as compared with control cows. It is likely, that in an intensively managed operation with a mixed ration meeting NRC trace mineral recommendations, such as the dairy in the Vanegas et al (2004) study, adding a trace mineral injection did not increase the trace mineral status enough to observe increases in pregnancy rates to FTAI.

In an attempt to understand the elevated pregnancy rates in the Trace Mineral group, as compared to the Control group, it is important to note the anecdotal observation of potential vaginal infection following removal of the reused CIDRs on d 26 of the study. As CIDRs were removed, small amounts of pink tinged vaginal discharge were observed both on CIDRs and vulvas of heifers in both treatment groups. We speculate that the reused CIDRs may have irritated the vaginal mucosa and possibly led to the introduction of bacteria and potential infection. We speculate that a potential infection may have affected ability to conceive to FTAI,

reducing the overall pregnancy rate of the herd in the present study to a percentage much below the average for FTAI in heifers. Though different estrous synchronization protocols were used, pregnancy rates of heifers in this same herd in recent years have been approximately 50%. These recent herd pregnancy rates are within the expected range of FTAI in heifers (43-62%; Martinez et al., 2002; Busch et al., 2007).

Similar observations of an infection following a 7 d CIDR protocol were described by Fischer-Tenhagen et al. (2012) and Chenault et al. (2003). Both noted the presence of coliform bacteria in cows that experienced vaginal discharge characteristic of infection following a 7 day CIDR protocol. Although vaginal infection was noted, infection status did not appear to alter pregnancy rates (Fischer-Tenhagen et al., 2012). Coliform bacteria, although a known inhabitant of the genitourinary tract, may have contributed to infection in the present study. Injectable Se has been shown to enhance the immune system's response to coliform bacteria (Panousis et al., 2001). Panousis et al. (2001) demonstrated that dairy cows treated with injectable Se had improved production of antibodies against coliform bacteria. In the present study, one could consider the inclusion of Se in the injectable trace mineral product as a possible means for overcoming infection and contributing to the increased pregnancy rates of the Trace Mineral heifers.

Injection with a trace mineral product has been shown to elevate acute phase proteins in receiving calves following transport, specifically: haptoglobins, ceruloplasmins, and acid soluble proteins, all early indictors of innate immune system activation (Arthington et al., 2014). One can speculate that these markers of an inflammatory reaction may serve to activate the innate immune system and better prepare it for future assaults. In the present study, treatment with an injectable trace mineral product may have elevated acute phase proteins of Trace Mineral treated

heifers and allowed the heifers' immune system to better respond to possible infections that may have been introduced by a prolonged CIDR protocol. Although the heifers in the present study did not experience the stress of transport, the EDTA component of the trace mineral product has been speculated to elevated acute phase proteins as well (Arthington et al., 2014).

Improvement in immune status has been frequently reported among cattle treated with an injectable trace mineral product. Use of an injectable trace mineral product has been shown to improve production of neutralizing antibodies in calves receiving viral vaccinations, as well as improve feed efficiency in finishing steers (Clark et al., 2006; Arthington and Havenga, 2012). In a study evaluating the effects of a trace mineral injection on shipping stressed heifers, heifers receiving an injectable trace mineral product had lower antibiotic treatment costs, reduced morbidity to bovine respiratory disease, and improved feed conversion (Richeson et al., 2006). These effects pertaining to the immune system could also be in place in the present study. The prolonged CIDR protocol may have introduced potential infection. Heifers treated with an injectable trace mineral product may have been able to overcome potential infection because of heightened immune function.

The effect of estrous behavior (as indicated by estrous detection patch color at FTAI) on pregnancy status was as expected. Regardless of treatment, of heifers with a red patch at FTAI (indicating displayed estrus) 48.39% became pregnant. Of heifers with a grey patch at FTAI (indicating had not displayed estrus) only 31.25% became pregnant. Perry et al. (2007) concluded that heifers in standing estrus within 24 h of FTAI had greater pregnancy rates than those heifers not in estrous. These findings are replicated in the present study with heifers displaying estrous behavior as indicated by a red patch becoming pregnant more frequently than those not displaying estrous behavior.

