WINTER FEEDING STRATEGIES: IMPLICATIONS OF CORN SUPPLEMENTATION ON GESTATING BEEF COWS

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MASTER OF SCIENCE

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ABSTRACT

Overwintered, gestating beef cows fed low-quality forage are at risk of nutrient restriction which can lead to compromised growth and poor carcass quality in their offspring. In many species, poor maternal nutrition also results in reduced uteroplacental hemodynamics and fetal growth. To better understand how different nutritional paradigms influence the cow, our lab has examined the impacts of nutrient restriction and diet composition on uterine hemodynamics. Previous research suggests that dietary intake or protein supplementation alone does not increase uterine blood flow. The current study examined the effects of dietary starch supplementation and our findings indicate that increasing starch composition of the diet does not alter uterine hemodynamics or fetal growth. Perhaps, a more successful feeding strategy could include a balanced protein to energy ratio and *ad libitum* access to forage, allowing the cows to increase intake without the substitution effect documented in this study. These ideas merit further investigation.

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"*Never doubt that a small group of thoughtful, committed people can change the world. Indeed, it is the only thing that ever has*." - unknown

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LIST OF ABBREVIATIONS

ADF.....acid detergent fiber

ADG .....average daily gain

BCS .....body condition score

BNC .....binucleate cell

BW .....body weight

CON .....control

CP .....crude protein

CRL .....crown-rump length

CSB .....concentrated separator by-product

DAG.....diacylglycerol

DDGS.....dried distiller's grains plus solubles

DHIA .....dairy herd improvement association

DMI .....dry-matter intake

E_2.....estradiol 17β

eNOS .....endothelial nitric oxide synthase

G to F .....gain to feed

HCW .....hot carcass weight

HG .....heart-girth

HR .....heart rate

IP_3.....inositol trisphosphate

IUGR .....intrauterine growth restriction

ME.....metabolizable energy

NDF .....neutral detergent fiber

NEFA .....non-esterified fatty acid

NEm .....net energy of maintenance

NO.....nitric oxide

NRC .....national research council

PI.....pulsatility index

PIP_2.....phosphatidylinositol 4,5-bisphosphate

RFI .....residual feed intake

RI.....resistance index

RIA.....radioimmunoassay

RIC.....roughage intake control

SUP .....supplemented

T3 .....triiodothyronine

T4 .....thyroxine

TDN .....total digestible nutrients

TMR.....total mixed ration

VAD.....vascular area density

VSD.....vascular surface density

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CHAPTER 1. LITERATURE REVIEW

The placenta: a historical perspective

Humanity has long been fascinated with the origins of life, recognizing the placenta was intimately involved in the miracle of pregnancy (Power and Schulkin, 2012). Mystery and superstition has shrouded this biologically marvelous transient organ, including the belief that the placenta and its former owner shared a connection after birth (Ploss & Bartels, 1935; reviewed in Longo and Reynolds, 2010). The ancient Egyptian culture preserved the placenta to mummify placentas as part of the "external soul", burying them with their owners to accompany them into the afterlife (Seligman and Murray, 1911; Power and Schulkin, 2012). Additionally, another theory states that later pharaohs believed their placentas were their stillborn twin, and as such, also considered divine and to bring prosperity is even depicted in art (Seligman and Murray, 1911).

The Egyptians were not alone in their beliefs as many cultures in Pacific Islands, Australasia, and Africa also considered the placenta to possess supernatural powers or an immaterial portion of the human soul and were often buried with their deceased owner or near the family residence (Longo, 1963; Power and Schulkin, 2012). Even the Hebrew scriptures refer to the placenta as the "bundle of life" (Stirrat, 1998). In addition to its proposed supernatural properties, the placenta was steeped in superstition, believed to bring luck to the individual in possession (Longo and Reynolds, 2010). Sailors from the 1700s until the early 1900s purchased "cauls", or amniotic tissue as a good luck charm to protect them from drowning (Forbes, 1953).

From a scientific perspective, Ionian physician, Diogenes of Apollonia (c. 480 BC) was the first documented individual to postulate that the placenta was the organ of fetal nutrition (Power and Schulkin, 2012). Aristotle joined the philosophical and physiological discussion of

the function of the placenta. He suggested that the placenta worked like a yolk sac he observed in chick embryos, further suggesting the metabolic role of the placenta in development of the fetus (reviewed in Power and Schulkin, 2012). In fact, his comparative anatomy descriptions and drawings amusingly led him to depict the gravid human uterus as having a ruminant cotyledonary placenta (Longo and Reynolds, 2010). Grecian physician Claudius Glaenus or Galen (c 130-201 A.D.) agreed with Aristotle's suggested function of the placenta, suggesting the fetus is nourished by direct contact with the maternal blood through the unification of the maternal and fetal vasculature to supply nutrients via the umbilical cord (Galen, 1914; De Witt, 1959).

It wasn't until the 1700's that John and William Hunter demonstrated that maternal and fetal blood supplies were not shared (Power and Schulkin, 2012). However, the importance of maternal vascular supply of blood to the maternofetal interface for the transport of nutrients and gasses to occur is still critical for the prevention of intrauterine growth restriction pregnancies.

Regardless of historical superstition or cultural significance, the placenta still captivates scientists across the world today, promising to play an important part in improving global food production and improving human health. It is not surprising that Nathanielsz (2006) implicated the placenta as playing a key role in the developmental programming, in his 10 principles of developmental programming for the developmental origins of adult diseases.

Placental development and function in the bovine

The placenta is remarkable transient organ which serves as an artificial lung, kidney, and liver for the developing fetus as well as protects the conceptus from attacks from the maternal immune system. Because cattle utilized for meat production spend over one-third of their lives *in utero*, understanding how the bovine placenta develops and vascularizes over pregnancy is

critical for targeting therapeutics nutritional interventions that could potentially improve later carcass quality.

Approximately 4 to 5 days after ovulation, the bovine morula enters the uterus and is transformed into a blastocyst (Rowson and Moor, 1966). At 9-10 days after estrus, the bovine blastocysts hatches after loss of the zona pellucida (Flechon and Renard, 1978). The conceptus elongates and produces of bovine interferon τ as the maternal recognition of pregnancy signal approximately 12 to 16 days after conception (reviewed in Wooding and Burton, 2008). The conceptus then immobilizes itself by extending cellular protrusions called trophectodermal papillae down into the uterine glands (Wooding and Staples, 1981). Approximately 16 days after fertilization, the trophectoderm and uterine epithelium develop microvillar interdigitation over the central region of the conceptus (reviewed in Wooding and Burton, 2008).

The synepitheliochorial placental structure of the cow is characterized by presence of binucleate cells (BNC) which migrate during implantation and fusion with the caruncular epithelium to cause a shift from cellular to maternofetal syncytial plaques (Wooding, 1984; Wango et al., 1990). BNCs have two principal functions: 1) to create the maternofetal syncytium necessary for proper implantation and 2) to promote placentomal growth, produce and deliver a variety of steroid and protein hormones (Wooding, 1992).

Cotyledonary placenta

The bovine cotyledonary placenta is characterized by attachment of the fetal placenta (cotyledon) to discrete, aglandular locations along the uterine wall described as caruncles (reviewed in Vonnahme et al., 2013). These caruncles are present in the non-pregnant female, organized into 2 dorsal and 2 ventral rows that extend into both uterine horns (Ford, 1999). The cotyledonary-caruncular unit is referred to as a placentome serves as the site of maternal-fetal

exchange as well as transient as an endocrine organ to help sustain pregnancy (reviewed in Vonnahme et al., 2013).

The key evolutionary advantage of concentrating the fetal villi into these compact placentomes is increased (5 to 10x) surface area for maternal-fetal exchange (Baur, 1977). The villous development of the fetal cotyledon begins approximately d 28 to 30 post-conception (King et al., 1979) concurrent with chorioallantoic fusion (reviewed in Wooding and Burton, 2008). This event can be detected by the presence of clusters of binucleate cells (BNC) in the trophectodermal endothelium and the fetal villi (reviewed in Wooding and Burton, 2008). Patterns of vascularity are established as subepithelial maternal capillaries push the maternofetal syncytium and trophectoderm back toward the chorion (reviewed in Wooding and Burton, 2008). The production of angiogenic and growth factors by both the maternal and fetal vascular systems promote mutual growth of the caruncular and cotyledonary portions of the placentome (Wooding and Burton, 2008). The villus vasculature of the cow is complex in nature, most closely resembling the goat with countercurrent and/or cross-current flow in the fetal cotyledon (Wooding and Burton, 2008).

Vascularization of the bovine placenta over gestation

The formation of the uteroplacental unit is one of the earliest and most critical requirements during development of the conceptus (Reynolds and Redmer, 1995). Uteroplacental blood flow has been demonstrated to be directly related to placental nutrient transport efficiency and, consequently, fetal growth restriction is highly correlated to aberrations in uteroplacental growth and development (Reynolds and Redmer, 1995). Both uterine blood flow and uterine epithelial growth increase to prepare for implantation of the embryo (Reynolds and Redmer, 1992). Unlike the sheep placenta, cotyledonary growth in bovine increases throughout gestation

(Vonnahme et al., 2007) and thus factors influencing vascularity could impact cotyledonary function throughout gestation. Sheep and cattle have different placentomal shapes (concave vs. convex) and the convex bovine placentome has been suggested to be more efficient because its shape lends itself to a more highly vascularized maternofetal interface per gram of tissue (Reynolds et al., 2005). These characteristics are attributed to greater complexity of the branching of terminal fetal villi which are vascularized by central arterioles extending to the tops of the fetal villi and branching to create extensive small lateral capillary beds approximately 150 to 300 μm (reviewed in Wooding and Burton, 2008). These capillary networks are longer and more prominent than sheep, resembling a starfish, and are drained by central venules (reviewed in Wooding and Burton, 2008).

On the maternal side, the villi of the peripheral capillary beds are supplied by blood from the central arterioles and drained by the venules at the base or center of the villi (reviewed in Wooding and Burton, 2008). The relationship of maternal and fetal blood flow is similar to other ruminants aside from maternal blood drainage from the central arteriole in contrast to the sheep which the maternal capillary cascade exists between individual terminal villi to continuously drain back to the basal veins (reviewed in Wooding and Burton, 2008).

Vascular changes in capillary area density are well pronounced in sheep with 200 and 400% increases in caruncular and cotyledonary tissue from mid- to late-gestation (Borowicz et al., 2007). In comparison, bovine sees more modest changes with a 30% decrease in caruncular vascularity and a 190% increase in cotyledonary vascularity during mid- to late-gestation (Vonnahme et al., 2007). In contrast to the sheep, the bovine placentome continues to grow all of pregnancy. Caruncular and cotyledonary tissue increased by 530% and 640% in mass respectively from mid- to late-gestation (Vonnahme et al., 2007). These differences in placental

growth patterns could potentially explain why the bovine placenta is not as sensitive to nutritional deficiencies as the ovine placenta (Ferrell, 1989; Greenwood and Café, 2007).

Maternofetal exchange

The effective transport of nutrients and factors is a fundamental function of the placenta. For blood borne molecules to reach fetal circulation, it must cross up to 5 to 6 layers of separation between maternal and fetal circulation. Several transport mechanisms exist to aid the transport of desired factors to and from fetal circulation: 1) simple diffusion; 2) facilitated diffusion; 3) ATP-required transport processes of active transport; and 4) the processes endocytosis or phagocytosis can allow factors to be taken up by the placenta and enter fetal circulation (reviewed in Wooding and Burton, 2008). While these processes might seem simple, true control of nutrient transfer is often complex.

