MATERNAL INJECTABLE MINERAL DURING EARLY GESTATION IMPACTS PLACENTAL FUNCTION AND CALF PERFORMANCE

by
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A thesis submitted in partial fulfillment

of the requirements for the degree

of

Master of Science

in

Animal and Range Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 2022

ACKNOWLEDGEMENTS

I would like to thank my committee members for all of their support and advice. Thank you to my advisor Dr. McCoski for all of your advice and support during my time in your lab, especially for answering my all questions. Thank you for always being there when I needed something or had a hundred questions or thoughts, and for believing in me when I doubted myself. A big thank you to Dr. Marques for all your help with calf biopsies and all my craziness of questions. Your help, advice, and words of encouragement during calving season and this past year have been amazing and I've been able to grow and gain confidence in my abilities to complete tasks and not doubt myself. Thank you to Dr. Boss, for your support and the opportunity to work with you and everyone at NARC. I would like to express my thanks to Dr. Sanford for your guidance and support while I completed the study. Additionally, thank you to Dr. Tom Geary, your words of encouragement, advice, support, and your mentoring has helped me to grow and become more confident in myself over the past several years.

I would also like to thank you to Julia Dafoe and the livestock crew at NARC for all of their support and advice over the course of the study, especially with color doppler scans and during calving season with the biopsy days. Additionally, I would like to thank Sidney Stratton for all of your help and assistance during calving and the craziness that came with it. Lastly, but most importantly, I would like to thank my family for their continued support as I worked through my master's program. Growing up you all showed me the importance of hard work and dedication in order to achieve what you wanted in life, and I wouldn't have made it as far as I have without those skills and the continued support.

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ABSTRACT

Limited research has evaluated the effects of maternal mineral supplementation during early gestation on placental function and progeny growth. For this study, Angus and SimAngus cows (n = 52) were bred via artificial insemination. At day (d) 60 of gestation, cows were assigned to one of two treatment groups; an injectable group (INJ, n = 26) receiving a single subcutaneous mineral injection and a control group (CON; n = 26). A subset of cows (CON n = 10; INJ n = 6) were selected for liver biopsy at d60 and 209±1 of gestation. At d139.5±0.5 and 209±1 of gestation, uterine artery measurements were collected using Color Doppler ultrasonography. Placentas, liver tissue, and blood samples were collected from the first 11 cows from the subset group that calved (n = 11; CON, n = 5; INJ, n = 6). Analysis revealed a TRT*d interaction at d139.5±0.5 for circulating Co concentrations which were greater in INJ ($P = 0.05$) compared to CON cows. Circulating Zn concentrations tended to be decreased at d209±1 ($P = 0.06$) in INJ cows compared to the CON cows. Circulating Cu tended to be increased (P-value = 0.09) and Mn was decreased ($P = 0.04$) in INJ cows throughout the study. A TRT*d interaction was observed at d139.5±0.5 for non-gravid uterine artery area, diameter, and circumference to be increased in the INJ cows ($P = 0.004$, 0.006, and 0.006, respectively). Additionally, pulsatility index of the gravid uterine artery tended to be increased ($P = 0.09$) in the INJ cows. Hepatic Fe concentrations were decreased in the INJ cows ($P = 0.01$) at d209±1. Blood and liver samples were collected from calves (CON n = 24; INJ n = 26). INJ calves had greater liver Se concentrations ($P = 0.001$), lower Fe concentrations (P=0.04), and tended to have increased liver concentrations of Zn ($P = 0.09$) and Mn ($P = 0.08$) compared to CON calves. Finally, INJ calves tended to have elevated levels of serum Se ($P = 0.09$) compared to CON calves. These results suggest that injectable mineral administration during early gestation altered placental function and calf performance.

CHAPTER ONE

INTRODUCTION

Successful beef production systems must have the ability to raise and economically produce both cattle and beef products that meet the needs of the consumer and market demands (Greenwood et al., 2017). Much of the land in the western region of the United States (U.S.) is used for cow-calf production, the environment and landscape are more suitable for livestock production due to the lack of rainfall and harsh terrain which make crop production more difficult. Nearly 66.2% of Montana farm and ranch land is pasture and rangeland, according to the USDA National Agricultural Statistics Services (USDA NASS, 2021b). During the drought of 2021, nearly 56% of the grazing land in Montana fell into the "very poor" to "poor" pasture and range conditions (USDA Livestock, Dairy, & Poultry Outlook, 2021a). The "very poor" to "poor" pasture and range conditions are associated with the lack of rainfall during 2021 which impacted the forage growing season. Even with drought conditions, pastures and rangelands in Montana are home to 1.4 million beef cows, 380,000 replacement heifers, and 100,000 bulls, 200,000 sheep and lambs, and various wildlife species (USDA NASS, 2021b). The cow-calf pairs that graze rangelands in the western U.S. receive little supplemental dietary inputs, such as grain and hay (Davis, 2021). Still, cows may be culled due to loss of pregnancy, body composition, disposition, illness, injury, age, or lameness. Proper mineral supplementation programs and development of replacement heifers is critical to the performance and longevity of the females in the cow herd. Thus maternal nutrition is key for proper fetal, muscle development, and long-term efficiency of the offspring (Du et al., 2010).

CHAPTER TWO

LITERATURE REVEIW

The Importance of Trace Minerals in Cattle Diets

Though relatively low quantities of trace minerals are needed to meet the requirements of animal diets, they are essential to physiological function. Several trace minerals, including copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) have varying functions in the animal, from production of hormones to enzyme production (National Academies of Sciences, 2016).

Copper

Copper requirements are higher in ruminant species compared to non-ruminants which is due to the ruminal environment and antagonistic interactions (Spears et al., 2008). Multiple factors are known to affect Cu absorption in the animal, some of these factors are cattle breed, antagonists, and type of forage (National Academies of Sciences, 2016). Copper is known to have various other antagonistic interactions with other minerals such as sulfur (S) and molybdenum (Mo); many Cu deficiencies are associated with high concentrations of Mo (Ahuja and Parmar, 2017). These antagonistic interactions can impact Cu availability of the animal causing Cu deficiencies. Gooneratne et al. (1994) reported that Angus and Simmental cattle have different Cu requirements, and Simmental cattle excreted greater quantities of Cu in bile compared to Angus. Therefore, Simmental require greater quantities of Cu than Angus cattle as they are more susceptible to a deficiency (National Academies of Sciences, 2016). A Cu

deficiency can have severe consequences to the animal, including early embryonic mortality, necrosis of muscle and nerves, fetal reabsorption, delay or suppression of estrus expression, retained placenta, impaired ovarian function, infertility in dairy cows, decreased growth of the animal, changes in physical appearance of hair and growth, and anemia (National Academies of Sciences, 2016; Ahuja and Parmar, 2017). Copper toxicity can occur when feeds contain high concentrations of Cu or excessive supplementation of Cu occurs (National Academies of Sciences, 2016). The liver can accumulate high concentrations of Cu prior to signs of toxicity, and the clinical signs of toxicity occur when Cu is released from the liver. Once high concentrations of Cu are released, jaundice, hemolysis, widespread necrosis, and death can occur (National Academies of Sciences, 1980, 2005). Copper is essential for many biological functions, including growth and reproduction in cattle; however, due to the complex antagonistic interactions with other minerals, Cu availability to the animal can vary depending on other mineral concentrations in the animal.

Manganese

Similar to Cu, Mn is a component in multiple enzymes and acts as an activator for other enzymes (Hurley and Keen, 1987). Manganese is needed for both maximal growth of the animal and skeletal growth, however, Mn requirements are less for maximal growth than skeletal growth (National Academies of Sciences, 2016). Manganese also influences the reproductive system, although the exact pathway of Mn involvement in the reproductive system is unknown (Kumar et al., 2011). A Mn deficiency in older females may cause irregular estrus, abortions, stillbirths, and low birth weights (National Academies of Sciences, 2016). Additionally, in younger animals a Mn deficiency can result in limb stiffness, twisted legs, enlarged joints, decreased bone strength, and other skeletal abnormalities (Hurley and Keen, 1987).

Selenium

Organically bound Se is a component of two different amino acids (AA); cysteine which forms selenocysteine and methionine which forms selenomethionine (National Academies of Sciences, 2016). Selenocysteine aids in the formation of selenoproteins, while selenomethionine aids in the increase in the selenium located in the muscle of the animal (National Academies of Sciences, 2016). Once the selenomethionine is catabolized the leftover Se will be combined with the selenocysteine to form selenoproteins (National Academies of Sciences, 2016). Selenoproteins aid in physiological functions of Se in the animal (Juszczuk-Kubiak et al., 2016). Selenoproteins impact the hormone metabolism, protein metabolism, Se transport, redox signaling, and peroxidase and reductase activity (Juszczuk-Kubiak et al., 2016). Factors that define Se requirements in cattle have not been well defined; however, there is a known relationship with vitamin E (National Academies of Sciences, 2016). When a diet is low in vitamin E, the Se requirement increases to prevent muscular abnormalities, such as muscular dystrophy, also known as white muscle disease (National Academies of Sciences, 2016). Absorption of Se in ruminants is lower than in monogastric animals, as the majority of Se absorption occurs in the duodenum (Wright and Bell, 1966). In young animals, white muscle disease is a known indication of Se deficiency, though livestock may exhibit other signs as well. Deficiency can be expressed as reduced thriftiness (Underwood and Suttle, 1999), death, and decreased weaning weights (Spears et al., 1986). Toxicity occurs during times of excessive supplementation and consumption of plants containing high Se concentrations (National Academies of Sciences, 2016). There are different categories of Se toxicity, chronic and acute. Chronic toxicity occurs with consumption of forages containing 5 to 40 mg/kg of Se (National Academies of Sciences, 2016). Signs of chronic toxicity include lameness, sore feet, deformed,

cracked, and/or elongated hooves, and anorexia. Acute toxicity occurs when the animal exhibits signs of labored breathing, ataxia, diarrhea, and death caused by respiratory failure (National Academies of Sciences, 1980, 2005). Various factors can affect Se absorption, requirement by the animal, deficiencies, and toxicities, which can ultimately affect the binding of Se to amino acids and formation of selenoproteins. Se and trace mineral supplementation is ideal for proper function of the animal, growth, and reproductive performance.

Zinc

Like many trace minerals, Zn plays a major role in the metalloenzymes which effect metabolism of proteins, carbohydrates, lipids, and nucleic acids (Hambidge et al., 1986). Additionally, many Zn enzymes are associated with carbohydrate metabolism, protein metabolism, nucleic acid, and reproduction of the animal (Hambidge et al., 1986; Graham, 1991). During Zn absorption, which occurs in both the small intestine and abomasum (Miller and Cragle, 1965), absorption is determined by the stage of production such as lactation or growth (Miller, 1975). Depending on the stage of production that the animal is in, the requirement for Zn may be greater dependent upon the nutrient requirements, for example during lactation, pregnancy, or growth. A Zn deficiency in cattle results in decreased feed intake, decreased feed efficiency, and growth of the animal (National Academies of Sciences, 2016). Additionally, Zn impacts reproductive success (Van Emon et al., 2020). A deficiency of Zn concentrations in an animal can cause pregnancy failure, decreased testicular growth, impact normal luteal phase activity, and the postpartum period (National Academies of Sciences, 2016; Van Emon et al., 2020).

Forms of Mineral Supplementation

Mineral supplementation is often critical for optimal growth, reproductive performance, lactation, and immunity in livestock (Arthington and Ranches, 2021). There are two main ways minerals can be supplemented to beef cattle; indirect and direct mineral supplementation (McDowell, 1996). Direct supplementation consists of oral drenching, ruminal boluses, intramuscular injection of minerals, minerals added to a water source, free-choice, or energy/protein feed source (Greene, 2000). Indirect supplementation is changing of soil pH, fertilizers containing minerals, and planting of forage species known to contain minerals of interest (Greene, 2000). A majority of mineral supplementation occurs through free-choice minerals (Greene, 2000), as other forms of supplementation are not economical if cattle are grazing large areas.

Free-Choice Minerals

Many commercially available trace mineral mixes include salt in the formation of the mineral mix to limit the consumption of trace minerals (Arthington and Ranches, 2021). It is worth noting that with this route of mineral supplementation there is a variation in individual animal intake. The free-choice, salt-based mineral supplements are commercially available in both block and loose form. Another form of free-choice mineral is through the addition of energy and protein supplements. This type of supplementation is beneficial in times of drought, when forage quality or quantity is insufficient (Arthington and Ranches, 2021). Mineral supplementation when provided with energy or protein sources, reduced intake variation (Bowman and Sowell, 1997). Other methods such as incorporating injectable mineral, or a high-

Mn form of mineral may be more appealing to producers depending on the management scheme, regional and seasonal differences, and overall goal of supplementation program.

