THESIS

THE INFLUENCE OF TRACE MINERAL SOURCE ON REPRODUCTIVE PERFORMANCE IN RECIPIENT MULTIPAROUS BEEF COWS

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ABSTRACT

THE INFLUENCE OF TRACE MINERAL SOURCE ON REPRODUCTIVE PERFORMANCE IN RECIPIENT MULTIPAROUS BEEF COWS

An experiment was conducted to determine the effects of copper (Cu), cobalt (Co), manganese (Mn), selenium (Se), and zinc (Zn) source on the reproductive parameters of multiparous beef cows. Fifty cow-calf pairs were divided into ten groups (n=5 cow-calf pairs per group) and balanced across groups for animal source, cow weight, calf age, calf sex, and breed type. Each group was randomly assigned to one of the following treatments: 1) Organic trace minerals: 75 mg of Cu/d from Cu proteinate, 8 mg of Co/d from Co proteinate, 105 mg of Mn/d from Mn proteinate, 3 mg of Se/d from Sel-Plex, and 220 mg of Zn/d from Zn proteinate; and 2) Inorganic trace minerals (at two times the NASEM (2016) requirements): 255 mg of Cu/d from $CuSO_4 \cdot 5H_20$, 2.6 mg of Co/d from $CoCO_3$, 1018 mg of Mn/d from $MnSO_4$, 3 mg Se/d from Na_2SeO_4, and 763 mg of Zn from $ZnSO_4 \cdot 5H_2O$. Animals were fed a corn silage – corn stalk-based diet that met or exceeded the NASEM (2016) requirements for gestating beef cows with the exception of Cu, Co, Mn, Se, and Zn. Cows were individually supplemented with their appropriate treatments daily for 89 days, five days after the initiation of estrus synchronization (day 0).

Estrus synchronization was achieved through implantation of a progesterone controlled internal drug release (CIDR; Zoetis; impregnated with 1.38 g progesterone) device and 2ml of gonadotropin-releasing hormone (GnRH; Factrel from Zoetis) administered intramuscularly. After seven days, the CIDR was removed, 2 ml lutalyse (HighCon from Zoetis) was

administered intramuscularly, and an Estrotect patch was applied for estrus detection. After two additional days, recipient cows were given a second dose (2ml) of GnRH. Eight additional days later, all recipient cows that were synchronized were palpated, and those with a viable corpus luteum (CL) received an embryo as well as a CIDR. The CIDR was removed 15 days later and an Estrotect patch was applied. Expression of estrus was evaluated for the following four days of the experiment. Cows that did not express estrus, and cows that did not maintain a pregnancy from the first embryo, underwent the same estrus synchronization protocol a subsequent time. A licensed veterinarian made pregnancy diagnoses on August 18th, September 12th, and October 8th using an ultrasound, recording each cow as either pregnant or open.

Blood samples were collected via jugular venipuncture from each animal on days 0, 16, 43, 68, and 94 of the experiment and analyzed for progesterone, luteinizing hormone, anti-mullerian hormone, follicle-stimulating hormone, and estradiol. However, as animals in this study varied by pregnancy status and days pregnant, mineral status and hormone status were evaluated by time points (TP) defined as TP0: prior to embryo transfer; TP1: day of embryo transfer; TP2: 28-33 days post embryo transfer; TP3: 58-60 days post embryo transfer; and TP4: 84 days post to embryo transfer. There was a treatment by time effect for plasma Cu concentrations ($P < .03$), with Cu concentrations of the inorganic treatment decreasing (0.89 to 0.76 mg Cu/L) and those of the organic treatment increasing (1.01 to 1.19 mg Cu/L) as the experiment progressed. There were no other significant time or treatment by time effects for plasma trace mineral concentrations. Cows receiving organic trace minerals had greater plasma Se and Cu ($P < .0001$) concentrations when compared to cows receiving the inorganic trace mineral. Plasma cobalt, manganese, and zinc concentrations were similar between the two treatments ($P > 0.1$). Pregnancy rates of the inorganic and organic treatments were similar at the

conclusion of the trial (66.6% and 62.5%, respectively). Treatment outcomes were also similar ($P > 0.10$) for embryo transfer attempts, calf birth weights, and days pregnant. There were no treatment or treatment by time interactions for serum progesterone, luteinizing hormone, anti-mullerian hormone, follicle-stimulating hormone, or estradiol concentrations ($P > 0.10$). The differences in circulating trace elements between treatments did not appear to impact the hormone concentrations or reproductive outcomes in this trial. Further research is needed to further understand the impact of mineral source on reproductive outcomes.

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CHAPTER 1: REVIEW OF LITERATURE

TRACE MINERAL METABOLISM

Introduction

There are 16 minerals required for normal biological function in ruminants. Of the 16 required minerals, seven are referred to as "trace" minerals because they are required in small quantities in the diet (generally less than 100 mg/kg DM). These minerals include cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn), each of which will be discussed in this review. The effects of minerals on beef cattle production efficiency have been studied, to a large extent, for well over a century. The following section aims to describe the progress made in the understanding of mineral metabolism.

Biological Processes of Trace Minerals

Trace minerals perform functions in the body that can be categorized as: structural, physiological, catalytic, and regulatory (Suttle, 2010). These broad functions are involved in various biological processes like reproduction (Corah and Ives, 1991), immunity (Arthington, 2006), and growth (Mills, 1981). These biological processes can be affected by both deficiencies and toxicities of a given trace mineral (Smart et al., 1981; López-Alonso, 2012).

Deficiencies of a given trace mineral can be a product of either a primary deficiency, where the quantity of given mineral being consumed by the animal is below the animal's requirement, or a secondary deficiency. A secondary deficiency is not caused by insufficient dietary quantities of the given mineral but instead, due to other biological conditions. For example, a secondary mineral deficiency can be due to mineral interactions with antagonists that limit the ability of a specific essential mineral to be absorbed. A well-documented mineral interaction is the copper-molybdenum-sulfur interaction that limits copper absorption (O'Dell,

1989). Sulfur in the rumen is reduced and binds to molybdenum forming a thiomolybdate. Thiomolybdates have a high binding affinity for copper. When bound, copper becomes insoluble and therefore not available for absorption in the gut (Gould and Kendall, 2011). The absence of other important micronutrients can also lead to a secondary mineral deficiency. For example, the lack of adequate dietary vitamin E concentration can reduce the overall selenium status in an animal (McDowell et al., 1996). Changes in these biological processes can impact the health (Dorton et al., 2006), reproduction (Corah and Ives, 1991), and growth capability of an animal. This review covers five minerals that are involved in metabolism, cellular functions, and enzyme activity: cobalt, copper, manganese, selenium, and zinc.

Cobalt

Cobalt is an essential trace mineral for ruminants (Neal and Ahmann, 1937). Underwood and Filmer (1935) first discovered the essentiality of cobalt in ruminant diets. They investigated the effectiveness of cobalt supplementation on suppressing symptoms of enzootic marasmus (nutritional anemia), a wasting disease also called bush sickness (Becker and Gaddum, 1937). Sheep with enzootic marasmus lose weight, have decreased hemoglobin, and death occurs without supplementation of cobalt or cobalt-contaminated trace minerals (Underwood and Filmer, 1935). Later it was determined that these shared symptoms of various diseases were due to a dietary cobalt deficiency (Jones and Anthony, 1970).

Another disease referred to as polioencephalomalacia, has been observed in ruminants, where lesions appear on the brain. The animals can be seen applying pressure to their heads by pressing against fence posts (Mullins et al., 1958). This disease is most often a direct result of elevated sulfur concentrations in the diet of ruminants (Gould et al., 1997). However, the pathology of this disease includes depression of thiamine production (Rammell and Hill, 1986),

which is produced in the rumen by microbes (Breves et al., 1980). Microbial production of thiamine can also be inhibited by microbial thiaminases (Edwin and Jackman, 1981) or by low cobalt concentrations (MacPherson et al., 1976). Hartley et al. (1962) separated wethers into two groups while grazing cobalt-deficient pastures in New Zealand. One group received cobalt and the control group received no supplemental cobalt. These researchers observed that the control group developed what appeared to be polioencephalomalacia, including vision loss, decreased body weight gain, and some died, which agreed with findings in the 1958 report above.

Similarly, MacPherson et al., (1976) reported that sheep given a cobalt-deficient diet developed polioencephalomalacia symptoms and that the animals had developed thiamine deficiency. These researchers noted that elevated pyruvate, glutamic-oxaloacetic transaminase, and pyruvate kinase were present in the circulatory system of sheep that had developed thiamine deficiency. However, more recently, these findings were called into question by Sargison et al. (2001), due to the lack of common polioencephalomalacia symptoms, the lack of response to thiamine supplementation, and the sufficient concentration of B_{12} (cobalamin) after inducing cobalt deficiency in sheep. These researchers diagnosed the symptoms of their work and the previous work as hepatic encephalopathy, instead of polioencephalomalacia, as they found degeneration of the hepatocytes and status spongiosum of the brain. Vellema et al. (1999) also made connections between cobalt deficiency and liver damage but noted a deficiency of vitamin B_{12} during cobalt deficiency. A disease similar to hepatic encephalopathy was previously discovered and termed white liver disease (Clark et al., 1978), due to the appearance of the pale, fatty livers. White liver disease was induced by a deficiency in vitamin B_{12}, coinciding with an accumulation of methylmalonic acids which are mis-incorporated into fatty acids in the liver, as described by Kennedy et al. (1994). Kennedy et al. (1997) subsequently conducted further

investigations with results that suggested the buildup of fatty acids is likely due to decreases in the activity of B_{12} dependent enzymes causing lipid peroxidation, including methylmalonyl CoA mutase and methionine synthase. Therefore, the current data would suggest that a cobalt deficiency does not induce a true polioencephalomalacia, but rather induces liver and brain damage via vitamin B_{12} and thiamine deficiencies.

Other research conducted in sheep demonstrated that B_{12} deficiency caused a buildup of circulating methylmalonyl-CoA in whole blood, coinciding with a marked decrease in the conversion of propionate to succinyl-CoA by the liver (Marston et al., 1972). These data suggest that ewes deficient in cobalt had a reduced the ability to metabolize propionate. Additionally, to evaluate the ability of the cobalt deficient and cobalt supplemented animals to metabolize organic acids, these researchers measured the concentrations of short-chain fatty acids in the blood stream after feeding, as well as after intravenous injection of either formate, acetate, or propionate. Based on other findings by Marston et al. (1961), the authors hypothesized that the failure of the cobalt deficient animals to metabolize propionate at a regular rate was likely due to the lack of cobalamin and subsequently a lack of 5'-deoxy-adenosylcobalamin.

When comparing the effects of concentrations of cobalt supplementation on feedlot steers during growing and finishing phases, Tiffany et al. (2003) observed that animals not supplemented with cobalt had greater circulating concentrations of methylmalonic acid during a portion of the growing phase and for the entirety of the finishing phase when compared to animals supplemented with cobalt. The conversion of methylmalonyl-CoA (the co-enzyme-linked form of methylmalonic acid) to succinyl-CoA in the Krebs cycle, is vitamin B_{12} dependent and a vital step in gluconeogenesis and energy creation (Girard and Matte, 2006). A vitamin B_{12} deficiency, and a subsequent decrease in methylmalonic acid conversion to succinyl-CoA, causes

propionic acid and methylmalonyl-CoA concentrations to increase in the blood (as discussed in a recent review by González-Montaña et al. 2020). The authors summarize by stating that the metabolism of propionic acid is critical for glucose homeostasis in ruminants. Due to the B_{12} deficiency-induced metabolic changes, it is evident that cobalt, through its essentiality for the synthesis of vitamin-B_{12} (Stangl et al., 2000), plays a major role in animal health, specifically through enzymatic pathways. All forms of B_{12} have cobalt in the center of the corrin ring, which is formed in the rumen through microbial fermentation (Girard et al., 2009). The conversion of the corrin ring to cobalamin continues by the addition of α-substituents. It then can be absorbed into the mucosal cells of the small intestine by way of glycoproteins (Mills, 1981). It has been determined that the addition of α-substituents relies on vitamin E (Pappu et al., 1978), and that the lack of vitamin-E may cause cobalamin to be oxidized and become non-functional.

Copper

Copper essentiality in ruminants was first demonstrated by supplementing copper to cattle that were diagnosed with "Salt Sickness" (nutritional anemia) in Florida (Becker et al., 1932). This wasting disease had many similar symptoms to cobalt deficiency, enzootic marasmus, including weight loss, low hemoglobin, and eventual death. The cause of this nutritional anemia was likely iron deficiency (Reeves and DeMars, 2004), caused by copper deficiency. Copper-induced iron deficiency occurs when there is impaired hematopoiesis due to the reduction of ferroxidase enzymes such as hephaestin (Chen et al., 2006) and ceruloplasmin (Ferroxidase I; Evans and Abraham, 1973). Both ferroxidase enzymes are copper-dependent enzymes involved in the oxidation of iron. Inappropriate oxidation of iron impairs incorporation of iron into hemoglobin which results in iron deficiency symptoms.

Heart muscle (myocardial) function and cardiovascular health have also been linked to copper deficiency. "Falling Disease" was a prevalent disease in cattle in Western Australia in the early 1900s, where 5-40% of the cattle in specific herds were reported to die seasonally from July to September (Bennetts and Hall, 1939). There were few visual symptoms before sudden death in lactating cows. Cows with this disease were found to be anemic and the animals and the soil had below-average copper status. This copper deficiency was further studied in cows by Bennetts et al. (1941), where low soil copper concentrations (1.1 to 3.2 mg Cu/kg DM) were associated with falling disease and anemia and the disease prevalence was improved with copper supplementation to the animals (100 mg/day). These researchers also explored the primary pathology of falling disease and its relation to low copper status. The researchers found that cows with the condition shared a few key traits: hemosiderosis, anemia, and red blood cells anisocytosis.

In a subsequent paper, Bennetts et al. (1948) further studied the pathology of falling disease by collecting livers, hearts, kidneys, and spleens of cows not supplemented with copper to evaluate the progression of the disease. Heart lesions had developed due to a lack of copper supplementation. Lesions of the heart were accompanied by a pale and soft appearance suggestive of a loss of muscle tone. Myocardial atrophy was shown to be progressive in cows slaughtered at different stages of the trial: some atrophy was observed early in the trial, whereas later stages of the trial showed many cardiac lesions and fibrosis. These data demonstrated that copper deficiency led to heart lesions, heart failure, and the instantaneous death of the cows.

Copper has also been reported to be involved in lipid metabolism in cattle. Engle and Spears (2000) investigated the interaction of copper and lipid metabolism by supplementing steers with either 0 mg, 10 mg (NRC requirement) or 20 mg (2x NRC requirement) Cu/kg DM.

The researchers measured cholesterol, copper status, in-vitro VFA production, backfat thickness, and intramuscular fat. None of the animals, including the control group, were copper deficient. However, the animals with higher copper supplementation had less backfat, lower cholesterol, and increased unsaturated and decreased saturated fatty acid content of the muscles. In a review, Engle (2011) discussed the potential pathways that copper could impact lipid metabolism, some of which were cellular mechanisms of lipolysis, an increase in oxidized glutathione, enhanced fatty acid desaturase systems, altered rumen biohydrogenation, impaired ability to convert dopamine to norepinephrine, and expression of copper chaperon proteins.

Copper has also been shown to affect animal immunity in numerous studies. Leukocytes killing capacity (Jones and Suttle, 1981), blood neutrophil concentration (Arthington et al., 1996), as well as B lymphocyte production (Cerone et al., 1998) are affected by an animal's copper status. Jones and Suttle, (1981) reported that when ewes were copper deficient, the ability for leukocytes to neutralize *Candida albicans* was severely inhibited during in-vivo testing. In addition, the researchers found that leukocyte superoxide dismutase (LSOD) and erythrocyte superoxide dismutase (ESOD), two copper-dependent superoxide dismutase (SOD) enzymes, were disrupted. These enzymes are involved in protecting the host's cells from oxidative stress by converting superoxide radicals to a less toxic hydrogen peroxide (Sordillo and Aitken, 2009).

The previously mentioned changes in SOD production, from copper-deficiency, may induce DNA damage. Using cytogenetic analysis, Picco (2004) found a negative association between blood copper concentrations and chromosomal aberrations (a change in the sequence of nucleic acids of a chromosome), in copper-deficient cattle. Additionally, the researchers found that DNA from copper-deficient animals, was fragmented into smaller DNA fragments indicating greater DNA damage. Virginia (2012) continued this research to discover the cause of

the DNA damage. Using molybdenum and sulfur-induced copper-deficiency in Holstein cattle, these researchers measured plasma copper status, erythrocyte copper content, SOD activity, DNA integrity, and fatty acid composition of the erythrocyte membranes. Data from these experiments suggest the following: molybdenum and sulfur induced copper deficiency decreased production of SOD, which increased circulating free radicals that appear to have created oxidative stress on saturated fats. This most likely caused changes in fatty acid composition of erythrocyte membranes as well as DNA, which led to the observed DNA damage.

In another study, the immune system of beef heifers was challenged by the inoculation of Bovine Herpesvirus-1 (BHV-1), where the animals either had secondary copper deficiency induced by supplementation of molybdenum and sulfur, or had no molybdenum supplemented (Arthington et al., 1996). This experiment demonstrated that cattle deficient in copper had delayed ceruloplasmin response, indications of inflammation via increased acute-phase protein presence, as well as increased neutrophil numbers.