Simple diffusion

While a large variety of factors can influence simple diffusion rates, blood flow rates are deemed of the utmost importance due to the high diffusing capacity of oxygen and the high exchange reaction with hemoglobin (reviewed in Wooding and Burton, 2008). Rates of diffusion can be altered by not only concentration gradients, but also diffusion distance, utilization of transported molecules by the placental tissue, and the rate and pattern of maternal and fetal hemodynamics (Wooding and Burton, 2008). The anatomical relationship between maternal and fetal blood flows (directionality) plays a key role in efficiency of transfer (i.e., concurrent and cross-current exchanges are less efficient than countercurrent exchanges; Silver and Steven, 1994; Leiser and Kaufmann, 1994). Distance between maternal and fetal vascular beds further limits the efficiency of transfer of molecules (Allen and Stewart, 2001). However, as gestation advances, maternal and fetal capillaries compress the uterine epithelium and trophectoderm

respectively, reducing the distance required for diffusion (reviewed in Wooding and Burton, 2008). While this effect is observed across all species studied thus far, the sheep placenta is much more efficient (as measured by distance for molecules to diffuse) when compared to the bovine placenta (1.0 vs. 6.0 μm; Wooding and Fowden, 2006). Additionally, utilization of molecules (like oxygen) by the placenta also plays a role in the efficiency of its transfer (Silver and Comline, 1975; Silver and Steven, 1975). Biologically relevant molecules that are transported through simple diffusion include molecular oxygen and carbon dioxide (reviewed in Wooding and Burton, 2008).

Facilitated diffusion

Facilitate diffusion can greatly increase the speed of transport of membrane transporter specific molecules compared to simple diffusion. Control of this process includes relative concentration differences between maternal and fetal circulation, kinetic efficiency, and transporter density, ligand affinity, stereospecificity and saturation (reviewed in Wooding and Burton, 2008). These variables can change with the metabolic demands of the growing conceptus as gestation advances. One example of the necessity of active transport is due to the extensive water demand of the fetus which creates a continuous flow driven by osmotic and hydrostatic differences via aquaporin transporter channels (Atkinson et al., 2006). Biologically relevant molecules that are transported through mechanism include glucose and lactate (reviewed in Wooding and Burton, 2008). In fact, the requirements of glucose vary by species and are lower in the developing bovine, than porcine and equine (Fowden, 1997).

Active transport

The transport of molecules against gradients (electrochemical gradient, concentration) necessities energy, although species-specific variation exists between maternofetal ratios of key

nutrients (reviewed in Wooding and Burton, 2008). An additional layer of complication exists as many of the molecules being transported via active transport have diverse physical properties and are currently understudied in the bovine. Important biological molecules that are transported through active transport include amino acids, ions like sodium, potassium, chloride and phosphates, as well as free fatty acids, glycerol and cholesterol.

Endocytosis and phagocytosis

The placental areolae are the portion of the bovine placenta responsible for the uptake of histotrophic secretions via small endocytotic vesicles or larger phagocytotic vesicles which is critical during early pregnancy (Spencer et al., 2004a, b). While the role of the histotroph in later pregnancy is understudied, it is hypothesized to continue to supply micronutrient to the uteroplacental unit such as calcium ions

Brief overview of developmental programming classical literature

Walton and Hammond (1938) were some of the first to suggest that maternal uterine environment, influenced by many factors including nutrition, could contribute to the growth of the offspring through their famous study involving reciprocal crosses of Shire and Shetland horses. One of the first documented studies in livestock species that highlighted the role of maternal nutrition in proper fetal and placental development was Wallace (1948) who demonstrated how ewes on different planes of nutrition during gestation produced lambs that grew at different rates. Foote et al. (1959) conducted similar studies by nutrient restricting various yearling ewe lambs. While they documented mixed effects of maternal nutrition on fetal growth, the placenta was consistently impacted by altered caruncular and cotyledonary growth (Foote et al., 1959). The famous 1962 “thrifty genotype” for diabetes mellitus was postulated by James Neel to describe large infants born to mothers with diabetes, then later developed diabetes

mellitus in adult life. He suggested that this "thrifty genotype" could have been beneficial to hunter/gatherer ancestors as the extra adiposity of that individual could help them survive longer during periods of food shortages (Neel, 1962).

The infamous "Dutch Hunger Winter" is a historical cohort study of the Dutch famine during 1944-1945 of the perfect storm of horrific environmental conditions. In response to a failed strike on a Nazi occupied bridge, the Germans retaliated by restricting Holland's food supply. This restriction of food (as few as 450 calories at the most severe time) was accompanied by an inordinately frigid winter and the psychological stresses of war (Ravelli et al., 1976; Nathanielsz, 2006). The complex interaction of these harsh environmental factors compromised the health of children *in utero* during this time, resulting in greater incidence of chronic metabolic diseases like obesity and diabetes (Ravelli et al., 1976; Nathanielsz, 2006). While severity of each child's symptoms depended on stage of gestation during maternal nutrient restriction and location in Holland, this humanitarian crisis is an example of the previously discussed "thrifty hypothesis" and how maternal physiology was helping prepare her offspring for the postnatal environment it would face.

This idea that improper maternal nutrition in late pregnancy decreases offspring performance had already been suggested by Wallace (1948) and because the majority (75%) of fetal growth occurs over the last two months of gestation (Robinson et al., 1977) it is logical to understand why adequate maternal nutrition during late gestation is critical for maintaining fetal growth. These deleterious effects observed in offspring of nutrient restricted dams are not limited to neonatal growth and health, rather this "programmed" effect has a profound effect on lifelong growth and increases the likelihood of developing non-communicable diseases later in life (Barker et al., 1993; Godfrey and Barker, 2000). This concept of the fetal origins of health and

disease was proposed by Dr. David Barker and has since given rise to the area of research referred to as fetal or developmental programming (Barker et al., 1993; Godfrey and Barker, 2000). Barker observed that low birth weight babies were pre-dispositioned towards cardiovascular disease, hypertension and other metabolically driven disorders like obesity and diabetes (Barker et al., 1993; Godfrey and Barker, 2000; Hales and Barker, 2001)

Even more recently are the proposed concepts of the lactocrine hypothesis and the second-hits phenotype. The lactocrine hypothesis was proposed by Skip Bartol and describes another developmental programming mechanisms that allows the dam to modify her progenies metabolism through non-nutritive milk-borne bioactive factors through nursing (Bartol et al., 2008; Bagnell et al., 2017). The original study using milk-borne relaxin to investigate the reprogramming of the porcine endometrium was among the first evidence for this hypothesis (Bartol et al., 2008). This exciting new field promises to offer further explanation into developmental programming mechanisms and should be further investigated across all species.

Another recently pioneered concept is the idea of the second-hits phenotype. Growing epidemiological evidence suggests that developmental programming effects can be numerous, multigenerational, and occur across the lifetime of the offspring (Nathanielsz, 2006; Messer et al., 2015). It has been hypothesized that heightened sensitivity is one of the mechanisms exacerbated by multiple, "second-hit" exposures across generations that result in poorer health outcomes (Messer et al., 2015). Several rodent studies have evaluated the "second-hit phenotype" for offspring after subjection to two adverse environments and the interaction between the two insults caused phenotypic alterations and reduced health and fitness (Messer et al., 2015). While the field of developmental programming has been recognized for some time,

much is still yet to be understood about the consequences of various maternal insults as well as developing therapeutics for these stressors, especially if more than one can occur.

In addition to the evident need for developmental programming research in biomedical science, improper maternal nutrition during pregnancy also has profound consequences on livestock offspring health and performance (Wu et al., 2004). Compounding on offspring altered growth trajectories, improper maternal nutrition also leads to a decrease in the carcass quality of the offspring, including altered fat deposition, muscle fiber type and reduced meat quality (Wu et al., 2006).

Intrauterine growth restriction and experimental models

Intrauterine growth restriction (IUGR) is a significant health complication that prevents the fetus from reaching its full growth potential *in utero* (Anthony et al., 2003). Not only does IUGR affect approximately 8% of human pregnancies worldwide, it is the second leading cause of infant mortality and morbidity preceded only by premature birth (Anthony et al., 2003; Carr et al., 2013; Swanson and David, 2015). As evidenced by epidemiological studies across the years, humanitarian crises like the Dutch Hunger Winter provided insights into the lifelong consequences of developmental programming effects and necessitate further research to develop therapeutics to ameliorate such effects. In addition to the obvious ethical limitations in recreating these conditions in humans to develop therapeutic interventions, it is also difficult to tease apart the many environmental conditions affecting the Dutch Hunger Winter cohort survivors. Thus, animal models for fetal growth restriction have been widely utilized as they can provide the biological complexity to more effectively model human IUGR with the potential to repeatedly sample both maternal and fetal tissues to test how they respond to various physiological stressors.

As gestation progresses, uterine and umbilical blood flows increase exponentially (Reynolds and Redmer, 1995). This increase is imperative to supply the metabolic needs of fetus and promote normal fetal growth as uptake of glucose, oxygen, and water must all increase as gestation advances to sustain fetal growth (Meschia, 1983; Ferrell, 1989; Reynolds and Redmer, 1995). The placenta's key function is to provide for the physiological exchange between the mother and the developing fetus (Reynolds and Redmer, 1995). In fact, placental insufficiency is the primary cause of inadequate transfer of nutrients to the fetus, asymmetrical fetal growth and in severe cases, it can induce fetal hypoxia, hypoglycemia, and acidosis (Anthony et al., 2003). Glucose uptake by gravid uteroplacental tissue is reduced as placental mass and blood flow decrease in compromised pregnancies (Reynolds et al., 1985; Wallace et al., 2002). Notably, IUGR pregnancies characterized by marked reductions in uteroplacental blood flow, and the only prevention is to maintain appropriate blood flow for adequate nutrient transport to the fetus (Reynolds and Redmer, 1995; Anthony et al., 2003; Reynolds et al., 2005).

A variety of physiological and environmental factors have been implicated in reducing utero-placental blood flow, increasing vascular resistance, and altering fetal oxygen and nutrient uptake (North et al., 1994). Factors such as maternal nutrition, fetus number, maternal age, physiological stressors such as glucocorticoids, and environmental factors such as heat stress and altitude (Reynolds and Redmer, 1995; Anthony et al., 2003; Reynolds et al., 2006).

Improper maternal nutrition

The most prevalent studies on developmental programming in animal models have concentrated on various aspects improper nutrition including both maternal undernutrition and overnutrition of the adolescent mother. A summary of these studies can be found in Appendix Table A.1, A.2, and A.7. Timing of nutritional insult is also important as most fetal growth is in

the last 2 months of gestation (Robinson, 1977). While most fetal growth occurs during late gestation, inadequate nutrition during early gestation can have profound effects on placental development, vascularization, and fetal organogenesis (Funston et al., 2010a). Adult sheep that are nutrient restricted during late gestation experience a 17 to 32% decrease in uterine blood flow, decreased caruncular capillary area density as well as reduced fetal weight (Anthony et al., 2003; reviewed in Reynolds et al., 2006). Additionally, underfed multiparous cows that were nutrient restricted during mid and late gestation experienced significant reductions in calf birth weights (reviewed in Greenwood and Café, 2007).

Underfed adolescent animals often respond differently than mature animals. Heifers that were nutrient restricted during gestation experienced more extreme birth weight reductions in their calves than mature beef cows (reviewed in Greenwood and Café, 2007). Additionally, overfed adolescent sheep experienced a reduction in fetal weights and a decrease in caruncular capillary area density (Luther et al., 2005). The effects of nutrient restriction and subsequent supplementation on birth weight are highly variable due to variation in forage quality, composition of the diet, timing of restriction, severity of restriction. Additionally, placental alterations can occur due to altered maternal nutrition during early-to mid-gestation without impacting fetal weights (Rasby et al., 1990). The bovine placenta also appears to be sensitive to protein supplementation during early- to mid-gestation but calf birth weight was not impacted (Perry et al., 1999; Perry et al., 2002). However, because the bovine placenta continues to grow throughout gestation (Prior and Laster, 1979; Ferrell, 1989) it has been suggested, that the bovine placenta is not as sensitive to nutritional deficiencies as the ovine placenta (Ferrell, 1989; Greenwood and Café, 2007).

While undernutrition can dramatically impact fetal growth, overnutrition of adolescent sheep can also have detrimental impacts on fetal weight, placental weight, uterine and umbilical blood flows, and total capillary volume (Wallace et al., 2002; Redmer et al., 2004). Additionally, when multiparous beef cows were overfed (150% NRC) during gestation, expression of genes influencing meat quality were altered in the offspring longissimus dorsi (Duarte et al., 2014). The calves from gestationally overfed dams had decreased expression of the myogenic marker, β- catenin, as well as increased the adipogenic markers, zing finger protein-424, CCAAT-enhancer-binding proteins α, and peroxisome proliferator-activated receptor γ (Duarte et al., 2014). Furthermore, fibrogenic markers such as transforming growth factor-β and collagen III were also increased in calves from overfed dams (Duarte et al., 2014). Unlike overfed adolescent sheep, the impact of overfeeding heifers during mid-gestation seems to be more limited with no influence on fetal growth (Jennings et al., 2016). Maternal overnutrition of heifers did increase preadipocyte factor-1 and μ-calpain expression in fetal loin muscle at 180 of gestation (Jennings et al., 2016). Currently, the consequences of overnutrition in the heifer and impacts on offspring carcass quality, fertility, and health are under-investigated and more work should be done in this area.