Although the effect of estrous behavior on pregnancy status was expected, the percentage of heifers displaying estrous behavior between treatments was not as clear. A similar percentage of red patches were observed for both Trace Mineral heifers (30.77%; 12/39) and Control heifers (47.50%; 19/40) at time of FTAI. The increase in pregnancy rates of treated heifers does not appear to be related to estrous behavior

Additional research is needed to further evaluate the effect of a trace mineral product on reproductive function. Because of the possibility of infection introduced by the synchronization protocol used in the present study, a more common estrous synchronization protocol would be of benefit. Evaluating heifer progesterone data prior to synchronization could also be of benefit to evaluate cyclicity. Serum concentrations of Zn, Cu, Mn, and Se would be beneficial at various time points throughout the study to add to the body of knowledge regarding the circulation of injectable trace minerals.

If a synchronization protocol similar to the one used in the present study were to be repeated, it would be very interesting to collect data pertaining to a potential infection such as acute phase proteins, inflammatory cytokines, and bacterial populations.

The goal of this study was to evaluate the effect of a trace mineral injection on pregnancy rates to first service FTAI. Our findings indicate that treatment with an injectable trace mineral product, in addition to a dietary mineral program, to developing beef heifers 28 d prior to FTAI can be successful at increasing pregnancy rates.

Implications

Under conditions of the present study, a supplemental trace mineral injection, administered in conjunction with a dietary mineral program, to developing beef heifers 28 d prior to FTAI is successful at increasing pregnancy rates, but does not affect estrous behavior.

Figures

Figure 3.1 Modified 7-11 Co-Synch with CIDR protocol used to synchronize estrous cycles of Trace Mineral and Control heifers following treatment.