Similarly, Cerone et al. (1998) induced secondary copper deficiency by feeding calves 30 ppm molybdenum and 225 ppm sulfate five days a week to evaluate the impact on immune function. The researchers reported that copper-deficient animals had a marked increase in monocyte count, as well as a 40% decrease in B lymphocytes. In addition, the ability of neutrophils to function was reduced, even though the cell count was similar between the treatment groups. Each of these cell types have a role in immune system function.

Manganese

Manganese is an essential trace mineral involved in fetal development, estrous cycle, bone formation, carbohydrate metabolism, lipid metabolism, and the synthesis of many enzymes as discussed in a review by Hidiroglou (1979). Skinner et al. (1932) conducted two experiments

to further understand why the reproduction of milk-fed rats became abnormal, even after supplementation of copper and iron. The first trial demonstrated that animals supplemented with manganese reached sexual maturity sooner and the second trial found that the primary determinate of estrus was energy content of the ration. Still, the addition of manganese further improved how often an estrous cycle occurred. Bentley and Phillips (1951) sought to test the aforementioned effect of manganese deficiency on fertility in dairy cattle. Two experiments were conducted using heifer calves fed either a manganese deficient diet (3.2 to 4.5 ppm/kg manganese) or the manganese deficient diet supplemented with manganese sulfate at 13.6 mg Mn/kg DM in experiment one and at either 14.1or 27.2 mg Mn/kg DM in experiment two. In both experiments, manganese-supplemented cattle came into the first estrous cycle earlier (roughly two months), had slightly fewer breeding services per conception, and had a 50% increase in ovarian manganese concentrations.

Rojas et al. (1965) discovered that significant manganese deficiency, due to dietary inclusion of less than 16 mg Mn/kg DM causes neonatal deformities in Hereford cattle. Every calf born to a manganese-deficient cow was deformed: enlarged joints, stiffness, and twisted legs were noticed along with muscle weakness. Similar results were previously reported by Bentley and Phillips (1951). These calves also had weaker bones with a breaking strength nearly half that of the adequate manganese group.

In Canada, "Congenital Joint Laxity" (CGJL) was a term used to describe a skeletal deformity in calves (Ribble and Janzen, 1987). These birth defects were described as calves that had shortened limbs, joint laxity, and rear legs that appeared "banana-shaped" when the animal was standing. With the hypothesis that this deformity could be nutritionally related, Ribble et al. (1989) began several feeding trials at three ranches to determine the epidemiology of CGJL.

Briefly, for one trial, Ribble et al. (1989) fed either silage (50% grass, 35% red clover, and 15% oats) or grain and hay in addition to the silage. Cows fed only silage had greater risk of having CGJL calves. These findings were used to design another trial which demonstrated that when all cows were fed the silage with grain and hay, there were significantly fewer CGJL calves. These trials demonstrated that calves born to cows that receive silage were far more likely to be born with deformities. In another trial, Ribble et al. (1989) compared cows fed only red-clover-based silage or rolled-barley in addition to the silage. Cows receiving only red-clover silage had a five-times greater risk of having CGJL calves. In a final trial, cows were fed either (1) grass silage, (2) red-clover silage, or (3) grass hay. Cows fed grass hay only were more than 15 times less likely to have a CGJL calf. The results of these trials suggest that grains or hay decreased the chances of CGJL. The authors indicate that CGJL is most likely a manganese deficiency and that feeding feedstuffs (grains and hays) with elevated manganese reduced the incidences of CGJL.

After the completion of the above research, Hidiroglou et al. (1990) reported that serum manganese concentrations of animals fed either grass silage or red clover silage were significantly lower than those fed hay (2.06 and 1.99 versus 2.60 ng/mL, respectively), and that numbers of CGJL calves for each group were 28% in the grass silage treatment, 38% in the red clover silage treatment, and 0% in the hay treatment. In a more recent study, Hansen et al. (2006) found similar defects in 5 of 7 offspring born to heifers fed manganese deficient diets, namely dwarfism, weakness, and enlarged joints. These researchers hypothesize this is due to the manganese requirement for glycosyltransferase and its role in cartilage metabolism.

The effects of manganese that have been discussed prior, and numerous other examples of manganese deficiency, are rooted in the role of manganese in the function of certain enzymes. In a review, Utter (1976) discusses that manganese has two essential functions in mammals: as a

dissociable co-factor of an enzyme, or as a part of a bound metalloprotein that assists the function or structure of an enzyme. An example given of manganese as a co-factor is mucopolysaccharide (glycosaminoglycan) biosynthesis. Glycosaminoglycans are long-chain sugar molecules that are commonly found in mucus and joints. Leach and Muenster (1962) conducted a studied perosis in chickens by feeding either manganese sufficient or manganese deficient diets and observing the glycosaminoglycans content of cartilage decreased in manganese deficient chickens.

Manganese is also an essential part of the metalloprotein pyruvate carboxylase (Mildvan et al., 1966), an enzyme that is involved in gluconeogenesis. This relationship was studied in young developing rats, specifically during a period where the rat transitions from using a continuously supplied diet (nursing) to an intermittent diet and is required to perform gluconeogenesis to maintain blood glucose concentrations (Baly et al., 1985). The pups from manganese deficient mothers that were weaned and fed a manganese deficient diet (1 μg/g DM) experienced 99% mortality within three days of birth. Additionally, liver pyruvate carboxylase activity drastically increased from day 0 to 4, likely due to impaired storage and/or mobilization of glycogen to compensate for inadequate blood glucose concentrations. Furthermore, blood glucose concentrations were decreased during the first two days of life, in manganese deficient pups, most likely due to impaired glycogenolysis and gluconeogenesis.

Selenium

The primary role of selenium is the creation of selenoproteins (Wichtel, 1998). Researchers had historically believed that selenium was used exclusively in the selenoprotein glutathione peroxidase. However, researchers have discovered selenium to be involved in the

other selenoproteins, specifically within the immune system (Arthur et al., 2003) and thyroid hormone production (Larsen and Berry, 1995).

Nutritional muscular dystrophy (also known as white muscle disease, nutritional myopathy, and stiff lamb disease) was a first recognized in sheep in the 1920s, and later it was observed in cattle (Muth, 1956). This disease occurs mainly in young animals located in the northwestern United States during any season. The disease is recognized as extreme muscle loss, where the animal loses strength and is often unable to stand. In addition, the heart can be affected, and death occurs soon after, leaving the heart appearing bleached with spots or streaks. After observing rats being supplemented with selenium to treat liver necrosis, Muth et al., (1958) attempted supplementing selenium to ewes, in addition to Vitamin E, to prevent white muscle disease. All ewes were fed a diet of clover, alfalfa hay, and 114 g of oats daily, with treated ewes receiving either: (1) 770 I.U. of a-tocopherol administered parenterally each week, (2) 100 I.U. of a-tocopherol fed with oats each day, or (3) 0.1 mg Se/kg DM with oats. The hay used in this study contained less than 0.1 mg Se/kg DM. The researchers reported that animals given feedstuffs with additional selenium acquired white muscle disease at a lower rate (1 of 16), whereas supplementing vitamin E orally or parenterally had limited improvements when compared to the basal only group (16 of 20 and 11 of 15 verses 11 of 15, respectively).

Several years later, Whanger et al. (1977) conducted three trials where pregnant ewes were fed selenium and vitamin-E deficient (purified) diets supplemented with: 1) selenium; 2) vitamin-E; 3) or both selenium and vitamin E, to evaluate the impact on white muscle disease prevalence of lambs. In the first trial, sheep received either: 1) injections of 5 mg sodium selenite twice a week and 750 IU of vitamin E weekly; 2) vitamin E weekly; 3) sodium selenite two times a week; or 4) or neither before lambing. All animals receiving selenium, vitamin E, of both

had decreases in the prevalence of lesions compared to those animals not receiving selenium or vitamin E. The second trial consisted of a similar design, except for slight changes of the diet including removal of calcium carbonate and the addition of sodium phosphate. The findings were similar to the previous trial, with the ewes receiving neither supplement having lambs with white muscle disease and the group receiving selenium and/or vitamin E had no lambs with white muscle disease. The final trial had ewes gradually introduced to the same diets as describe in trial 2, but after parturition. All lambs from the treatment receiving no supplemental selenium or vitamin E died by 12 weeks of age and three lambs from each of the other three treatments died by 12 weeks. These trials demonstrated that both selenium and vitamin E prevent white muscle disease. However, it appears that selenium prevents the disease for a limited time and has fewer protective effects when compared to vitamin E.

When comparing lambs supplemented selenium to those on selenium deficient-rations, Juszczuk-Kubiak et al. (2016) observed significant differential expression of 7 and 6 of 12 selenoprotein genes in the liver and longissimus dorsi of sheep supplemented 0.5 mg/kg selenium. Some of these selenoproteins are involved in redox reactions, such as glutathione peroxidase, thioredoxin reductase, and iodothyronine deiodinase as discussed in a review by Brigelius-Flohé and Maiorino (2013). In an early study, Rotruck et al. (1972) measured an increase in erythrocyte hemolysis through oxidative damage in weanling rats given a selenium-deficient diet. Subsequently, Rotruck et al. (1973) demonstrated that induced selenium deficiency decreased glutathione peroxidase synthesis when rats were fed selenium-deficient diets. The authors hypothesized that the absence of glutathione peroxidase, and the subsequent inability to reduce hydrogen peroxide to water and oxygen, was causing the cellular hemolysis.

Another example of selenium's essentiality is its integral role in thioredoxin reductase, an enzyme required to maintain the antioxidant protein thioredoxin in its reduced state to help with preventing oxidative damage in tissues. Hill et al. (1997) observed that rats fed selenium-deficient diets had circulating thioredoxin reductase levels at 4.5% of the levels of the group given adequately supplemented group. Additionally, Sordillo et al. (2007) measured antioxidant potential in pluriparous dairy cows from 21 days before calving to 21 days in milk. During this time thioredoxin reductase activity levels decreased as well as antioxidant potential in peripheral blood mononuclear cells. This study suggests that thioredoxin reductase plays a vital role in antioxidant systems. In a mammary cell culture study, Guo et al. (2018) initiated oxidative stress to bovine mammary cells and subsequently supplemented selenium to one treatment group. The inclusion of selenium in the cultured mammary cells up-regulated the production of thioredoxin reductase and decreased oxidative damage compared to the group not supplemented selenium.

In early human research on the topic of hypothyroidism, Pitt-Rivers et al. (1955) demonstrated that triiodothyronine (T3) was derived from thyroxine (T4) via hepatic cellular conversion, without a functioning thyroid. Later, it was reported that selenium was required for this conversion (Beckett et al., 1987). Subsequently, Beckett et al. (1989) elucidated that the cause of the inhibited T4 to T3 conversion in the liver was due to a decrease in iodothyronine deiodinases, an enzyme produced by the thyroid gland, which requires selenium. The researchers also reported that renal 5'-deiodination was decreased in selenium-deficient kidney cells. This effect of selenium deficiency has been substantiated in Friesian steers by feeding a selenium-deficient (< 0.015 mg/selenium/kg DM), torula yeast-based diet (Arthur et al., 1988). After 23 weeks, the researchers reported a 62% increase in plasma T4 concentration, a 35% decrease in plasma T3 concentrations in selenium-deficient animals, greater plasma urea and creatinine, and

a reduction in plasma alkaline phosphatase activity. Similar work by Contreras et al. (2002) was published on pregnant Friesian cows receiving either a diet supplying 18% of daily selenium requirements (0.05 mg selenium/kg DM) or the same diet with 1.0 mg selenium/kg barium selenite via a subcutaneous injection. The resulting data described a decrease in circulating T3 in cattle fed a selenium deficient diet without supplementation, however no significant differences between groups were detected for T4.

Zinc

Zinc is a component of more than 300 metalloenzymes in various species (Vallee and Auld, 1990; McCall et al., 2000), including carboxypeptidase A in bovine (Rees et al., 1983). Hormones that support immune function, like thymic hormone (Iwata et al., 1979), as well as gene stability and expression (Dreosti, 2001) also require zinc for production and appropriate function (Langova et al., 2020).

Using a rat model, Follis et al. (1941) induced a skin condition with thickening of the epidermis of the esophagus (parakeratosis) and loss of hair follicles from the skin (hyper-keratinization) by supplying a zinc deficient diet. From the years of 1957 to 1959, Legg and Sears (1960) recorded findings of cattle with lesions and parakeratosis, similar to those found in the previously reported rat experiment. These researchers also noted that new hair growth was observed within one week when the cattle were given zinc orally (2 g/week) or injection (1 g/week). Further feed analysis indicated poor zinc absorption from feedstuff and low zinc content.

In another study completed in Scotland, Mills et al. (1967a) reported numerous additional side effects of zinc deficiency in lambs and calves, namely: halted weight gain, large quantities of frothy saliva, pale color of the tongue, weakening of hoof wall and horns (lambs), enlargement

of hock bones (calves), and restless hind legs (calves). Similar effects were demonstrated in Holstein calves by Miller and Miller (1960), where the rumen had accumulated excessive cell over-growth and keratin formation. In addition, there were numerous external lesions and, notably, a decrease in circulating carbonic anhydrase, which will be discussed further below.

The lesions recorded in studies, like those described above, lead Williams and Chesters (1970) to research zinc deficiency effects on protein and DNA synthesis in an effort to better understand the etiology of the deficiency. Rats fed a zinc-deficient diet had a significant decrease in H-3 thymidine incorporation, a labeled thymidine (DNA Nucleoside "T"), into DNA. There was also a significant decrease in labeled lysine (C-14 Lysine) incorporation into proteins. Additionally, growth was ceased in the zinc-deficient rats, which the researchers hypothesized may have been due to the decrease in DNA synthesis. Engle et al. (1997) evaluated the effects of marginal zinc deficiency on calves fed 17 mg Zn/kg of dry matter compared to a control group (40 mg Zn/kg). In both commercial beef heifers and Holstein Friesian steers, cattle fed zinc-deficient diets had a decrease in average daily gain as well as feed efficiency and a subsequent improvement in feed efficiency when zinc concentrations were repleted. Additionally, there was a decrease in ending body weight (130 ± 1.0 vs. 137 ± 2.8 kg) in zinc-deficient cattle.

Fraker et al. (1977) used a mouse model to test if zinc deficiency influenced immune response. The researchers measured liver, kidney, spleen, and thymus weight after 28 days of consuming a zinc-deficient (0.7 μg zinc/g DM). They found that the thymus of zinc-deficient mice had atrophied more than any other organs, followed by spleens which were smaller than those of the low zinc (4.4 μg zinc/g DM) and high zinc supplemented mice (69 μg zinc/g DM; 64 mg zinc/kg DM versus 120 mg zinc/kg DM and 119 mg zinc/kg DM, respectively). Additionally, by immunizing mice with purified sheep red blood cells, it was determined that

mice fed zinc-deficient diets (0.5 μg zinc/g DM) produced one-sixth the immunoglobulin G (IgG), as well as impaired T-cell helper function, when compared to zinc supplemented mice (25 μg zinc/g DM).

It is likely that many of the pathological causes of observed zinc-deficiency diseases are due to enzymatic changes, specifically changes of zinc metalloenzymes (Miller, 1970). As mentioned previously, zinc is essential element for more than 300 enzymes and serves typically as a cofactor. An example of a zinc requiring enzyme is carbonic anhydrase, as briefly mentioned above. This enzyme is essential in carbon dioxide equilibrium and acid balance in the blood through the reversible hydration of carbon dioxide to bicarbonate and protons (Lindskog, 1997). Although not the first to isolate the enzyme, Keilin and Mann (1939) had successfully isolated carbonic anhydrase, and notably discovered a significant zinc content in carbonic anhydrase, using the cells derived from whole blood obtained from an ox.

Keilin and Mann (1940) went on to be the first to classify carbonic anhydrase as a metalloprotein, a subset of metalloenzymes, after collecting evidence of the nature of the zinc in the enzyme. This evidence included: potassium cyanide-induced inhibition suggesting the enzyme had an active metal group and zinc content in tissues correlated to the level of enzymatic activity. Miller and Miller (1962) also reported a high correlation between blood zinc concentration and carbonic anhydrase activity in Holstein calves. Animals fed a zinc-deficient semi-purified diet (0.02 mg zinc/kg DM) had lesser blood zinc concentrations and carbonic anhydrase activity when compared to values in animals supplemented with zinc (260 mg of zinc/day).

Absorption and Transport Mechanisms of Trace Minerals

Cobalt

Suttle (2010) states that there is no evidence that any species requires cobalt other than for the production of cobalamin (Vitamin B_{12}); thus, this portion of the review will follow the process of the cobalt conversion to cobalamin and the storage and excretion of the product. When rats were given oral doses of cobalt, it was retained at 0.27 of 10 µg (< 5%) when recovered from the rat tissues after four days of cobalt administration (Copp and Greenberg, 1941). Comar et al. (1946) found that about 94% of a jugular cobalt treatment disappeared from the blood after mere hours and bolus cobalt treatment decreased to 1% remaining in rumen after ten days.

Within the rumen, bacteria and cocci sequester a large portion (≈ 80%) of the available dietary cobalt from their external rumen environment (Tosic and Mitchell, 1948). The bacteria then convert cobalt into cobalamin (Johnson et al., 1956). This conversion of cobalt into cobalamin has two major routes, aerobic and anaerobic. The former being the route of ruminal bacterial (P. shermanii and S. typhimurium) conversion of uroporphyrinogen III to adenosylcobinamide, including the addition of the corin (cobalt) ring, and the final formation of cobalamin (Roth et al., 1996). Bacterial conversion of cobalt to cobalamin ranges from 3-13% depending on the source, delivery method, and supplementation level of cobalt, with supplementation decreasing the conversion rate (Smith and Marston, 1970). Andrews et al. (1960) found that depending on the animal's cobalt status, the proportion of cobalt used to synthesize cobalamin can be altered. Some of the cobalt is converted to insoluble cobalamin analogs which bypass the GI tract and are eventually excreted (McDowell, 2003). Additionally,

some cobalt binds to methionine, creating a complex, and escaping the GI tract unabsorbed (NASEM, 2016).