Age and parity

Due to the size and metabolic requirements of a growing animal, age can influence fetal growth. Adolescent sheep produced smaller offspring with reduced placental weights compared to mature sheep (Borowicz et al., 2005). Additionally, heifers give birth to smaller calves than cows (Holland and Odde, 1992) and are more susceptible to nutrient restriction especially if they are carrying male calves from high birth weight offspring (Hennessy et al., 2002). Currently, not much is known about overnutrition of beef heifers and its influence on birth weights.

Fetal number

While twin calves are not a common occurrence with traditional beef cattle management strategy, twin pregnancies reduce individual calf weights, as well as reduced placentome numbers and placental weights (Greenwood et al., 2000; reviewed in Greenwood and Café, 2007). Additionally, cows carrying twins are more sensitive to nutritional insults than dams carrying singleton pregnancies (Wilkins et al., 1994). In sheep, ewes carrying triplets compared to singleton pregnancies gave birth to smaller lambs, experienced reductions in placental weight, and uterine blood flow (Christenson and Prior, 1978; Grazul-Bilska et al., 2006; Vonnahme et al., 2008). Interestingly, cows carrying twin calves had greater circulating levels of circulating plasma pregnancy associated glycoproteins and vascular endothelial growth factor (Echternkamp et al., 2006). Fetal number can also influence carcass quality as twin calves were lighter at weaning and had reduced HCW but had improved marbling scores and a greater percentage graded choice (Echtenkamp and Gregory, 2002).

Heat stress

Environmental conditions such as ambient temperature can regulate blood flow to the peripheral tissues and the lungs resulting in altered blood flow and nutrient supply to the gravid uterus (Reynolds et al., 1985). Chronic heat stress in sheep reduces fetal growth and placental weight, as well as decreases uterine and umbilical blood flows (Bell et al., 1987; Regnault et al., 2003). Not only does chronic heat stress in sheep reduced lamb birth weights and alter uteroplacental hemodynamics, it is also detrimental to calf birth weights. Beef cows that were chronically heat stressed during mid-pregnancy experienced an 18% reduction in fetal BW (Reynolds et al., 1985). Both uterine and umbilical hemodynamics were reduced in heat stressed cows as well as uterine arterial circulating oxygen, lactate, and glucose (Reynolds et al., 1985).

Furthermore, fetuses from heat-stressed cows had reduced liver, heart and spleen weights and reduced RNA and protein in fetal liver and heart (Reynolds et al., 1985). However, umbilical arterial nutrient flux remained unaltered by heat stress (Reynolds et al., 1985). When shade was provided to gestating beef cows, birth weights increased by 3.1 kg (Collier et al., 1982). Conversely, failing to meet the nutritional requirements of overwintered, gestating beef cows that are exposed to severe cold can also reduce fetal growth as more energy requirements for the dam are greater to maintain body temperature (Andreoli et al., 1988).

Glucocorticoid

The administration of exogenous glucocorticoids has been established to reduce umbilical blood flow in sheep and reduce the fetal hemodynamic response to acute hypoxemic stress (Jellyman et al., 2004). Furthermore, elevated cortisol has been documented as a physiological response to heat stress in late-gestating beef cows (Wright et al., 2014). Circulating cortisol in gestating sheep can also decreased by nutrient restricting (60% NRC) and increased by overfeeding (140% NRC) in late gestation (Vonnahme et al., 2013; Lemley et al., 2014). Furthermore, the injection of exogenous cortisol into the d 130 sheep fetus decreases binucleate cell production, although the mechanisms involved have yet to be determined (Wooding and Burton, 2008). Additionally, the impacts of chronic exogenous glucocorticoid administration across pregnancy in beef cows has still yet to be investigated.

Altitude

Pregnancies that occur in a high-altitude environment often become IUGR due to hypoxic conditions and having high oxidative stress (Robinson et al., 1995; Parraguez et al., 2015). Women living in high-altitude environments during pregnancy often give birth to smaller babies which suffer from greater infant mortality (reviewed in Parraguez et al., 2015). When pregnant

women were exposed to hypoxic conditions in late pregnancy, a 35% reduction of uterine blood flow was documented (Zamudio et al., 1995). While sheep fetal weights were not always reduced in fetal hypoxia models (hypobaric stress), capillary area density was increased, suggesting that the placenta adapted to preserve fetal nutrient supply (Krebs et al., 1997; Parraguez et al., 2006). The effects of high altitude in beef cows include late feedlot death associated with right heart failure because of vascular remodeling attributed to being born and raised in high-altitude (Neary et al., 2015). Perhaps this could also be influenced by maternal exposure to chronic high-altitude which could influence uterine hemodynamics and more research should be conducted in this area.

Bovine models of fetal programming

Global nutrient restriction

Global nutrient restriction on the dam during various stages of gestation can impair placental function and calf growth depending on the severity of the restriction, timing of nutritional insult, parity, and pregnancy type. A summary of these studies can be found in Appendix Tables A.1, A.2, and A.7. In comparison to sheep, the developing bovine fetus that is subjected to maternal nutrient restriction during mid- to late-gestation is more susceptible to alterations in myogenesis and adipogenesis (Greenwood and Café, 2007) whereas early nutritional restrictions can have subtle effects on organogenesis, causing long-term health complications (Greenwood and Bell, 2003; Bell et al., 2005). Pre-breeding management of heifers influenced uterine hemodynamics as low input (fed to 45 to 55% of mature BW at breeding) had increased uterine blood flow when adjusted for maternal BW when compared to conventionally fed heifers (65 to 70% of mature BW at breeding), suggesting that this increase in

uterine blood flow helped compensate for the inadequate heifer management practice (Cain et al., 2017).

When heifers were fed diets that did not meet their daily nutritional requirements of either ME or CP during early- to mid-gestation, a reduction in fetal weights was observed (Micke et al., 2010a). The reduction in fetal weights corresponded to a decrease in umbilical diameter and circulating progesterone in the nutrient restricted animals coupled with increased estrone sulfate and bovine placental lactogen during early gestation (Sullivan et al., 2009; Micke et al., 2010a). Interestingly, heifers during mid-gestation that were not fed to meet their nutritional requirements saw an increase in umbilical diameter compared to adequately fed heifers (Sullivan et al., 2009; Micket et al., 2010a). Perhaps this was a compensatory mechanism to support fetal growth, as evidenced by greater fetal abdominal circumference and crown-nose length and thoracic diameter (Sullivan et al., 2009; Micket et al., 2010a). However, the decrease in performance due to nutrient restriction does not always occur as heifers that were nutrient restricted to 60% of their NRC requirements in early gestation (conception to d 60 gestation) then were realimented had normal calf birth weights and placental weights (Spiegler et al., 2014).

In contrast, multiparous beef cows that were nutrient restricted (60% NRC) in early pregnancy (d 30 to 85) experienced a greater BW percentage change, elevated cholesterol and lactate in maternal circulation, and produced altered fetal growth (Camacho et al., 2014a; Camacho., 2013). These fetuses displayed a greater ponderal index, larger livers and pancreata, as well as placentas with more placentomes (Camacho et al., 2014a and Camacho, 2013). When the nutrient restriction was continued until mid-gestation (d 85 to 140), cows had reductions in maternal BW, circulating maternal fructose, umbilical arterial blood flow: fetal BW as well as

increased placentome number and decreased placental efficiency (Camacho et al., 2014a and Camacho, 2013). However, upon realimentation in late gestation (d 140 to 254), previously restricted cows saw greater uterine blood flow which allowed fetal growth to compensate for previous nutrient restriction (Camacho et al., 2014b). Additionally, cows that were realimented after early- to mid-gestation (d 30 to 140) nutrient restriction saw an increase in ipsilateral uterine blood flow although total uterine arterial blood flow was unaffected (Camacho et al., 2014b).

However, when global nutrient restriction occurs during times of greater fetal growth without realimentation, dams that were fed inadequate nutrition over mid- to late-pregnancy (d 118 to term) and progressively lost body condition over pregnancy, not surprisingly saw a reduction in calf birth weights (Freetly et al., 2000). It seems that the combined mid- to late-gestation nutrient restriction causes more changes in calf weight at birth because heifers that were nutrient restricted (60% NRC) during late gestation (d 213 to term) produced calves with normal birth weights and placentas (Spiegler et al., 2014). This would agree with a study by Corah et al. (1975) where heifers were nutrient restricted to 57% of their NRC requirements and saw a reduction in fetal BW. In contrast, no birth weight differences were detected when mature cows were nutrient restricted to 55% of their NRC requirements (Hough et al., 1990). Perhaps, multiparous cows have a compensatory mechanism for maintaining proper fetal growth.

The placenta's ability to adapt to maternal insults is critical for maintaining normal fetal growth, and global nutrient restriction in early pregnancy demonstrates this compensatory mechanism by increasing markers of vascular permeability such as placental growth factor and fms-like tyrosine kinase I in multiparous beef cows (Vonnahme et al., 2007). Furthermore, the bovine placenta demonstrates a placental programming effect when early pregnancy (d 30 to125)

global nutrient restricted cows are realimented and then fed a diet meeting their NRC requirements until d 250 of pregnancy as caruncular capillary surface density increases (Vonnahme et al., 2007). Interestingly, the corresponding cotyledonary tissue saw decreases vascularity as capillary area density, capillary number density, and capillary surface density in the fetuses that were nutrient restricted in early pregnancy (Vonnahme et al., 2007).

Those same animals not only saw reductions in vascularity, but reductions in both cotyledonary and caruncular tissue at both d 125 and d 250 as well as a covalent (phosphorylation) upregulation of protein kinase B and mitogen-activated protein kinase 1 in nutrient restricted cows in d 125 (Zhu et al., 2006). Additionally, not all the global nutrient restricted cows had reduced birth weight pregnancies, some were able to compensate for the nutritional inadequacies of their diet and sustain normal fetal growth (Long et al., 2009). The mechanism that allows some cows to physiologically compensate for nutritional inadequacies and sustain fetal growth could unlock potential therapeutic targets for bovine IUGR pregnancies and should be further investigated.

Global nutrient restriction can not only influence bovine fetal growth and placental function, it can have profound impacts on postnatal growth and ultimately, red meat production. When heifers were nutrient restricted (low ME and CP) during early and mid-gestation then later realimented saw not only reduced birth weights, but reduced leptin and insulin-like growth factor-1 and 2 receptor expression in adipose tissue (Micke et al., 2010b). Furthermore, heifers that only received 55% of their NRC requirements during early gestation (d 32 to 115) saw reduced lung and trachea weights in steers with lower DNA concentration and increased muscle fiber diameter and faster glucose clearance; however, feed efficiency was unaffected (Long et al., 2010a; Long et al., 2010b). Interestingly, weaning weights and carcass composition and quality

of those cattle were unaffected (Long et al., 2010a; Long et al., 2010b). Gonzales et al. (2013) could rescue muscle fiber size and muscle progenitor cell numbers through realimentation of late-gestating beef cows that were previously nutrient restricted during early pregnancy.

By increasing the forage quality of late gestating beef cows, Underwood et al. (2010) improved offspring hot carcass weights although earlier differences in neonatal performance were not detected until weaning. The steers from the improved pasture trial also had higher average daily gain and more 12th rib subcutaneous fat at slaughter (Underwood et al., 2010). This would agree with a study by Mohrhauser et al. (2013) that observed when beef cattle were fed diets exceeding their energy requirements during mid-gestation, their offspring had fatter, lower yielding carcasses compared to offspring from dams fed a negative energy diet.

A study by Greenwood et al. (2004) demonstrated that nutrient restricted gestating beef cows resulted in offspring with reduced carcass weights. These findings were congruent with another study examining nutrient restriction in cows and heifers from d 80 of gestation until term, noting that nutrient restricted dams produced lighter weighing calves at birth with lower average daily gain (ADG) and dry matter intake (DMI) in the feed yard, and fatter carcasses at 30 months of age (Café et al., 2006; Café et al., 2009).