Injectable Minerals

A benefit of an injectable mineral is that a known concentration of the trace mineral is administered to each animal (Arthington et al., 2014). Several studies (Mundell et al., 2012; Pogge et al., 2012; Arthington et al., 2014; Preedy et al., 2018; Stokes et al., 2018; Stokes et al., 2019a; Stokes et al., 2019b) have evaluated the effects of injectable mineral on mineral status, reproductive performance, and calf performance, however, conflicting results were reported. Arthington et al. (2014) observed no differences in average daily gain of calves that were administered injectable mineral within 24 hours of birth or saline throughout the duration of the study. Additionally, authors found increased hepatic concentrations of Se at d150 of age, and of Cu throughout the study (Arthington et al., 2014). Stokes et al. (2019b) reported an increase in average daily gain of the control calves when compared to calves whose dams had received injectable mineral during pregnancy from day 28-42 of the study. Stokes et al. (2019a) observed differences in calf hepatic Cu and Se concentrations days after birth when dams received repeated injections of trace mineral during pregnancy. Authors also did not observe any differences in weaning weights of the calves whose dams received repeated injections of trace minerals or the control heifers (Stokes et al., 2019a).

Conflicting results have been reported on the impacts of injectable trace mineral on reproductive parameters in both cows and heifers (Mundell et al., 2012; Arthington et al., 2014; Stokes et al., 2018). Arthington et. al (2014) did not detect any differences in age at puberty or percent pregnant in heifers that received the injectable trace mineral during the development

phase of these heifers. Whereas Stokes et al. (2018) reported that fewer heifers that received repeated trace mineral injections were exhibiting estrous cycles than the control heifers, there were no differences in antral follicle counts between treatments. Conversely, Mundell et al. (2012) reported an increase in pregnancy rates of cows that received the injectable trace mineral 105 d prior to calving and again 30 d prior to breeding. Free-choice mineral supplementation should not be replaced by administration of injectable mineral; however, it could be used in addition to free-choice mineral supplementation during stressful events such as breeding, calving, and weaning (Arthington and Ranches, 2021).

The Estrous Cycle

The start of the estrous cycle is initiated by the onset of puberty, generally occurring between 6-12 months of age in heifers (Forde et al., 2011a). The estrous cycle in cattle varies in length, ranging from 17-24 days (O'Connor and Senger, 1997), consisting of 2 phases, luteal and follicular phase (Forde et al., 2011a). The luteal phase is approximately 14-18 days long, and begins following ovulation with the formation of the corpus luteum [CL; (Forde et al., 2011a)]. The follicular phase is about 4-6 days, beginning at the time of luteolysis, also known as the regression of the CL, and ending at the time of ovulation (Forde et al., 2011a).

The luteal phase consists of 2 stages, metestrus and diestrus. Metestrus typically lasts for approximately 3-4 days, beginning with the formation of the CL where the follicle ovulated from the ovary (Forde et al., 2011a). As the dominant follicle becomes more sensitive to LH will undergo atresia (death) causing a decrease in E2 and inhibin, initiating resumption of FSH

secretion and new follicular waves occur (Forde et al., 2011a). The new follicular wave follows the same pattern as described above, during the new follicular wave when the dominant follicle produces greater concentrations of estradiol, the dominant follicle will cause a GnRH surge from the hypothalamus. The increase in E2 also stimulates the GnRH surge resulting in an LH surge, which allows for final maturation of the dominant follicle and ovulation signaling the end of the follicular phase (Sunderland et al., 1994). The ovulated oocyte has the potential to be fertilized if the female was bred during estrus, however, many factors within the maternal uterine environment can impact the success of pregnancy.

Immediately following ovulation, progesterone (P4) concentrations increase with the formation of the CL (theca and granulosa cells from the ovulated follicle become luteinized and begin producing P4). Progesterone prepares the uterine environment for establishing and maintaining pregnancy or the resumption of the estrous cycle (Forde et al., 2011a). The luteal phase ends with regression of the CL and decreased P4 concentrations, to signal return of the follicular phase (Hansel and Convey, 1983). The follicular phase is approximately 4-6 days in length, as estradiol (E2) concentrations increase (Forde et al., 2011a). The follicular phase consists of the two stages: pro-estrus and estrus. Pro-estrus occurs as the CL regresses, P4 concentrations decrease, and estradiol (E2) increases due to the rapid growth of the dominant follicle (Forde et al., 2011a). As the P4 levels decrease, the pituitary gland will release more frequent pulses of gonadotropin-releasing hormone (GnRH). These pulses of GnRH stimulate the release of FSH and luteinizing hormone [LH; (Sunderland et al., 1994)]. Luteinizing hormone and FSH play an important role in follicle growth, and the dominant follicle increases in size and becomes the healthiest follicle from the cohort of follicles (Gougeon and Lefèvre, 1983). The

dominant follicle secretes increased quantities of E2 resulting in estrus expression, the visual display of estrus by the female standing to be mounted (Ireland, 1987).

Embryo and Conceptus Development

Embryo Development

Prior to the bovine embryo entering the uterus, the oviduct is the primary location for capacitation of sperm, transport, fertilization, and eventually the early stages of embryonic development (Leese et al., 2007; Rodriguez-Martinez, 2007). The 16-cell embryo enters the uterus around d4 of gestation (Lonergan and Forde, 2014). By d7 of pregnancy, the embryo has formed a blastocyst (Lonergan and Forde, 2014). The blastocyst consists of an inner cell mass and the trophectoderm. The inner cell mas (ICM) will eventually form the fetus, while the trophectoderm forms the placenta (Lonergan and Forde, 2014). Around d8 of pregnancy, the embryo has formed an expanded blastocyst, which is when the diameter has increased and zona pellucida has thinned (Lindner and Wright, 1983). Within several days following the expanded blastocyst forming, the embryo hatches from the zona pellucida and develops a filamentous form, known as a conceptus (Spencer et al., 2016).

Conceptus Development

Elongation of the embryo begins around d9-10 of pregnancy, as the embryo undergoes physical changes after hatching from the zona pellucida (Forde et al., 2014), prior to implantation initiating around d19-20 (Bazer et al., 2009). Following the hatching of the blastocyst, the conceptus forms a spherical shape and begins to elongate (Green et al., 2021).

Various germ layers will differentiate during the gastrulation, to form the amnion, allantoic membranes, chorion, and the yolk sac (Perry, 1981). As the conceptus elongates, the mesoderm migrates from the intracellular membrane (ICM) in-between the endoderm and the trophectoderm to begin forming the trophoblast layer (Green et al., 2021). As the conceptus continues to elongate, the trophectoderm that surrounds the ICM will be lost (Betteridge and Fléchon, 1988). Day 16-18 of gestation, the embryonic disc will begin to surround about the conceptus, with the formation of the trophoblast layer (Green et al., 2021). The embryonic disc is a rounded sphere which is located on the surface of an elongated conceptus around d16 of pregnancy (Green et al., 2021). As the trophoblast closes around the embryonic disc this leads to the formation of the amnion and the amniotic sac which will accumulate fluid (Green et al., 2021). Around d18-23 of gestation, the yolk sac begins developing (Wrobel and Süss, 1998). The yolk sac is a vascular component that aids in transfer of nutrients from the chorion to the embryo and conceptus prior to the attachment of the placenta (Green et al., 2021). The trophoblast cells of the elongating conceptus will also secrete interferon tau (IFNT) which is caused by cellular proliferation (Bazer and Thatcher, 2017). Interferon tau is the maternal recognition of pregnancy (MRP) signal for the dam and inhibits luteolysis via the lack of oxytocin receptors which prolongs the P4 exposure to the conceptus (Martins et al., 2018; Sánchez et al., 2019). By d23 of gestation, the bovine conceptus is loosely attached to the epithelium of the uterine wall (King et al., 1980). As the chorion continues to develop and differentiate, a more secure attachment of the placenta will occur as the microvilli becomes more intertwined (interdigitation) within the caruncle epithelium of the uterine lining (King et al., 1982). Day 26-60 of gestation, the chorion and allantois mesenchyme will combine to form the chorioallantois placenta (Green et al., 2021).

As fluid accumulates within the allantois, the allantois will continue to expand with fluid as pregnancy progresses causing the chorioallantois membranes to fuse (Green et al., 2021). Disruption of the mechanisms involved in conceptus elongation can be catastrophic as the process is crucial to pregnancy success.

Pregnancy Establishment & Maintenance

Both the blastocyst and the conceptus secrete IFNT as the maternal recognition of pregnancy (MRP) signal from the embryo and/or conceptus (Martins et al., 2018). Interferon tau production alters the transcriptome in the endometrium, while the conceptus produces proteins and greater IFNT production (Forde et al., 2015; Mathew et al., 2018; Sánchez et al., 2019). It will inhibit oxytocin receptor formation in the endometrium (Hansen et al., 2017), thus there is a lack of prostaglandin F2alpha production, and the CL will be maintained and continues to produce P4 (Estepa et al., 2020). The continued exposure of P4, to the conceptus, aids in implantation and uterine receptivity for establishing pregnancy (Martins et al., 2018).

Uterine Environment/Histotroph

Histotroph is the fluid that is secreted into the uterine lumen (Martins et al., 2018), and it consists of hormones, enzymes, transport proteins, ions, growth factors, glucose, lipids, and amino acids (Gray et al., 2001; Gray et al., 2002; Spencer et al., 2006). Histotroph and uterine secretions provide nutritive components for development and survival of the conceptus (Diskin and Sreenan, 1980; Diskin et al., 2011; Wiltbank et al., 2016). The protein content within the bovine uterus is not well defined (Spencer et al., 2008). However, it is known that the histotroph

can impact the growth and development during the pre-implantation phase of embryonic development (Forde et al., 2014).

Forde et al. (2014) investigated which genes and proteins were present in the histotroph during the time of pregnancy recognition. Authors reported that the histotroph at d16 of pregnancy, had nearly 177 over-represented biological processes that are associated with proteins. The top 5 pathways of which the proteins were associated with included lysosomes, antigen processing and presentation, leukocyte trans-endothelial migration, regulation of actin and cytoskeleton, and glycolysis/gluconeogenesis (Forde et al., 2014). Authors suggested that since the majority of proteins reported at d16 of pregnancy were associated with glycolysis/gluconeogenesis, that these proteins likely aid in cellular metabolism to promote elongation of the conceptus (Forde et al., 2014). Results indicate that the conceptus may require greater energy for cellular metabolism when elongating just prior to the implantation period. As the conceptus begins to elongate, the endometrium will simultaneously undergo changes which prime the uterus for implantation (Forde et al., 2014). Uterine secretions support conceptus growth, the secretions are produced by the epithelial cells which line the endometrium. The epithelial cells are highly secretory and are involved in the expression of nutrient transporters prior to implantation of the conceptus (Guillomot et al., 1981). The rate of conceptus elongation is influenced by the epithelial secretions and P4 within the endometrium (Spencer et al., 2008; Lonergan, 2011; Forde et al., 2012).

Progesterone will activate a set of genes within the endometrium during pregnancy and the estrous cycle that establish uterine receptivity. Uterine receptivity is a physiological state in which the uterus is capable of supporting growth and implantation of a conceptus (Spencer et al.,

2016). The actions of P4 causes modifications to the endometrium and the composition of the uterine lumen fluid (Spencer et al., 2016). In cattle the reduction of P4 from the CL caused a delay in temporal changes of the endometrial transcriptome which compromised elongation of the conceptus (Forde et al., 2010; Forde et al., 2011b). This evidence supports the idea that P4 causes changes in the endometrial transcriptome (Forde et al., 2009) meaning that P4 has an indirect effect on conceptus elongation (Carter et al., 2008; Clemente et al., 2009) through changing the transcriptome of the endometrium. The uterine environment plays a crucial role in supporting and maintaining pregnancy, especially during the elongation period of the conceptus and implantation period.

The presence of preovulatory E2 impacts the oocyte, gamete transport, preparation of the uterine environment for establishment of pregnancy, follicular cells, and the establishment and maintenance of pregnancy (Pohler et al., 2012). Estradiol is known to affect the expression of uterine proteins and induce receptors of the endometrium (Bartol et al., 1981). Oocytes express estrogen receptor beta and are expressed in the cumulus cells (Beker-van Woudenberg et al., 2004), exposure to high concentrations of preovulatory E2 could be acting via nuclear receptors (Su et al., 2009).

Placental Growth & Development

Placental Growth

The placenta is an multipurpose organ that is involved in endocrine, autocrine, and paracrine functions (Gootwine, 2004). These functions allow for a range of production of peptide and steroid hormones which aid in the development of the placenta (Gootwine, 2004). the

majority of the placental growth in the cow occurs during early- and mid-gestation, however, the placenta will continue to grow through late-gestation at a slower pace in comparison to fetal growth (Reynolds and Redmer, 1995). Around d19 and 20 of gestation the earliest stage of placentation begins in the cow (King et al., 1980; Wathes and Wooding, 1980). The placenta attaches to sites on the uterine wall known as caruncles. These are aglandular sites along the uterine wall which will attach to the placental membranes on the fetal side of the placenta (Vonnahme, 2012). The fetal membranes that attach to the caruncles are known as cotyledons, and the caruncle-cotyledon unit is called a placentome (Vonnahme, 2012). As gestation progresses, placentome size increases as the fetal weight increases (Reynolds and Redmer, 1995). The main function of the placentome is the exchange of nutrients between the maternal and fetal circulations (Vonnahme, 2012). An increase in placental function as the pregnancy progresses is necessary to meet the metabolic requirements of the growing fetus (Metcalfe, 1988; Ferrell, 1989). The ability to meet the metabolic requirements of the fetus are accomplished via the vascularization and growth of the caruncles, cotyledons, uterine blood flow (BF), and umbilical BF (Reynolds and Redmer, 1995).