After synthesis, approximately 5% of cobalamin is absorbed by bacteria which are eventually digested in the lower gut, with the majority of the remaining cobalamin being excreted in the feces (≈ 93%). Free cobalamin in the rumen is then bound/transported by the gastric intrinsic factor (IF) produced in parietal cells of the abomasum (Smith and Marston, 1970; McKay and McLeay, 1981). This bound cobalamin-IF compound crosses the epithelial cell membrane of the small intestine via the intrinsic factor-cobalamin receptor (IFCR; Seetharam et al., 1985, Seetharam et al., 1988), also referred to as gp280 (Sahali et al., 1988; Seetharam et al., 1997). After passage through the cellular wall, the cobalamin-IF compound is bound to transcobalamin (TC II; Seetharam and Li, 2000). Transcobalamin II is used in intracellular transport and the extracellular import of cobalamin from circulating plasma into the epithelial cells via transcobalamin II-receptor (TC II-R; Bose et al., 1995).

Copper

Copper absorption can be altered by the presence of elements like sulfur and molybdenum forming thiomolybdates in the rumen (Dick, 1953; Bird, 1970; Suttle, 1991), iron (Campbell et al., 1974), and zinc (Cousins, 1985). Through meta-analysis of 12 published studies, (Dias et al., 2013) demonstrated that plasma copper concentrations were negatively affected by dietary molybdenum and sulfur. Additionally, anti-protozoal ionophores increase copper absorption and accumulation in the liver (Ryssen and Barrowman, 1987); this is possibly due to the impact ionophores have on removing some species of protozoa, namely the Entodinium species (Olumeyan et al., 1986). Protozoa produce sulfide, which can bind to copper to form copper sulfide (CuS; Ivan et al., 1986). Removing the protozoa would prevent CuS

formation and therefore, increase copper availability to the animal (Olumeyan et al., 1986; Spears, 1990). Copper absorption rate can also be intrinsically upregulated or downregulated depending on the mineral status of the animal (Los Alamos Medical Research Group, 1986), presumably by altering the levels of a family of proteins called copper transport proteins (Kuo et al., 2006).

Copper absorption relies on both copper-specific transporters, namely copper transport proteins Ctr1 and Ctr2, as well as a non-specific transporter, divalent metal transporter 1 (DMT1). Although the entire copper absorption process is not entirely understood, it appears that Ctr1 and Ctr2 are primarily responsible for the import of copper from the small intestine, through the mucosal cell wall and into the cell cytoplasm of the enterocyte via endocytosis (Peña et al., 1999; EFSA, 2016.

Another copper absorption route is the divalent metal transporter-1 (DMT1), a non-specific transporter of eight trace minerals, including copper and iron (Garrick et al., 2003). Divalent metal transporter-1 is located on the surface of the small intestine's enterocytes, where the copper is bound and absorbed into the cell via apical uptake (Trinder et al., 2000). Intestinal cell culture experiments have demonstrated that inhibiting DMT1 decreases the uptake of copper and iron by 48% and 80%, respectively (Arredondo et al., 2003). There are at least four isoforms of the DMT1 transporter. However, the copper-specific transporter has an additional 25 amino acid residues on the N-terminal and is encoded by mRNA that lacks the iron response element (IRE). Additionally, the transporter has an N-terminal extension of 30 amino acid residues with the first “AUG” at exon 2, giving it the 2/-IRE designation. This process of apical copper absorption appears to be related to this 2/-IRE isoform of DMT1 (Arredondo et al., 2014).

Manganese

Manganese binds to both alpha-2-macroglobulin and transferrin in the bloodstream (Gibbons et al., 1976). The level of binding of manganese to each protein can be affected by the ambient temperature during electrophoresis, which the authors state suggests a required enzymatic oxidative reaction. The researchers noted that alpha-2-macroglobulin transports manganese in its reduced Mn^{2+} form. It was reported that transferrin could only bind to manganese in the oxidized form (Mn^{3+}; Davidsson et al., 1989), possibly relying on the presence of an oxidizing agent (ceruloplasmin; Gibbons et al., 1976). Once oxidized, manganese is bound to transferrin and imported through the portal vein and into the liver by the transferrin receptors of the hepatocytes (Goff, 2018).

The transferrin pathway of manganese uptake is markedly slower than other forms of manganese uptake and also competes with iron uptake, as they appear to share similar pathways (Gunter et al., 2013). Limited evidence suggests that transferrin also is the primary transporter of manganese across the blood-brain barrier (Aschner and Aschner, 1991). Albumin has also been shown to bind to manganese but is not considered a major transporter (Mildvan and Cohn, 1963). Transmanganin, a transporter proposed by both Bertinchamps et al. (1966) and Cotzias and Bertinchamps (1960), was not able to be differentiated from transferrin but seems to have a small role as a transporter of manganese (Scheuhammer and Cherian, 1985).

Across all pathways of absorption, animals absorb manganese at a rate of between one and seven percent (Greenberg and Campbell, 1940; Greenberg and Campbell, 1940; Sansom et al., 1978; Weiss and Socha, 2005). It appears that the body has strong homeostatic control of manganese concentrations in various tissues, where increasing the dosage of concentration of manganese supplemented decreases the quantity found in the duodenum and jejunum. These

researchers suggest that variable absorption rates partially control tissue manganese concentrations (Abrams et al., 1976). Manganese absorption also interacts with the absorption of calcium and phosphorus, where when fed in combination, manganese prevents the negative impact on weight gain from high levels of monocalcium phosphate (Hawkins et al., 1955).

Selenium

Orally dosed selenium is absorbed inefficiently by ruminants (≈ 29%) compared to non-ruminants (≈ 77%; Wright and Bell, 1966). However, an intravenous dose was retained at the same levels (70%) in both species. Diet influences selenium absorption efficiency in ruminants, where a concentrate-based diet had greater rates of selenium absorption than high forage diets (Koenig et al., 1997). The various forms of selenium have differing rates of metabolism (Finley and Davis, 2001), specifically when comparing inorganic (selenate, selenite) and organic forms (selenomethionine, selenocysteine; Finley, 2006). Greater biological values and net retention of selenium were observed in lambs orally supplemented selenomethionine compared to those supplemented with selenite (Se^{4+}; Ehlig et al., 1967). Other researchers reported that selenite was more available than amino acid-bound selenium yeast in sheep (Koenig et al., 1997).

A large quantity of selenium (49% and greater) leaves the rumen as part of the bacterial fraction within the chyme (Koenig et al., 1997). In sheep, no absorption of selenium was found in the rumen, with limited amounts in the abomasum, and while significant absorption occurred throughout the duodenum and the cecum (Wright and Bell, 1966). Absorbed selenium is commonly associated with selenoprotein P (Sepp1) in serum and hemoglobin in whole blood (F. T. Awadeh et al., 1998). Selenium is then rapidly removed from circulation by the liver, through the portal vein, within 30 minutes after intrajugular injection (Symonds et al., 1981). The decrease in circulating concentrations of selenium was followed by a subsequent increase as the

liver released 30-40% of the selenium back into circulation via the hepatic vein. A large portion of the selenium is found incorporated in the liver, bound to hepatic proteins, with all selenium being found in a reduced selenide (Se2-) state (Ehlig et al., 1967). A large portion of selenium released from the liver is in the form of selenoprotein P (Sepp1; Hill et al. 2012), where it is primarily produced and used for whole-body transport of selenium (Burk and Hill, 2009).

Differing absorption pathways for each of the chemical forms of selenium impacts the bioavailability of elemental selenium (Fairweather-Tait et al., 2010). Selenomethionine is imported into the cell by intestinal methionine transporters, as demonstrated in swine (Wolffram et al., 1989), and may compete with selenocysteine absorption. In rats, the conversion of selenomethionine into selenocysteine occurs within the transsulfuration pathway (Esaki et al., 1981). Selenocysteine is also brought across the epithelial cell membrane by the neutral and basic amino acid transport protein (rBAT) in frog-oocytes (Nickel et al., 2009).

Zinc

In calves, zinc concentrations increase within the duodenal, kidney, and liver tissues and plasma with an increased dose of mineral supplemented (Wright and Spears, 2004). Furthermore, apparent absorption increases in sheep fed a zinc deficient diet when compared to sheep fed a zinc adequate diet (VanValin et al., 2018). In dairy cattle, zinc is absorbed throughout the ruminant digestive tract (Miller and Cragle, 1965). Zinc uptake has been shown to occur in ruminal tissues, however it does not appear to enter the bloodstream from this location (Wright et al., 2008).

In rats, dietary zinc absorption into the bloodstream from the digestive tract primarily takes place in the small intestine via cysteine-rich intestinal binding protein and metallothionein (MT; Hempe and Cousins, 1992). Zinc absorption into the enterocyte is also achieved by ZIP4

(Liuzzi and Cousins, 2004) and DMT1 (Harris, 2002) transporters. However, zinc must compete with iron and manganese to bind with the DMT1 transporter (Goff, 2018). In rats, ZIP4 proteins were shown to increase prevalence during a dietary zinc deficiency (Dufner-Beattie et al., 2003). After absorption into intestinal mucosa, the zinc is transported into the bloodstream (Miller, 1969). Specifically, zinc is primarily transported within the blood stream by albumin in humans and swine (Chesters and Will, 1981). In rats, transferrin transfers zinc from albumin into the liver (intraportal; Evans and Winter, 1975). Additionally, using bovine pulmonary endothelial cells, albumin was shown to transport zinc from circulation into the hepatocyte through receptor-mediated cotransport as well as non-selective transcytosis (Tibaduiza and Bobilya, 1996). From zinc absorption into the enterocyte through the apical membrane (ZnT5b and Zip4), to the export of zinc through the basolateral membrane (ZnT1 and Zip5), zinc transfer is primarily controlled by the family of ZnT and Zip transporters (Wang and Zhou, 2010).

In rats, absorption of vitamin E was hindered when animals were fed a zinc-deficient diet (Kim et al., 1998). The effects of zinc deficiency in chickens were decreased when supplemented with vitamin E (Bettger et al., 1980). This suggests a positive interaction between zinc and vitamin E. However, zinc bioavailability was decreased by consumption of a combination of phytic acid and calcium, in both chickens and pigs (Mills, 1964). Additionally, high dietary cadmium has been reported to decrease zinc absorption and zinc tissue concentrations in Holstein calves (Powell et al., 1967).

Storage and Excretion of Trace Minerals

Cobalt

Most cobalt is stored as cobalamin (Vitamin B_{12}), with a lesser portion stored as elemental cobalt. Cobalt is stored in high quantities in the muscle and bone in humans (43% and 14% of body cobalt, respectively) and similarly with ruminant and nonruminant species, with the remainder having a presence in tissues such as the liver, pancreas, and kidneys (Underwood, 1977). Research in rats seems to support the idea of the kidney being a location of storage for cobalt (Bose et al., 1995). However, the administration technique influences the location and concentration of cobalt storage. Cobalt concentrations are greater in intestinal mucosa than muscle tissue when cobalt is injected versus consumed (1.5 vs. 2.1 times, respectively; Comar and Davis, 1947). In another trial, age was shown to affect cobalt retention in the liver in dairy cows (Kincaid et al., 2003).

The source can influence the stored form of cobalt (supplemental vs cobalt within feedstuffs; Andrews et al., 1959). Animals given supplemental cobalt had a lower ratio of cobalamin to cobalt than those who did not require supplementary cobalt. Henry et al. (1997) reported that two separate pools of cobalt storage exist within the liver. The researchers hypothesized that this may be due to each pool having different kinetics or that there are multiple absorption pathways for cobalt. There appears to be a homeostatic mechanism that is engaged when supplemental cobalt surpasses 40 ppm DM (Kawashima et al., 1997). Additionally, Marston (1970) observed a limited maximum production of vitamin B_{12} that was seemingly limited of the capacity of the liver, which caused a noticeable negative correlation between the increases in Vitamin B_{12} and total cobalt concentrations over time.

Urine excretion of cobalt in sheep is between 6-8% of ingested cobalt (Rothery et al., 1953). Comar et al. (1946) found that approximately 1% of injected cobalt was excreted in the feces. Still, roughly 65% of orally supplemented cobalt appeared in the feces after 32 hours, with minimal quantities found in the urine and saliva. Over 60% of cobalt administered orally, in rats, indicated that supplemented cobalt was excreted in feces, suggesting less than half was absorbed (Copp and Greenberg, 1941).

Copper

The liver stores a significant portion of the copper in the body of ruminants (Bingley and Dufty, 1972). Compared to many monogastric animals, including pigs and rats, ruminants have greater levels of liver concentrations (Beck, 1956). The form of copper stored within the liver was elucidated by Bremner and Marshall (1974), where it was found to be stored in three distinct fractions. Fraction one being associated with superoxide dismutase (SOD), two potentially being associated with hepatocuprein (likely ceruloplasmin), and three seemed to be associated with a protein similar to metallothionein (MT). Superoxide dismutase is an enzyme that contains copper and is responsible for the conversion of superoxide radicals (0_2-) to H_20 and 0_2 (McCord and Fridovich, 1969). Hepatocuprein is a term for copper-containing proteins that are found within liver cells (Mann and Keilin, 1938), which includes the enzyme ceruloplasmin, a copper transporter (Cousins, 1985). MT is a transport protein involved in intestinal absorption of copper and delivery to the liver (Cousins, 1985). As observed by López-Alonso et al. (2005), MT concentration in the liver is not correlated with copper but instead with zinc concentration in the liver. However, copper-MT is directly related to MT levels in the liver, demonstrating that copper is a poor inducer of the binding protein while zinc is, limiting the copper-MT storage ability in mammals.

As discussed in a review by Luza and Speisky, (1996), the initial storage of copper seems to be primarily associated with the MT complex and subsequently transferred to ceruloplasmin within a few hours. In a rat model, it appears that there are two phases of copper storage: first transfer into the liver and to a lesser extent, the kidneys. Second, from the liver to other tissues via ceruloplasmin (Linder and Hazegh-Azam, 1996). López-Alonso et al. (2006) assessed the use of circulating ceruloplasmin as a method for testing the accumulation of copper in the liver of cattle. Still, they found that it was not an accurate representation of liver copper values.

McCord and Fridovich (1969) observed that SOD contained two copper molecules per mol in bovine erythrocytes. It was also discovered that this superoxide-inhibiting enzyme was the same product observed previously by Mann and Keilin (1938) and was called hemocuprein. Wong et al. (2000) demonstrated that in mice, the copper chaperone for superoxide dismutase (CCS) is a vital protein for delivery of copper to SOD and the incorporation of copper into SOD. Recently, Hepburn et al. (2009) found that the quantity of CCS increased in copper deficient cattle when compared to those who were copper sufficient, offering a viable option for testing copper status.

There is a strong correlation between the concentration of copper in the liver and the copper status of the animal, which is why liver biopsies are the most wildly adopted technique to determine copper status in ruminants (Claypool et al., 1975). However, liver copper concentrations are not consistent across breeds of the same species (Littledike et al., 1995). When comparing the total liver copper across nine different breeds of cows, the limousine cows had significantly higher concentrations than all other breeds, other than Angus. In another paper by Du et al. (1996), there was a significant difference between Jersey and Holstein cows supplemented 80 mg copper/kg DM. However, lower doses of copper (5 mg copper/kg DM)

showed no differences between breeds. Bohman et al. (1984) tested forms and amounts of copper injections to determine the effect on mineral status. They reported that regardless of source, any dose below 60 mg of copper had little effect on liver concentration, whereas 120 mg of copper has a significant impact, and 240 mg of copper caused severe damage to red blood cells.

Using a rat hepatic cell culture model, Aoki and Suzuki, (1985) observed that copper was excreted at an increasing rate as time progressed. Additionally, the concentration of MT forms (MT I vs MT II) decreased at different rates, and although MT II was more common at the start of the trial, MT I was the most common at the end of the trial. The authors suggest this is due to faster degradation of MTI than MTII, or a greater synthesis of MTI in the liver. In a review of copper homeostasis, Evans (1973) discussed that bile is a major pathway of copper excretion in mammals. Essentially, copper is excreted with bile from the liver to the gallbladder, through the bile duct, and released into the small intestine where it associates with amino acids and peptides. This copper cannot be absorbed due to the inability of protein-bound biliary copper to be reabsorbed. Additionally, copper excretion rate is affected by elevated levels of supplemented molybdenum and sulfur, which increases the activity of both urinary (Marcilese et al., 1970) and biliary excretory pathways for copper (Gooneratne et al., 1994).

Manganese

Hidiroglou et al. (1978) reported that manganese concentrations are greater in tissues with greater mitochondria count, like the liver and pancreas. However, manganese was not primarily concentrated in any one organ. In their experiment, the sheep appeared to preferentially store manganese in the liver, as pancreas concentrations were maintained for only a short time. After ingestion of manganese by cows, Gibbons et al. (1976) reported that manganese absorbed into circulation was predominantly associated with α2-macroglobulin before it was transported to

the liver. Transferrin was also commonly associated with manganese, which is likely oxidized manganese that was excreted by the liver. Additionally, the researchers observed that the cow liver appeared have a large capacity for removing manganese from circulation and excreting it, while also maintaining a constant concentration of 5-10 μg copper/L in circulation. Friedberg (1975) observed in an in-vitro model that there was a tendency for manganese to selectively bind with albumin in human and rabbit blood when manganese was introduced to the cells.