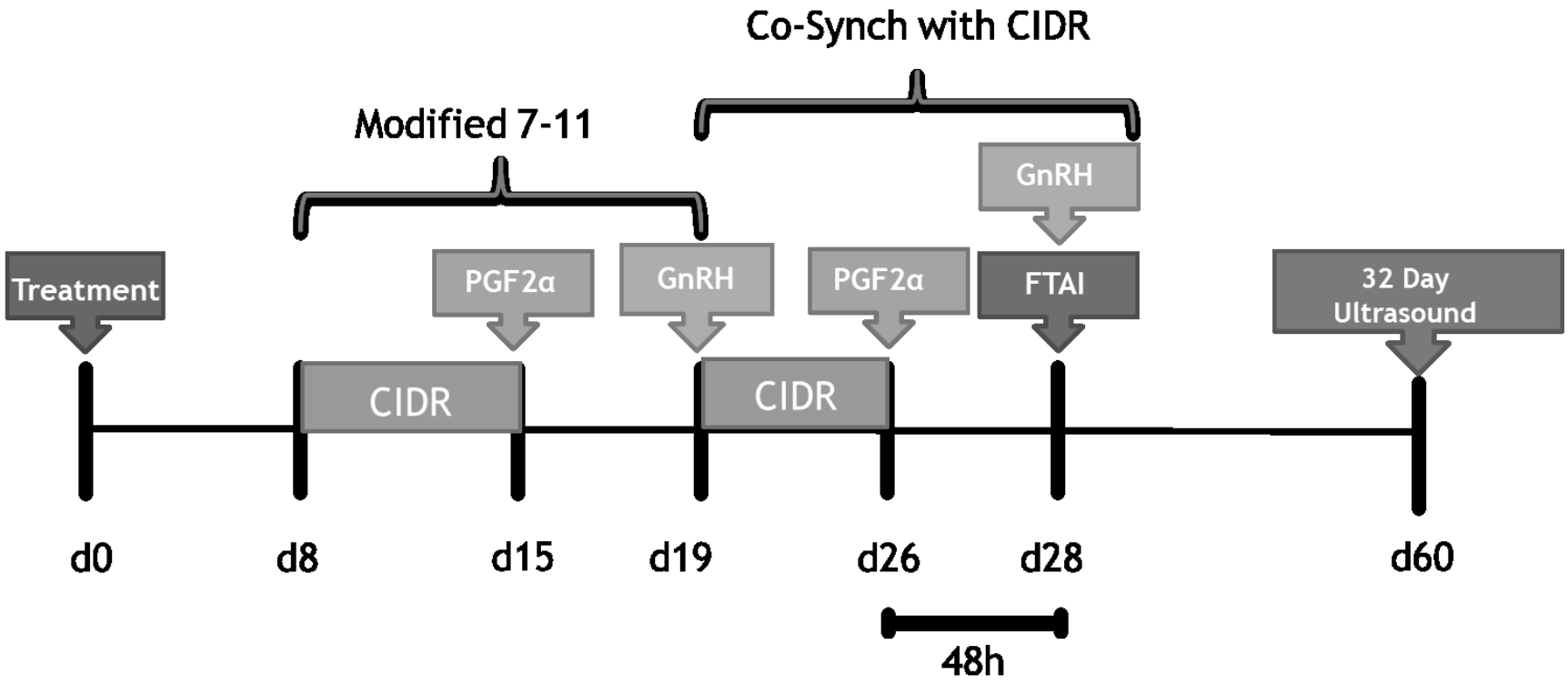


Figure 3.2 Pregnancy rate at 32 d post FTAI of Control and Trace Mineral heifers administered an injectable trace mineral product (Trace Mineral; n = 39) or saline (Control; n = 43); *treatment (*P* = 0.02).

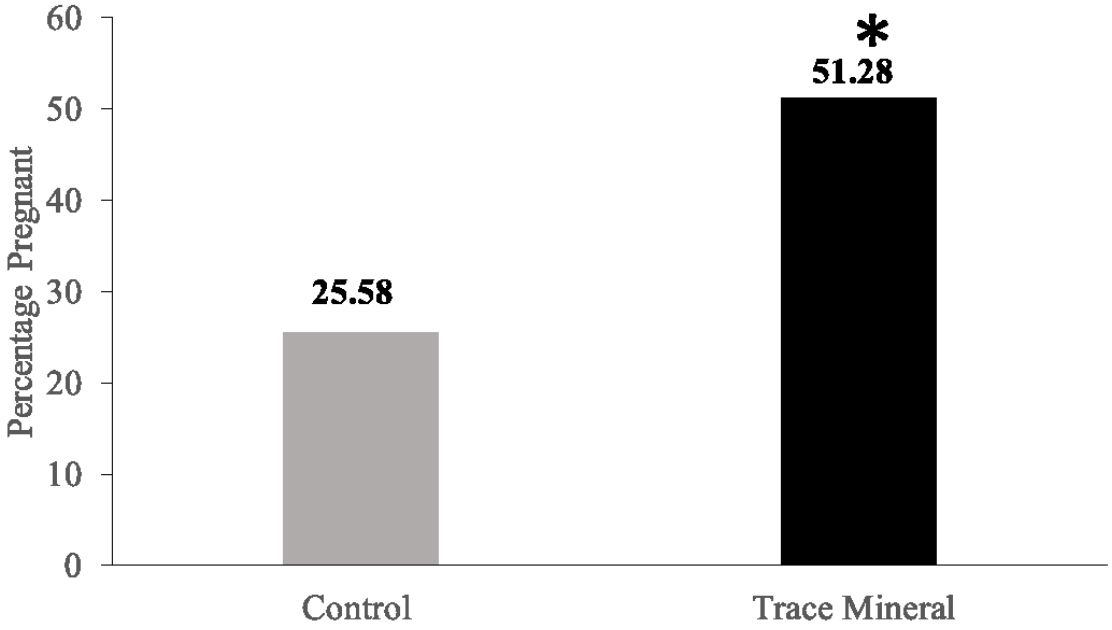


Figure 3.3 Percentage Control and Trace Mineral heifers administered an injectable trace mineral product (Trace Mineral; n = 39) or saline (Control; n = 43) that displayed estrous behavior as indicated by presence of a red estrous detection patch at FTAI *(P* = 0.13)