Currently, there is a shortage of information about the developmental programming consequences of *in utero* global nutrient restriction on bull and heifer fertility without intervention of protein or energy supplementation of their dam. Martin et al. (2007) did find that dams who were nutrient deficient during late gestation and early lactation produced heifers with lower fertility rates although age at puberty was not altered. Corah et al. (1975) similarly observed that age at puberty of heifers was not altered by maternal nutrient restriction (65%

NRC). However, this area is under-investigated in the bovine, especially in the bull and merits further research.

Dietary maternal protein restriction

Not only does global nutrient restriction reprogram the bovine placenta, causing transgenerational impacts in health and performance, imbalances of key macronutrients like protein also can have similar outcomes. When cows were fed a diet deficient in CP (7% CP vs. 14% CP) but meeting other energetic dietary requirements, they saw a reduction in placental blood vessel density if the cows were moved from 7% CP early to 14% CP in mid-pregnancy and vice versa without impacting birth weights (Perry et al. 1999). Furthermore, dietary CP preconception and during early gestation seems to have sexually dimorphic impacts on uterine hemodynamics and neonatal blood pressure. Heifers that were supplemented with high (14% CP) and low (7% CP) protein during periconception (d -60 to d 23 of gestation) or post-conception (d 23 to 198 of gestation) had reduced uterine blood flow in low periconception protein males at d 150 of gestation but this effect disappeared by d 210 of gestation and interestingly, low periconceptual protein increased male birth weights (Hernandez-Medrano et al., 2015). While heifer birth weights were similar regardless of maternal protein, high protein females were observed to have heavier fetal heart weights at d 98 and reduced HR at birth (Hernandez-Medrano et al., 2015). However, low CP periconceptual diets did increase postnatal blood pressure in heifers (Hernandez-Medrano et al., 2015).

Poor maternal nutrition such as low protein reduces offspring postnatal growth and alters intramuscular fat accretion (Stalker et al., 2006; Funston et al., 2010). These effects extend across species as lambs born to nutrient restricted mothers also had altered muscle formation as well as increased connective tissue and intramuscular adiposity (Zhu et al., 2006). When heifers

receiving adequate nutrition (100% NRC for ME) were protein restricted during early-to mid-gestation, they not only saw reductions in fetal birth weights but had sexually dimorphic differences in postnatal growth and carcass quality (Micke et al., 2010).

Timing of protein restriction as well as fetal sex impacted growth of the offspring as steer calves that were protein restricted during early gestation were heavier at weaning opposed to female offspring that were lighter following protein restriction (Micke et al., 2010). Additionally, female offspring from lower CP and ME dams produced lighter carcasses at slaughter than their high CP and ME counterparts, but this effect was not observed in steers (Micke et al., 2010). Conversely, only high CP and ME male offspring saw increased 12^{th} rib subcutaneous fat at slaughter compared to lower CP and ME steers, and this difference was not observed in females (Micke et al., 2010).

Additionally, when dams received only 70% of their NRC requirements including protein, their offspring displayed larger adipocytes after birth with altered solute carrier family 2-member 4 (glucose transporter) mRNA expression as well as produced lower yielding carcasses (Long et al., 2012). However, when the cows receiving 70% NRC also received additional essential amino acids to bring them up to 100% NRC requirements of protein, this effect was not observed suggesting that protein restriction alters postnatal adiposity and ultimately, red meat production (Long et al., 2012). Additionally, *in utero* protein restriction of heifer calves reduces their performance in the feed yard by reducing their DMI as well as their fertility (Martin et al., 2007). The mechanisms responsible for this effect still need to be elucidated.

Maternal supplementation strategies

Throughout the Midwest, extensively managed cattle operations are still common which can lead to nutrient restriction via low quality forage diets in over-wintered gestating beef cows.

While the specific amino acid requirements are unknown, the supplementation of dietary protein and energy supplements have been examined in the bovine with mixed success. A summary of these studies can be found on Appendix Table A.3 and A.4. When cows that were supplemented with high ME and high CP during early gestation saw decreases in circulating maternal estrone sulfate and bovine placental lactogen while maternal progesterone increased (Sullivan et al., 2009). While greater levels of estrone sulfate was observed to be associated with cotyledon number and weight as well as total placental weight during late gestation, reduced ME and CP diets reduced dam body weights and corresponding calf birth weights (Sullivan et al., 2009; Micke et al., 2010).

Dietary protein seems to have variable effects on umbilical size. When dams received diets high in CP and ME, greater umbilical diameter was observed in early gestation (Micke et al., 2010). However, in mid-gestation, conceptuses from dams consuming low CP and ME diets had larger umbilical diameter than high CP and ME conceptuses (Micke et al., 2010). Furthermore, cows that were supplemented with varying levels of DDGS during late gestation into early lactation saw increases in calf birth weights proportional to the level of maternal supplementation, however, this effect disappeared by weaning (Winterholler et al., 2012). This would agree with Radunz et al. (2010) that observed that dams supplemented with DDGS during late gestation gave rise to calves that were heavier than hay fed offspring at birth but not at weaning. Conversely, Kennedy et al. (2016) observed this increase in birth weight coupled with heavier weaning weights of calves whose dams received DDGS supplementation at 0.3% BW during late gestation (d 200 to 270). This difference is likely due to the improved roughage intakes of DDGS supplemented beef cows and corresponding increase in uterine arterial blood flow (Kennedy et al., 2016).

However, it appears that *ad libitum* access to forage is a critical component of this increase as cows that were supplemented with hay at 2% of BW and DDGS at 1.7 g/ kg of BW daily did not increase calf birth weights or weaning weights and decreased uterine arterial blood flow (Mordhorst et al., 2016). An overabundance of dietary protein during late gestation to heifers was also documented to improve calf birth weights (Gunn et al., 2014). Protein supplementation can also influence milk production as milk composition was altered because of DDGS supplementation as positive energy balance animals and low levels of DDGS supplementation resulted in lower milk protein than high DDGS supplementation levels (Winterholler et al., 2012). This would agree with Kennedy et al. (2017) who also observed that DDGS supplemented altered milk production as both colostrum and milk production were improved in DDGS supplemented dams although total mammary blood flow was unaltered. The supplementation of late-gestational DDGS also did not influence mammary blood flow in the study by Mordhorst et al. (2016).

To examine effective timing of dam protein supplementation and improved forage strategies, multiparous beef cows were provided with either a protein supplement at 0.45 kg/d or no supplement during late gestation and grazed on either subirrigated meadow or cool-season grass hay during lactation (Stalker et al., 2006; Martin et al., 2007). While no differences were detected early in life as calf birth weight, maternal milk production and cow rebreeding rates did not differ with maternal dietary supplementation, calves of protein supplemented dams who grazed subirrigated meadow during lactation saw improved weaning weights (Stalker et al., 2006; Martin et al., 2007). While no differences in carcass composition, carcass quality, or red meat production were detected amongst the steers (Stalker et al., 2006), the heifers of protein supplemented dams saw improved fertility rates and increased DMI (Martin et al., 2007).

Additionally, supplementation of protein to overwintered dams during mid- to late-gestation that were grazed on either winter range or corn residue and were provided with either a protein supplement or no supplement altered postnatal performance (Larson et al. 2009; Funston et al., 2010). Steers calves from dams who grazed winter range and received the protein supplement had heavier weaning weights although feed efficiency traits were not altered (Larson et al., 2009). Furthermore, steer hot carcass weight and USDA quality grades were improved because of protein supplementation (Larson et al., 2009).

In contrast with their previous work, protein supplementation without subirrigated meadow grazing did not alter heifer fertility but did improve ADG and gain to feed in heifers from dams that grazed corn residue with protein supplementation (Funston et al., 2010). When heifers were offered *ad libitum* access to grass hay and supplement at low levels of DDGS (0.83 kg/d) calf weights birth and weaning (Summers et al., 2015a). However, the calves from DDGS supplemented dams had lower feed efficiency as they had higher residual feed intake (RFI) which contrasts with other DDGS supplementation projects (Summers et al., 2015b). Furthermore, the calves from DDGS supplemented dams also had decreased marbling compared to offspring from grass fed only dams (Summers et al., 2015b).

Like many other protein supplementation studies with multiparous beef cows during late gestation, limited impacts of protein supplementation were detected on early life measurements such as calf birth weight and milk production, but differences in postnatal performance were detected by weaning (Shoup et al., 2015a; Shoup et al., 2015b). Age at weaning can also impact how maternal diet influences calf performance. When calves were weaned early (d 78 vs. d 186), calves from protein supplemented saw increased weaning weights compared to non-supplemented calves (Shoup et al., 2015a). However, it was calves from dams on medium plane

of nutrition during pregnancy that excelled in feed lot performance by gaining the most weight (Shoup et al., 2015b) and at the molecular level, experienced an up regulation of proadipogenic markers (miR-103, miR-143, & miR-21) and a down regulation of anti-adopogenic marker, miR-34a (Moisa et al., 2016). Nevertheless, the only carcass traits affected by level of maternal protein supplementation was quality grade as offspring from high supplementation cows had a higher percentage of average choice compared to offspring whose dams did not receive supplementation (Shoup et al., 2015b).

Similarly, Radunz et al. (2012) did not observe any differences in feed efficiency or carcass composition because of the maternal DDGS supplementation. Gunn et al. (2017) also observed limited effects of DDGS supplementation with diets meeting NRC requirements for ME. Calves from dams supplemented with DDGS did not differ in feed efficiency, glucose tolerance, or any carcass characteristics (Gunn et al., 2017). However, milk composition was altered by maternal diet as gestation DDGS supplementation decreased milk fat percentage and total solids while increasing milk urea (Gunn et al., 2014). Calves from protein supplemented dams were also heavier at weaning and heifers receiving a diet greater in protein had larger antral follicles (Gunn et al., 2014; Gunn et al., 2017).

Maternal protein supplementation to mature beef cows at low levels (454 g/d) during late gestation has limited impacts on postnatal performance as weaning weights, feed efficiency, carcass quality, composition and total red meat was not impacted by maternal diet (Mullininks et al., 2013). Additionally, even more protein supplementation can have limited impacts on postnatal performance as multiparous beef cows supplemented with 2.1 kg of DDGS pellets per day did not see improvements in any neonatal measurements of birth weight or milk production, or postnatal measurements of weaning weight, feed efficiency, carcass quality or red meat

production although offspring from non-supplemented cows tended to have more 12th rib subcutaneous fat (Wilson et al., 2015b). There is much variation in nutritional developmental programming models' due to inconsistency between timing of insults (stage of gestation), severity of nutritional insult, and components of the diet (Greenwood and Café, 2007).

Although it is difficult to completely tease apart dietary protein vs. energy requirements in the bovine, due to both nutritional sources converging in volatile fatty acid and microbial protein production, we still do not know individual amino acid requirements for the bovine. While crude concepts of meeting metabolizable protein requirements can prevent negative pregnancy outcomes, it is still reasonable to acknowledge that amino acids act as signaling molecules to play a role in other metabolic processes outside of their function as a nutritional source and thus, the specific roles of individual amino acids and pregnancy in the bovine should be examined.

Impacts of supplemental starch on pregnancy and postnatal outcomes

Due to the convergence of energy and protein metabolism in the ruminant, much of the variation in starch supplementation outcomes on pregnancy and the neonate are due to inclusion level of corn or other high starch supplements. A summary of these studies can be found on Appendix Table A.5 and A.6. Loerch (1996) conducted several studies examining the economic benefits of various levels of corn supplementation to the dam during mid- to late-gestation (over winter feeding). He observed that corn supplementation did not consistently improve dam BW, produce heavier calves at birth or at weaning. (Loerch, 1996). Despite variability of calf growth improvements, he did conclude that corn supplementation was a safe and a more economical way for the beef producer to supply the nutritional demands of their gestating beef cows during winter months (Loerch, 1996). This is congruent with the findings of Schoonmaker et al. (2003)

that also did not observe birth weight or weaning weight improvements of offspring from dams supplemented with corn in addition to roughage. They did observe dam BW improvements after calving with prepartum corn supplementation. However, when 5.3 kg of corn plus 1 kg of supplemental pellet and 2.1 kg of hay were provided to beef cows during late-gestation, calf birth weights were improved compared to hay fed only dams (Radunz et al., 2010). While corn supplementation did not influence colostrum production or cow rebreeding rates, it did improve offspring weaning weights when compared to offspring from hay fed dams (Radunz et al., 2010).