When supplementing calves elevated manganese levels (15 ppm vs 0.5 ppm DM), the rate of bile excretion increased 30-fold and the manganese levels in the gall bladder and liver increased 2-fold (Carter et al., 1974). Papavasiliou et al. (1966) attempted to stop manganese excretion by blocking the release of bile from the bile duct and observed that manganese was found in the feces, suggesting that there is another pathway for manganese excretion. Bertinchamps et al. (1966) observed two phases of manganese excretion in a rat model, corresponding with either a direct transfer of manganese from blood to bile or manganese excreted directly into the small intestine. Additionally, the excretion of manganese occurs primarily in the jejunum and duodenum of the small intestine. By supplementing various manganese concentrations to steers with a surgically altered duodenum for bile collection, Hall and Symonds (1981) discovered that the quantity of manganese excreted in the bile did not account for all the manganese intake of the animal, suggesting there are other excretory pathways. In subsequent research, Symonds et al. (1982) discovered that the flow of bile increased as the animals weight increased Manganese excretion varied throughout the day, which appeared to be related to feeding activity, roughly 7 hours after consuming ration.

Selenium

Whole blood, serum, and plasma are reported to be accurate representations of selenium status as they are fast to respond to changes in selenium intake (Stowe and Herdt, 1992). However, various other measures have been used to determine selenium status (e.g., urine, circulating glutathione peroxidase levels in whole blood samples, and biopsies of the liver, kidney, and muscles). When studying selenium tissue distribution, Jacobsson (1966a) observed that sheep tended to accumulate selenium to the highest degree in the kidney. However, selenium uptake into the pancreas was increased when animals were supplemented with selenomethionine or selenocysteine. Additionally, the percentage of supplemented selenium retained in the tissues increased as the dosage decreased. Later, Echevarria et al. (1988) reported that the liver, kidneys, and serum selenium concentrations were the most sensitive to changes in dietary selenium. It was also reported that selenium levels increased linearly with increased selenium dosages and that the levels continued to increase across time points, from day 10 to day 30.

Rotruck et al. (1973) observed that when rat red blood cells were cultured with selenium isotopes and then processed through gel electrophoresis, roughly 70% of the isotope was incorporated into the enzyme glutathione peroxidase. The researchers suggested that the mineral is an essential part of the protein (selenoprotein). Later it was discovered that selenium is associated with other proteins (Herrman, 1977). After injecting selenium isotopes into rats, these researchers collected a serum protein bound to the isotope that was not glutathione peroxidase. Yang et al. (1987) later demonstrated in rats that this new selenoprotein (selenoprotein P) had greater increases in activity from the introduction of selenium to the diet compared to glutathione peroxidase and subsequently.

Behne et al. (1991) performed gel electrophoresis on rat muscle and liver tissues after oral administration of selenite or selenomethionine. They detected 13 proteins or protein subunits that contained selenium but that selenomethionine had been incorporated nonspecifically into numerous proteins. Awadeh et al. (1998) observed that, in cattle serum, selenium was associated with glutathione peroxidase (11.8%), albumin (19.2%), and selenoprotein P (68.9%), with selenoprotein P representing the largest fraction of selenium. These researchers also determined that supplemented selenomethionine had been incorporated non-specifically into other proteins, increasing the serum's selenium concentration. These data suggest that selenium is associated with many proteins in circulation and in tissues, and that the assessment of mineral status of an animal is multi-factorial, which includes tissue type and mineral source.

Excretion of selenium has been observed in the urine, feces, and lungs, where excretion of selenium by the lungs is typically used in times of severe selenium toxicity (Rosenfeld and Beath, 1964). Sheep given doses of selenium by either intraruminal, intravenously, or subcutaneous methods all demonstrated fecal and urine excretion of selenium (Jacobsson, 1966b). Additionally, subcutaneous delivery in sheep showed greater excretion through the urine than animals dosed equal quantities intraruminally. However, there were no significant differences in the total amount of selenium excreted between different delivery methods of the same dosage. Finally, biliary excretion in two sheep given intravenous selenium was 1.4% and 3.7% of the administered doses.

In a subsequent research paper, Jacobsson and Lindberg (1968) set out to understand why increases in selenium status are inconsistent when animals were supplemented selenium with a variety of dosages and delivery methods. Utilizing sheep, the researchers observed that urinary excretion reached the highest concentration, a 10-fold increase, immediately after injection with

selenite. After each “flushing dose" of non-radioactive selenium, this excretion was increased 10% and 3%, respectively. In the first three days after the selenite injection, 19% of the dose was excreted, suggesting that increases in circulating selenium-initiated exchange of stored selenium.

As discussed by Ganther (1986), the final major excretory pathway is the respiration of dimethyl selenide. Respiration of this compound occurs when hydrogen selenide undergoes methylation. At the third stage of methylation dimethylselenide becomes rate-limiting due to large amounts of selenium entering this chemical reaction. Dimethylselenide escapes into circulation and is eventually exhaled.

Zinc

Over six decades ago, zinc was discovered to be bound to protein fractions in the kidney of horses, along with cadmium (Margoshes and Vallee, 1957). This protein was later characterized to have 2.2% zinc, 5.9 % cadmium, and 8.5% sulfur, and subsequently termed metallothionein (MT) because of its metallic properties (Kagi and Vallee, 1960; Kagi and Vallee, 1961). When analyzing MT, Kägi et al. (1974) found that the protein contained different levels of bound zinc, depending on if it was in the liver or kidneys, with the livers having slightly greater quantities of zinc (6g atoms per molecule). Research by Bremner and Marshall (1974) determined there were three fractions of bound zinc in the liver of cattle that coincided with bound copper. This is likely due to copper and zinc sharing common transporters, namely a hepatocuprein (likely superoxide dismutase), as well as what appeared to be MT. Fraction 3 was similar to the previously mentioned MT, fraction 2 appeared to be a superoxide dismutase, and fraction 1 was not determined, although it was previously observed in rats. A recent review discusses the role of MT, specifically its role in controlling zinc concentrations through binding zinc and sequestering it in hepatocytes until signaled for release (Maret, 2000). The release of

zinc appears to require a conformation change in the protein structure, as MT surrounds the element when bound. In another review Davis and Cousins (2000) summarize MT research, stating that zinc tissue accumulation has been correlated with its synthesis, further strengthening the hypothesis that the transport protein is involved in the zinc homeostasis.

As mentioned by Bremner and Marshall (1974), the protein called hepatocuprein or superoxide dismutase (SOD) more recently, has been shown to contain two atoms of zinc per molecule in human livers (cytocuprein; Carrico and Deutsch, 1970). However, the original discovery of SOD, as discussed in the copper section, only indicated that the protein contained copper (Mann and Keilin, 1938). This protein was also called erythrocuprein in early literature, including that of Weser et al. (1971), who demonstrated the presence of roughly two zinc atoms per mole of bovine SOD found in the blood. This finding of zinc bound as part of SOD was subsequently reinforced by research from Bannister et al. (1971), who made similar observations in ox blood around the time of the submission of the previous paper.

When studying zinc accumulation in cows and calves, Kincaid et al. (1976) measured a considerable increase in the concentration of zinc in the calves' liver and pancreas (600% and 1400%, respectively) when the supplemented zinc at 600 ppm DM for 12 days. In contrast, the cows showed no change in zinc concentrations in the liver or pancreas when supplemented with the same amount. In another study, Whanger et al. (1981) reported that zinc accumulated primarily with MT in the liver, kidneys, pancreas, and the epithelial cells of small and large intestines. Zinc concentration of livers of necropsied cattle, aged ≤ 1, was associated linearly with age (Puschner et al., 2004). As the age of the animal increased, there was a subsequent decrease in the concentration of zinc in the liver.

Researchers studying zinc status and excretion observed that animals given supplemental zinc had lower blood zinc concentrations than those not supplemented with zinc, and had greater total zinc in the liver, heart, lung, kidney, spleen, testicles, and skin (Miller et al., 1968). As discussed in the transport section of this paper, researchers have demonstrated that an animal excretes zinc in the upper section of the small intestine (Miller, 1969). Additionally, a large portion of the zinc excreted is in an unabsorbed dietary form as described in a review by Underwood (1977). Cows and young calves were supplemented with 150, 100, or 50 μc zinc daily, for 10 days. Known routes of excretion, including blood, urine, and fecal matter were monitored throughout the experiment, and a necropsy was conducted on the 11th day (Miller and Cragle, 1965). These researchers observed that the feces were a major route of zinc excretion during the final four days, representing 72% and 86% of the daily dose administered to the of calves and cows, respectively. Additionally, urinary zinc excretion was determined to be insignificant during the same study. Powell et al. (1967) found that despite interventions of EDTA and cadmium to alter the excretion of zinc in calves and goats, intestinal excretion was the primary excretory pathway. Some changes were observed though, where cadmium delayed excretion and EDTA increased in the urinary excretion of zinc.

Summary of Trace Mineral Metabolism

Mineral absorption, transport, storage, and excretion are complex processes involving enzymes, mineral interactions, and transport proteins. In many instances, there are transporters shared by numerous minerals, and multiple transporters for a single mineral, further creating complexity around how the mammalian body can regulate mineral concentrations. Trace mineral homeostasis relies on both the rates of absorption and excretion, which as discussed, have many routes depending on the specific mineral. Disruption of an adequate mineral supply can lead to

disease, which has often led to scientists' discovery of important micronutrient pathways. These diseases generally result from mineral-dependent steps in biological processes, ranging from aerobic metabolism to free radical scavenging. In contrast to the disease pathology, when trace minerals are supplied at appropriate levels, there can be marked increases in animal performance, health, and productivity can be observed.

TRACE MINERAL SOURCE

The effect of mineral source on the health and productivity of an animal has garnered considerable interest in recent years, including in ruminant nutrition (Spears, 1996). This section will offer a brief overview of studies comparing the effects of mineral source on digestion, fertility and production, immunity, and genetics. However, it should be noted that there are various organic mineral sources available and used within these trials, including metal-amino acid complexes, proteinates, amino acid chelates, and metal-polysaccharide complexes. A primary benefit of the use of organic-trace mineral sources is increased mineral bioavailability (Cao et al., 2000; Pal et al., 2010; Caldera et al., 2019; Sun et al., 2019). This increase in bioavailability may be due to a corresponding decrease in mineral interactions within in the digestive tract, fewer interactions with other dietary factors like polyphenols, and less precipitation through processes like hydroxypolymerization (Andrieu, 2008).

Mineral Status

Animal mineral status is a complex area of research as it can have inconsistent results and can be difficult to compare and extrapolate results from independent studies. This topic of trace mineral status intentionally precedes the later subsections because all body processes rely upon adequate concentrations of essential minerals to properly function. This complexity and importance make the task of determining animal nutritional requirements especially difficult as

the recommendation must avoid both nutrient deficiency and toxicity. Common sample types for testing trace mineral status in livestock include serum, plasma, and liver tissue (Claypool et al., 1975; Mills, 1987). Blood samples may be used as they are a less invasive measure to test for mineral status than, for example, liver biopsies. However, the liver most accurately represents the current mineral status for cattle when testing for concentrations of cobalt (or B_{12}), copper, manganese, and selenium, but not for zinc (Herdt and Hoff, 2011).

It is vital to have a commonly accepted baseline concentration for comparison to determine adequate mineral status. Based on a depletion and repletion trial, Mills et al. (1967b) suggest that zinc deficiency is diagnosed once plasma zinc levels fall below 0.4 ug zinc/mL for longer than one week. Mills (1987) proposed that, generally, copper sufficient cattle will have a plasma copper concentration of greater than 0.6 mg copper/L and erythrocyte SOD levels of greater than 0.3 mg copper/g of hemoglobin. Jones and Suttle (1981) stated that copper sufficient sheep should have more than 8 μmol per liter of leucocyte in their plasma. Gibbons et al. (1976) reported that typical blood plasma levels of manganese are found to be between 5 and 10 ng/mL Dargatz and Ross (1996) reported a sufficient whole blood selenium concentration in cattle as being greater than 0.080 mg/L. Cobalt concentration is considered adequate if serum vitamin B_{12} concentrations are between 0.4 and 0.9 ppm or if liver vitamin B_{12} concentrations are between 0.25 and 0.5 ppm (Corah and Arthington, 1994).

When studying the effect of mineral source on the mineral status of crossbred cows, Ahola et al. (2004) discovered that organic mineral sources of Cu, Mn, and Zn, fed at the same level as inorganic mineral sources, will increase liver mineral status to a greater extent. Additionally, organic trace minerals supplemented at lower dosages exhibited greater plasma mineral status of selenium, zinc, copper, and manganese than cattle supplemented greater and

equal amounts of sulfate minerals during a standard feedlot fattening period (186 days; Rossi et al., 2020). Jalali et al. (2020) reported that over the course of a 2-year study, hydroxy trace minerals supplemented to crossbred beef cattle had greater liver mineral status than animals receiving a 1:3 mix of organic and sulfate mineral for copper in years 1 and 2 and for zinc in year 2, however manganese status was similar across all treatments.

Caramalac et al. (2017) reported that in pre-weaning calves, regardless of mineral source (hydroxy or sulfate), liver mineral status for copper, manganese and zinc was similar when supplemented at equal rates for 84 days. Similarly, in a more recent paper, there were no significant differences in the liver mineral status of cows or calves when comparing hydroxy and sulfate trace mineral sources after 20 weeks of consuming similar quantities (Cows, 69.5 vs. 60.7g; Calves, 15 vs. 16.6g; Arthington et al., 2021). Trace-mineral delivery method, however, may influence the mineral status of an animal. In a study by Pogge et al. (20120), mineral adequate steers were injected with trace minerals, delivering 15 mg copper, 60 mg zinc, 10 mg manganese, and 5 mg selenium per mL at a rate of 1 mL for each kg of body weight. Steers that were injected with these trace minerals had greater liver copper and selenium, and tended to have greater zinc than animals who did not receive the mineral injection at each sampling time of the 15-day trial.

As discussed in previous sections, there are also interactions between the various trace minerals that can decrease the utilization of other trace minerals and thus affect mineral status. For example, McDowell (2003) discusses this thoroughly regarding molybdenum, sulfur, and iron and their ability to limit intestinal absorption of copper and subsequently negatively affect the copper status of the animal. Littledike et al. (1995) notes a positive correlation between copper and zinc liver concentrations, where liver copper concentration increases 0.13 ± 0.03 ug

copper/g DM for each unit increase in liver zinc. Trace mineral status is not only impacted by interactions between trace minerals, as discussed by Kabaija and Smith (1988), where increasing levels of dietary fiber in the ration of sheep affected apparent absorption of all minerals, which can hinder mineral status.

To establish the efficacy of serum for assessing selenium mineral status, Maas et al. (1992) used serum samples to predict the plasma selenium status of cattle. They were unsuccessful as the prediction intervals were too wide to be used accurately. The authors suggest that a better method for testing selenium status of an animal is by way of glutathione peroxidase activity (GSH-Px) or plasma selenium concentrations. Alternatively, blood plasma may be a useful sample matrix for determining manganese status as it appears to be a more responsive predictor at deficient levels of manganese intake compared to tissue samples (Hidiroglou et al., 1978).

Andrews et al. (1960) suggest that B_{12} should be used to compare cobalt levels rather than circulating cobalt. However, Suttle (1986) discusses that assessing cobalt status through liver B_{12} and serum B_{12} is not consistently accurate and that concentrations of other constituents, like methylmalonic acid, should be used instead. During a mineral deficiency, circulating mineral forms and blood components disappear at varying rates. For example, cobalt deficiency may result in a more significant decrease in circulating B_{12} than in circulating cobalt (Andrews et al., 1959). A proposed solution by Mills (1987) includes two alternative methods for measuring cobalt status, including metabolism of propionate and the transfer of reactive methyl groups. Both methods are based on measuring intermediates in body fluids.

Many other environmental and genetic factors can also impact mineral status. An example is the effect of cattle breed on mineral intake and retention. Littledike et al. (1995)

discovered that Limousin cattle had significantly higher liver copper concentrations when compared to Braunvieh, Charolais, Gelbvieh, Hereford, Red Poll, Pinzgauer, and Simmental cattle. In a similar study, Ward et al. (1995) reported that Simmental and Hereford cattle have limited ability to maintain copper mineral status compared to Angus, further suggesting differences in mineral requirements between cattle breeds. Finally, Small et al. (1997) reported that during the estrous cycle, cows had an increased concentration of serum copper when compared to 21 days after breeding service.

Digestion

The source of fed trace mineral supplements impact ruminal fermentation and metabolism, as demonstrated by Genther and Hansen. (2015). These researchers found that supplementing sulfate trace minerals (copper, manganese, and zinc) negatively impacted dry matter disappearance, while hydroxy trace minerals, at the same doses, did not impact digestion. Alternatively, in another study with fistulated steers, Guimaraes et al. (2019) found differences between sulfate and hydroxy trace minerals (copper, manganese, and zinc) and their effect on neutral detergent fiber (NDF) digestibility, suggesting that all trace mineral sources may affect ruminal digestion. Cows used in a Latin square design were given sulfate and hydroxy minerals (copper, manganese, and zinc) for 28 days on each 56-day treatment (Faulkner and Weiss, 2017). These treatments did not effect the dry matter intake or total digestible nutrients; however, the hydroxy treatment did increase the neutral detergent fiber digestion slightly (48.5% vs 46.4%). Similarly, Caldera et al. (2019) found that dry matter digestibility was not affected by the mineral source, but that NDF digestibility tended to be greater in the organic supplemented group. Miller et al. (2020) found only insignificant effects of mineral source (sulfate versus hydroxy copper, manganese, and zinc), on rumen pH, fermentation, turnover, or particle passage

rates. These researchers note that silage type had a more significant effect on digestion than the mineral source.