Vascularization of the placentome is responsible for most of the nutrient transfer during pregnancy. This is the result of angiogenesis or formation of vascular beds and blood vessels of the placenta which are essential to the growth and formation of the placenta (Hudlika, 1984; Folkman and Klagsbrun, 1987; Klagsbrun and D'Amore, 1991; Reynolds et al., 1992). Angiogenesis is the major component in the increase of uterine and umbilical BF as pregnancy progresses (Reynolds et al., 1992; Reynolds and Redmer, 1995; Magness, 1998; Charnock-Jones et al., 2004; Kaufmann et al., 2004; Mayhew et al., 2004; Reynolds et al., 2005b; Reynolds et al.,

2005c; Borowicz et al., 2007). Several angiogenic factors have been identified for their role in placental vascularization. These factors include vascular endothelial growth factor, fibroblast growth factor, and angiopoietin protein families and their receptors (Yancopoulos et al., 2000; Koh et al., 2002; Charnock-Jones et al., 2004; Reynolds et al., 2005b; Reynolds et al., 2005c; Borowicz et al., 2007). Changes to the maternal environment are known to affect vascular development, and the change in vascular development is known to alter the expression of angiogenic factors and receptors (Redmer et al., 2005; Reynolds et al., 2005a; Reynolds et al., 2005b; Reynolds et al., 2005c; Vonnahme et al., 2007; Vonnahme et al., 2008). These environmental changes can be facilitated through the diet of the dam such as nutrient restriction (Vonnahme et al., 2007; Zhu et al., 2007). Both studies revealed, that during mid-gestation with maternal nutrient restriction, placental vascularization was altered affecting both placentome and fetal growth (Vonnahme et al., 2007; Zhu et al., 2007). Therefore, angiogenesis of the placenta is a key component to placental function and impact nutrient exchange between the maternal and fetal circulation.

Blood Flow

Much of the uterine and umbilical BF is dependent upon the development of the placental vascular beds, which depends on the angiogenesis of the placenta (Bassingthwaighte, 1984; Hudlika, 1984). In cows, BF of the uterine artery will increase between 3 to 4-fold from mid- to late-gestation (Rosenfeld et al., 1974; Reynolds et al., 1986; Reynolds and Redmer, 1995). The nutrient exchange between the maternal and fetal circulation is dependent upon the proper vascularization of the placenta, uterine, and umbilical BF (Redmer et al., 2004). During pregnancy there will be an increase in the oxygen uptake by the bovine fetus from mid- to late-

gestation. This is supported by the increase in the uterine BF (Reynolds et al., 1986). Therefore, BF to the fetus is crucial for proper oxygen transfer from mid- to late-gestation to support fetal growth.

Placental Transfer of Nutrients

The placenta is responsible for nutrient and waste exchange between the maternal and fetal circulation; therefore, altered placental function can impact fetal growth and development (Reynolds and Redmer, 1995, 2001). An increase in nutrient exchange in an adequately developed placenta will support the rapid fetal growth in the last third of gestation (Eley et al., 1978; Prior and Laster, 1979). Nutrient transfer and placental size are involved in the growth trajectory of the fetus during pregnancy and long-term growth of the progeny following birth (Redmer et al., 2004).

Transfer of nutrients are partitioned according to the most metabolically active tissues to the least metabolically active tissues (Redmer et al., 2004). Hence a decrease in nutrients could be transferred to the fetus when fewer nutrients are available to the dam, for example a heifer is still growing therefore, may require more nutrients to continue to reach maturity (Redmer et al., 2004). During pregnancy, the fetus relies solely on the maternal circulation for nutrients, including trace minerals (Hidiroglou and Knipfel, 1981). Fetal requirements for Cu increase as gestation progresses (Hidiroglou and Knipfel, 1981). In the ovine model, Cu will be absorbed by the ewe and when Cu enters the maternal liver, it is converted to hepatocuprein and then into haemocuprein (Hidiroglou and Knipfel, 1981). Once converted to haemocurpein, it will enter the blood stream and then can be transferred to the fetus (Hidiroglou and Knipfel, 1981). In the bovine fetal liver, Cu concentrations were higher than the dam (Pryor, 1964), whereas, the ovine

fetal liver had a variation in the Cu stores from ewes with reduced Cu concentrations (Suttle and Field, 1969). When Mn was administered to the ewe within 12 hours following the injection of Mn, concentrations of Mn in the placenta were nearly 50% of the amount administered to the dam (Hansard, 1973). Authors reported, that around 168 hours post-injection, Mn concentration in the placenta had decreased to approximately 25% within the fetal compartment (Hansard, 1973). The results reported by Hansard (1973) suggest that Mn can be transferred rapidly from the dam to the fetus during pregnancy. Cows that received Mn supplementation delivered calves with greater Mn concentrations in the muscle and liver when compared to calves born to dams with low or adequate concentrations of Mn in the diet (Rojas et al., 1965; Howes and Dyer, 1971). Hidiroglou et al. (1994) reported cows that were administered an injection of Se, had greater Se placental transfer compared to the cows that received no supplemental Se. These results are corroborated by similar Se concentration of both the dams that received the Se supplementation and the calves, which demonstrated Se transfer across the placenta (Hidiroglou et al., 1969; Hidiroglou et al., 1994). Placental transfer of trace minerals during pregnancy are crucial to fetal development of the nervous, reproductive, and immune systems (Hostetler et al., 2003; Pepper and Black, 2011).

Fetal Growth

Many factors are known to regulate placental growth, which affects fetal growth and development (Wu et al., 2006). Factors such as the epigenetic state such as histone methylation and acetylation, genetics of both the sire and dam, environmental factors, maturity of the dam, and placental growth can all affect the transfer of nutrients and fetal growth (Wu et al., 2006).

Fetal growth may be compromised by inadequate placental function and availability of nutrients in the maternal circulation which directly limits the fetal nutrient supply. During early gestation, placental growth and organogenesis of the fetus occurs simultaneously (Vonnahme et al., 2018). In cattle, the fetal heartbeat is evident around d21-22 of pregnancy (Funston et al., 2010), while development of the limbs begins by d25 of pregnancy (Hubbert et al., 1972). Reproductive organ development begins in bull calves by d45 of pregnancy when testicles begin to form (Funston et al., 2010) and ovarian development will occur between d50-60 of gestation when ovarian weight increases (Erickson, 1966). Tissue growth is dependent on the stressors of the dam at specific time points of gestation, which can impact organ development and affect fetal growth and development (Funston et al., 2010). As gestation progresses in the bovine, placental growth slows down while fetal growth is rapid during late gestation (Reynolds and Redmer, 1995). This suggests that placental growth, although it slows down, has reached optimal size to keep up with the transport of nutrients needed for the rapid growth of the fetus.

Fetal Programming

Fetal or developmental programming has been defined as the maternal stimulus or insult that occurs at the critical period during fetal development that has long-term or permanent impacts on the offspring (Barker et al., 1993; Godfrey and Barker, 2000). Changes to the maternal environment such as nutrition, disease, or exposure to toxins or prolonged stress, and age of dam can impact the size of the placenta and nutrient transfer from maternal to fetal circulations (Wu et al., 2006; Greenwood et al., 2017).These changes to the environment of the dam impacted fetal muscle development and formation (Russell and Oteruelo, 1981; Stannard

and Johnson, 2004; Zhu et al., 2006), growth (Marques et al., 2016), and limited research has indicated changes in reproductive performance of the offspring (Mossa et al., 2013; Harvey et al., 2021b).

During pregnancy, skeletal muscle development and growth in the fetus is critical since there is a finite number of muscle fibers following birth (Stickland, 1978; Zhu et al., 2004). A decrease in number of muscle fibers could alter offspring growth and performance (Du et al., 2015), and gestation is crucial to the formation of intramuscular adipocytes which will ultimately affect intramuscular fat deposition within the muscle (Tong et al., 2008). Most of the muscle fiber development within the bovine fetus occurs during early- and mid-gestation (Russell and Oteruelo, 1981). When the dam is exposed to nutrient restriction during gestation, muscle growth is impaired and fat levels increase (Greenwood and Cafe, 2007; Funston et al., 2010; Funston and Summers, 2013), resulting in impaired growth and performance of the offspring. Glucose is the primary energy substrate for the fetus during gestation (Bell and Ehrhardt, 2002). Transport of glucose to the fetus during pregnancy, is dependent upon the plasma glucose concentration gradient of the placenta (Simmons et al., 1979; DiGiacomo and Hay, 1990; Hay et al., 1990). Maternal nutrient restriction during early- and mid-gestation increased glucose exchange across the placenta (Heasman et al., 1998; Dandrea et al., 2001). An increase of placental exchange of glucose results in an increase in sensitivity of insulin-like growth factor-1 [IGF;(Symonds et al., 2012)]. Insulin-like growth factor-1 is known to promote the development of adipose tissue in the animal (Symonds et al., 2012), which can result in an increase of fat thickness in the offspring (Du et al., 2015). Much of the fetal programming research has investigated the effects of maternal nutrition on overall growth and muscle development of the offspring, however, less

research has focused on reproductive performance of the offspring when maternal nutrition was affected during early or mid-gestation (Mossa et al., 2013; Harvey et al., 2021b).

Throughout gestation, both the testes and ovaries will differentiate in the bovine fetus (Erickson, 1966; Funston et al., 2010). By d45 of gestation the testes have begun forming (Funston et al., 2010) and around d50 to d60 of gestation, ovarian weight increases (Erickson, 1966). Nutritional insults during this time likely impact the development and later function of these structures. Mossa et al. (2013) reported that heifer calves from the dams that received nutrient restrictive diet during early gestation had reduced antral follicle counts (AFC). Additionally, authors reported that anti-Mullerian hormone (AMH) concentrations were decreased in the progeny from dams that were nutrient restricted (Mossa et al., 2013). Anti-Mullerian hormone is produced by the granulosa cells of pre-antral follicles on the ovary (Rico et al., 2011). Authors stated that the decrease in AMH and decrease in AFC suggest that the nutrient restrictive diet impaired ovarian reserves (Mossa et al., 2013). However, it is unknown how the impaired ovarian reserves impacted attainment of puberty and pregnancy rates. In another study, Harvey et al. (2021b) fed cows organic, inorganic, or no additional sources of mineral during mid and late gestation. Harvey et al. (2021b) observed that heifer progeny from the cows supplemented with organic mineral sources attained puberty at an earlier age. Effects of fetal programming on male reproductive performance are investigated even less. In the ovine model, ewes were fed to approximately 70% of the energy requirements from about d100 of gestation until birth. Authors reported that ram lambs from the nutrient restricted group had lower testicular weight at 60 days of age while the ewe lambs from the same treatment group had reduced uterine weights (Hoffman et al., 2018). Limited research evaluating the developmental

programming effects on reproductive performance is available, therefore, additional research is needed to determine any potential effects from the maternal diet during gestation on reproductive performance.

Mineral Effects on Calf Performance

Trace mineral deficiencies during the fetal period of life are known to influence the growth, immune, and morphological development for a variety of fetal and neonatal tissues (Underwood and Suttle, 1999). Minerals such as Cu, Mn, Co, and Zn are required for proper development of the fetal nervous, reproductive, and immune system of the fetus (Hostetler et al., 2003; Pepper and Black, 2011). These minerals are critical to the fetal development through alteration of hormones, growth factors, and cell signaling pathways that are involved in nutrient uptake by the fetus (Ashworth and Antipatis, 2001; Wu et al., 2006; Du et al., 2017). Alteration of growth factors, cell signaling pathways, and hormones due to insufficient mineral availability to the fetus during gestation could alter growth and performance of the calf.

Birth Weights

When cows were supplemented with trace minerals during gestation, various studies (Hansen et al., 2006; Marques et al., 2016; Stokes et al., 2019a; Harvey et al., 2021a) reported no differences in birth weight of calves. One study reported calves born to heifers that were supplemented with Mn had decreased birth weights (Hansen et al., 2006). Similar results were observed when heifers were supplemented with injectable mineral throughout gestation (Stokes et al., 2019a). Though cows were supplemented during either mid- and late- gestation or solely

during late gestation, authors (Marques et al., 2016; Harvey et al., 2021a) found no differences in birth weights of calves born to cows supplemented with various sources of trace minerals.