In addition to heavier weaning weights, Radunz et al. (2012) found that beef cows limit-fed corn produced calves that had greater fasting glucose, and corn-fed female offspring had the fastest glucose disappearance rate. At slaughter, offspring of dams fed corn had carcasses with less intramuscular fat than calves from hay fed dams (Radunz et al., 2012). Furthermore, steers from dams that were supplemented with corn gluten feed (0.83 kg/d) and *ad libitum* access to grass hay had lower residual feed intake (RFI) and leaner, higher yielding carcasses than offspring from non-supplemented cows (Summers et al., 2015b). Some effects of maternal diet on muscle quality have also been documented at the molecular level which can be useful for providing insights into the cellular mechanisms altered by maternal diet. Wang et al. (2015) revealed that beef cows fed corn based diets during pregnancy had altered the expression of imprinted genes and DNA methyltransferases in the skeletal muscle of their calves shortly after birth. Combined with the results of a similarly designed study by Lan et al. (2013), lambs from dams fed diets high in starch (corn) during pregnancy also had altered expression of imprinted genes in skeletal muscle and subcutaneous fat.

In addition, by using bisulfite sequencing, Lan et al. (2013) showed DNA methylation pattern differences in the skeletal muscle of lambs from ewes fed corn vs. hay diets during

pregnancy. As intriguing as these molecular changes can be, it should be acknowledged that there is still much variation in terms of how composition of the diet seems to effect carcass quality. An example of this is a study by Wilson and colleagues (2015), who limit- fed a corn coproduct to beef cows during late-gestation, reported improved birth weights but unaltered carcass composition and quality when compared to offspring from hay-fed dams.

Uteroplacental hemodynamics

Uterine arterial blood flow is modulated by two mechanisms: phasic contractility and tonic contractility (Ford, 1995). Phasic contractility modulates short-term contractions of the uterine arterial smooth muscle (5 to 10 minutes) due to factors like maternal stress (reviewed in Vonnahme et al., 2014). Tonic contractility of the uterine arterial smooth muscles promotes an extended contractile state which influences arterial diameter and alters baseline rates of flow (reviewed in Vonnahme et al., 2014).

Catecholamines

Catecholamines are hormones secreted by the adrenal medulla as a sympathetic nervous response (Griffin and Ojeda, 1996). They target α-or β- adrenergic receptors which are responsible for phasic and tonic contractility of the uterine vasculature (Ford, 1995; Grffin and Ojeda, 1996). Phasic contractility is activated by the binding of catecholamines to the α-1 adrenergic receptor which hydrolyzes phosphatidylinositol 4,5- bisphosphate (PIP_2) into inositol triphosphate (IP_3) and diacylglycerol (DAG; Ford, 1995). This stimulates a flood of intracellular calcium thereby activating the calmodulin pathway resulting in muscle contractions and subsequent relaxation after depletion of the free cytosolic calcium (Ford, 1995).

In contrast, tonic contractility is modulated by DAG that was previously produced through PIP_2 hydrolysis binds to protein kinase C (PKC) in the presence of intracellular calcium,

consequently shortening the actin filaments (Ford, 1995). When α-2 adrenergic receptor stimulation occurs, more intracellular calcium is taken up by the potential-sensitive channels which increases the activity of the membrane bound PKC-DAG complex and sustains a tonic vascular contraction (Ford, 1995).

Adrenergic control of uterine blood flow during gestation is primarily controlled through catecholamine stimulation of α-1 adrenergic receptor and decreased activity of α-2 adrenergic receptor which results in reduced uterine vascular tone and increases vasorelaxation (reviewed in Vonnahme et al., 2014). There also appears to be a pregnancy related decrease in sensitivity to α-2 adrenergic receptor to allow sustained decrease in vascular tone and an increase in caruncular blood flow (reviewed in Vonnahme et al., 2014). Catecholamines elicit a vascular response by modulating a strong phasic contractility, resulting in a 90% reduction in uterine blood flow (reviewed in Vonnahme et al., 2014).

The α-2 adrenergic receptor's action is believed to be through the stimulation of calcium influx through potential-sensitive channels along the uterine arterial smooth muscle membranes thereby increasing vascular tone and decreasing caruncular arterial diameter (Ford, 1995). This process is necessary during acute maternal stress to shunt blood away from the viscera, toward the periphery (skeletal muscle for the fight or flight response; Vonnahme et al., 2014). However, because this response is temporary, the fetus should remain viable until baseline uteroplacental blood flow reestablishes equilibrium (reviewed in Vonnahme et al., 2014).

Estradiol 17β

Estradiol 17β is a potent vasodilator that can also alter cardiovascular parameters by increasing maternal heart rate and cardiac output and decreasing vascular resistance (Magness and Rosenfeld, 1989). Estrogens decrease uterine arterial tone thereby increasing arterial

diameter and flow through their conversion to catechol estrogens by peroxidase in uterine lymphatic fluid (Ford, 1995). These catechol estrogens directly inhibit calcium reuptake through potential-sensitive channels, thus reducing the activity of PKC- DAG complexes (Ford, 1995).

The administration of exogenous estradiol 17β systemically increases uterine blood flow but local infusion results in local increases in flow without systemic effects suggesting that estrogen modulations of blood flow occur locally (reviewed in Chang and Zhang, 2008). Estradiol 17β is not the only biologically active estrogen that can elicit increases in uterine blood flow as estrone and estriol have also been documented to have the same effects (reviewed in Chang and Zhang, 2008). A variety of studies have implicated nitric oxide as the main mediator of estrogen induced vasodilation as the inhibition of eNOS reduces the estradiol-mediated vasodilation by 60-70% (reviewed in Chang and Zhang, 2008). In addition to eNOS regulation through calcium-activated calmodulin binding, eNOS is also covalently regulated through phosphorylation from several kinases (reviewed in Chang and Zhang, 2008). Furthermore, a membrane bound protein known as caveolin-1 is thought to regulated estrogen-mediated regulation of estrogen receptor which acts as an endogenous eNOS inhibitor (reviewed in Chang and Zhang, 2008).

Estrogen has also been suggested to increase vascular smooth muscle dependent pathways through cyclic guanosine monophosphate pathway and vascular smooth muscle potassium channels which is a likely mechanism that is responsible for increases in estrous cycle fluctuations in non-pregnant uterine blood flow but is not as critical in the pregnant animal (Rosenfeld et al., 1996). Both genomic and nongenomic responses of estrogens are thought to influence vasoactivity but the genomic impacts are currently understudied.

Progesterone

Progesterone maintains the phasic contractility of the vascular endothelium of the uterine artery by increasing adrenergic receptor-α1 receptors (Ford, 1995). When exogenous progesterone is administered alone, it has no vasodilatory effects but when administered as a cocktail with estrogen, it has an inhibitory effect (Resnek et al., 1977). Progesterone favors caruncular blood flow and is thought to play a role in the increase in caruncular blood flow over pregnancy (Anderson et al., 1977; Rosenfeld et al., 1974). Estrogens increases progesterone receptor transcription but progesterone downregulates its own receptor (reviewed in Chang and Zhang, 2008). Progesterone increase the expression of eNOS in the uterine arterial epithelium but estrogen increases eNOS more robustly which is believed to be due to a downregulation in cavelolin-1 (reviewed in Chang and Zhang, 2008).

Cortisol

Cortisol has also been suggested to play a role in mediating the uterine arterial blood flow increase during late gestation, as adrenalectomized ewes entering late gestation failed to increase uterine blood flow compared to normal and high cortisol ewes (Jensen et al., 2005). In fact, abnormal levels of circulating cortisol are often indicative of IUGR and often results in increased morbidity and mortality (Jensen et al., 2005). Cortisol has been suggested to illicit responses locally and systemically to alter uteroplacental perfusion by altering maternal plasma volume during late gestation in the ewe (Jensen et al., 2002; Jensen et al., 2005).

Interestingly, evidence that these mechanisms are attenuated in pregnant animals compared to non-pregnant animals is suggested in the sheep by the pregnant state altering ligand-receptor affinity and 11-beta-hydroxysteroid dehydrogenase 1 activity (Xiao et al., 2002). Although cortisol in the current study was elevated in the control group, total uterine blood flow

was not altered so it appears that circulating cortisol levels on this trial were not enough to induce vasoconstriction in the control cows or alter fetal growth as calves of both treatments weighed similar amounts. In fact, the control cows which had higher levels of cortisol also had greater cotyledonary vascularity. Additionally, no treatment differences in fetal cortisol because of maternal gestational diet but levels do decrease after birth which agree with Osorio et al. (2013) which also found that maternal nutrition (restricted vs. control) did not impact calf cortisol but calf cortisol did decrease after birth in both treatments.

Conversely, glucocorticoids well recognized for their role in inducing growth restriction pregnancies as they are well documented potentiators of angiotensin II, vasopressin, and norepinephrine, inducing vasconstriction (Yagil and Krakoff, 1988; Xia et al., 2002). They are also shown to decrease eNOS expression and subsequent NO release in both pregnant and non-pregnant animals, although this effect is still not well understood (Malek et al., 1999; Li et al., 2007).

Dietary factors that increase uterine blood flow

During gestation, uterine blood flow increase in three distinct stages: 1) increase in microvascular volume and vasodilation of the uterine artery to aid in an environment suitable for implantation, 2) angiogenesis and vascular remodeling of uterine vascular beds, and 3) a third trimester exponential increase in uterine blood flow and uterine artery vasodilation to support fetal growth (Chang and Zhang, 2008). Maternal nutritional plane has been implicated in altering hemodynamics to the uteroplacental unit (Leketz et al., 2011; Lemley et al., 2012; Kennedy et al., 2016) so proper maternal nutrition can be considered as a potential therapeutic strategy to improve IUGR pregnancy outcomes.

In fact, ewes that were nutrient restricted to 60% of their NRC requirements from mid- to late-gestation experienced a decrease in both umbilical and uterine blood flows (Lemley et al. 2012). Interestingly, when beef cows were nutrient restricted to 60% of their daily NRC requirements during early (d 30 to 140) pregnancy, uterine blood flow was unchanged (Camacho et al., 2014). Perhaps this is due to differences in stage of pregnancy or inherent species-specific differences. Additionally, the supplementation of protein to beef cows in late gestation (d 180 to 246) also increases uterine hemodynamics to the uteroplacental unit and positively benefits fetal growth as calf birth weights were improved (Kennedy et al., 2016). Cows were provided *ad libitum* access to forage and limit fed a DDGS protein supplement (0.3% of BW), dry matter intake was increased (Kennedy et al., 2016a) and uterine arterial blood flows and calf birth weights were both improved (Kennedy et al., 2016b). These studies suggest that perhaps it is dietary metabolizable protein that is driving these changes in uterine blood flow rather than total energy intake.

Not only does protein supplementation have a positive impact on birth weight, but other studies report that maternal dietary protein concentration during gestation effects calf growth and subsequent carcass quality. For example, Shoup et al. (2015b) examined the effects of maternal dietary protein supplementation on offspring carcass quality and found that dams receiving additional protein during late gestation produced calves with increased marbling and subcutaneous fat at slaughter.

However, it also appears that protein supplementation alone is not solely driving blood flow and increased fetal growth. When beef cows were limit-fed forage while being fed a DDGS protein supplement in late gestation (d 190 to d 240), a decrease in uterine blood flows occurred in supplemented cows (Mordhorst et al., 2016). Surprisingly, calf birth weight was unaffected

despite the decrease in uterine blood flow (Mordhorst et al., 2016). This observation suggests that providing a dam with a dietary protein supplement (DDGS) is only effective when complemented by *ad libitum* access to low quality forage as seen by Kennedy et al. (2016). This idea is supported by Lemley et al. (2014) who found that higher dry-matter intakes are linked to higher insulin-like growth factor 1 and perhaps, could increase glucose and amino-acid uptake in both fetal and maternal tissues. Further studies should be conducted to better understand the relationship between nutrient type (energy, MP) and stage of gestation corresponding uterine blood flow changes.