Fertility

When conducting a meta-analysis, researchers observed that supplementation of organic trace minerals decreased a cows' days open by 13.5 days (weighted mean average) and decreased the number of breeding services per conception by 0.27 (weighted mean difference) in dairy cows (Rabiee et al., 2010). However, mineral source did not affect days until first service or 21-day pregnancy rate. Dantas et al. (2019) reported that cows supplemented organic trace minerals had greater 28-day pregnancy rate after embryo transfer than the inorganic treatment (64.71 and 52.90%, respectively). Additionally, there was a 53% greater likelihood of recovering a Cumulus-Oocyte Complex from cows in the organic treatment. Campbell et al. (1999) demonstrated that organic trace minerals (cobalt, copper, manganese, and zinc) decreased days until first estrus, however the mineral source showed no effect on days until first service or body condition score.

Chester-Jones et al. (2013) studied the effect of copper, manganese, and zinc source on reproductive performance. These researchers determined that first service conception rates were highest in cattle supplemented exclusively metal-polysaccharide complexes when compared to either exclusively sulfate trace minerals, a combination of 67% sulfate and 33% amino acid chelates, or a combination of 67% sulfate and 33% polysaccharides (58.3 vs. 36.4, 25.0, and 35.7%. respectively). In another study, Hackbart et al. (2010) found no significant differences in ovarian function, embryo development measures, or progesterone concentrations when comparing organic and inorganic mineral treatments. In fact, organic mineral supplemented cows tended to have smaller corpus luteum.

Copper source was tested for its role in cow reproduction rate, calf health and immunity, and mineral status of both cows and calves (Muehlenbein et al., 2001). The researchers reported that organic mineral-treated cows had a three-fold increase in liver copper storage levels when compared to inorganic treated animals as well as an increase in IgG titers in the colostrum. In a dairy cow model, Ballantine et al. (2002) reported that replacing inorganic trace minerals with organic trace minerals (zinc, manganese, copper, cobalt) decreased the days open by 22 days. However, Siciliano-Jones et al. (2008) found no difference in any measured fertility proxy in dairy cows fed either organic or inorganic trace mineral (zinc, manganese, copper, cobalt) sources.

Hormones

An essential factor of animal fertility is the concentration of reproductive hormones, namely progesterone, estradiol, follicle-stimulating hormone, luteinizing hormone, and anti-mullerian hormone. These hormones have a multitude of roles during reproduction that are discussed in a later section. However, this section will discuss the effect of trace mineral source on concentrations of reproductive hormones. There is limited research in this area, with few papers concerning the connection between these parameters in cattle.

Cerny et al. (2016) reported that cows supplemented with 35 ppm of a 1:1 ratio of organic and inorganic selenium sources had greater concentrations of plasma progesterone on day 6 post-estrus compared to an inorganic treatment supplement at the same selenium concentration. However, there was no difference between the organic treatment of the same concentration and the mixed or inorganic treatments. The same researchers reported no effect on plasma estradiol from any mineral treatment. In a subsequent experiment, Carr et al. (2020) reported a similar outcome, where cows supplemented 35 ppm of a 1:1 ratio of organic and

inorganic selenium sources had greater plasma progesterone concentrations than the inorganic treatment on day 7 post-estrus but not on day 4 or 10. It was also reported that cows in the mixed mineral source treatment had greater progesterone levels during months 1, 3, 5, and 7 of gestation.

Immunity

Holder et al. (2016) used high-risk feedlot steers to study the effect of copper, cobalt, manganese, and zinc source on immunity and animal health (morbidity and mortality). The researchers observed a decrease in mortality of 57% when comparing the organic to the inorganic trace mineral supplemented cattle (4.78 vs. 2.05%) but no other health metric differed. Kulow et al. (2017) reported that feeding organic sources of cobalt, copper, manganese, and zinc decreased the probability of feedlot steers having active hoof lesions and increased the average carcass grade, when compared to animals supplemented inorganic minerals. Engle et al. (1999) reported, in two trials, that mineral sources of copper, manganese and zinc had little effect on the immune response to IBRV inoculation and did not affect feedlot performance of steers.

Marques et al. (2016) observed that calves born to cows supplemented with amino acid chelates of cobalt, copper, manganese, and zinc were treated for BRD symptoms less often when compared to cows supplemented inorganic forms. Additionally, these calves were >20 kg heavier from weaning to slaughter when compared to offspring of cows supplemented with inorganic minerals. Formigoni et al. (2011) partially replaced inorganic mineral sources of copper, manganese, and zinc with organic sources. These researchers observed that dairy cows supplemented with the organic source had greater colostrum immunoglobulin and less incidence of offspring mortality at calving, when compared to the inorganic treatment. When testing the source of zinc in the diets in 6-week-old calves, Kincaid et al. (1997) reported that both 150 ppm

and 300 ppm of zinc lysine and zinc methionine had neither a beneficial nor consequential effect on the calf immune system or performance. In another study, Dorton et al. (2006) reported no significant differences in the morbidity nor number of treatments per morbid animal during the receiving phase of a feedlot, regardless of source of cobalt, copper, manganese, and zinc.

Summary of Trace Mineral Source

The effect of mineral source on mineral status, digestion, fertility, and immunity has been highly studied yet these studies have developed relatively inconsistent results. This phenomenon may be due to minerals other than those being tested impacting processes within digestion, fertility, and immunity. Additionally, some factors are not controlled in or are dissimilar between many of these studies. These factors include those of the animal's internal and external environment, including stress, age, genetics, weather, altitude, ration composition, hydration, activity level, and numerous other sources of variation. However, possibly the most impactful factor is the individual animal's mineral status before supplementation of any trace mineral source. An animal that is mineral sufficient will likely not benefit from additional mineral supplementation unless the source impacts the animal in other biologically significant ways. This aspect of mineral source is still to be fully elucidated.

REPRODUCTIVE HORMONES AS EVALUATIONS OF FERTILITY

Progesterone

It is useful to have a commonly accepted plasma progesterone concentration to survey cows for optimal fertility and chance for a successful pregnancy. Researchers have attempted to determine this value, including Starbuck et al. (2004), who observed that cows with circulating plasma progesterone concentrations of equal to or less than 2.8 ng/mL at week 5 of pregnancy had a 50% decreased chance for a successful pregnancy. These researchers also determined that,

on average, cows who maintained their pregnancy to week 9 had progesterone concentrations of 6 ng/mL. Kenyon et al. (2013) found that an increase in progesterone concentration from day 0 to 14 days post-conception was positively associated with pregnancy outcomes. Specifically, day 63 pregnancy was positively affected by the rise in progesterone from day 0 to 7, as well as day 7 to 14 post-conception. These researchers also discuss that pregnancy is less likely to be maintained until day 63 if progesterone concentrations are less than 5.0 ng/mL on day 14. These data suggest that to optimize cattle fertility, progesterone levels should be above 2.8 ng/mL and preferably increase consistently to above 5.0 ng/mL by week 2 of pregnancy.

Garrett et al. (1988) reported that cows given exogenous progesterone had greater conceptus length on day 14 of pregnancy than control cows receiving no exogenous progesterone (37.3 ± 14.9 vs. 3.8 ± 1.9 nm, respectively). These data agree with recent work demonstrating increases in embryo length in cows with greater progesterone concentrations on both day 13 and 16 of pregnancy (Carter et al., 2008). The timing of elevated progesterone concentrations can influence embryo development as well. When using an intravaginal CIDR (controlled internal drug release) device to deliver exogenous progesterone in mature cows during embryo transfer, Mann et al. (2006) determined that earlier increases in progesterone benefit trophoblast length, whereas later increases had little effect (5-9 days after ovulation, versus 12–16 days). However, Parr et al. (2012) reported that animals with progesterone concentrations that exceeded the deduced optimal range of 2.5 ng/mL on day 4 and 5.2 ng/mL on day 7 post-conception had decreased pregnancy rates. When evaluating the effect of progesterone concentration before ovulation, Stevenson and Pulley (2016) observed that cows with low progesterone concentrations became pregnant more often than those that had high progesterone levels (P4 < .45 ng/mL vs. > .53 pg/mL; P = .02).

A possible indicator of fertility and pregnancy success is the change in progesterone concentrations during days immediately following insemination. Parr et al. (2012) reported that a 3.5 ng/mL change in progesterone concentration between days 4 and 7 was considered optimal and had an 80% chance of a successful pregnancy via artificial insemination. Kenyon et al. (2013) also detailed a time by progesterone concentration interaction, where no cows were found pregnant on day 63 if they had an increase in progesterone concentrations of less than 2.7 from day 0 to 7 and less than 1.48 from days 7 to 14.

Clemente et al. (2009) demonstrated that embryo elongation did not change when supplementing progesterone to embryos in-vitro, but that elongation increased significantly when progesterone was supplemented to cows before embryo transfer. The differential growth and pregnancy success were further investigated by transferring embryos into cows with elevated or unaltered progesterone concentrations and comparing the embryo transcriptome of each treatment (Carter et al., 2010). These researchers observed that 46% of changes in the embryo's transcriptome were related to cellular metabolic and biosynthetic processes. These data suggest that increases in embryo elongation occur because of changes in the maternal endometrium which subsequently affect embryo development.

Animals given exogenous progesterone had increased expression of genes relating to energy sources (e.g., DGAT2, which is involved in the formation of triglycerides, and MSTN, which regulates glucose secretion; Forde et al., 2009). This result agrees with another recent article by Mullen et al. (2014), which described an increased concentration of amino acids and glucose in the histotroph as the pregnancy progressed (days 0 - 13). Additionally, as the concentration of circulating progesterone increased, amino acids increased in concentration. It is well summarized by Lonergan and Forde (2014) that endometrial changes are required for a

successful pregnancy. These changes are likely to increase nutritional capacity of the maternal histotroph to better support the elongating embryos and suggest that increased embryo elongation, from cows with above-average progesterone levels may be due to greater nutritional availability by the histotroph.

Estradiol

Dominant ovarian follicles, the most developed of the follicles during proestrus, are responsible for releasing an oocyte during ovulation. Ovulation of the follicle occurs when the dominant follicle secretes enough estradiol to signal the hypothalamus, and eventually the pituitary gland, to increase circulating GnRH concentrations (Rispoli and Nett, 2005). This increase in GnRH causes a subsequent rise in luteinizing hormone, called the "LH surge." The LH surge is a critical step in ovulation, the delivery of a viable oocyte, and the fertility of the animal (Schoenemann et al., 1985). Through these mechanisms, estradiol appears to control follicular waves in cows, as demonstrated through use of estradiol to suppress follicle development causing emergence of a new follicular wave 4.3 days later, on average (Bo et al., 1995).

Circulating estradiol decreases after an LH surge, likely because the LH surge causes the granulosa cells to increase progesterone secretion and decrease secretion of estradiol (Hansel and Convey, 1983). This increase in progesterone after ovulation may be related to the impact of estradiol on progesterone receptors prior to ovulation (Ing and Belen Tornesi, 1997). Cows with high circulating concentrations of estradiol and estrone during early pregnancy, measured through increased urine excretions, showed inhibited pregnancy success compared to cows with lower estrogen concentrations (Stott et al., 1971). This agrees with Atkins et al. (2013), where

plasma progesterone concentrations 7 days post-ovulation were enhanced by greater estradiol concentrations on the day of ovulation.

While researching oocyte competency and pregnancy outcomes, Atkins et al. (2013) discovered there was a strong correlation between estradiol concentration and follicle size on the day of estrus synchronization and that successful fertilization was increased in recipient cows with greater estradiol. The authors also reported that the size of the follicle after ovulation and just prior to embryo transfer is correlated to serum estradiol concentration of donor cows. Additionally, mean estradiol concentrations were greater in donor cows that had fertilized embryos, and recipients that were pregnant on day 27 of the project. This agrees with Larimore et al. (2016), where cows with high estradiol also had larger follicles on day of estrus synchronization and at follicular aspiration. Vasconcelos et al. (1999) observed that pregnancy rates were greater in dairy cows with smaller and younger follicles.

To document normal levels for estradiol, Wettemann and Hafs (1973) measured concentrations of the hormone during estrus and early pregnancy, finding estradiol was 12.6 pg/mL at estrus, decreased to 8.4 pg/mL on day 4 of pregnancy, and averaged between 6.2 and 8.6 pg/mL from days 7 to 75 of pregnancy (except for day 40). In another study, estradiol was measured during the final days of pregnancy, twenty-six days prior to parturition estradiol levels average around 32 pg/mL, increased to 293 pg/mL two days before parturition, and then rapidly decreased to an average of 52 pg/mL after parturition (Smith et al., 1973).

Follicle Stimulating Hormone

Within a given estrous cycle, there are multiple follicular waves, each occurring in 7-to-10-day intervals that include stages called selection, dominance, and atresia (Evans et al., 1994; Ginther et al., 1996b; Ireland et al., 2000). Each of these follicular waves coincides with an

increase in basal concentrations of FSH which induces the growth of the antral follicles (Fortune, 1994). As follicular growth progresses, the FSH concentrations decrease causing only a few of the follicles to continue growing (selection stage), one of which deviates by becoming larger than the rest (dominance stage) and is given the term “dominant follicle” (Sunderland et al., 1994; Ginther et al., 1996b). The remaining follicles, which are significantly smaller than the dominant follicle, cease to grow, go through atresia, and are termed “subordinate follicles” (Ginther et al., 2001). Researchers hypothesize that smaller follicles have not yet reached an advanced enough developmental stage to survive with decreased FSH concentration. If the follicular wave is unsuccessful in producing ovulation, the dominant follicle will begin to regress 3-4 days after the emergence of the follicular wave (Ginther et al., 1989). These follicular waves occur even after a successful pregnancy is established, including the surges in FSH concentration, which reportedly do not differ significantly between months of pregnancy (Ginther et al., 1996a). When evaluating the consistency of FSH concentrations, Lopez et al. (2005) reported great variation between individual animals’ concentration but very minimal difference between a single cows’ various estrous cycles.

There are two FSH “surges” for each of the follicular waves, with the first being the preovulatory surge, which typically occurs just over one day prior to ovulation (Haughian et al., 2004), and is associated with the number of antral follicles in ewes (Cahill et al., 1981). The subsequent increase, called the postovulatory rise or secondary FSH surge, occurs roughly one day after the preovulatory surge in unaltered estrous cycles of cattle (Turzillo and Fortune, 1990). Adams et al. (1992) suggests that it is the loss of the dominant follicle that causes the increase in FSH, as animals given exogenous doses of FSH did not have an immediate decline in follicle size. A negative correlation has been observed between level of peak FSH concentration

and the diameter of the largest follicle (Ginther et al., 1996a). Additionally, Burns et al. (2005) observed that animals with a more follicles had lower FSH concentrations. This agrees with Ireland et al. (2007), where high follicle count animals tended to have lower basal FSH concentrations than the low follicle count animals.

In an experiment to test the effect of FSH secretion on follicle numbers, Mossa et al. (2010) performed ovariectomies on low and high follicle count cows (≤ 15 & ≥ 30 follicles, respectively). The objective of this experiment was to determine if FSH concentrations were due to greater gonadotropin negative feedback or inherent differences in FSH production ability. Both groups (low and high) had an equal increase in FSH, demonstrating that the difference was not intrinsic gonadotropin production. It has been recognized that altered concentrations of FSH can hinder the reproductive performance of animals, where over-suppression of FSH inhibits follicular development, either exogenous (Turzillo and Fortune, 1990) or endogenous (Haughian et al., 2004). Also, increased secretion of FSH has been shown to coincide with decreased follicle numbers, indicative of low ovarian reserve in older cows (Malhi et al., 2005). In a meta-analysis of 243 cows (448 oocyte collection records), researchers found that the FSH treated cows had fewer small follicles ($P < .01$), greater number of medium follicles, and similar total follicle and large follicle count (Sarwar et al., 2020). Additionally, the quality grades of oocytes and the number of transferable embryos were greater in FSH-treated cows.

Inhibin, a glycoprotein that appears to selectively downregulate the rate of secretion of FSH (Franchimont et al., 1980), was successfully isolated from bovine follicular fluid (FF; Robertson et al., 1985). In another study, researchers administered 10 mL of FF either intravenously or subcutaneously, and a control group was dosed with 10 mL saline intravenously. These researchers observed a delayed estrus by an average of 2.3 days, being 8

days in 2 of 3 given intravenous doses and 9 days in 1 of 3 given subcutaneous doses compared to the control cows (Quirk and Fortune, 1986). Additionally, when cows were dosed with 20 mL FF intravenously, treated cattle had an average FSH level that was 28% lower than control animals given 20 mL intravenous saline from hours 24-48. In a similar experiment, Adams et al. (1992) found that FF could be used to decrease the circulating FSH concentrations of treated animals for longer, as seen on days 0-3, 3-6, 6-11 (6.5 vs 9.9, 3.6 vs 9.3, and 61 vs 100, for treated and untreated respectively). In other research, treatments with follicular fluid prevented the postovulatory FSH surge and retained low FSH levels until the termination of the treatment when FSH levels subsequently rose above the control levels (Turzillo and Fortune, 1990). These researchers also found that the modulation of FSH caused a delay in the follicular wave and dominant follicle emergence.