Weaning Weights

Several studies (Stanton et al., 2000; Marques et al., 2016) reported that mineral supplementation during late gestation improved weaning weights, while other studies (Stokes et al., 2019a; Harvey et al., 2021a) concluded that mineral supplementation during mid and/or late-gestation did not influence weaning weights. Stanton and others (2000) observed that supplementing late-gestating beef cows with high organic-complexed Cu, Zn, Co, and Mn improved calf weaning weights when compared to the calves from the sulfate-supplemented group (Stanton et al., 2000). Similar results were observed in another study, both weaning weights and 205-day weights were greater in calves receiving an organic source of minerals when compared to the control calves when dams were not supplemented with any additional Cu, Co, Mn, and Zn (Marques et al., 2016). However, when cows were supplemented with either organic or inorganic sources of trace minerals during mid-gestation until calving, no difference in weaning weights was observed between the treatment groups (Harvey et al., 2021a). Stokes et al. (2019a) also observed no differences in calf weaning weights when dams were supplemented with multiple injections of trace mineral throughout pregnancy. Still, there is no known mechanism for the differences in weaning weights when the dam was supplemented during mid- or late-gestation.

Long-Term Effects

Mineral supplementation during gestation has been reported to have long-term effects on both male and female calf performance (Marques et al., 2016; Shao et al., 2020; Harvey et al.,

2021b). Harvey et al. (2021b) observed that the female progeny from an organic trace mineral supplemented group of cows reached puberty at an earlier age when compared to the heifers of the group of cows supplemented with sulfate minerals. Females that obtain puberty earlier are more likely to become pregnant earlier in life, and have been shown to stay in the herd and be a more productive cow (Day and Nogueira, 2013).

There is some research investigating the effects of steer calf performance in the feedlot and carcass characteristics, however, many of these studies have differing results (Marques et al., 2016; Shao et al., 2020; Rodríguez et al., 2021). Steer calf performance in the feedlot was enhanced through maternal mineral supplementation during later gestation (Marques et al., 2016). Marques et al. (2016) concluded that calves from the organic source of trace mineral supplemented cows, had greater weaning weights, live weight when sent to slaughter, and hot carcass weights. These calves were sent to slaughter with fewer days on feed when compared to the calves from the control group (Marques et al., 2016). However, Rodríguez et al. (2021) and Shao et al. (2020) did not observe any differences in weights of calves at the end of the feedlot phase when the dams were supplemented during gestation. Although no differences were observed in final live weight prior to slaughter, authors reported a greater percentage of calves graded choice from the trace mineral supplemented group when compared to the controls (Shao et al., 2020). Additionally, authors concluded that administration of injectable trace mineral calves had increased Cu and Se shortly after birth that it could have contributed to greater adipogenesis when the calves were younger (Shao et al., 2020). Although results differ between studies, further research is needed to determine the mechanisms that contributed to the growth of calves in the feedlot and carcass characteristics.

Conclusion

Trace minerals are required in small quantities yet essential for physiological function (National Academies of Sciences, 2016). Mineral concentrations can be influenced by the antagonistic interactions, bioavailability, toxicity, and deficiencies within the animal (National Academies of Sciences, 2016). Thus, mineral supplementation is imperative to growth, health, and reproduction in cattle in some instances (Arthington and Ranches, 2021). Trace minerals are critically important during the preimplantation and post-implantation stages of embryogenesis (Ashworth and Antipatis, 2001). During pregnancy, the fetus relies completely on the dam for nutrients, including trace minerals which are needed for growth and development (Hidiroglou and Knipfel, 1981). Studies examining the impacts of maternal trace mineral supplementation on calf performance report contradicting results (Stanton et al., 2000; Marques et al., 2016; Stokes et al., 2019a; Harvey et al., 2021a). Many of these studies focused on supplementing the cow during the last two-thirds of gestation when exponential fetal growth occurs (Stanton et al., 2000; Marques et al., 2016; Stokes et al., 2019a; Shao et al., 2020; Harvey et al., 2021a; Rodríguez et al., 2021). Therefore, investigating how maternal mineral supplementation during early gestation affects calf growth and performance is needed to determine developmental programming effects on calf performance. The work discussed in the remaining chapter will focus on the effects of injectable mineral administration to the dam during gestation on placental development and calf performance.

CHAPTER THREE

INJECTABLE MINERAL ADMINISTRATION DURING EARLY GESTATION ALTERS PLACENTAL FUNCTION AND CALF GROWTH AND PERFORMANCE

Introduction

Maternal nutrition can affect offspring growth and performance and placental growth and development (Caton et al., 2020). The majority of placental development and growth takes place during early- and mid-gestation, so the placenta is capable of providing nutrient transport when fetal growth is occurring rapidly (Reynolds and Redmer, 1995). During pregnancy, the fetus is dependent on the dam for nutrients, including trace minerals, for proper growth and development of the nervous, reproductive, and immune systems (Hidiroglou and Knipfel, 1981; Hostetler et al., 2003; Pepper and Black, 2011). Trace minerals are crucial to the preimplantation period of gestation and the post-implantation period (Ashworth and Antipatis, 2001). Copper, Fe, Mn, Se, and Zn are all transferred across the placenta (Rojas et al., 1965; Hansard, 1969; Howes and Dyer, 1971; Gooneratne and Christensen, 1989; Hidiroglou et al., 1994). Both Mn and Se transfer across the placenta resulted in greater hepatic Mn and Se concentrations in the progeny from supplemented dams (Rojas et al., 1965; Howes and Dyer, 1971; Hansard, 1973; Hidiroglou et al., 1994). Thus, maternal mineral supplementation can impact the fetal liver concentrations of trace minerals (Gooneratne and Christensen, 1989).

Maternal nutrition during pregnancy and the effects on progeny performance is a broad field that has been extensively studied. This is termed developmental or fetal programming

occurring when an insult or stimulus to the maternal environment results in altered progeny performance (Barker et al., 1993; Godfrey and Barker, 2000). However, developmental programming research evaluating the effects of trace mineral supplementation is limited, although there is a growing body of literature available (Marques et al., 2016; Stokes et al., 2019a; Stokes et al., 2019b; Shao et al., 2020; Harvey et al., 2021b, a; Rodríguez et al., 2021). Marques et al. (2016) reported that calves from dams that were supplemented with organic trace minerals during late gestation weighed heavier at weaning than calves from non-supplemented dams. Conversely, other authors have not observed differences in weaning weights when dams were supplemented with trace mineral injections or organic trace minerals during mid- and/or late-gestation (Stokes et al., 2019a; Stokes et al., 2019b; Harvey et al., 2021a; Rodríguez et al., 2021). Still, none of these studies evaluated the effects of a single injection of trace minerals during early-gestation, when organogenesis and placental development is occurring (Vonnahme et al., 2018). Therefore, we hypothesized that maternal injectable mineral administration would improve placental function causing developmental programming effects that result in enhanced calf performance. Our main objectives were to 1) evaluate the effects of injectable mineral (zinc, copper, manganese, selenium, and copper) in first trimester beef cows on body weight, condition, and placental function; 2) evaluate the effects of maternal injected mineral on calf mineral status at birth and growth through weaning.

Materials and Methods

This experiment was conducted at the Northern Agricultural Research Center (NARC), Montana State University (Havre, MT, USA). All experimental procedures and collections were

approved by the Animal Care and Use Committee of Montana State University (protocol # 2020-AA01).

Cow Management

One hundred and two late-calving, primi- and multiparous, lactating Angus and SimAngus cows were exposed to the 7 & 7 estrus synchronization protocol and fixed-timed artificially inseminated (FTAI) to 1 of 5 sires at day (d) 0 of the study. Seven days following FTAI, cows were exposed to 2 clean-up bulls for a 45-day breeding season. Body weights and body condition scores (BCS) were recorded by 2 trained individuals at day (d) -16, -9, -2, 0 (FTAI),45, 60, 139.5±0.5, 209±1, and at calving. Pregnancy status was determined at d45 of gestation via rectal ultrasonography. Cows with the presence of a fetus of the appropriate size, were determined to pregnant to FTAI (n = 52; sire 1, n = 15; sire 2, n = 3; sire 3, n = 8; sire 4, n = 19; sire 5, n = 17). Cows failing to conceive by FTAI were removed from the study.

All cows were maintained to meet NRC mineral requirements prior to study initiation (National Academies of Sciences, 2016). Prior to treatment administration, cows were blocked by parity, breed, AI sire, BW (control group BW = 537.51 kg; injectable group BW = 566.35 kg), BCS (control group BCS = 5.5; injectable group BCS= 5.4), and non-esterified fatty acid (NEFA) concentration. Cows were then assigned to 1 of 2 treatment groups; 1) control group (CON; n = 26) received free-choice mineral supplement (Ultramin ® 12-6+ Pressed Block, PayBack CHS Inc., Sioux Falls, SD, USA; see Appendix A); or 2) injectable group (INJ; n = 26) which had access to free-choice minerals (Ultramin ® 12-6+ Pressed Block) and received a single subcutaneous injection of trace minerals (1 milliliter per 200 pounds of body weight) at d60 of gestation (Cu, 15 mg/ml; Mn, 10 mg/ml; Zn, 60 mg/ml; Se, 5 mg/ml).

Cows were managed as a single herd from d-16 until completion of the study. Cows grazed on a mixture of cool and warm season forages and were provided free-choice minerals. Cows were provided a maintenance diet, and a protein supplement with chelated minerals (New Generation Supplements, Belle Fourche, SD, USA) at ad libitum from August (d50 of gestation) through October (d140 of gestation). Beginning at approximately d140 of gestation, cows were provided grass-alfalfa hay and free-choice compressed mineral supplement block (Ultramin ® 12-6+ Pressed Block) due to drought conditions. At approximately d200 of gestation, cows were supplemented daily with 2.5 lbs of protein/cow and grass hay, and alternating days of the week cows were fed straw or alfalfa pea hay (see Appendix B for nutrient analysis). Cows were maintained on this diet until d260 of gestation, when the diet was adjusted to prepare for calving. Cows were supplemented with a mix of corn silage, hay, grass hay, and protein supplement twice a day until approximately 69 d post-calving (see Appendix C for nutrient analysis). At d209±1 of gestation, all cows (n = 52; CON, n = 26; INJ, n = 26) received pre-calving vaccinations (Scourguard) and topical treatment for lice control (Cleanup 2). Seven cows were removed after initiation of the study (CON = 3; INJ = 4) due to death, pregnancy loss, bred to cleanup bull, or BCS ≤ 3. Data from these cows were not included in the results. Cows and calves were transported to the Thackery Ranch (Havre, MT) and managed together, grazing cool season grasses until weaning, at approximately 163.9 d post-calving.

Blood Sampling Analysis

Blood samples were collected from all cows at d-9, -2, 45, 60, 139.5±0.5, and 209±1. The first 5 cows/treatment to calve from the subset group were selected for blood sample collections at calving. Samples were collected via jugular venipuncture. Following collection, samples were

immediately placed on ice until transport to the laboratory. Samples were centrifuged at 2,500 rpms for 15 minutes at 4ºC for blood serum and plasma isolation. Serum samples were aliquoted and frozen at -20ºC until they were shipped to Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI, USA) for mineral analysis. Plasma samples were analyzed for non-esterified fatty acid (NEFA) concentrations (Fuji Film Health Care Solution) and frozen at -20ºC until analysis.

Tissue Sampling Collection and Analysis

A subset of cows (n=16; CON = 10; INJ = 6) were randomly selected from the herd for liver tissue collection at d60 and 209±1 of gestation. Liver biopsies were completed as previously described (Arthington and Corah, 1995). Liver tissue samples were placed in a cryovial and placed on ice prior to being shipped to Michigan State University Veterinary Diagnostic Laboratory for mineral analysis. The first 11 cows (CON, n = 5; INJ, n = 6) that calved from the subset group were selected for liver tissue and blood sample collection at calving. The same 11 cows were also selected for placental cotyledon (COT) collection. Immediately upon retrieval of the placenta, the 5 largest COT were identified and dissected from intercotyledonary tissue using dissection scissors. Samples were then washed in nanopure water for 5 minutes. Cotyledon were frozen at -20º C until they were dried at 65º C for 24 hours and subsequently stored at -80º C. Samples were then shipped to Michigan State University Veterinary Diagnostic Laboratory for mineral analysis.

Color Doppler Ultrasonography

Hemodynamic measurements of the uterine artery of both the gravid and non-gravid horns were completed at d139.5±0.5 and 209±1 of gestation (CON, n = 26; INJ, n = 26).

Approximately 3 cardiac wave cycles from 3 different scans were used to calculate the following information: maximum velocity (Vmax; cm/s), pulsatility index, resistance index, diameter (DIA; cm), artery area, artery circumference, blood flow (BF; L/min), and heart rate (HR) average (beats per minute; bpm).

Calf Management

Approximately 24 hours after birth, calves (n= 52; CON= 26; INJ = 26) were given an individual animal identification number, weights were recorded, blood samples were collected for mineral analysis, and all calves were vaccinated with Alpha 7. Calf sex was identified and recorded and all male calves (n = 27; CON, n = 17; INJ, n = 10) were castrated. Cow-calf pairs were managed together. Immediately following collection, blood samples were placed on ice and centrifuged at 2,500 rpms for 15 minutes at 4º C. Serum was isolated and frozen at -20º C until samples could be shipped to Michigan State University Veterinary Diagnostic Laboratory for mineral analysis. Two calves (CON = 2) were removed from study 24 hours after birth due to rejection by dam and/or lack of milk production, data from these calves were removed from all further analysis.