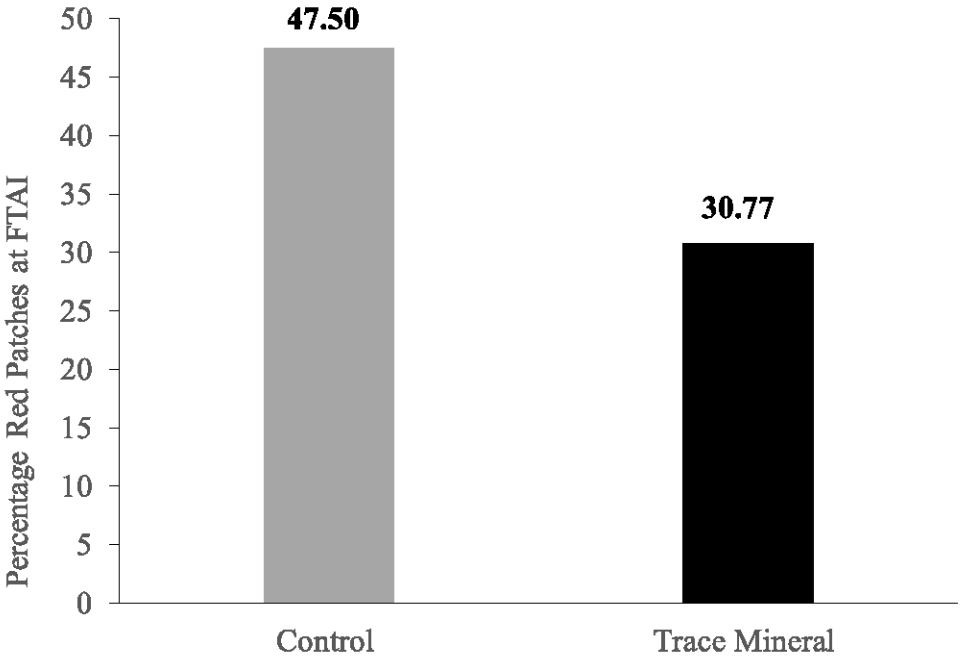
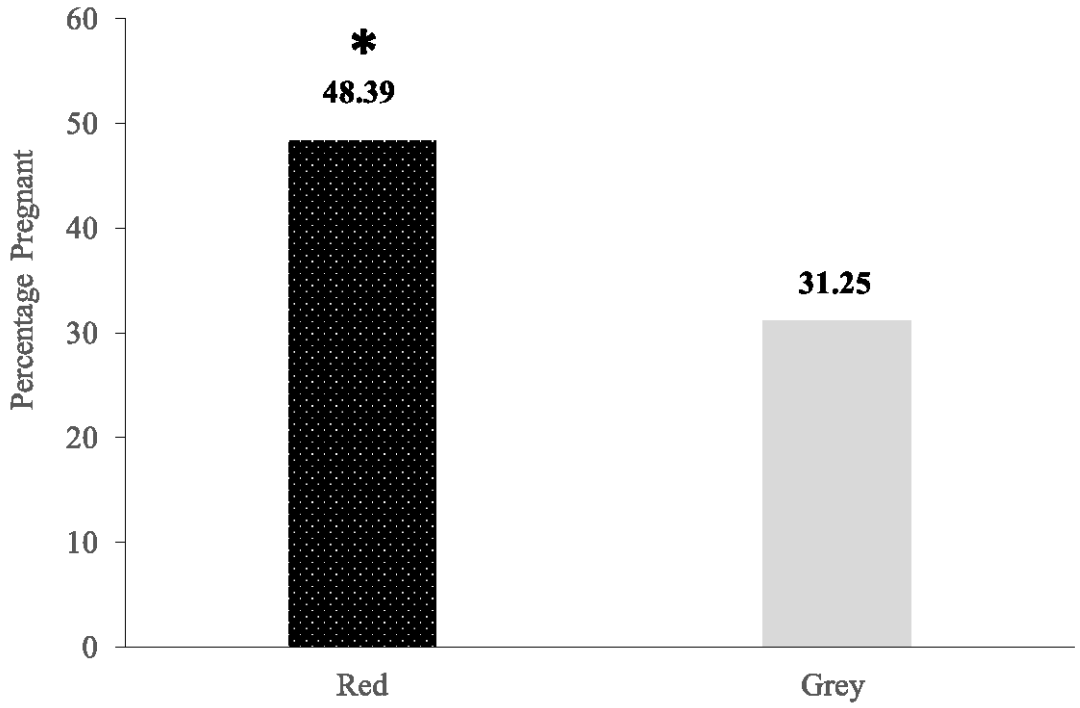


Figure 3.4 Pregnancy rate to FTAI of heifers that displayed estrous behavior (Red patch; n = 31) and those that did not display estrous behavior (Grey patch; n = 48); *patch (*P* = 0.04).



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