Administration of exogenous FSH resulted in the second FSH surge and the emergence of a new follicular wave to occur two days earlier than in control heifers, suggesting the FSH surge directly causes the emergence of a dominant follicle (Ginther et al., 2002). Additionally, similar research completed by Mihm et al. (1997) found that treating cattle with FSH increased the time in growth before atresia of follicles and delayed the deviation of the dominant follicle by 2.2-days. In a similar study, Adams et al. (1993) administered FSH two days prior to selection of the dominant follicle and saw a subsequent delay in the divergence of the dominant follicle. These data combined suggest that a surge in FSH is required to induce the emergence of a follicular wave, and if FSH is altered, the wave will be delayed. FSH is involved in the selection of the dominant follicle, follicle size and count, and subsequently in the fertility of a cow.

Luteinizing Hormone

Luteinizing hormone (LH) is derived from the pituitary gland and is produced after stimulation by GnRH (Day et al., 1986; Rao, 2001). During an estrous cycle, there is a drastic increase in the quantity of LH, called an "LH surge" (Clapper et al., 1990), which occurs near the time of a surge in estradiol. It was discussed by Rao (2001) that early LH research used human chorionic gonadotropin (hCG) to stimulate LH in reproduction studies. Wiltbank et al. (1961) studied the effect of LH (hCG) on the maintenance of a corpus luteum. They reported that four of five treated heifers had increased time in estrus (32.4 versus 17.7 days for treated and control, respectively) and maintained their corpus luteum until the treatments ceased (26 days). Another experiment reported that when heifers were treated with hCG, 23 of 71 observed estrous cycles produced an additional corpus luteum (Price and Webb, 1989). These studies demonstrated that LH had a role in bovine reproduction, specifically related to the corpus luteum.

Reames et al. (2011) treated ovariectomized cows with intravenous progesterone and estradiol and measured the change in LH and presence of estrus. The LH surge consistently occurred 16 hours after the start of the infusion of progesterone, with estrus occurring roughly 3 hours before to 3 hours after the LH surge in this study. Adriaens et al. (2019) compared the ability of milk progesterone, activity, and blood LH concentrations to capture the onset of estrus. These researchers reported that LH surge values were reliable indicators of ovulation to determine timing of insemination. In a study on prepubertal heifers, Melvin et al. (1999) observed that LH had pulsatile nature which increased in frequency through prepuberty, especially between 8 and 6 months and between 3- and 1- month(s) before puberty. These papers and others (Rawlings et al., 2003) have established that LH is intimately involved in ovulation and development of the reproductive tract.

Nutritional factors can influence the concentrations of LH. Jordan and Swanson (1979) reported that cows fed 12.7% crude protein had lower plasma LH concentrations than those fed 16.3% and 19.3% crude protein. Cows receiving reduced nutrient intakes (losing 1% bodyweight each week) had fewer LH pulses than those fed maintenance nutrient levels (maintain bodyweight), and significantly affected ovarian activity and estrus (Richards et al., 1989). Schillo (1992) also discusses the impact of energy-deficient diets, specifically regarding the disruption of LH secretion, altering estrus, and reduction of ovarian follicle development. In an earlier paper, Schillo (1982) discussed how a combination of exogenous estradiol and LH had decreased blood concentrations of LH in ovariectomized cows when compared to cows given exogenous LH only (Schillo, 1992). There are also documented interactions of LH with other hormones, like progesterone, as described by Procknor et al. (1986). These researchers found correlations between LH and progesterone concentrations, suggesting that progesterone is reliant on the pulsatile pattern of LH. The role of LH is vital to reproductive success of animals, with roles in estrus behavior, folliculogenesis, ovulation, and embryo maturation (Clemens et al., 1995).

Anti-Mullerian Hormone

Anti-mullerian hormone (AMH), previously called Mullerian Inhibiting Substance, is a glycoprotein isolated from newborn bovine testis (Cate et al., 1986). The researchers isolated the gene for AMH and observed its expression in the ovaries and the testes during early embryonic development. Using bovine ovarian tissues, researchers used a staining technique to discover that AMH is secreted by granulosa cells of ovarian follicles, as described by Takahashi et al. (1986). More recently, it was demonstrated in cows that the highest concentrations of AMH are found in the small antral follicles, and the concentration of the hormone decreases once follicles surpass 5

mm in diameter (Monniaux et al., 2008). The researchers hypothesize this may have an important function on follicular development, through it has not been fully elucidated.

Anti-mullerian hormone has been used as a proxy for cattle fertility in recent years, with research finding high repeatability and correlation of the hormone to follicular response from gonadotropin treatments (Rico et al., 2012). These researchers reported that cows with plasma AMH concentrations below 87 pg/mL produced 15 fewer large ovarian follicles at each super-ovulated estrus, suggesting a minimum plasma AMH concentration. Additionally, Jimenez-Krassel et al. (2015) reported that cows with bottom quartile (<19 pg/mL) AMH concentrations also had decreased productivity, specifically 196 fewer days of production when compared to the middle two quartiles, as well as reduced first calf survival rate and highest culling rate, based on animal herd life. Other research agrees that AMH is correlated with response of follicles to superovulation and fertility (Rico et al., 2009).

The concentration of AMH has been correlated with and can predict fertility factors, like ovarian reserve (Ireland et al., 2011), which is a measure of remaining number of oocytes available for ovulation in the "ovarian follicle pool." Researchers reported cows with AMH levels of 100-200 and > 200 pg/mL produced a greater quantity of embryos than those with < 100 pg/mL (Monniaux et al., 2010), a standard metric to assess ovarian reserve (Burns et al., 2005). Monniaux et al. (2010) hypothesized that the elevated concentrations of plasma AMH demonstrates greater AMH production from all small antral follicles, which is a sign of greater number of follicles produced and follicles available.

Plasma AMH concentrations of cows can be affected by factors such as parity number, nutrition, breed, and the animals' genetics of an individual animal (Umer et al., 2019). Researchers studied 460 genotyped cows to evaluate the relationship between fertility,

quantitative loci, and AMH concentrations (Gobikrushanth et al., 2018). These researchers demonstrated that AMH levels might be affected by variants or regulatory regions upstream of the AMH gene causing variability in the quantity of AMH produced, like the AMH variant rs43505519, or the variation that was observed at BTA11. Nutrition can also affect the circulating AMH concentrations. Mossa et al. (2013) fed pregnant cows 60% of maternal nutritional requirements to evaluate the effect of undernutrition on the ovarian reserve of their offspring, assessed by antral follicle count and AMH concentrations. Their findings were as follows, lower antral follicle count at 7 and 18 weeks of age, follicle diameter was similar, and circulating AMH was decreased in the nutritional restricted calves when compared to the control group.

Breed differences for plasma AMH concentrations were demonstrated when comparing Nelore (Bos indicus) to Holstein (Bos taurus) breeds by Batista et al. (2014). Antral follicle count was higher in the Nelore cattle at all collection timepoints (18 to 85 and 8 to 51 for Nelore and Holstein, respectively). Additionally, AMH concentrations were highly correlated to antral follicle count, regardless of breed, but Nelore cattle had greater average concentrations. Similar research by Ribeiro et al. (2014) also found that breed – Jersey, Holstein, and crossbred – had a large effect on AMH concentration and reported a significant impact on lactation number.

Summary of Hormones as Evaluations of Fertility

Reproductive Hormones, namely progesterone, estradiol, luteinizing hormone, follicle stimulating hormone, and anti-mullerian hormone, have a vital role in ensuring proper growth for both the follicle and the fetus, as well as orchestrating the complex process of the estrous cycle. However, the concentration of specific hormones can change due to stress, malnutrition, age, genetics, and pregnancy status, as well as differing from animal to animal. These interactions

complicate the use of hormones as a measure of fertility. Although, hormones have previously been used as proxies: AMH has been used successfully to create ranges for number of ovarian follicles, LH surges are a reliable indicator of ovulation, FSH concentration has been used to predict follicle count and size, estradiol concentration can be used as a predictor of early pregnancy success, and progesterone concentrations have been correlated fetus growth.

SUMMARY OF LITERATURE REVIEW

This literature review serves to introduce topics that are investigated in the following experiment, namely the effect of mineral source on the fertility of multiparous cows. Combined, the previous chapters have described the absorption, transport, and storage of each trace minerals included in the proceeding experiment and how each element is involved in mammalian physiology and metabolic processes. Additionally, this literature review describes the role of reproductive hormones, typical concentrations, and how they can be used to predict fertility. Previous research evaluated fertility uses proxies like pregnancy rate, reproductive hormones, follicular growth, and fetal development. However, there is limited research investigating the impact of mineral source on hormone concentrations. The following research was aimed at providing a multifactorial dataset to examine the influence of trace mineral source on reproductive performance and mineral status in embryo transfer recipient beef cows.

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CHAPTER 2: THE INFLUENCE OF TRACE MINERAL SOURCE ON REPRODUCTIVE PERFORMANCE IN RECIPIENT MULTIPAROUS BEEF COWS.

SUMMARY

An experiment was conducted to determine the effects of copper (Cu), cobalt (Co), manganese (Mn), selenium (Se), and zinc (Zn) source on the reproductive parameters of multiparous beef cows. Fifty cow-calf pairs were divided into ten groups (n=5 cow-calf pairs per group) and balanced across groups for animal source, cow weight, calf age, calf sex, and breed type. Each group was randomly assigned to one of the following treatments: 1) Organic trace minerals: 75 mg of Cu/d from Cu proteinate, 8 mg of Co/d from Co proteinate, 105 mg of Mn/d from Mn proteinate, 3 mg of Se/d from Sel-Plex, and 220 mg of Zn/d from Zn proteinate; and 2) Inorganic trace minerals (at two times the NASEM (2016) requirements): 255 mg of Cu/d from $CuSO_4 \cdot 5H_20$, 2.6 mg of Co/d from $CoCO_3$, 1018 mg of Mn/d from $MnSO_4$, 3 mg Se/d from Na_2SeO_4, and 763 mg of Zn from $ZnSO_4 \cdot 5H_2O$. Animals were fed a corn silage – corn stalk-based diet that met or exceeded the NASEM (2016) requirements for gestating beef cows with the exception of Cu, Co, Mn, Se, and Zn. Cows were individually supplemented with their appropriate treatments daily for 89 days, five days after the initiation of estrus synchronization (day 0).

Estrus synchronization was achieved through implantation of a progesterone controlled internal drug release (CIDR; Zoetis; impregnated with 1.38 g progesterone) device and 2ml of gonadotropin-releasing hormone (GnRH; Factrel from Zoetis) administered intramuscularly. After seven days, the CIDR was removed, 2 ml lutalyse (HighCon from Zoetis) was administered intramuscularly, and an Estrotect patch was applied for estrus detection. After two additional days, recipient cows were given a second dose (2ml) of GnRH. Eight additional days

later, all recipient cows that were synchronized were palpated, and those with a viable corpus luteum (CL) received an embryo as well as a CIDR. The CIDR was removed 15 days later and an Estrotect patch was applied. Expression of estrus was evaluated for the following four days of the experiment. Cows that did not express estrus, and cows that did not maintain a pregnancy from the first embryo, underwent the same estrus synchronization protocol a subsequent time. A licensed veterinarian made pregnancy diagnoses on August 18th, September 12th, and October 8th using an ultrasound, recording each cow as either pregnant or open.

Blood samples were collected via jugular venipuncture from each animal on days 0, 16, 43, 68, and 94 of the experiment and analyzed for progesterone, luteinizing hormone, anti-mullerian hormone, follicle-stimulating hormone, and estradiol. However, as animals in this study varied by pregnancy status and days pregnant, mineral status and hormone status were evaluated by time points (TP) defined as TP0: prior to embryo transfer; TP1: day of embryo transfer; TP2: 28-33 days post embryo transfer; TP3: 58-60 days post embryo transfer; and TP4: 84 days post to embryo transfer. There was a treatment by time effect for plasma Cu concentrations ($P < .03$), with Cu concentrations of the inorganic treatment decreasing (0.89 to 0.76 mg Cu/L) and those of the organic treatment increasing (1.01 to 1.19 mg Cu/L) as the experiment progressed. There were no other significant time or treatment by time effects for plasma trace mineral concentrations. Cows receiving organic trace minerals had greater plasma Se and Cu ($P < .0001$) concentrations when compared to cows receiving the inorganic trace mineral. Plasma cobalt, manganese, and zinc concentrations were similar between the two treatments ($P > 0.1$). Pregnancy rates of the inorganic and organic treatments were similar at the conclusion of the trial (66.6% and 62.5%, respectively). Treatment outcomes were also similar ($P > 0.10$) for embryo transfer attempts, calf birth weights, and days pregnant. There were no

treatment or treatment by time interactions for serum progesterone, luteinizing hormone, anti-mullerian hormone, follicle-stimulating hormone, or estradiol concentrations ($P > 0.10$). The differences in circulating trace elements between treatments did not appear to impact the hormone concentrations or reproductive outcomes in this trial. Further research is needed to further understand the impact of mineral source on reproductive outcomes.

INTRODUCTION

In an embryo transfer system, recipient cattle are expected to accept donor embryos, establish a successful pregnancy, and deliver a live calf. The consistent occurrence of pregnancy in these systems is essential to the productivity of the cow and the profitability of the operation. A successful pregnancy requires the recipient cow to be in good health and have their nutritional requirements met, part of which are the trace minerals: copper, cobalt, manganese, selenium, and zinc. These trace minerals support various critical metabolic processes, some of which are essential for reproduction (Suttle, 2010). Historically, trace minerals have been primarily supplemented to cattle in an inorganic form. Today, other sources are available to the producer, like organic trace minerals. Organic trace minerals are chelated or complexed with amino acids or polysaccharides and have been shown to have greater bioavailability (Spears, 2003), suggesting their use could be beneficial. Recently, however, research has shown that mineral source may also affect the performance of cows. Organic supplementation improved reproductive performance in both timed AI systems (Stanton et al., 2000) and donor cattle in embryo transfer systems (Dantas et al., 2019). These data suggest that supplementing organic trace minerals to recipient cattle could improve reproductive performance. The present research was designed to compare the effects of mineral source on recipient cattle receiving embryos in an embryo transfer system.

MATERIALS AND METHODS

Prior to project initiation, all animal care, handling, and procedures described herein were approved by the Colorado State University Animal Care and Use Committee (IACUC approval # 1171).

Animals

Fifty multiparous beef cow/calf pairs were transported to the Colorado State University Agriculture Research, Development, and Community Education Center (ARDEC). Eighteen cows were from the resident cow herd at ARDEC, and 32 were from the Eastern Colorado Research Center in Akron, Colorado. The cows from these two locations were a mix of Hereford (n= 6), crossbred (Hereford x Angus; n=35), and Angus (n= 9) breeds. All cows were aged between 3 and 9.

Two days after arrival, cows and calves were individually weighed in a hydraulic squeeze chute fitted with a certified livestock scale. All 50 cow-calf pairs were divided into ten groups (n=5 cow-calf pairs per group). All groups were balanced for animal source, cow weight, calf age, calf sex, and breed type. Each of the ten groups was then randomly assigned a corresponding number from 1 to 10 and placed in their respective dry lot pens. Each pen was 6.1m x 40m and contained a concrete feed bunk, a 3 m concrete bunk pad, and an automatic waterer. Every other pen was supplemented with a corresponding treatment, with even-numbered pens receiving the inorganic trace mineral supplement and the odd-numbered pens receiving the organic trace mineral supplement.

Treatments

Each cow was individually supplemented their respective treatment in rubber feed pans, daily. Cows were supplemented for 89 days, beginning on day 5 of the trial and continuing

through day 94 of the trial (Figure 1). Supplementation occurred each morning before the total mixed ration was fed. Each cow was given 0.9 kg of extruded range pellets formulated to daily amounts of Cu, Mn, Se, and Zn. Treatments included: 1) Organic trace minerals: 75 mg of Cu/d from Cu proteinate, 8 mg of Co/d from Co proteinate, 105 mg of Mn/d from Mn proteinate, 3 mg of Se/d from Sel-Plex, and 220 mg of Zn/d from Zn proteinate; and 2) Inorganic trace minerals at two times the NASEM (2016) requirements: 255 mg of Cu/d from $CuSO_4 \cdot 5H_20$, 2.6 mg of Co/d from $CoCO_3$, 1018 mg of Mn/d from $MnSO_4$, 3 mg Se/d from Na_2SeO_4, and 763 mg of Zn/d from $ZnSO_4 \cdot 5H_2O$ (Table 1). Cows and their calves were monitored for consumption to prevent calves from consuming supplements and pans from spilling. Cows were given approximately 10 minutes to consume all of their supplement. In the rare event that a cow did not consume all of their supplement, orts were quantified.

Basal diet

All cows were provided the same total mixed ration between 07:00 and 09:00 daily. The basal diet was provided in amounts to allow for ad libitum access to feed over a 24 h period. The feed was delivered via feed truck and offloaded into the concrete feed bunks. Each pen consumed 111.5 kg/d DM, on average, based on the quantity of feed dispensed into feed bunks each day (22.3 kg/head/d DM), however the feed remaining in the bunks each day was not accounted for. The total mixed ration consisted of silage, corn stalks, whole corn, limestone, salt, and a liquid supplement (Table 2).