Approximately 4-8 d after birth, body weight, heart girth, and crown to rump length were recorded (n = 50; CON = 24; INJ = 26). Immediately following the neonatal measurements, liver tissue samples were collected and processed as previously described. Samples after collection were stored at -20º C and then shipped to Michigan State University Veterinary Diagnostic Laboratory for mineral analysis.

Calves (n = 50; CON = 24; INJ = 26) were weighed and vaccinated with One Shot Ultra 7 at 120.5±0.5 d of age. Calves were weaned at approximately 163.9 d of age, administered a

booster vaccination of One Shot Ultra 7, weaning weights (WW) were recorded, and average daily gain and weight gained from birth to weaning was calculated.

Feeds, Forages, Water Sampling & Analysis

Forage samples were collected when cows entered a pasture and again when they were removed from the pasture. Samples (n = 5-15) were collected using a 1 sq. meter frame, and forages were cut to 3.71 centimeters in length. Samples were then weighed and were then dried for 72 hours at 35º C. After drying, samples were allowed to cool for a minimum of one hour before weighing. Samples were then stored in a cool, dry room until they underwent nutrient and mineral analysis (Ward Laboratories Inc., Kearny, Nebraska, USA; Appendix D-N). A sample of the mineral block was also collected for mineral analysis (Ward Laboratories Inc.; Appendix A).

Feed samples were taken from each feed stuff that was supplemented to the cows over the course of the study. Hay, barley straw, grass hay, and pea hay samples were submitted to North Border Analytics (Chinook, MT USA, 59523) for analysis (Appendix B and C). The remaining samples (30-9 protein pellet, corn silage from 2021, lactation intake protein pellet, first cutting hay, and corn silage from 2020) were submitted to Midwest Laboratories (Omaha, NE USA, 68144) for analysis (Appendix B and C). Water samples were collected from two water sources that were accessible to study animals. Samples were collected into a 500 ml bottle and kept in a cool, dark place until samples were sent off for analysis (Energy One Laboratories, Helena, MT, USA; Appendix O).

Statistics

Both cow and calf variables were analyzed with cow as the experimental unit, and cow(treatment) as the random variable. Quantitative data was analyzed using the MIXED

procedure of SAS (SAS Inst. Inc., Cary, NC), binary data was analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.), and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Model statements for the cow-related responses included the effects of the treatment. Model statements for calf-related responses included the effects of treatment, calf gender as independent covariate, as well as day and treatment × day interaction for plasma & liver variable. Results are be reported as least square means, covariately adjusted to calf gender when applicable and separated using PDIFF. Significance was set at $P \leq 0.05$, and tendencies were determined if $0.5 < P \leq 0.10$.

Results

Cow Performance

No differences were observed in cow BW, BCS, or wt. gained or lost over the course of the study ($P = 0.40$ and 0.26 respectively; Table 3.1). No differences were detected in plasma non-esterified fatty acid (NEFA) concentrations at d45, 139.5±0.5, and 209±1 of gestation (*P*-value = 0.21), intra and inter CVs were 8.18% & 34.3% respectively. Furthermore, no differences were observed in gestation length between treatment groups ($P = 0.22$).

There was a decrease in hepatic Fe concentrations at d209±1 of gestation in the INJ group ($P = 0.01$; Table 3.2). No differences were observed in hepatic Co, Cu, Mn, Mo, Se, or Zn concentrations at d209±1 of gestation ($P \geq 0.34$; Table 3.2). Additionally, at calving there were no differences observed in hepatic Co, Cu, Fe, Mn, Mo, Se, or Zn concentrations between the different treatment groups ($P \geq 0.28$).

There was a treatment by day (TRT x d) interaction for serum Co at d139.5±0.5 of gestation, the INJ cows had greater circulating Co concentrations than the CON cows ($P = 0.04$; Table 3.3). There was a tendency for a TRT x d interaction at d139.5±0.5 for Se to be increased ($P = 0.07$) Additionally, there was a tendency for TRT x d interaction for Zn to be decreased in the serum of the INJ cows at d209±1 of gestation ($P = 0.07$). At d139.5±0.5 there was a tendency for circulating Se to be increased the INJ cows ($P = 0.07$). There was a tendency for a treatment effect for the INJ group to have greater circulating Cu concentrations ($P = 0.09$). Additionally, circulating Mn was lower for the INJ cows throughout the study when compared to the CON cows ($P = 0.04$). At the time of calving, circulating molybdenum (Mo) concentrations were decreased in the INJ cows ($P = 0.04$), but no differences were detected in circulating Se, Cu, Co, Zn, or Mn between treatment groups ($P \geq 0.28$; Table 3.4).

Measurements of Placental Function

A TRT x d interaction was detected at d139.5±0.5 of gestation for the non-gravid uterine artery diameter ($P = 0.004$), area ($P = 0.006$), and circumference ($P = 0.006$) which was increased in the INJ cows (Table 3.5). No differences ($P > 0.10$) were detected in the non-gravid uterine artery area, diameter, or circumference at d209±1. There was a tendency for a treatment effect for pulsatility (PI) and resistance indices (RI) to be increased in the gravid uterine artery in the INJ cows throughout gestation (P-value = 0.09; Table 3.5). No effects were detected for maximum velocity (V_{max}), area, diameter, circumference, and blood flow of the gravid uterine artery (P-value ≥ 0.75; Table 3.5). No effects were detected in Vmax, PI, RI, and blood flow of the non-gravid uterine artery, (P-value ≥ 0.53; Table 3.5).

No differences were observed in the cotyledon Co, Cu, Fe, Mn, Mo, Se, or Zn, when analyzed ($P \geq 0.14$; Table 3.6). Additionally, no differences were observed in cotyledon length, circumference, diameter, area, wet weight, or total dry weight between treatment groups ($P \geq 0.28$; Table 3.6).

Calf growth and performance data

No differences were observed in calf birth weight, calf body weight at 4 – 8 d after birth, heart girth, crown to rump length, weight gain, or average daily gain (ADG) from birth until 4-8 d after birth ($P \geq 0.35$; Table 3.7). A tendency was observed for circulating Se concentrations to be increased in the INJ calves compared to the CON calves ($P = 0.09$; Figure 3.1). There were no differences between the CON and INJ groups for mineral concentrations in the serum for Cu, Mn, Mo, or Zn ($P \geq 0.29$) at 24 hours after birth (Figure 3.1).

INJ calves had greater hepatic Se concentrations ($P = 0.009$) and reduced liver Fe concentrations (P-value = 0.04) at 4-8 d after birth compared to the CON calves (Figure 3.2). There was a tendency for the INJ calves to have increased liver Mn concentrations ($P = 0.08$) respectively compared to the CON calves (Figure 3.2). There were no differences between the groups for liver Cu, Mo, or Co ($P \geq 0.15$; Figure 3.2) at 4 – 8 d after birth.

No differences were observed in calf body weight, weight gained, or ADG ($P \geq 0.35$ see table 3.8) when calves were 120.5±0.5 of age. Calves were 163.9 days of age at the time of weaning. No differences were observed for weaning weights, weight gained since birth, and ADG ($P \geq 0.17$; See table 3.9).

Discussion

All cows were of adequate mineral status upon initiation of the study, this study was designed to evaluate the effects of supranutritional mineral supplementation effects on placental function and the potential changes to calf performance and growth. Both feed and mineral intake was not measured on an individual animal basis during the current study. There is a gap in current literature evaluating individual animal mineral intake and how intake can affect placental development and ultimately impact progeny growth and performance.

A TRT x d interaction was detected at d139.5±0.5 of gestation for circulating Co to be increased in the INJ group compared to the CON cows. Interestingly, there was a TRT x d interaction at d209±1 for circulating Zn to be decreased in the INJ cows. These results differ from Stokes et al. (2019a) who did not observe a difference in plasma Zn concentrations at the time of calving. The injectable did not contain Co in the trace mineral injection, however, the free-choice mineral did contain Co (APPENDIX A), thus this interaction could be due to increased consumption of free-choice minerals or diet provided. Although the injectable mineral had Cu, Mn, Se, and Zn, the INJ cows still had decreased Zn concentrations at d209±1 of gestation. Additionally, a tendency for circulating Se to be increased at d139.5±0.5 in the INJ cows was observed. This is similar to Ranches et al. (2017), who reported that cows supplemented with Se fortified hay had increased plasma Se concentrations 4 d following calving when supplementation began approximately 30 d prior to calving. Conversely, Stokes et al. (2019a) observed a tendency for circulating Se to be increased at calving among dams that received multiple trace mineral injections.

A tendency was observed for the INJ cows to have greater circulating serum Cu concentrations, conversely, there was a decrease in Mn in the INJ cows throughout the study.

Stokes et al. (2019a) observed a difference in dam serum Cu concentrations and a tendency for increased Se concentrations of the group that received multiple injections of trace minerals at calving. A possible reason no difference was detected in serum or hepatic mineral concentrations at calving in the current study is cows were approximately 149.5±0.5- (d209±1 of gestation) and 220-d post-injection (at calving) based on the administration of injectable trace mineral at d60 of gestation. According to the manufacturer, the injectable mineral is only detectable in the liver for up to 30-90-d post-injection and measurable in the serum up to 29-d post-injection. A decrease in circulating Mn could indicate a potential increase in placental transfer of Mn to the growing fetus during pregnancy (Hansard, 1973). Hansard (1973) reported that 12 hours post-injection, ewes that were administered Mn had approximately 50% increase of Mn in the placenta (Hansard, 1973), thus indicating rapid placental transfer of Mn.

No differences were observed in hepatic Co, Cu, Mn, Mo, Se, or Zn, at calving in the cows between the treatment groups. These results differ from previous studies where both Marques et al. (2016) and Stokes et al. (2019a) observed a difference in hepatic Cu, and Marques and coworkers observed a difference in Zn at the time of calving. Although more recent studies have begun evaluating the effects of maternal mineral supplementation on calf growth, little evidence is available to understand the role trace minerals play on placental function or if trace minerals affect placental function.

Limited information is available on the role that trace minerals play in placental development or function and uterine artery blood flow. The current study is the first study, to our knowledge, that evaluates the role of trace mineral supplementation on placental function via uterine artery blood flow measurements. Research indicates placental transfer of trace minerals

during gestation (Rojas et al., 1965; Hidiroglou et al., 1969; Hansard, 1973), however, it is unknown if trace minerals affect placental development and function. Other studies (Lemley et al., 2018; McCarty et al., 2018; Sanford et al., 2021) have shown that nutritional changes and the use of exogenous hormones impact placenta function. The non-gravid uterine artery diameter, area, and circumference was increased at d139.5±0.5 of gestation in the INJ cows. Similar results were reported when McCarty et al. (2018), who measured both gravid and non-gravid uterine artery diameter at d240 of gestation in heifers which received melatonin implants. Conversely, Sanford et al. (2021) did not observe a difference in the gravid or non-gravid uterine artery diameter when cows were supplemented bi-weekly with bovine somatotropin injections. Neither author reported area and circumference of the uterine artery when cows were supplemented with melatonin or bovine somatotropin (McCarty et al., 2018; Sanford et al., 2021). Additionally, an increase in non-gravid uterine artery area, diameter, and circumference may have impacted cotyledon size of the non-gravid horn even though it did not increase non-gravid uterine artery blood flow. Cotyledons were removed from what was considered to be the gravid side of the placenta because it has been shown that the largest, most functional cotyledons are closest to the fetus during gestation indicating greatest nutrient transfer (Senger, 2012). Thus, an increase in non-gravid uterine artery area, diameter, and circumference may have impacted the non-gravid side of the placenta.

There was a tendency for a treatment effect for the PI and RI of the gravid uterine artery to be increased in the INJ group of cows. This translates to an increase in uterine artery resistance of the blood vessel. Sanford et al. (2021) reported a tendency for PI and a difference in RI to be decreased during early gestation in the cows that were administered bovine

somatotropin every 14 d throughout the first trimester of gestation compared to the control cows. McCarty et al. (2018) and Lemley et al. (2018) did not observe any differences in PI or RI of the gravid or non-gravid uterine arteries among cows and heifers that received either a melatonin implant or were under nutrient restricted. Sanford et al. (2021) reported differences during late gestation in gravid uterine artery blood flow of cows that were administered bovine somatotropin during early gestation. Authors speculated that the decrease in PI and RI during early gestation could have altered vascularity early on in pregnancy, which could explain the increase in gravid uterine artery blood flow during late gestation (Sanford et al., 2021). Thus, the tendency for PI and RI to be increased throughout gestation in the INJ cows of the current study, could potentially have impacted the blood flow of the gravid uterine artery. Regardless, the uterine artery blood flow appears sensitive to nutritional and exogenous hormone changes.