Biophysical factors influencing blood flow

Blood flow is defined as the quantity of blood that passes a specific location within circulation at a specific timepoint (Guyton and Hall, 2015). The distribution of blood flow is driven by tissue specific needs and, thus, can shift during physiological changes. Two principal factors control blood flow through a vessel: 1) pressure difference of blood between entering and leaving a vessel and 2) the impediment to flow through the vessel (Guyton and Hall, 2015). The first factor referred to as a pressure gradient is responsible for pushing the blood through the vessel (Guyton and Hall, 2015). The second factor referred to as vascular resistance is caused by friction between the vascular endothelium and moving blood but cannot be measured directly (Guyton and Hall, 2015). Cardiac output is defined as the quantity of blood pumped into the aorta by the heart during a specific time and is controlled by the sum need of tissue specific needs (Guyton and Hall, 2015). Thus, it is logical that cardiac output should increase as gestation progresses and blood volume expands.

To calculate flow (F) through a vessel, Ohms law ($F = \frac{\Delta P}{R}$) can be utilized which accounts for change in vessel pressure (ΔP) and resistance (R) (Guyton and Hall, 2015)

Depending on the velocity of flow and other factors such as arterial pressure, size of the vessel, etc, blood can either exhibit laminar or turbulent flow (Guyton and Hall, 2015). Laminar flow is characterized by streamline behavior of each layer of blood flowing the same distance from the vessel wall (Guyton and Hall, 2015). In contrast, turbulent flow occurs when blood flow velocity is too great or the linear shape of the vessel is altered (sharp turns, obstructions, etc.) and is characterized by the formation of whorls or eddy currents in the blood (Guyton and Hall, 2015). Turbulence can be further influenced by the pulsatile behavior of the flow and rapid changes in vessel diameter (Guyton and Hall, 2015). To calculate the turbulence of blood flow, the Reynold's number (Re) can be calculated as $Re = \frac{\nu \cdot d \cdot \rho}{\eta}$ where ν represents velocity, d is vessel diameter (cm), ρ is density (g/mL) and η is the viscosity (in poise) of the blood (Guyton and Hall, 2015)

One of the most important driving factors of blood conductance through a vessel is vessel diameter. Briefly, conductance is defined as a measure of blood flow through a vessel for a given change in pressure and it is the reciprocal of resistance (Guyton and Hall, 2015). Conductance is extremely sensitive to changes in vessel diameter and a 4-fold increase in vessel diameter causes a 256-fold increase in blood flow (Guyton and Hall, 2015). This relationship can be explained by Poiseuille's law which is represented by the following equation $F = \frac{\pi \Delta P r^4}{8 \eta l}$ where F represents rate of blood flow, ΔP is change in pressure, r is the radius of the vessel, l is vessel length, and η is the blood viscosity (Guyton and Hall, 2015). Blood viscosity changes over pregnancy due to a blood volume expansion and the human literature supports the primary driver of this expansion is a plasma volume increase during the first two thirds of pregnancy (Longo, 1983).

Methods of tracking changes in uterine hemodynamics

As pregnancy progresses, the dam must increase both her blood volume and cardiac output by 40-50% to effectively perfuse the uteroplacental unit (Silver et al., 1982). This is due in part to a 30 to 40% increase in plasma volume and a 35% increase in stroke volume and 15% increase in heart rate (Rosenfeld, 1984). In the cow, uterine blood flow increases 4-fold and umbilical blood flow increases 21-fold to support fetal growth from d 137 to d 250 of pregnancy (Reynolds and Ferrell, 1987). As a percentage of distribution of increased gravid uterine blood flow, 85% is directed toward the caruncular tissue for the transfer of oxygen and nutrients through the cotyledon as to supply the needs of the growing fetus (Rosenfeld and Fixler, 1977).

As gestation progresses, measurements of resistance (RI and PI) decrease in parallel with fetal growth (reviewed in Wooding and Burton, 2008). This decrease in vascular resistance theory suggest that decreasing vascular resistance is a mechanism that may allow blood volume expansion (Schrier and Briner, 1991; Duvekot et al., 1993). This increase in uteroplacental hemodynamics and decreases in arterial resistance are the primary mechanisms for promoting maternofetal exchange as the oxygen extraction rate and arteriovenous differences in nutrient concentration do not fluctuate in response to the increase in uteroplacental flow (Reynolds et al., 2005, 2006). Measuring changes in uterine hemodynamics is important to diagnosis IUGR pregnancies as well as test the efficacy of therapeutic treatments of IUGR.

Heavy water

Deuterium oxide is a non-invasive technique to estimate mean tissue blood flow and is less hazardous than radioactive tracers (Furuya et al., 1997). This technique was utilized in pregnant beef cattle to track changes in uterine hemodynamics during mid and late gestation (Reynolds et al., 1986; Reynolds and Ferrell, 1987; Ferrell, 1991; Reynolds and Ferrell, 1992).

Some disadvantages of deuterium oxide include cost as well as less sensitivity than alternative methods of blood flow measurements that provide an accurate estimate of changes in uterine arterial blood flow.

Electromagnetic or Doppler cuff flow probes

Electromagnetic and Doppler flow probes allow internal measurements of arterial hemodynamics to be quantified without opening a vessel by placing a cuff around the artery of interest. Several studies in the bovine have successfully measured changes in uterine arterial blood flow for short periods of time across pregnancy (Ford, 1979; Ferrell and Ford, 1980). Electromagnetic flow probes utilize Faraday's Law of Induction, generating a magnetic field that captures the electromotive force (voltage) from the iron in the red blood cells passing through the vessel (Scott and Sandler, 1978).

A blood vessel is placed between the poles of a strong magnet and electrodes are placed opposite to each other around the vessel, perpendicular to the magnetic line of force (Guyton and Hall, 2015). As blood passes through the vessel with the electromagnetic flow probe, electrical voltage is produced proportional to the rate of flow and recorded using a voltmeter (Guyton and Hall, 2015). The size of the vessel, magnetic field strength and average flow velocity are all factors in calculating the voltage and estimating flow (Scott and Sandler, 1978). An advantage of electromagnetic flow probe is its sensitivity to document changes in flow in less than 1/100 of a second (Guyton and Hall, 2015).

In contrast, Doppler cuff flow probes utilizes piezoelectric crystals that are energized to transmit ultrasound at a desired frequency (Guyton and Hall, 2015). This technique utilizes the Doppler effect (see below) which is the change in frequency of energy waves due to motion

between the source (red blood cells) of the wave and the ultrasound receiver (Abramowicz and Sheriner, 2008; Maulik, 2005).

Doppler ultrasound

Doppler ultrasound is a safe, non-invasive, repeatable way to track changes in uterine hemodynamics over time to detect the consequences of poor maternal nutrition and target when therapeutic interventions should be applied (Vonnahme and Lemley, 2011). Doppler ultrasound utilizes the Doppler effect which is the change in frequency of energy waves due to motion between the source of the wave and the ultrasound receiver (Abramowicz and Sheriner, 2008; Maulik, 2005). The reflected ultrasound waves bounce back toward the crystals (in the cuff or transducer) and have a lower frequency than the transmitted wave because the red blood cells are moving away from the transmitter (Guyton and Hall, 2015). This change in frequency is referred to as the Doppler frequency shift which is calculated as fd (Doppler frequency) = ft (transmitted frequency) – fr (received frequency (Maulik, 2005).

To utilize this effect to measure changes in uterine blood flow, the transducer is the stationary object receiving the waves and the red blood cells are the moving source of the waves through the uterine vasculature that produce returning echoes (Ginther and Utt, 2004). The flow probe (either cuff or finger probe) intermittently cut off transmission of the ultrasound waves so the reflected wave can be received back on to the crystals and frequency differences determined (Guyton and Hall, 2015).

Doppler ultrasonography has been effectively utilized in beef cattle studies across all stages of gestation (Bollwein et al., 2002; Panarance et al., 2006; Herzog et al., 2011; Camacho et al., 2014; Kennedy et al., 2016b; Mordhorst et al., 2016; Cain et al., 2017). In fact, Doppler ultrasound is the only method that can perform repeated measurements on a single animal

throughout the entire pregnancy. Doppler ultrasound can also be utilized to record measurements of vascular resistance. Measures of arterial resistance include pulastility index (PI) a commonly utilized index to indicate tissue perfusion and resistance indices (RI) is an indicator of arterial resistance (Ginther, 2007). Some advantages of transrectal Doppler ultrasonography compared to Doppler cuff flow probes include not requiring an invasive surgery for installation of the flow probes as well as the rigid nature of the probe does not allow the vessel to undergo the necessary vasodilation to support pregnancy (Meschia, 1989).

Contrast-enhanced ultrasound

An alternative to glass microspheres (not discussed in this review) which cause tissue occlusion are a technology known as contrast-enhanced ultrasound or "microbubble" technology. As defined by Forsberg et al. (1999), microbubble-based contrast agents are comprised of microbubbles of gas, encapsulated by a shell of a different composition ranging in diameter from 2 to 6 μm. These microbubbles have been used in human medicine for the past 20 years with Doppler ultrasound to diagnose pathologies, and provide enhanced vasculature visualization. Furthermore, because of the metabolically inert state of microbubbles, contrast-enhanced ultrasound has been used as a vehicle to deliver drugs or gene therapies directly to a cell.

In addition to its application in the delivery of pharmacological agents or biological materials, contrast-enhanced ultrasound can be used detect pathologies during pregnancy. Studies in rodents have used contrast-enhanced ultrasound to examine placental function throughout pregnancy, as well as to look at the permeability of the placental barrier (Xie et al., 2008; Hua et al., 2009; Foster et al., 2011; Yan et al., 2013;). Further studies in humans and macaques have used contrast-enhanced ultrasound to quantify placental perfusion as well as test the efficacy of novel treatments of pre-eclampsia along with Doppler ultrasonography of in

rodents (Verlohren et al 2010; Foster et al., 2011; Zhou et al., 2013; Roberts et a., 2016). It has even been suggested that contrast-enhanced ultrasound could be inducing angiogenesis in an *in vitro* study of bovine aortic endothelial cells (Mizrahi et al., 2007). The plethora of biomedical and agricultural applications of microbubble-based technologies are certainly promising and merit further investigation as their potential to improve livestock health and pregnancy outcomes has yet to be fully realized.

Development of the mammary gland during pregnancy

The lactation cycle in the adult bovine is divided into 4 stages which are all hormonally regulated: 1) mammogenesis, 2) lactogenesis, 3) galactopoiesis, and 4) involution. In these stages, 3 classifications of hormones are responsible for the physiological changes in the mammary including 1) reproductive hormones (estrogen, progesterone, placental lactogen, prolactin, and oxytocin), 2) metabolic hormones (growth hormone, corticosteroids, thyroid hormones, insulin, and GI hormones), and 3) endocrine hormones such as parathyroid hormone-related peptide, insulin-like growth factor 1, growth hormone, and leptin; (reviewed in Svennersten-Sjaunja and Olsson, 2005). This review will focus on the vascular development and the nutritional influences that affect vascular development and blood flow of the mammary gland.

Vascularization of the mammary

Before pregnancy, the mammary parenchyma and capillary network develops in parallel at a slow rate, followed by growth of the mammary vasculature while branching and ductal development occurs (Svennersten-Sjaunja and Olsson, 2005). Shortly after parturition, the vascular system redirects most of the uteroplacental blood flow to the mammary to support lactation, although full development of the capillary network and metabolic activity may not be

complete at parturition, it is reached within the first few days of lactation as measured by carbonic anhydrase activity (reviewed in Svennersten-Sjaunja and Olsson, 2005). In the goat, the mammary production of CO_2 has been attributed to mammary blood flow and is a good indicator of metabolic activity (reviewed in Svennersten-Sjaunja and Olsson, 2005). Mammary blood flow and subsequent milk production has been positively correlated (reviewed in Svennersten-Sjaunja and Olsson, 2005; Berger et al., 2016). Like the uteroplacental unit, the vascular responses of the mammary are stimulated by similar factors. Vasodilation in the mammary can be caused by nitric oxide and atrial natriuretic peptide and vasoconstriction can be caused by catecholamines, angiotensin II, and vasopressin (reviewed in Svennersten-Sjaunja and Olsson, 2005).