Sample Collection

Blood samples were collected via jugular venipuncture from each animal on days 0, 16, 43, 68, and 94 of the experiment. Animals in this study varied by pregnancy status and days pregnant, so mineral status and hormone concentrations were evaluated by time points

(TP),defined as TP0(prior to embryo transfer); TP1 (day of embryo transfer); TP2 (28-33 days post embryo transfer); TP3 (58-60 days post embryo transfer); and TP4 (84 days post to embryo transfer). At the time of blood collection, two 10 mL vacutainer tubes were used to collect blood samples. One vacutainer tube contained the anticoagulant K_2EDTA (BD Vacutainer; Becton, Dickenson, and Company; 1 Becton Drive, Franklin Lakes, NJ 07417-1880), and the other vacutainer tube had no additive (Monoject; Covidien; 710 Medtronic Parkway, Minneapolis, MN 55432-5604). All samples were centrifuged (1,100 x *g* at 4°C for 15 minutes; Beckman Tj-6 Benchtop Centrifuge; Beckman Coulter Diagnostics; 5350 Lakeview Parkway S Drive, Indianapolis, IN 46268) within 4 hours of collection. After centrifugation, plasma and serum were removed and aliquoted into four separate 0.5 ml acid-washed polypropylene storage tubes per sample type and stored at -80°C until analysis was performed. Only a portion of cows were sampled on day 43 of the study, corresponding to cows that had embryos transferred and had not shown signs of estrus. All animals were sampled at all other blood collection time points. Total mixed ration and treatment supplement samples were collected every 2-3 weeks for nutrient analysis. All feed samples were stored in plastic Ziploc bags at -20ºC.

Hormones

The serum samples that were collected on each of the sampling days, as previously discussed, were stored at -80ºC until analyzed. Progesterone concentration was quantified using a double-antibody radioimmunoassay procedure was used (Niswender, 1973). Intra-and inter-assay coefficients of variation were 9.3 and 10.2%, respectively. Luteinizing hormone concentration was quantitated by radioimmunoassay as described by (Niswender et al., 1969). Intra-and inter-assay coefficients of variation were 6.2 and 14.1%, respectively. Estradiol concentration was quantified by radioimmunoassay (Oxender et al., 1977; Stellflug, 1977). Intra-

and inter-assay coefficients of variation were 2.8 and 12.9%, respectively. FSH was determined via radioimmunoassay (Acosta et al., 1983). The serum AMH concentration was analyzed using an AMH (Bovine) ELISA kit (Ansh Labs, TX, USA). Intra-and inter-assay coefficients of variation were 2.8 and 6.1%, respectively.

Mineral Status

As previously discussed, plasma samples were collected on each sampling day and stored at -80ºC until analyzed. Mineral status was evaluated using ICP-RS (per manufacturer's recommendations; CEM Microwave Accelerated Reaction System MARS6, and Thermo Fisher Scientific iCAP 6300 Inductively Coupled Plasma Radial Spectrometer), and selenium mineral analysis (Wahlen et al., 2005).

Feed and Supplement Analysis

All feed ingredients of the basal diet, the total mixed rations samples, and treatment supplements were composited by month and analyzed as follows; dry matter (Goering and Soest, 1970), Crude Protein (Method 990.03; AOAC, 2006), ADF (Method 973.18; AOAC, 2006), NDF (Van Soest et al., 1991), mineral analysis (per manufacturer's recommendations; CEM Microwave Accelerated Reaction System MARS6, and Thermo Fisher Scientific iCAP 6300 Inductively Coupled Plasma Radial Spectrometer), and selenium mineral analysis as described by Wahlen et al.. (2005).

Estrus Synchronization, Embryo Transfer, and Pregnancy Diagnosis

Five days before initiation of the trace mineral treatments (day 0 of the experiment), the first blood samples were collected, progesterone controlled internal drug release (CIDR; Zoetis; impregnated with 1.38 g progesterone) devices were implanted into each of the 50 cows, and 2ml of gonadotropin-releasing hormone (GnRH; Factrel from Zoetis) was administered

intramuscularly. After seven days (day 7 of the experiment), the CIDR was removed from each of the cows, and 2 ml lutalyse (HighCon from Zoetis) was administered intramuscularly, and an Estrotect patch was applied for estrus detection. After two additional days, recipient cows received a second dose (2ml) of GnRH. All synchronized recipient cows were palpated on day 17 of the experiment, and those with a viable corpus luteum (CL) received an embryo and a CIDR. The CIDR was removed on day 32 of the experiment, and an Estrotect patch was applied. Expression of estrus was evaluated from day 33 to day 36 of the experiment (Figure 1).

The recipient cows that did not receive an embryo on day 17 of the experiment received a CIDR. GnRH was administered on day 26 of the experiment, the CIDR was removed, 2 ml lutalyse administered, and an Estrotect patch was applied on day 33 of the experiment. Recipient cows then received a GnRH injection on day 35 of the experiment. On days 41, 42, and 43 of the experiment, embryos were transferred to recipients that had received an embryo previously but did not become pregnant expressed a subsequent estrus (observations occurring morning, noon, and evening on days 33 through 36 of the trial) and had a viable corpus luteum, as well as recipients that had not yet received an embryo and had a viable corpus luteum (Figure 1). There were no further embryo transfer attempts. Embryos transferred were either Angus or Hereford purebred embryos from varying origins. All transferred embryos were obtained by In-Vitro Fertilization (IVF) and frozen. Each embryo was thawed immediately before transfer by a licensed technician, loaded into an embryo transfer gun, and deposited into the left or right ovary uterine horn ipsilateral to the ovary with the viable CL.

A state-licensed veterinarian and embryo transfer specialist determined pregnancy status via rectal ultrasonography (Aloka 500V equipped with 5.0-MHz linear array transducer, Corometrics Medical Systems, Wallingford, CT) as either pregnant or open on days 43, 68, and

94 of the experiment. All animals that received embryos on days 17, 41, 42, or 43 of the experiment were assessed for pregnancy each subsequent month.

Statistical Analysis

All statistical analysis was conducted in R (version 4.0.5). The current analysis used the animal as the experimental unit, as each animal was individually supplemented. A chi-square test was used to compare the difference in pregnancy rate between the two treatments after cows were subjected to an embryo recipient synchronization protocol. Hormone concentration and mineral status were assessed using a mixed-effect model from the lme4 package for R (Bates et al., 2015) and emmeans from the emmeans package for R (Lenth, 2021).

Animal ID was used as the random effect variable for each hormone and mineral assessed using mixed-effect models. The fixed effects for each hormone model included a treatment by timepoint interaction and days pregnant as a covariate. Backwards elimination was then used to select additional predictor variables: the anti-mullerian hormone model included preliminary calf weaning weight and days postpartum; and the estradiol model included cow age. Similarly, each model for mineral status included a treatment by timepoint interaction and days pregnant as a covariate. Backwards elimination was used to select the following additional predictor variables: the cobalt model included cow age; the manganese model included cow age; and the zinc model included preliminary calf weaning weight, and cow age.

The effects of treatment, time, and the treatment by time interaction for the mixed-effect models were evaluated with a Type 3 Anova from the car package for R (Fox and Weisberg, 2019). The two-sample t-test was used to compare average days pregnant at the end of supplementation for the two treatment groups. Finally, linear regression, Type 3 Anova, and

emmeans were used to evaluate average embryo transfer attempts per animal, average embryo transfer attempts for a successful pregnancy, and average calf birthweight for each treatment.

As discussed above, only a portion of cows had blood samples collected on day 43 of the experiment, corresponding to cows that had embryos transferred and had not shown signs of estrus. All other collections times contained all animals in the study. Two animals were bred via AI during this experiment. Hormone and mineral concentration data from these animals were included in the statistical analysis until they became pregnant and were removed from all other evaluations.

RESULTS

Table 3 shows the effect of trace mineral source on pregnancy outcomes. Average days pregnant was similar across treatments. There were no differences ($P > .05$) regarding reproductive outcomes between the two treatment groups. Numerically, cows supplemented with organic trace minerals required more embryo transfer attempts to achieve a successful pregnancy when compared to the inorganic treatment, averaging 1.27 and 1.06, respectively. Twenty-two cows from the organic and inorganic treatment received embryos during the first transfer. However, five cows from the inorganic treatment and twelve from the inorganic treatment received an embryo during the second transfer.

Table 4 shows the treatment effects on serum hormone concentrations in multiparous beef cattle during embryo transfer. There were no treatment-by-time interactions for any measured hormone. Therefore, the main effects of treatment serum hormone concentrations are presented in Table 4. Cows supplemented with inorganic trace minerals had numerically greater progesterone concentrations than the organic treatment (9.30 and 8.90 ng/mL, respectively). Serum luteinizing hormone concentrations were numerically greater in cows supplemented with

organic trace minerals than the inorganic treatment (4996.00 and 4697.00 pg/mL, respectively). However, there were no significant differences between treatments for any hormone measured. There was a significant effect of time on the concentration of luteinizing hormone ($P < 0.0002$) and follicle-stimulating hormone ($P < 0.0001$), as well as a tendency for time to affect progesterone concentration ($P < 0.06$). There was no effect of time on the anti-mullerian hormone or estradiol serum concentrations.

Table 5 shows the effects of mineral source on plasma mineral concentrations for each treatment. There was a treatment by time effect for plasma copper concentrations ($P < .03$), with copper concentrations of the inorganic treatment decreasing (0.89 to 0.76 mg Cu/L) and those of the organic treatment increasing (1.01 to 1.19 mg Cu/L) as the experiment progressed. Cows receiving organic trace minerals had significantly greater plasma selenium and copper ($P < .0001$) concentrations when compared to cows supplemented with inorganic trace minerals. Plasma cobalt, manganese, and zinc concentrations were similar across treatments.

DISCUSSION

This experiment aimed to investigate the influence of trace mineral source on reproductive performance in recipient multiparous beef cows. The onset of estrus, the concentration of reproductive hormones, and the continuation of pregnancy were used as proxies for reproductive performance. The supplemented treatment were either 1) Organic trace minerals: 75 mg of Cu/d from Cu proteinate, 8 mg of Co/d from Co proteinate, 105 mg of Mn/d from Mn proteinate, 3 mg of Se/d from Sel-Plex, and 220 mg of Zn/d from Zn proteinate; and 2) Inorganic trace minerals (at two times the NASEM (2016) requirements): 255 mg of Cu/d from $CuSO_4·5H_20$, 2.6 mg of Co/d from $CoCO_3$, 1018 mg of Mn/d from $MnSO_4$, 3 mg Se/d from Na_2SeO_4, and 763 mg of Zn/d from $ZnSO_4·5H_2O$. These treatments were selected to represent

concentrations of mineral supplements common in the cattle industry, with the inorganic mineral representing supplementation above the current recommended dosage of trace minerals (NASEM, 2016). This inclusion level is similar to previous studies, like that of Engle and Spears (2000). The organic treatment inclusion was formulated to equal that sold in the Alltech Blueprint® mineral supplements (Alltech; 3031 Catnip Hill Road, Nicholasville, Kentucky 40356). Organic trace minerals, like Blueprint®, can be more bioavailable than inorganic trace minerals, which is further discussed below and in Spears (2003). Greater bioavailability potentially allows for lower inclusion rates of organic trace minerals while still fulfilling the trace mineral requirements of the animal.

Mineral Concentrations

The plasma mineral concentrations of all treatments within the current research were greater than previously described mineral sufficiency ranges. Adequate serum cobalt values are between 0.3 and 1.1 ng/mL (Herdt and Hoff, 2011), representing an order of magnitude greater than the corresponding cobalamin (B_{12}) concentrations. B_{12} is the biologically active derivative of cobalt, and it is the value pertinent to the productivity of ruminants consuming cobalt. However, circulating cobalt is an inconsistent measure of B_{12} status, as a large portion of liver and serum cobalt is not associated with the vitamin, making interpretation difficult (Herdt and Hoff, 2011). The animals in the current experiment had plasma cobalt values greater than 1.1 ng/mL, suggesting they were cobalt sufficient prior to embryo transfer and throughout the project. However, the values in the current experiment were obtained from plasma, whereas the previously established values were using serum cobalt concentrations. The circulatory transport of cobalt to tissues is primarily conducted by transcobalamin. However, serum proteins also transport cobalt (Herdt and Hoff, 2011), suggesting that plasma and serum cobalt may be

differentially concentrated when separated and analyzed. Additionally, plasma copper, manganese, selenium, and zinc concentrations were sufficient before and throughout the experiment with concentrations of copper greater than 0.5 mg/L (Claypool et al., 1975), manganese greater than 10 ng/mL (Gibbons et al., 1976), selenium greater than .08 mg/L (Dargatz and Ross, 1996), and zinc greater than .4 mg/L (Mills et al., 1967).

In the current research, the inclusion of organic trace minerals was lower for milligrams of copper (75 mg versus 255 mg Cu/d), manganese (105 mg versus 1018 mg Mn/d), and zinc (220 mg versus 763 mg Zn/d), greater for cobalt (8 versus 2.6 mg Co/d), and equivalent for selenium (3 mg Se/d) across treatments. The similar plasma cobalt concentrations between the two treatments were surprising as the organic trace minerals were supplemented at a greater dosage (8 mg/d) than the inorganic treatment (2.6 mg/d). This finding does not agree with previous research, where cobalt status was greatest in cattle supplemented organic trace minerals compared to an inorganic cobalt treatment. Marques et al. (2016) found concentrations were similar between the liver cobalt concentration of cows supplemented either organic cobalt (undisclosed dosage) or inorganic cobalt (undisclosed dosage) for the first ten days, but that the organic group had greater liver cobalt concentrations on day 75. However, researchers found that both organic (either 0.05 or 0.10 mg Co/kg) and inorganic mineral sources (either 0.05, 0.10 or 1.0 mg Co/kg) were used to a similar extent for microbial production of B_{12} (Tiffany et al., 2003). The current research finding may be due to the aforementioned low accuracy of plasma cobalt status in predicting the biologically relevant B_{12} status compared to liver cobalt concentration or serum B_{12}.

In the current research, greater plasma copper concentrations of cattle receiving organic trace minerals, supplemented at a lower rate than the inorganic treatment, suggests greater

bioavailability. Previous research by Spears et al. (2004) indicated that organic forms of copper were more available when diets contained high sulfur (0.15% added S on a DM basis) and molybdenum. The ration in the current experiment contained 0.12% sulfur, and all feed ingredients used contained less than 1 mg Mo/kg DM. Although the dietary concentrations of sulfur and molybdenum in the current experiment were lower than those used by Spears et al. (2004), possible interactions between copper and sulfur could have reduced copper absorption of copper from $CuSO_4$. Suttle (1974) conducted a series of experiments that fed sheep copper repleted diets (1.5 mg Cu/kg) and supplemented with varying sources of sulfur and delivery methods of copper. The general findings of these trials were that sulfur inclusion in the diet led to decreased copper utilization, likely through the formation of CuS in the digestive tract. Additionally, the observation that dietary sulfur inclusion did not impact intravenous doses of copper supports the theory that copper becomes less bioavailable by interacting with sulfur in the digestive tract.

Copper is transported from the small intestine to the liver and kidneys by albumin and transcuprein (Linder et al., 1998). Once in the liver, copper is bound to metallothionein for storage. Subsequently, copper is predominantly incorporated into ceruloplasmin, the primary blood copper transporter, representing 70-95% of serum copper (Cerone et al., 2000). Previous research has shown a linear relationship between ceruloplasmin activity and plasma copper, with ceruloplasmin increasing as copper concentration increases (Stoszek et al., 1986). Furthermore, as ceruloplasmin functions as a copper transporter from the liver to peripheral tissues, an increase in the transporter also represents an increase in circulating copper. Additionally, previous research demonstrated no effect of copper source, organic versus inorganic, on ceruloplasmin activity (Mullis et al., 2003). However, in the same study, ceruloplasmin

concentrations decreased with the copper levels as the study progressed. These data suggest that elevated plasma copper concentrations in the current research, may be due to greater ceruloplasmin activity from an increased concentration of copper being absorbed from the small intestine.

Plasma manganese concentrations were similar across treatments, even though there was approximately 10-fold less manganese in the organic trace mineral supplement. This finding suggests that the organic manganese may have been more bioavailable, agreeing with previous research by Henry et al. (1992). These researchers reported that lambs supplemented manganese methionine had greater tissue concentrations (liver, kidney, and bone) of manganese compared to manganese oxide and manganese sulfate of equivalent inclusion rates. Previous research suggests calcium and phosphorus can alter the bioavailability of manganese in cattle, especially when manganese supplementation is low and when dietary calcium or phosphorus concentrations exceed dietary requirements (Hawkins et al., 1955; Rojas et al., 1965). Although the calcium and phosphorus concentrations in the current research did not exceed dietary recommendations, the current data suggest decreased metabolic interactions of manganese.

Plasma selenium concentrations were greater in organic trace mineral supplemented cows when compared to inorganic supplemented cows. This finding is intriguing as dietary selenium concentrations were similar across both treatments, with both groups receiving 3 mg Se/d. These findings agree with Ortman and Pehrson (1999), where organic selenium (selenium yeast) was more effective at increasing plasma selenium concentrations of cows than similar doses of selenite or selenate. Similarly, Slavik et al. (2008) reported that selenium yeast supplementation of 50 mg Se/kg of premix increased whole blood selenium of cows more effectively when compared to sodium selenite supplemented at the same inclusion level. Additionally, Ehlig et al.