A small body of literature is available investigating the effects of trace mineral supplementation on placental function and development (Marques et al., 2016; Harvey et al., 2021a; McCarthy et al., 2022). The largest COT are thought to have the greatest nutrient transfer during pregnancy (Senger, 2012). No differences were observed in COT Co, Cu, Fe, Mn, Mo, Se, or Zn concentrations in the present study. Harvey et al. (2021a) had similar results when cows were supplemented during mid- and late-gestation with sulfate or organic complexed trace minerals. However, Marques et al. (2016) reported cows supplemented with organic complexed trace minerals during late gestation had greater Co and Zn concentrations and a tendency for Cu to be increased in the cotyledons. Additionally, no differences were observed in the COT length, circumference, diameter, area, total wet weight, and total dry weight. Further research is needed to determine if these results are due to small sample size, mineral supplementation occurring in

early and mid-gestation does not alter the trace mineral concentrations of the cotyledons, or if there is simply no effect of injectable trace mineral supplementation on COT mineral concentrations.

Circulating Se concentrations of the INJ calves at 24 hours after birth tended to be increased compared to the CON calves. This is similar to the results observed by Ranches et al. (2017), who reported increased plasma Se concentrations in calves from dams that received Se fortified hay or selenium selenite. However, these results differed from Stokes et al. (2019a) when authors observed no differences in plasma Se in calves from dams receiving repeated trace mineral injections. Hepatic Se was increased in the INJ calves at 4-8 d after birth, similar to the results observed by Stokes et al. (2019a) and McCarthy et al. (2022), among dams that received repeated trace mineral injections or vitamin and mineral supplementation during pregnancy, however, Stokes and coworkers also observed greater hepatic Cu concentrations which was not observed in the current study. McCarthy et al. (2022) observed greater Se concentrations in the fetal liver when collected at d83 of pregnancy, among the dams that received a vitamin and mineral supplementation. Conversely, Ranches et al. (2017) did not observe any differences of the hepatic Se concentrations in calves from cows that received either Se fortified hay or selenium selenite mineral. This is potentially due to Se transfer across the placenta, since Hidiroglou et al. (1994) determined that cows which received injectable Se had greater Se placental transfer than the cows receiving no supplemental Se. Although Se placental transfer was not measured directly in the current study, greater Se transfer across the placenta could be a potential mechanism that resulted in greater hepatic Se and tended to increase serum Se in the INJ calves.

Fe deficiencies are not commonly observed in livestock (Arthington et al., 2002), however, the antagonistic interaction between Fe, Zn, Mn, and Cu is likely to occur (Arthington and Ranches, 2021). Injectable trace mineral calves had decreased hepatic Fe concentrations when compared to CON calves at 4-8 d after birth, although none of the calves in this study were considered deficient. Arthington et al. (2014) administered injectable mineral consisting of Cu, Mn, Se, and Zn to calves within 24 hours after birth and also reported similar results for hepatic Fe to be decreased in the treated calves throughout the study. Additionally, the CON cows in this study, had greater hepatic Fe concentrations at d209±1 of gestation, although no differences were observed in cotyledon Fe concentrations. In the ewe, when Fe was administered intravenously, authors reported that Fe was transported across the placenta throughout gestation and was transferred to meet the demands of the developing fetal tissues (Hoskins and Hansard, 1964). Additionally, much of the Fe that the fetus acquires is from the plasma of the dam (Hidiroglou, 1980). The transfer of Fe across the placenta via plasma from the dam, could be the cause of the increased in hepatic Fe concentrations in the CON calves following birth.

The INJ calves had a tendency for hepatic Mn to be increased at 4-8 d after birth. These results are similar to McCarthy et al. (2022) who observed increased Mn concentrations in the fetal liver at d83 of gestation when the dams received vitamin and mineral supplementation. Conversely, Marques et al. (2016), Stokes et al. (2019a), and (Harvey et al., 2021a), did not observe any differences in hepatic Mn of calves. Previous studies (Rojas et al., 1965; Howes and Dyer, 1971) determined that when dams had either low or adequate levels of Mn in the diet, calves had greater Mn concentrations in the liver and muscle. Hansard (1973) determined that Mn could be rapidly transferred across the placenta in the ovine model. When ewes were

administered Mn injection approximately 50% of Mn administered was present in the placenta within 12 hours post-injection (Hansard, 1973). Although the INJ cows tended to have lower circulating Mn concentrations, this may be caused by greater placental transfer of Mn resulting in a tendency for INJ calves to have greater hepatic Mn concentrations.

The lack of a difference in weaning weights of the CON and INJ calves, agrees with previous studies when dams received either multiple or single injections of trace mineral or fed various sources of trace minerals (Stokes et al., 2019a; Harvey et al., 2021a; Rodríguez et al., 2021). Conversely, Marques et al. (2016) reported an increase in weaning weights in calves from dams that received organic complexed trace minerals when comparted to calves that received no additional mineral supplementation. Additionally, based on the data in the current study and previous work, injectable mineral supplementation had no effects on the growth of the calves from dams that received injectable trace minerals.

Tables

Table 3.1. Cow body weight, body condition score, and weight gained or lost at d45, d60, d139.5±0.5, d209±1, and d281.

d45	[2]CON	INJ	SEM	[3]TRT Effect	Day Effect	TRT*day
[1]BW, kg	600.30	620.93	14.15	0.40	<0.0001	0.74
BCS	5.80	6.00	0.12	0.26	<0.0001	0.22
d60						
BW, kg	592.51	614.99	14.16	0.40	<0.0001	0.74
BCS	5.60	5.70	0.13	0.26	<0.0001	0.22
Wt. Change, kg	-8.93	-5.93	5.60	0.13	<0.0001	0.92
d139.5±0.5						
BW, kg	570.68	583.81	14.15	0.40	<0.0001	0.74
BCS	5.40	5.54	0.12	0.26	<0.0001	0.22
Wt. Change, kg	3.73	2.79	5.56	0.13	<0.0001	0.92
d209±1						
BW, kg	67.58	617.22	14.15	0.40	<0.0001	0.74
BCS	5.80	5.90	0.12	0.26	<0.0001	0.22
Wt. Change, kg	37.16	33.40	5.56	0.13	<0.0001	0.92
Calving						
BCS	5.00	4.90	0.12	0.26	<0.0001	0.22

[1]BW = body weight, BCS = body condition score, and kg = kilograms.
[2]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and had access to free-choice minerals.
[3] Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Table 3.2. Cow hepatic mineral concentrations at d60, 209±1 of gestation, and calving.

	[3]CON	INJ	SEM	[4]*P*-value
[1]d60				
[2]Co	0.22	0.21	0.01	0.63
Cu	84.44	111.20	14.50	0.21
Fe	408.80	485.70	39.20	0.19
Mn	9.91	9.78	0.52	0.86
Mo	4.18	4.30	0.26	0.74
Se	2.218	2.673	0.17	0.10
Zn	186.1	155.7	20.1	0.30
d209±1				
Co	0.18	0.19	0.001	0.45
Cu	97.06	91.94	19.80	0.86
Fe	432.26	307.22	27.90	0.01[a]
Mn	10.06	10.54	0.34	0.34
Mo	3.52	3.67	0.16	0.52
Se	1.58	1.53	0.04	0.39
Zn	125.50	118.60	5.91	0.43
Calving				
Co	0.23	0.22	0.02	0.80
Cu	148.70	96.40	36.70	0.35
Fe	545.50	527.90	46.40	0.81
Mn	8.90	8.06	0.47	0.28
Mo	3.45	3.39	0.20	0.84
Se	2.14	1.75	0.32	0.43
Zn	138.40	127.70	10.20	0.49

[1]d60 of gestation cows were administered injectable mineral; d209+/-1 of gestation is 149.5±0.5 d post-injection; Calving is 220 d post-injection.

[2]Co = cobalt; Cu = copper; Fe = iron; Mn = manganese; Mo = molybdenum; Se = selenium; Zn = zinc; ug/g = micrograms per gram

[3]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and had access to free-choice minerals.

[4]Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Table 3.3. Circulating mineral concentrations of the cow at d139.5±0.5 and 209±1 of gestation

	d139.5±0.5			d209±1			[3]*P*-value		
[1]Mineral	[2]CON	INJ	SEM	CON	INJ	SEM	TRT Effect	Day Effect	TRT x d
Co, ng/ml	0.69	0.85	0.06	0.59	0.56	0.05	0.34	<0.0001	0.04[a]
Cu, ug/ml	0.79	0.81	0.03	0.80	0.88	0.03	0.09	0.10	0.22
Mn, ng/ml	2.39	2.28	0.14	2.97	2.49	0.14	0.19	0.006	0.04[a]
Se, ng/ml	137.62	143.94	4.71	128.06	118.82	4.77	0.78	0.0002	0.07[†]
Zn, ug/ml	0.79	0.80	0.04	1.05	0.93	0.04	0.16	<0.0001	0.07[†]

[1]Co = cobalt; Cu = copper; Mn = manganese; Se = selenium; Zn = zinc; ng/ml = nanogram per milliliter; ug/ml = microgram per milliliter
[2]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and had access to free-choice minerals.
[3]Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Table 3.4. Circulating mineral concentrations of cows at calving.

[1]Minerals	[2]CON	INJ	SEM	[3]*P*-value
Co, ng/ml	0.87	0.61	0.15	0.25
Cu, ug/ml	0.65	0.67	0.03	0.59
Mn, ng/ml	2.30	2.55	0.41	0.70
Mo, ng/ml	43.95	17.84	7.32	0.04[a]
Se, ng/ml	104.13	100.58	3.26	0.47
Zn, ug/ml	0.69	0.67	0.05	0.81

[1]Co = cobalt; Cu = copper; Mn = manganese; Mo = molybdenum; Se = selenium; Zn = zinc; ng/ml = nanogram per milliliter; ug/ml = microgram per milliliter
[2]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and had access to free-choice minerals.
[3] Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Table 3.5. Uterine artery measurements at d139.5±0.5 and 209±1 of gestation

	d139.5±0.5			d209±1			*P*-value		
Non-Gravid	**[2]CON**	**INJ**	**SEM**	**CON**	**INJ**	**SEM**	**[3]TRT**	**Day**	**TRT x d**
[1]V_{max}, cm/s	113.27	106.33	7.41	134.08	133.07	7.34	0.65	0.0002	0.62
PI	1.45	1.42	0.08	1.50	1.51	0.08	0.91	0.43	0.81
RI	0.68	0.65	0.02	0.70	0.70	0.02	0.53	0.05	0.40
Diameter, cm	0.89	1.10	0.05	1.04	0.93	0.05	0.37	0.83	0.005[a]
Area, cm^2	0.44	0.67	0.05	0.56	0.47	0.05	0.17	0.44	0.006[a]
Circumference, cm	2.38	2.87	0.13	2.71	2.46	0.13	0.39	0.76	0.007[a]
BF, L/min	1.59	1.93	0.28	2.27	2.02	0.28	0.86	0.14	0.27

	day 139.5±0.5			day 209±1			*P*-value		
Gravid	**CON**	**INJ**	**SEM**	**CON**	**INJ**	**SEM**	**TRT Effect**	**Day Effect**	**TRT*d**
V_{max}, cm/s	158.40	153.96	12.52	169.07	181.06	12.52	0.75	0.16	0.54
PI	1.05	1.21	0.07	1.33	1.43	0.07	0.09†	0.0002	0.59
RI	0.58	0.63	0.02	0.67	0.68	0.02	0.09†	<0.0001	0.34
Diameter, cm	1.21	1.25	0.06	1.20	1.14	0.06	0.90	0.38	0.49
Area, cm^2	0.79	0.75	0.08	0.76	0.76	0.08	0.81	0.92	0.84
Circumference, cm	3.21	3.25	0.16	3.18	3.06	0.16	0.76	0.51	0.65
BF, L/min	4.20	3.67	0.67	4.23	4.78	0.67	0.99	0.45	0.45

[1]V_{max} = maximum velocity (cm/s), PI = pulsatility index, RI = resistance index, BF = blood flow (L/min)

[2]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and was offered free-choice minerals.

[3] Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Table 3.6. Cotyledon mineral concentrations and measurements at calving.

	[2]CON	INJ	SEM	[3]*P*-value
[1]Minerals				
Co	0.08	0.56	0.23	0.19
Cu	5.70	6.03	0.60	0.71
Fe	539.50	1237.1	377.00	0.22
Mn	4.90	26.87	10.10	0.17
Mo	0.39	0.39	0.04	0.98
Se	1.47	1.34	0.05	0.14
Zn	57.6	61.6	2.30	0.24
Measurements				
Length, cm	13.40	12.70	0.83	0.56
Circumference	39.40	41.32	2.80	0.60
DIA	11.64	12.03	0.82	0.74
Area	107.90	117.70	16.10	0.68
Total wet wt., g	364.60	364.92	77.30	0.99
Total dry wt., g	22.90	20.00	4.50	0.66

[1]Co = cobalt; Cu = copper; Fe = iron; Mn = manganese; Mo = molybdenum; Se = selenium; Zn = zinc; ug/g = micrograms per gram

[2]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and had access to free-choice minerals.