While gestational nutrition has shown limited impacts on mammary gland vascularity, growth and development in beef cattle, it has been shown to influence other species (reviewed in Vonnahme et al., 2015). In the sow, mammary gland growth and DNA content was altered by gestational maternal diets varying in energy and protein (reviewed in Vonnahme et al., 2015). Gestational dietary impacts on mammary gland development are also observed in the ewe. When overfed, ewes had an enhanced mammary gland alveolar epithelium proliferation index (Swanson et al., 2008). It has been suggested this is due to decreased estradiol-17β could allow increased glucocorticoid-binding protein which would allow cortisol to increase cellular differentiation in the mammary (reviewed in Vonnahme et al., 2015). Furthermore, altered progesterone can alter lobular alveolar growth in the mammary (reviewed in Vonnahme et al., 2015).

Although limited nutritional effects have been observed in mammary gland growth in the beef cow, much remains to be determined on the molecular level. A gross examination of lipids, protein, and sugars can be useful for understanding the crude nutritional value of the milk.

However, examining the role of maternal diet on nutrient transporters can provide more refinement for understanding the true effects on neonatal performance. The abundance of glucose, fructose, lactose and amino acid transporters or, perhaps, even influences the activity of the type I fatty acid synthase enzyme, type II thioesterase. This critical enzyme is responsible for the broad lipid diversity in the milk to provide the neonate with the wide variety of lipids it requires for growth (Ritchie et al., 2016). More studies in beef cattle examining specific transporters or lipid synthesis mechanisms could provide continued insight into how developmental programming continues to affect the health of the offspring through lactation or how the maternal system compensates for a previously inadequate environment.

Gestational dietary impacts on mammary hemodynamics and milk production

The plane of nutrition for heifers during late gestation appears to be important for postpartum milk production as heifers that were nutrient restricted (55% NRC) during late gestation saw a decrease in total milk produced compared to control (100% NRC) cows (Corah et al., 1975). Tucker (1981) also documented a decrease in milk production with either over or underfeeding pubertal heifers. Global nutrient restriction seems to effect milk production in heifers more than mature cows as late-gestating beef cows that were global nutrient restricted (57% NRC) did not experience a decrease in milk production even though they lost body weight (Hough et al., 1990).

Just as global nutrient restriction can reduce milk production, an overabundance of nutrition can also impact milk quality as mature beef that were maintained in a positive energy balance during late gestation and early lactation had reduced milk protein and higher milk urea compared to those in negative energy balance (Winterholler et al., 2012). However, milk fat and total volume produced were unaltered between energetic levels (Winterholler et al., 2012).

Exceeding the dietary needs of CP during late gestation also affects heifer milk quality by reducing milk fat and increasing milk urea (Gunn et al., 2014).

When multiparous beef cows were supplemented with DDGS at 0.3% of BW and were given *ad libitum* access to corn stover and silage, experienced greater contralateral mammary blood flow during late gestation and tended to produce a greater quantity of colostrum and milk (Kennedy et al., 2016b). Additionally, when dairy cows were supplemented with higher levels of digestible undegraded protein, they saw improvements in milk yields (Moorby et al., 1996). It appears that *ad libitum* access to hay is also important as the DDGS improve feed intake, likely allowing for an increase in milk production. This would agree with a study done by Radunz et al. (2010) where 4.1 kg of DDGS or 5.3 kg of corn daily were supplemented along with 2.1 kg of hay daily produced no changes in milk production. Mordhorst et al. (2016) also observed that DDGS supplementation had limited impacts on mammary blood flow.

Furthermore, the positive effects of protein supplementation also seem dependent on levels of supplementation as cows supplemented at lower levels during the same period of gestation did not observe alterations in milk production (Stalker et al., 2006; Martin et al., 2007; Larson et al., 2009; Wilson et al., 2015b). Even type of forage provided along with the protein supplement seems to determine the effectiveness of the protein supplement on improving milk production as multiparous cows grazed on tall fescue grass and red clover with high protein supplementation did not alter milk production (Shoup et al., 2015a).

Beef cow metabolism

Starch digestion in the ruminant

Feeding high starch diets to beef animals is ultimately driven by the production cost advantage of feeding starches over forages as well as the added energetic density of starch.

When a ruminant consumes a medium or high concentrate diet, 30% of the animal's glucose needs are met from glucose absorption from the starch, 50% of their needs are met from the absorption of organic acid that are substrates of gluconeogenesis, and the remaining 20% come from other sources (Huntington, 1997). Gluconeogenesis is sensitive to glucose absorption by the ruminant gut and will be reduced with higher circulating levels of glucose (Huntington, 1997). To improve starch utilization efficiency, grain based starches have the highest energy payoffs is ideally fermented in the rumen, along with the inclusion of dietary protein to capture the most glucose (Huntington, 1997). Corn is comprised of 72% starch content which can fluctuate with variety, location, year, management, and climate (Huntington, 1997).

While protozoa and fungi contribute to some starch fermentation, most fermentation is completed by ruminal bacteria of which 15 key strains of amylolytic bacteria are well documented, as well as 8 amylolytic enzymes (Huntington, 1997). The basic mechanism that allows bacteria to ferment starch is the adherence and colonization of grain particles in the rumen which allow the production of endo and exo-enzymes that specialize in the hydrolysis of α 1-4 and α1-6 glyosidic bonds of amylose and amylopectin (Huntington, 1997). Optimal conversion of starch to monosaccharides require cocultures of bacteria including 4 key bacteria: 1) *Streptococcus bovis,* 2) *Butyrivibrio fibrisolvens,* 3) *Prevotella bryanti,* and 4) *Selenomonas ruminantium* (Cotta, 1992).

In the small intestine, starch digestion resembles the non-ruminant as α-amylase secreted by the pancreas hydrolyzes amylose and amylopectin into limit dextrins and linear oligsachharides of a minimum of 2 or 3 glucose units (reviewed in Huntington, 1997). Starch digestion is completed by surface oligosaccharides on the brush border membrane of the enterocytes, but the ruminant does not have detectable sucrase activity (reviewed in Huntington,

1997). The ruminant relies on maltase and isomaltase activity to produce glucose for absorption (reviewed in Huntington, 1997).

Rate of starch digestion is influenced by source of starch, composition of the diet, rate of consumption, and any mechanical or chemical alterations to the carbohydrate source, and finally, how accustomed the ruminal microbes are to the diet (reviewed in Huntington, 1997). Factors that also influence kinetics of starch digestion in vivo include quantity of ruminal liquid, feed particle sizes and properties of each pool, rate of digestion, rate of passage, rate of dilution, and outflow of microbial protein (Owens and Goetsch, 1986). Factors that alter feed intake may alter how effectively the ruminant digests starch. Additionally, the high rate of absorption of ammonia by the ruminant due to asynchrony between energy and nitrogen in the diet suggests the limited use of available nitrogen to the rumen (Huntington, 1997).

Urea cycle

The ability for the ruminant to recycle urea as part of their N economy gives them a unique advantage to produce quality red meat with low quality forage. Through ruminal fermentation, the protein profile reaching the small intestine is altered and is primarily fueled by the fermentation of carbohydrates (reviewed in Reynolds and Kristensen, 2008). Nitrogen is recycled through the blood and gut luminal exchanges of urea and ammonia allowing the nitrogen to be reintroduced for more ruminal fermentation (reviewed in Reynolds and Kristensen, 2008). Unlike monogastrics, the ruminant can recycle ammonia produced by microbial degradation of urea as well as from the degradation of dietary and endogenous amino acids rather than simply excreting the urea (reviewed in Reynolds and Kristensen, 2008). Urea can be recycled to the gut via saliva, directly to the rumen across epithelial tissues via urea

transporters to be utilized for amino acid synthesis or be re-absorbed via the portal vein for further recycling or excretion (reviewed in Reynolds and Kristensen, 2008).

Urea cycle pathway

Briefly, the urea cycle consists of 4 steps 1) the formation of citrulline, 2) the formation of argininosuccinate, 3) formation of arginine, and 4) release of urea and regeneration of ornithine (Voet and Voet, 2011). First, the amine group that was previously added to the carbamoyl phosphate during amino acid catalysis is shuttled to ornithine (the last product of the urea cycle) by the enzyme ornithine transcarbmoylase to form citrulline which then passes into the cytosol from the mitochondrial matrix (Voet and Voet, 2011).

Next, the second source of nitrogen enters the urea cycle through the addition of aspartate that was formed previously by the addition of an amine group to oxaloacetate via the enzyme aspartate aminotransferase (Voet and Voet, 2011). Then, the radical oxygen in citrulline is activated through the addition of ATP and pyrophosphate production and replaced by the aspartate amino group to form the citrullyl-adenine monophosphate intermediate, ultimately forming argininosuccinate by the enzyme argininosuccinate sythetase (Voet and Voet, 2011).

Third, argininosuccinate is cleaved by argininosuccinase, releasing fumarate which enters the citric acid cycle and producing arginine (Voet and Voet, 2011). Last, the formation of urea occurs when arginine is cleaved by arginase, replenishing the intermediate ornithine and releasing urea (Voet and Voet, 2011).

Carbohydrates and supplementation strategies on urea utilization

The ruminant is impressive in their ability to consume inferior quality diets containing only non-protein nitrogen sources of N, and via their ruminal microbes, produce high quality protein (milk and meat) with low quality forage. However, this mechanism is still sensitive to

factors that affect microbial growth such as carbohydrate fermentation (reviewed in Reynolds and Kristensen, 2008). These mechanisms efficiencies are reduced by dietary protein deficiencies as well as asynchronous supplementation of either carbohydrates or protein ultimately effects N excretion and utilization (reviewed in Reynolds and Kristensen, 2008). The utilization of ammonia for microbial protein synthesis is energy-dependent and how quickly the carbohydrate source can be digested can influence ammonia absorption (reviewed in Reynolds and Kristensen, 2008).

Supplementation management strategies attempt to mitigate this effect through oscillatory dietary protein supplementation strategies, providing equal quantities of dietary protein to animals daily, helping sustain N recycling within the gut (reviewed in Reynolds and Kristensen, 2008). Even infrequent protein supplementation with no more than 3-day intervals did not hurt recycling urea to the gut (reviewed in Reynolds and Kristensen, 2008). It is because of the robust ruminant N recycling that helps them buffer the effects of irregularities in protein intake (reviewed in Reynolds and Kristensen, 2008).

Metabolic markers

Non-esterified fatty acid

Circulating concentrations of non-esterified fatty acids (NEFA) generally increases as caloric intake decreases and therefore can be used to indicate mobilization of lipid stores (Lucy et al., 1991). Interestingly, in beef cattle, this is not always true as Radunz et al. (2013) observed that hay-fed cows tended to have greater NEFA when compared to corn-fed dams during gestation. However, dietary treatment differences in plasma NEFA are not necessarily indicative of different energetic status (Althen et al., 1990; Rusche et al. 1993; Sullivan et al., 2009; Islas et

al. 2014) observed no differences in circulating NEFA while other metabolic parameters were modified.

Plasma urea concentration

Plasma urea can be an indicator of protein supplied in the diet, protein degradation in the rumen, and N utilization in the rumen (Reed et al. 2007). Circulating urea in the bovine can also indicate muscle catabolism due to nutrient deficiency (De Oliveria Franco et al., 2017). Interestingly, in beef cattle there was no observed relationship between ammonia absorption into the portal vein (portal-drained viscera measurement) and circulating plasma urea (Lapierre and Lobley, 2001). Perhaps, it is because these measurements did not include salivary urea transfer. However, this relationship is observed in ewes as Swanson et al. (2017) noted a decrease in urea in MP restricted (60% MP requirements) ewes compared to control ewes (100% MP requirements). Klein et al. (2014) also observed decreasing levels of urea in the blood of in cows with lower levels of protein in their diet. Furthermore, cattle that were supplemented with dietary protein also had greater circulating urea in their blood (Radunz et al., 2013; Klein et al., 2014; Islas et al., 2014; Mordhorst et al., 2016)

Plasma glucose

The principal source of glucose in the ruminant is from gluconeogenesis (Huntington, 1997). Interestingly, although beef cow diets differed in carbohydrate and protein inclusion in the diet, Radunz et al. (2013) also observed that there were no dietary differences in circulating glucose. This differs from sheep limit-fed high grain diets which had higher circulating levels of plasma glucose (Susin et al. 1995) and higher circulating glucose supply to the fetus improved lamb birth weights (Stevens et al., 1990). High-starch supplements seem to have limited impacts on circulating plasma glucose in beef cattle. However, protein supplementation has had variable

impacts on glucose depending on stage of life. In Islas et al. (2014) glucose was increased in protein supplemented growing steer calves which contradicts Radunz et al., (2013) which found glucose was not altered by protein supplementation in gestating beef cows.