(1967) reported increased selenium concentrations in the liver of sheep supplemented with organic selenium, compared to inorganic selenium supplemented at the same level (0.4 mg Se/d). These findings suggest greater bioavailability of the organic treatment, which may be due to greater whole-body retention of organic selenium, as demonstrated in a rat model (Mason and Weaver, 1986). Greater retention of organic selenium sources may be due to selenomethionine (organic selenium) being incorporated nonspecifically into body proteins in place of methionine (Behne et al., 1991). The current research and previous data demonstrate that organic minerals may have greater bioavailability and can meet and support adequate mineral status with lower inclusion levels.

Hormone Concentrations

Briefly, there are multiple follicular waves within a typical estrous cycle, occurring every 7-to-10-days, including three stages: selection, dominance, and atresia (Evans et al., 1994; Ginther et al., 1996). A typical estrous cycle will consist of 2 to 3 follicular waves (Adams et al., 1994), which is dependent mainly on the size of the follicle reached and an adequate elevation of estradiol concentrations (Noseir, 2003). Each follicular wave coincides with an increase in FSH concentrations, which induces the growth of antral follicles (Fortune, 1994). As follicular development continues, FSH levels decrease, causing only a limited number of the follicles to continue growing (selection stage), one of which deviates by becoming larger than the rest (dominance stage) and is called a “dominant follicle” (Sunderland et al., 1994; Ginther et al., 1996; Ireland et al., 2000). Meanwhile, there is a steady increase in estradiol secreted by the dominant follicle. When elevated adequately, estradiol signals the hypothalamus to increase pituitary gland secretion of GnRH, increasing circulating concentrations of GnRH (Rispoli and Nett, 2005). This increase in GnRH causes a subsequent increase in pituitary secretion of LH,

called an “LH surge,” which marks the onset of estrus and ovulation (~24 hours after surge; Chenault et al., 1975). During dominant follicle growth and after ovulation, the corpus luteum develops and secretes greater concentrations of progesterone as it increases in maturity, with maximum levels being reached 14-16 days post-estrus and a 40% decrease at 42-56 days of pregnancy (Gomes and Erb, 1965). As pregnancy progresses, progesterone secretion increases and estradiol secretion decreases, as Mann et al. (1995) described. Anti-mullerian hormone (AMH) is a reliable reproduction marker used to determine cow fertility (Umer et al., 2019). AMH has previously been used as a marker for the number of growing follicles in the ovarian pool of cattle (Rico et al., 2009). In mice, AMH was observed to have a role in recruiting primordial follicles with increasing numbers of follicles recruited as AMH concentration decreased (Durlinger et al., 1999). A low concentration of AMH is both a sign of diminished ovarian reserve (viable oocytes) and induces faster depletion of primordial follicles (Durlinger et al., 1999). A single blood sample can be used to reliably predict ovarian reserve and fertility, irrespective of the stage of estrus (Ireland et al., 2011).

The effect of time on hormone concentrations, other than for AMH, was expected. These hormones change as estrus and pregnancy progress, as discussed above. In the current research, there was a downward trend of plasma estradiol as early pregnancy progressed (data not shown), which agrees with that of Wettemann and Hafs (1973) and Mann et al. (1995). Mann et al. (1995) also described a decrease in plasma estradiol between 8 and 10 days after artificial insemination in cows given GnRH and not given GnRH. Additionally, it is noteworthy that the pregnant cows had a more drastic decrease in estradiol from day 10 to 13 and a subsequent increase in the plasma estradiol of the open animals after day 15. It has also been observed that heifers and cows with greater estradiol excretion in urine during early pregnancy had decreased

pregnancy success (Stott et al., 1971), having 140%, 180%, and 130% greater concentrations of estradiol in the urine of nonpregnant cows on day 0, 10 and 19 after breeding. These data suggest that a decrease in estradiol contributes to a successful early pregnancy. Although the organic treatment group has estradiol concentrations that indicate a more fertile hormonal environment, there was no significant difference in days pregnant at the end of the trial nor the embryo transfer attempts for a successful pregnancy.

Progesterone in the current research was observed to decrease slightly from the onset of the experiment to the final observation. This decrease may be related to supplemental progesterone introduced by the implanted CIDR devices prior to and 15 days following embryo transfer procedures but removed thereafter. Progesterone concentrations were similar between the organic and inorganic treatments in the present study ($P > 0.10$). This finding does not agree with previous research which demonstrated that mineral source could significantly impact progesterone concentrations (Cerny et al., 2016). For example, selenium sufficient cows supplemented additional organic selenium had greater plasma progesterone and whole blood selenium concentrations than those supplemented additional inorganic selenium. Kamada et al. (2014) reported that cows had greater plasma progesterone concentrations when supplemented with selenium. These data suggest that animals with greater selenium status have greater plasma progesterone. However, this does not agree with the current research as the inorganic and organic treatment had similar serum progesterone concentrations despite the organic treatment having greater plasma selenium concentrations.

Previous research suggests that progesterone concentrations greater than 2.8 ng/mL are associated with a 50% greater chance of a successful pregnancy than when less (Starbuck et al., 2004). Additionally, it was observed by Kenyon et al. (2013) that cows that maintained their

pregnancy had plasma progesterone levels greater than 5 ng/mL at day 14. The plasma progesterone concentrations of both treatments in the current research are greater than these values during pregnancy, suggesting an adequate hormonal environment for maintaining a pregnancy. However, recent research has demonstrated that cows with greater plasma progesterone concentrations prior to estrus have a lower pregnancy success rate than cows with lesser concentrations (Stevenson and Pulley, 2016). These data suggest that greater progesterone concentrations are beneficial for maintaining pregnancy but not prior to estrus. However, the current research showed no significant difference in the progesterone concentrations between the two treatment groups before estrus. Again, it is important to note that exogenous progesterone was supplemented to all cows for the first 15 days of pregnancy using CIDR devices as part of the embryo transfer protocol.

The inorganic treatment had numerically greater plasma concentrations of anti-mullerian hormone than the organic treatment. As discussed previously, this hormone has been utilized as a proxy for fertility in cattle (Rico et al., 2012), where cows with levels greater than 87 pg/mL have greater follicle production and donor potential. Additionally, Jimenez-Krassel et al. (2015) observed that cows with less than 30 pg/mL had reduced pregnancy rates and greater culling rates. However, there was no difference in anti-mullerian hormone concentrations, nor days pregnant and embryo transfer attempts, of the two treatments in the current research. Additionally, there is limited research on the impact of trace mineral source or mineral status on the concentration of plasma anti-mullerian hormone.

Ultimately, the current research project observed no significant treatment differences in plasma progesterone, luteinizing hormone, follicle stimulating hormone, estradiol, or anti-mullerian hormone concentrations. The current research analyzed hormone samples from

monthly collections, limiting the ability to capture more detailed hormone fluctuations during an estrous cycle, as estrus occurs in 7 to 10-day intervals (Ginther et al., 1996).

Reproductive Performance

In the current research, reproductive outcomes were similar between treatments, including days pregnant at the end of the trial, embryo transfer attempts per animal regardless of the outcome, embryo calf birth weight, and average embryo transfer attempts for a successful pregnancy. Similarly, Siciliano-Jones et al. (2008) reported that dairy cattle supplemented with organic trace minerals had similar services per conception of cows supplemented with inorganic minerals. Additionally, Muehlenbein et al. (2001) reported that cows supplemented organic and inorganic copper sources at 100 mg and 200 mg Cu/day, respectively, had similar 30-day and 60-day pregnancy rates. This finding also agrees with the current research, where treatments had a similar final percent pregnant (66.7 versus 62.5%, inorganic and organic, respectively). However, the inorganic treatment group required numerically fewer embryo transfer attempts for a successful pregnancy. This difference is difficult to elucidate as there is limited research on the effect of mineral source and status on embryo recipient success. There was no significant effect of trace mineral source on calf birth weight ($P > 0.10$). Price et al. (2017) also found no difference in the birth weight of calves when comparing inorganic and organic minerals supplemented during the last trimester of pregnancy. However, these researchers did observe that calves from the organic treatment had greater average daily gain and adjusted 205-day weaning weights. Additionally, Gunter et al. (2003) found no effect of selenium source on the birth weight of calves when supplemented with similar levels of organic or inorganic trace minerals.

CONCLUSION

Although there were differences in the copper and selenium mineral concentrations in the plasma of the organic and inorganic supplemented cows, these differences resulted in no significant changes in reproductive outcomes or serum hormone concentrations between the two groups. It is important to reiterate that there were different concentrations of the trace minerals in the two treatments. The organic treatment received less copper, manganese, and zinc but similar inclusion of selenium and greater inclusion of cobalt. Given the similar reproductive performance of the two treatments, these data suggest that lower inclusion of organic mineral sources for copper, manganese, and zinc can support similar reproductive performance compared to greater inclusion of an inorganic mineral source. However, future research investigating the impact of trace mineral source on hormone concentrations prior to and throughout pregnancy is warranted.

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TABLES AND FIGURES

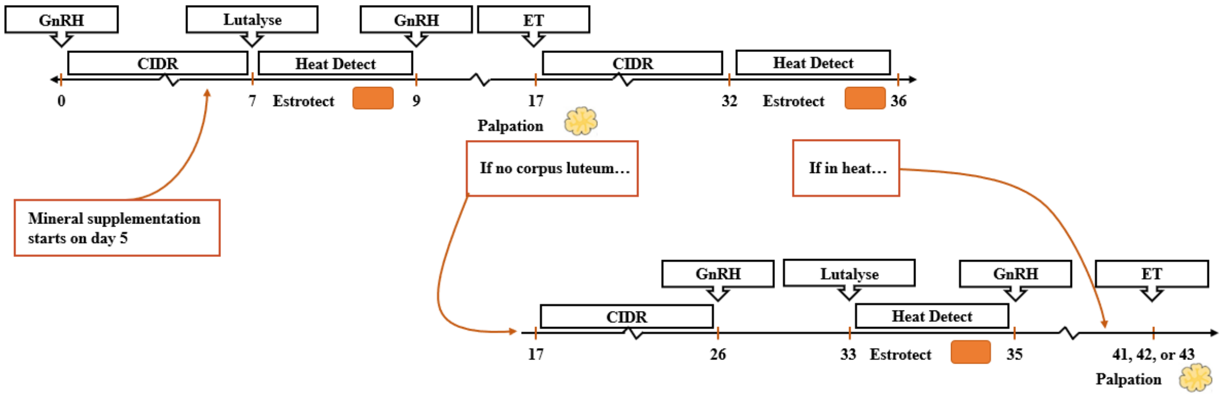

Figure 1. Estrous synchronization and embryo transfer timeline.

Table 1. Dry matter ingredient composition of basal diet.

Ingredient	%
Corn Silage	33.17%
Corn Stalks	47.23%
Whole Corn	15.77%
Limestone	0.59%
Salt	0.16%
Hay Treat[1]	3.09%
Analyzed nutrient composition	
Dry Matter % as fed	71.22%
Crude Protein, %	7.90%
Acid Detergent Fiber, %	28.64%
Neutral Detergent Fiber, %	45.04%
Total Digestible Nutrients, %	62.62%
Net Energy for Gain, Mcal/kg	0.82
Net Energy for Maintenance, Mcal/kg	1.41
Calcium, %	0.44%
Magnesium, %	0.24%
Phosphorus, %	0.18%
Potassium, %	1.41%
Sulfur, %	0.12%
Cobalt, mg/kg	0.79
Copper, mg/kg	5.45
Manganese, mg/kg	41.65
Selenium, mg/kg	0.04
Zinc, mg/kg	24.54

[1]Liquid supplement provided in a molasses suspension; DM, 55.2%; TDN, 73%; NEM, 0.327 Mcal/Kg; NEG, 0.227 Mcal/Kg; Crude fat, 0.1%; Crude protein, 53.8%; NPN, 47.8; .28% Ca; 1.10% P; 0.19% Ma; 4.04% K; 2.11% Na; .59% S; 688 mg Fe; 65 mg Zn; 19 mg Cu, 68 mg Mn; 12.2 mg Co; 0.59 mg Se; Vit A, 42161.81 IU/kg; vitamin D, 10998.63 IU/kg; vitamin E, 41.36 IU/kg; Rumensin, 162 Mg/kg.

Table 2. Ingredient composition of supplemental pellets.		
Analyzed Nutrient Composition	Inorganic Pellets	Organic Pellets
Dry Matter % as fed	88.97%	89.03%
Crude Protein, %	18.93%	20.07%
Acid Detergent Fiber, %	13.67%	14.67%
Neutral Detergent Fiber, %	30.73%	32.07%
Total Digestible Nutrients, %	77.33%	76.67%
Calcium, %	0.48%	0.45%
Magnesium, %	0.42%	0.42%
Phosphorus, %	1.00%	0.95%
Potassium, %	1.47%	1.46%
Sulfur, %	0.41%	0.31%
Cobalt, mg/kg	4.63	12.07
Copper, mg/kg	388.33	106.33
Manganese, mg/kg	1430.00	234.00
Selenium, mg/kg	4.90	4.93
Zinc, mg/kg	1102.00	491.33
Inorganic Treatment: 255 mg of Cu/d from $CuSO_4 \cdot 5H_20$, 2.6 mg of Co/d from $CoCO_3$, 1018 mg of Mn/d from $MnSO_4$, 3 mg Se/d from Na_2SeO_4, and 763 mg of Zn from $ZnSO_4 \cdot 5H_2O$.		
Organic Treatment, 75 mg of Cu/d from Cu proteinate, 8 mg of Co/d from Co proteinate, 105 mg of Mn/d from Mn proteinate, 3 mg of Se/d from Sel-Plex, and 220 mg of Zn/d from Zn proteinate.		

Table 3. Influence of trace mineral source on pregnancy outcomes after embryo transfer in multiparous beef cattle.				
	Treatment			P <
Item	Inorganic[a]	Organic[b]	SEM	Trt
n=	25	25	---	---
Percent pregnant	66.66	62.5	---	---
Days pregnant at end of trial	53.92	45.21	11.08	0.44
Calf birth weight, kg	31.16	25.85	6.94	0.45
Embryo transfer attempts	1.08	1.38	0.32	0.36
ET attempts to achieve pregnancy	1.06	1.27	0.39	0.60

Inorganic Treatment: 255 mg of Cu/d from $CuSO_4 \cdot 5H_20$, 2.6 mg of Co/d from $CoCO_3$, 1018 mg of Mn/d from $MnSO_4$, 3 mg Se/d from Na_2SeO_4, and 763 mg of Zn from $ZnSO_4 \cdot 5H_2O$.

Organic Treatment, 75 mg of Cu/d from Cu proteinate, 8 mg of Co/d from Co proteinate, 105 mg of Mn/d from Mn proteinate, 3 mg of Se/d from Sel-Plex, and 220 mg of Zn/d from Zn proteinate.

Table 4. Influence of trace mineral source on plasma hormone concentrations in multiparous beef cattle during embryo transfer.

	Treatment			P <		
Item	Inorganic[a]	Organic[b]	SEM	Trt	Time	Trt x Time
n=	25	25	---	---	---	---
Progesterone, ng/mL	9.30	8.90	0.485	0.42	0.057	0.93
Luteinizing Hormone, pg/mL	4697.00	4996.00	670.00	0.66	0.0001	0.87
Anti-Mullerian Hormone pg/mL	479.00	451.00	51.3	0.59	0.33	0.30
Follicle Stimulating Hormone, pg/mL	40.80	46.50	5.46	0.30	< 0.0001	0.90
Estradiol, pg/mL	2.58	2.44	0.151	0.33	0.19	0.88

Inorganic Treatment: 255 mg of Cu/d from $CuSO_4 \cdot 5H_20$, 2.6 mg of Co/d from $CoCO_3$, 1018 mg of Mn/d from $MnSO_4$, 3 mg Se/d from Na_2SeO_4, and 763 mg of Zn from $ZnSO_4 \cdot 5H_2O$.

Organic Treatment, 75 mg of Cu/d from Cu proteinate, 8 mg of Co/d from Co proteinate, 105 mg of Mn/d from Mn proteinate, 3 mg of Se/d from Sel-Plex, and 220 mg of Zn/d from Zn proteinate.

Table 5. Influence of trace mineral source on plasma mineral concentrations in multiparous beef cattle during embryo transfer.						
	Treatment			P <		
Item	Inorganic[a]	Organic[b]	SEM	Trt	Time	Trt x Time
n=	25	25	---	---	---	---
Copper, mg/L	0.87	1.19	0.05	< 0.0001	0.20	0.03
TP0	0.89	1.01	0.08	0.1025	---	---
TP1	0.88	1.21	0.08	0.0001	---	---
TP2	0.95	1.27	0.08	0.0002	---	---
TP3	0.86	1.29	0.08	<.0001	---	---
TP4	0.76	1.19	0.10	<.0001	---	---
Cobalt, mg/L	0.17	0.16	0.01	0.27	0.24	0.98
Manganese, ng/mL	19	19.6	0.714	0.40	0.30	0.13
Selenium, mg/L	19	24.1	0.944	<.0001	0.12	0.44
Zinc, mg/L	1.16	1.21	0.06	0.36	0.75	0.57
Inorganic Treatment: 255 mg of Cu/d from $CuSO_4 \cdot 5H_20$, 2.6 mg of Co/d from $CoCO_3$, 1018 mg of Mn/d from $MnSO_4$, 3 mg Se/d from Na_2SeO_4, and 763 mg of Zn from $ZnSO_4 \cdot 5H_2O$.						
Organic Treatment, 75 mg of Cu/d from Cu proteinate, 8 mg of Co/d from Co proteinate, 105 mg of Mn/d from Mn proteinate, 3 mg of Se/d from Sel-Plex, and 220 mg of Zn/d from Zn proteinate.						