[3] Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Table 3.7. Gestation length, birth weights, and neonatal measurements from birth until 4-8 d after birth.

	[2]CON	INJ	SEM	[3]*P*-Value
[1]Gestation Length	282.00	280.00	0.80	0.22
Calf birth wt., kg	39.62	41.30	1.31	0.32
Heart girth, cm	79.56	80.60	0.90	0.36
Crown to rump length, cm	84.40	84.80	0.75	0.67
Calf wt., kg	46.10	47.50	1.20	0.38
Average daily gain, kg	1.04	1.05	0.08	0.88

[1] kg= kilograms
[2]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and had access to free-choice minerals.
[3] Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Table 3.8. Calf body weights, weight gained, and average daily gain at 120.5±0.5 d old.

	[3]CON	INJ	SEM	[4]*P*-value
Age	120	121	0.7	0.08
[1]Calf body wt., kg	184.76	187.5	4.77	0.66
[2]Wt. Gained, kg	145.12	150.45	4.01	0.35
Average daily gain	1.21	1.23	0.03	0.55

[1]kg = kilograms
[2]Wt. gained since birth in kg
[3]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and had access to free-choice minerals.
[4]Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Table 3.9. Calf weaning weights, weight gained, and average daily gain at weaning.

	[2]CON	INJ	SEM	[3]*P*-Value
Age	163.00	164.85	0.75	0.08
[1]WW, kg	209.92	217.86	4.48	0.17
Wt. gained, kg	167.91	176.30	4.31	0.17
Average daily gain, kg	1.02	1.07	0.02	0.26

[1]kg = kilograms; Wt. gained = weight gained since birth
[2]CON = control group was offered free-choice minerals; INJ = injectable mineral group were administered injectable mineral at d60 of gestation and had access to free-choice minerals.
[3]Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. [a] is a difference and † is a tendency.

Figures

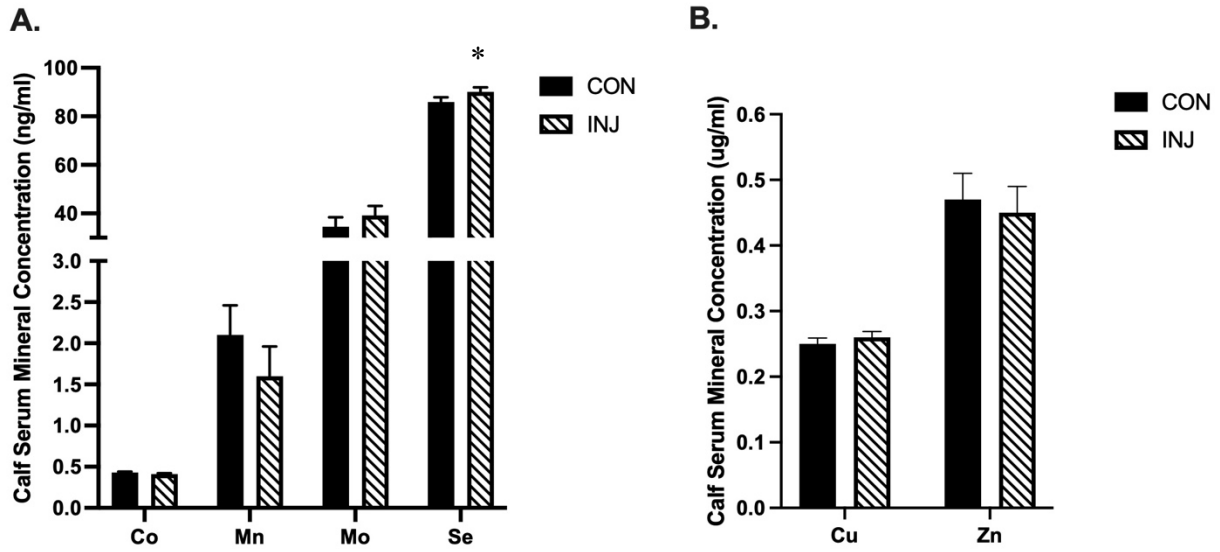

Figure 3.1. Circulating mineral concentrations of calves 24 hours after birth. Panel A) calf serum concentrations at 24 hours after birth. A tendency was detected for circulating Se to be increased in the INJ calves 24 hours after birth compared to the CON calves (*P*-value = 0.09). No differences were observed in Co, Mn, or Mo (*P*-value = 0.5, 0.29, and 0.36). Panel B) No differences were detected in circulating Cu or Zn concentrations (*P*-value = 0.85 and 0.77). Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. * is a difference and † is a tendency.

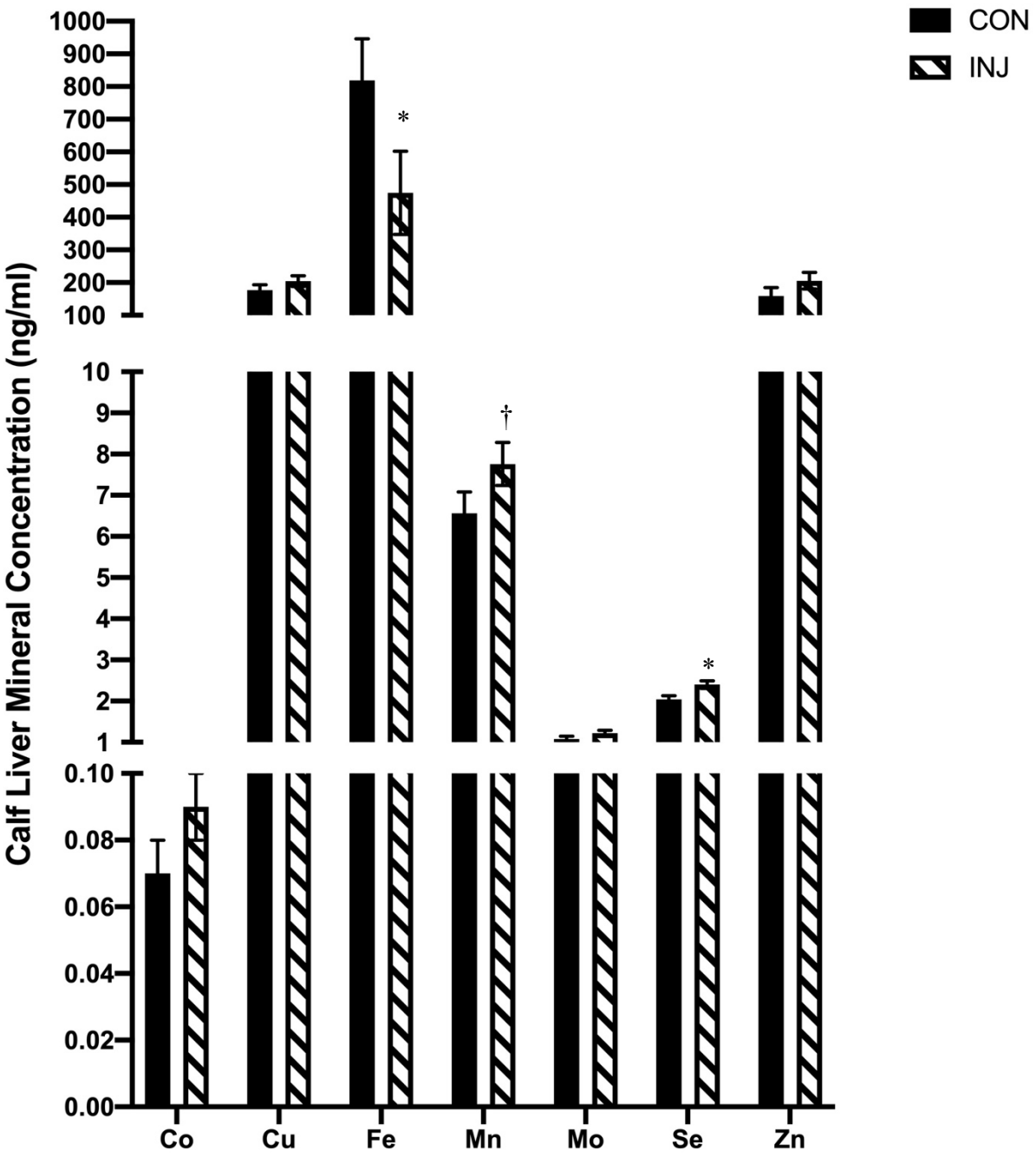

Figure 3.2. Hepatic mineral concentrations of calves 4 – 8 days after birth. Selenium was increased and Fe was lower in the INJ calves compared to the CON calves (*P*-value = 0.009 and 0.04). A tendency for hepatic Mn to be increased in the INJ calves was observed (*P*-value = 0.08). Difference defined as <0.05 & tendency defined as ≤0.05 and ≤0.09. * is a difference and † is a tendency.

CHAPTER FOUR

CONCLUSION

Maternal injectable mineral when applied during early gestation altered non-gravid uterine artery area, diameter, and circumference during early gestation, although not altering the gravid uterine horn or the uterine artery blood flow. Interestingly, injectable mineral administration did not influence maternal hepatic or circulating concentrations of Mn, Zn, or Se and tended to impact circulating Cu, however, this could be due to the number of days post-injection. Additionally, maternal injectable mineral influenced progeny serum Se and liver Se, Mn, and Fe concentrations. However, calf birth weights and weaning weights were not impacted by the maternal dietary treatment.

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APPENDICES

APPENDICES A. NUTRIENT ANALYSIS FOR MINERAL SUPPLEMENT.

[1,2]Item	[4]Dry Basis
[3]Moisture, %	0.00
DM, %	100.00
Ca, %	13.20
P, %	4.58
K, %	0.73
Mg, %	3.09
Zn, ppm	5693.90
Fe, ppm	1425.00
Cu, ppm	2802.00
Mn, ppm	2545.40
S, %	1.01
Na, %	1.84
Se, ppm	26.75
Co, ppm	31.16

[1]Mineral supplement Ultramin ® 12-6+ Pressed Block (PayBack, CHS Inc., Sioux Falls, SD, USA).

[2]Analyzed by Ward Laboratories Inc. (Kearny, NE, USA).

[3]DM = dry matter; Ca = calcium; P = phosphorus; K = potassium; Mg = magnesium; Zn = zinc; Fe = iron; Cu = copper; Mn = manganese; S = sulfur; Na = sodium; Se = selenium; Co = cobalt; ppm = parts per million.

[4]Samples were analyzed on a dry basis.

APPENDIX B. NUTRIENT ANALYSIS FOR MAINTENANCE DIET.

	[1]Grass Hay	Pea Hay	Barley Straw	[2]Lactation Pellet	30-9 Pellet	[3]Large Pellet	Small Pellet
[4]Moisture, %	95.20	92.60	89.50	NA	NA	NA	NA
Nitrate Content, ppm	NA	2208.00	738.40	NA	NA	NA	NA
CP, %	8.30	10.00	7.80	12.00	30.00	12.70	4.60
ADF, %	39.20	34.10	40.90	NA	NA	19.40	55.20
NDF, %	62.30	49.90	69.80	NA	NA	NA	NA
TDN, %	58.30	62.40	57.10	NA	NA	74.30	48.80
Ash	10.20	6.60	6.90	NA	NA	NA	NA
Crude Fiber	NA	NA	NA	25.00	9.00	NA	NA
Crude Fat	1.40	1.60	0.60	2.25	3.00	NA	NA
Calcium, %	NA	NA	NA	1.25	2.00	NA	NA
Calcium, %	NA	NA	NA	1.75	2.50	NA	NA
Phosphorous, %	NA	NA	NA	NA	0.20	1.00	NA
Salt, %	NA	NA	NA	NA	0.10	NA	NA
Salt, %	NA	NA	NA	NA	0.50	NA	NA
Potassium, %	NA	NA	NA	NA	1.75	0.75	NA
Selenium, ppm	NA	NA	NA	NA	0.13	NA	NA
Vitamin A, IU/lb	NA	NA	NA	NA	1,100.00	NA	NA
Vitamin D, IU/lb	NA	NA	NA	NA	110.00	NA	NA
Net Energy for Maintenance, Mcal/cwt	63.70	69.00	62.00	NA	NA	0.76	0.46
RFV	87.10	116.40	46.00	NA	NA	NA	NA
RFQ	91.40	122.10	79.70	NA	NA	NA	NA

[1]Grass hay, pea hay, & barely straw analyzed by North Border Analytics LLC (Havre, MT USA)
[2]Lactation protein pellet & 30-9 protein pellet were analyzed by CHS Inc. (Sioux Falls, SD USA)
[3]Large and small pellets were analyzed by Midwest Laboratories® (Omaha, NE USA)
[4]ppm = parts per million, CP = crude protein, ADF = acid detergent fibers, NDF = neutral detergent fibers, TDN = total digestible nutrients, CF = crude fat, RFV = relative feed value, RFQ = relative feed quality, NA = not available

APPENDIX C. NUTRIENT ANALYSIS FOR PRE-CALVING MAINTENANCE DIET FROM D260 OF GESTATION THROUGH 69-D POST- CALVING.

	[1]Hay	2021 Corn silage	[2]1st cutting hay	2020 Corn silage	[3]Lactation Pellet	30-9 Pellet	Large Pellet	Small Pellet
[4]Moisture, %	94.20	24.20	NA	NA	NA	NA	NA	NA
Nitrate Content, ppm	NA	9349.00	NA	NA	NA	NA	NA	NA
CP, %	8.50	7.60	9.42	10.90	12.00	30.00	12.70	4.60
ADF, %	43.30	38.30	37.60	27.30	NA	NA	19.40	55.20
NDF, %	62.80	54.30	NA	NA	NA	NA	NA	NA
TDN, %	55.20	59.10	59.70	68.90	NA	NA	74.30	48.80
Ash	9.50	3.90	NA	NA	NA	NA	NA	NA
Crude Fiber, %	NA	NA	NA	NA	25.00	9.00	NA	NA
Crude Fat, %	1.30	3.20	NA	NA	2.25	3.00	NA	NA
Calcium, %	NA	NA	NA	NA	1.25	2.00	NA	NA
Calcium, %	NA	NA	NA	NA	1.75	2.50		
Phosphorous, %	NA	NA	NA	NA	0.20	1.00	NA	NA
Salt, %	NA	NA	NA	NA	0.10	NA	NA	NA
Salt, %	NA	NA	NA	NA	0.50	NA	NA	NA
Potassium, %	NA	NA	NA	NA	1.75	0.75	NA	NA
Selenium, ppm	NA	NA	NA	NA	0.13	NA	NA	NA
Vitamin A, IU/lb	NA	NA	NA	NA	1,100.00	NA	NA	NA
Vitamin D, IU/lb	NA	NA	NA	NA	110.00	NA	NA	NA
Net Energy for Maintenance, Mcal/cwt	59.50	64.70	0.59	0.70	NA	NA	0.76	0.46
RFV	81.80	101.30	NA	NA	NA	NA	NA	NA
RFQ	85.80	106.20	NA	NA	NA	NA	NA	NA

[1]Hay & 2021 corn silage analyzed by North Border Analytics LLC (Havre, MT USA)

[2]First cutting hay, 2020 corn silage, large pellet, and small pellet were analyzed by Midwest Laboratories® (Omaha, NE USA)

[3]Lactation protein pellet, & 30-9 protein pellet was analyzed by CHS Inc. (Sioux Falls, SD USA)
[4]ppm = parts per million, CP = crude protein, ADF = acid detergent fibers, NDF = neutral detergent fibers, TDN = total digestible nutrients, IU/lb = international unit per pound; RFV= relative feed value, RFQ = relative feed quality, NA = not available

APPENDIX D. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PASTURE 1 FROM D0 UNTIL D28 OF GESTATION.

	[2,3]**Entering**	**Leaving**
Moisture, %	0.00	0.00
[1,4]DM, %	100.00	100.00
CP, %	8.70	4.80
ADF, %	35.30	40.20
NDF, %	66.40	73.10
TDN, %	62.30	56.70
Net Energy Maintenance, Mcal/lb	0.59	0.64
Relative Feed Value	86.00	73.00
Relative Forage Quality	120.00	78.00
Ca, %	0.26	0.29
P, %	0.18	0.11
K, %	1.07	0.72
Mg, %	0.12	0.14
Zn, ppm	18.40	18.90
Fe, ppm	154.00	260.00
Mn, ppm	33.00	40.00
Cu, ppm	6.00	5.40
S, %	0.12	0.11
Na, %	0.01	0.01
Mo, ppm	0.98	1.18

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX E. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PASTURE 2 FROM D28 UNTIL D36 OF GESTATION.

	[2,3]Entering	Leaving
Moisture, %	0.00	0.00
[1,4]DM, %	100.00	100.00
CP, %	7.10	5.40
ADF, %	39.80	41.40
NDF, %	71.80	72.60
TDN, %	57.10	45.00
Net Energy Maintenance, Mcal/lb	0.55	0.52
Relative Feed Value	75.00	73.00
Relative Forage Quality	94.00	83.00
Ca, %	0.22	0.27
P, %	0.19	0.12
K, %	1.99	1.42
Mg, %	0.12	0.14
Zn, ppm	13.10	12.10
Fe, ppm	119.00	197.00
Mn, ppm	27.00	26.00
Cu, ppm	5.40	5.00
S, %	0.11	0.12
Na, %	0.01	0.002
Mo, ppm	1.17	0.12

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX F. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PASTURE 3 FROM D28 UNTIL D41 OF GESTATION.

	[2,3]Entering	Leaving
Moisture, %	0.00	0.00
[1,4]DM, %	100.00	100.00
CP, %	7.40	5.80
ADF, %	38.50	43.00
NDF, %	70.90	76.50
TDN, %	58.60	52.50
Net Energy Maintenance, Mcal/lb	0.57	0.49
Relative Feed Value	77.00	67.00
Relative Forage Quality	96.00	71.00
Ca, %	0.31	0.22
P, %	0.14	0.16
K, %	1.28	1.58
Mg, %	0.14	0.10
Zn, ppm	16.10	14.40
Fe, ppm	93.00	81.00
Mn, ppm	23.00	25.00
Cu, ppm	5.40	5.70
S, %	0.15	0.10
Na, %	0.01	0.01
Mo, ppm	1.70	0.96

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX G. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PASTURE 4 FROM D28 UNTIL D41 OF GESTATION.

	[2,3]**Entering**	**Leaving**
Moisture, %	0	0
[1,4]DM, %	100	100
CP, %	15.4	7.2
ADF, %	34.7	43.5
NDF, %	61.2	75.9
TDN, %	63	52.9
Net Energy Maintenance, Mcal/lb	0.6398	0.4864
Relative Feed Value	94	67
Relative Forage Quality	108	78
Ca, %	0.5	0.26
P, %	0.2	0.17
K, %	3.53	2.32
Mg, %	0.46	0.13
Zn, ppm	18.6	18.1
Fe, ppm	111	112
Mn, ppm	44	40
Cu, ppm	7.9	5.8
S, %	0.25	0.11
Na, %	0.3	0.03
Mo, ppm	1.04	1.04

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX H. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PASTURE 5 FROM D40 UNTIL D62 OF GESTATION.

	[2,3]**Entering**	**Leaving**
Moisture, %	0	0
[1,4]DM, %	100	100
CP, %	8.3	5.2
ADF, %	37.8	41.9
NDF, %	71.2	76.4
TDN, %	59.5	54.8
Net Energy Maintenance, Mcal/lb	0.5873	0.5156
Relative Feed Value	78	68
Relative Forage Quality	105	73
Ca, %	0.31	0.24
P, %	0.23	0.13
K, %	1.49	0.93
Mg, %	0.1	0.08
Zn, ppm	25.9	333
Fe, ppm	14.6	130
Mn, ppm	25	19
Cu, ppm	5.9	4.7
S, %	0.13	0.07
Na, %	0.01	0.01
Mo, ppm	1.77	1.46

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX I. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PASTURE 6 FROM D28 UNTIL D41 OF GESTATION.

	[2,3]**Entering**	**Leaving**
Moisture, %	0	0
[1,4]DM, %	100	100
CP, %	4.7	5.2
ADF, %	40	43.5
NDF, %	71.1	74.9
TDN, %	57	52.9
Net Energy Maintenance, Mcal/lb	0.58	0.5349
Relative Feed Value	76	68
Relative Forage Quality	85	72
Ca, %	0.28	0.35
P, %	0.15	0.11
K, %	97	0.86
Mg, %	0.1	0.12
Zn, ppm	22.4	11.2
Fe, ppm	137	175
Mn, ppm	21	19
Cu, ppm	6.2	5.3
S, %	0.08	0.07
Na, %	0.01	0.01
Mo, ppm	1.66	0.84

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX J. FORAGE ANALYSIS FROM COWS ENETERING AND LEAVING PASTURE 7 FROM D46 UNTIL D56 OF GESTATION.

	[2,3]**Entering**	**Leaving**
Moisture, %	0	0
[1,4]DM, %	100	100
CP, %	8.5	8
ADF, %	43.8	41.9
NDF, %	68.3	67.7
TDN, %	52.6	54.7
Net Energy Maintenance, Mcal/lb	0.4817	0.5145
Relative Feed Value	75	77
Relative Forage Quality	79	84
Ca, %	0.63	0.61
P, %	0.18	0.11
K, %	1.36	1.25
Mg, %	0.2	0.18
Zn, ppm	18.2	14.5
Fe, ppm	150	198
Mn, ppm	35	29
Cu, ppm	6.2	5.8
S, %	0.12	0.12
Na, %	0.01	0.09
Mo, ppm	2.08	1.2

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX K. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PASTURE 8 FROM D56 UNTIL D67 OF GESTATION.

	[2,3]Entering	Leaving
Moisture, %	0	0
[1,4]DM, %	100	100
CP, %	7.2	5.1
ADF, %	39.8	42.5
NDF, %	73.7	75.2
TDN, %	57.1	54.1
Net Energy Maintenance, Mcal/lb	0.5513	0.5049
Relative Feed Value	73	69
Relative Forage Quality	92	74
Ca, %	0.27	0.37
P, %	0.14	0.11
K, %	1.27	0.69
Mg, %	0.12	0.13
Zn, ppm	14.3	17.3
Fe, ppm	220	134
Mn, ppm	29	30
Cu, ppm	5	5.6
S, %	0.11	0.09
Na, %	0.01	0.01
Mo, ppm	1.01	1.96

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX L. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PASTURE 9 AT D66 OF GESTATION.

	[2,3]**Entering**
Moisture, %	0
[1,4]DM, %	100
CP, %	5.3
ADF, %	42.4
NDF, %	72.6
TDN, %	59.9
Net Energy Maintenance, Mcal/lb	0.5062
Relative Feed Value	72
Relative Forage Quality	85
Ca, %	0.43
P, %	0.09
K, %	0.67
Mg, %	0.13
Zn, ppm	13
Fe, ppm	123
Mn, ppm	26
Cu, ppm	5.1
S, %	0.08
Na, %	0.01
Mo, ppm	1.63

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX M. FORAGE ANALYSIS FROM COWS ENTERING PASTURE 10 AT D94 OF GESTATION.

	[2,3]Entering
Moisture, %	0
[1,4]DM, %	100
CP, %	14.5
ADF, %	34.5
NDF, %	62.1
TDN, %	63.2
Net Energy Maintenance, Mcal/lb	0.6435
Relative Feed Value	93
Relative Forage Quality	121
Ca, %	0.46
P, %	0.22
K, %	2.11
Mg, %	0.19
Zn, ppm	18.7
Fe, ppm	253
Mn, ppm	43
Cu, ppm	8.6
S, %	0.18
Na, %	0.01
Mo, ppm	1.85

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX N. FORAGE ANALYSIS FROM COWS ENTERING AND LEAVING PATURE 11 FROM D102 UNTIL D138 OF GESTATION.

	[2,3]**Entering**	**Leaving**
Moisture, %	0	0
[1,4]DM, %	100	100
CP, %	4.9	3.2
ADF, %	44.6	44.3
NDF, %	75.9	78.4
TDN, %	51.7	52
Net Energy Maintenance, Mcal/lb	0.4673	0.4722
Relative Feed Value	66	65
Relative Forage Quality	78	62
Ca, %	0.38	0.36
P, %	0.07	0.07
K, %	0.46	0.49
Mg, %	0.12	0.14
Zn, ppm	12.5	8
Fe, ppm	177	131
Mn, ppm	44	50
Cu, ppm	5	4.2
S, %	0.08	0.06
Na, %	0.01	0.01
Mo, ppm	1.88	1.50

[1]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF =neutral detergent fibers, TDN = total digestible nutrients, Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Zn = zinc, Mn = manganese, Cu = copper, S = sulfur, Na = sodium, Mo = molybdenum; ppm = parts per million

[2]Samples were taken when cow entered and left each pasture

[3]Samples were analyzed by Ward Laboratories Inc (Kearney, NE USA)

[4]All samples were analyzed on a dry basis

APPENDIX O. WATER ANALYSIS FROM STANDING WATER SOURCE AND MOVING WATER SOURCES.

	[2]Standing water	Moving water
[1]pH	9.4	7.8
TDS, mg/L	2200	484
Alkalinity, mg/L	680	320
Bicarbonate, mg/L	610	390
Carbonate, mg/L	110	ND[1]
Chloride, mg/L	13	6
Sulfate, mg/L	839	94
Fluoride, mg/L	0.4	0.2
Hardness, mg/L	206	316
Nitrate + Nitrite, mg/L	[3]ND	ND
Ca, mg/L	20	71
Fe, mg/L	0.97	2.08
Mg, mg/L	38	34
K, mg/L	19	8
Na, mg/L	657	62

[1]TDS = total dissolved solids, Ca = calcium, Fe = iron, Mg = Magnesium, K = potassium, Na = sodium, mg/L = milligrams per liter

[2]Standing water sample was collected from a reservoir in the same pasture as the cows; Moving water sample was collected from a creek which ran through the pasture as the cows.

[3]ND = not detectable at reporting limit