Serum cortisol

Cortisol is well recognized for its regulatory effects of energy metabolism. It works in concert with other hormones, helping maintain blood glucose by enhancing gluconeogenesis through the covalent activation of phosphenolcarboxykinase (Ryan, 1975). In high concentrations, it has also been demonstrated to have catabolic effects on body protein reserves (Ryan, 1975). As reviewed in Chang and Zhang, (2008), maternal plasma cortisol has been demonstrated to double over gestation, suggesting its physiological relevance in fetal growth. However, cortisol levels can also increase from being handled which is well documented in beef cattle (Cooke, 2014). This effect was also observed in gestating ewes, independent of maternal nutritional plane (Lemley et al., 2014). Another well documented metabolic effect of cortisol is the liberation of lipids into circulation (Ryan, 1975).

Serum triiodothyronine and thyroxine

The biologically critical thyroid hormones triiodothyronine (T3) and thyroxine (T4) play many diverse roles in regulating adult protein and energy metabolism (Cassar-Malek et al., 2006). While these hormones are biologically critical for mobilizing maternal body reserves to supply nutrients to the developing conceptus, they cannot cross the placenta (Cappoen, 1989). In the bovine, limited studies have examined the metabolic role of T3 and T4 in the dam during gestation. In the human, maternal total T3 and T4 are elevated during weeks 17 through 36 of gestation in the human while all fetal thyroid hormones increased as gestation progressed and

reverse T3 decreased (Radunovic et al., 1991). Additionally, maternal total T3 was correlated with fetal T3 and T4 in the human (Radunovic et al., 1991).

While few studies have examined the role of T3 and T4 in the pregnant bovine, more is understood about the role of T3 and toT4 in the bovine fetus. The fetal thyroid gland differentiates between d 75 and 90 of gestation and has been shown to play a role in regulating the development and differentiation of the fetal liver, heart, and skeletal muscles as well as fetal adipose development (Cassar-Malek et al., 2006). This is because of the activation of the hypothalamic-pituitary- thyroid axis which is regulated by leptin (Micke et al., 2015). In fact, maternal diet can play a sex-specific role by controlling the fetal nutritional plane as male progeny exposed to a maternal protein restriction in-utero resulted in greater free T4 at birth (Micke et al., 2015). This is not terribly surprising as nutrient restricted calves had been previously shown to have higher gluconeogenic hormones such as cortisol and T3 at birth (Hough et al., 1990).

Interestingly, in some studies, maternal total T3 does not differ according to dietary treatment but decrease over gestation which is supported by what is observed in pregnant ewes (Lemley et al., 2014) but opposite to what is recorded in the human (Radunovic et al., 1991). The ratios of thyroid hormones and activities are affected by type-1 5'- deiodinase activity (Cassar-Malek et al, 2006). In fact, total T3: total T4 ratios were greater in heifer calves exposed to high protein diets during gestation (Micke et al., 2015).

The carbohydrate effect: the ugly side of carbohydrates

As previously discussed, the intake of dietary starch can influence N utilization by the ruminant as well as alter ruminal microbial populations, altering intake and feed utilization so care should be taken when selecting carbohydrate supplementation strategies. This negative

effect of corn supplementation on forage intake has been well demonstrated in beef cattle fed forage-based basal diets (Mould et al., 1983; Firkins et al., 1984; Lusby and Wagner, 1986; Chase and Hibberd, 1987; Sanson et al., 1990; Pordomingo et al., 1991; Loy et al., 2007; Leupp et al., 2009) This effect of reductions in forage intake resulting from energy supplementation is referred to as substitution (Caton and Dhuyvetter, 1997).

However, studies have suggested that corn supplementation less than 0.25% of BW does not decrease forage utilization (Pordomingo et al., 1991; Matejovsky and Sanson, 1995). Additionally, as reviewed in Caton and Dhuyvetter, (1997) several factors could be responsible for this effect including decreased ruminal pH, carbohydrate effect, and altered forage digestibility. It should be recognized that the variation in reductions in forage intake because of energy supplementation could be due differing basal forage quality and sources. While studies reviewed by Caton and Dhuyvetter varied greatly in their forage digestibility responses to energy supplementation, this could be in part due to the level of protein in their diets. In fact, a study by Sanson et al, (1990) suggest that when CP is limiting, energy supplementation alone might worsen the CP deficiency.

This substitution effect could potentially be explained by the likely shift in ruminal microbial populations with probable lower ruminal pH as energy supplementation drives bacteria towards greater amylolytic and lower cellulolytic populations (Caton and Dhuyvetter, 1997. This diet driven shift has been documented through reverse transcription quantitative polymerase chain reaction analysis in cannulated dairy steers that were first fed roughage based diets then transitioned to a ration with higher starch concentrations (Tajima et al., 2001).

Caution should be used when creating supplementation strategies involving corn. To navigate around this substitution effect on fiber digestion and still positively influence the energy

status of beef cows fed a low-quality forage diet, alternative energy sources with readily degradable fiber such as wheat midds and corn gluten have been shown to not decrease forage intake as much as high concentrate supplements (Carey et al., 1993). Furthermore, other studies that have fed corn to gestating beef cows have navigated around this effect by supplementing an additional protein supplement and urea to try to prevent a subsequent protein restriction (Loerch et al., 1996; Radunz et al., 2013).

Statement of the problem

Inadequate maternal nutrition during pregnancy can lead to a decrease in carcass quality of the offspring, including altered fat deposition, muscle fiber type and reduced meat quality (Wu et al., 2006). A study by Greenwood et al. (2004) and reviewed in Robinson et al. (2010) demonstrated that nutrient restricting gestating cows produced steers that had reduced carcass weights at 30 mo of age. Protein restriction of dams during late gestation also resulted in reduced carcass weights, postnatal growth and intramuscular fat accretion (Funston et al., 2012). By increasing the forage quality of late gestating beef cows, Underwood et al. (2010) improved steer hot carcass weights and Gonzales et al. (2013) rescued cattle muscle fiber size and muscle progenitor numbers through realimentation of cows who were nutrient restricted during early pregnancy. Evidently, negative carcass outcomes of progeny nutrient restricted in-utero can be resolved by improving the forage quality of beef cows during late gestation, but if that feed resource is not available, an alternate feeding strategy should be employed. In fact, nutrient restriction is still common in extensively managed, overwintered, gestating beef cows due to limited access to high quality forage. Supplementation of dietary protein or energy has been demonstrated to improve calf birth weights. In a study by Radunz et al. (2013), cows that were supplemented corn during late gestation saw improved calf birth weights and we observed that

supplementing DDGS at 0.3% BW during late gestation increased roughage intake and uterine hemodynamics which consequently improved calf birth weights (Kennedy et al., 2016a). One way to estimate nutrient supply to the gravid uterus is by measuring uterine arterial blood flow (Ferrell, 1991). Doppler ultrasound is a non-invasive, repeatable way to measure changes in uterine hemodynamics to detect the consequences of poor maternal nutrition and target when therapeutic interventions should be applied (Vonnahme and Lemley, 2011). We hypothesized that mid- to late-gestating beef cows receiving corn supplementation over winter will gain more BW and BCS over gestation, increase forage intake, indicate they are on a higher plane of nutrition with metabolic markers, have greater uterine and mammary arterial blood flow, increased placental function, greater colostrum production and give birth to heavier, faster growing calves. The objectives of this study were to evaluate the effects of winter corn supplementation on gestating beef cow feed intake and behavior, BW and BCS, metabolic profiles, uterine and mammary hemodynamics, placental morphology, efficiency and vascularity, colostrum production and neonatal growth

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CHAPTER 2. USING CORN SUPPLEMENTATION FOR OVERWINTERED BEEF COWS DURING MID- TO LATE- GESTATION ALTERS MATERNAL FEED INTAKE, FEEDING BEHAVIOR, AND METABOLIC PROFILES

Abstract

Improper maternal nutrition during pregnancy can lead to compromised growth and poor carcass quality in offspring. Extensively managed, gestating beef cows fed low-quality forage are often at risk of nutrient restriction. Thus, the objective of this study was to evaluate the effects of feeding supplemental energy (corn) with low-quality forage to gestating beef cows by tracking feed intake and behavior, BW and condition, metabolic markers, and neonatal growth. We hypothesized gestating beef cows receiving corn supplementation would have increased caloric intake and altered feeding behavior, gain more BW and condition, have less negative energy balance, and give birth to faster growing calves. Forty-seven multiparous Angus-based beef cows carrying bull calves were assigned randomly to dietary treatments receiving corn supplementation at 0.2% of BW (SUP; n = 24) or no supplement (CON; n = 23). All cows were fed the same basal diet (60% hay, 30% wheat straw, and 10% CSB; dry basis). Intake and feeding behavior was monitored individually with Insentec feeders from d 110 of gestation through calving. Cow BW, BCS, and jugular blood was collected every 28 d until d 240 of pregnancy, at parturition, and 3 wk postpartum. Calves were weighed at birth, 3 wk postpartum, and weaning (d 168). Data were analyzed with generalized least squares using the mixed procedure of SAS with repeated measures. Corn supplementation suppressed ($P < 0.01$) roughage intake in SUP cows; however, daily energy intake tended ($P = 0.06$) to be increased in SUP cows. Treatment interacted ($P < 0.05$) with day for cow BW and BCS where SUP cows increased ADG ($P < 0.001$) and body condition ($P = 0.06$) compared with CON cows. Corn

supplementation reduced ($P < 0.01$) forage intake but did not alter ($P = 0.75$) total intake. While SUP cows consumed forage at a slower rate ($P < 0.01$), they ate more roughage meals ($P = 0.04$) that were smaller ($P < 0.01$) in time and size. After feeding a lactation diet to all cows beginning at d 265 of gestation, no behavioral differences ($P > 0.10$) were observed aside from meal size which remained smaller ($P = 0.02$) in SUP dams. Corn supplementation did not alter ($P = 0.12$) plasma glucose but did decrease plasma NEFA ($P < 0.01$) and plasma urea ($P = 0.02$) in supplemented cows during gestation. These effects, however, disappeared ($P > 0.10$) at calving. The SUP cows tended to have reduced ($P = 0.07$) gestation lengths. Despite altering maternal metabolism and feeding behavior, corn supplementation did not affect ($P = 0.87$) calf birth BW. However, at 3 wk postpartum, calves from SUP dams were heavier ($P = 0.05$) but this growth advantage disappeared ($P = 0.64$) by weaning. While corn did decrease maternal roughage intake, it does appear to be a good substitute for hay as it does not have negative impacts on calf growth. Depending on the cost of feed inputs, this feeding strategy could be economically advantageous for the producer.

Introduction

It is well recognized that maternal nutritional status is one of the key external factors influencing nutrient partitioning for fetal growth, and that compromised fetal growth impairs lifelong health (Wallace, 1948; Godfrey and Barker, 2000). Inadequate maternal nutrition during pregnancy can lead to a decrease in carcass quality of the offspring, including altered fat deposition, muscle fiber type, and reduced meat quality (Wu et al., 2006). A study by Greenwood et al. (2004) and reviewed in Robinson et al. (2010) demonstrated nutrient restricting gestating cows produced steers that had reduced carcass weights at 30 mo of age. Protein restriction of dams during late gestation also resulted in reduced carcass weights, rate of gain,

and intramuscular fat accretion (Funston et al., 2012). By increasing the forage quality fed to beef cows during late gestation, Underwood et al. (2010) increased steer hot carcass weights and Gonzales et al. (2013) rescued cattle muscle fiber size and muscle progenitor numbers through realimentation of cows who were nutrient restricted during early pregnancy. Evidently, negative carcass outcomes of progeny nutrient restricted *in utero* can be resolved by improving the forage quality fed to beef cows during late gestation, but if that feed resource is not available, an alternate feeding strategy should be employed. Supplementation of dietary protein or energy has been demonstrated to increase calf birth weights. In a study by Radunz et al. (2013), cows supplemented corn during late gestation produced heavier calves at birth. We observed supplementing DDGS at 0.3% of BW during late gestation increased roughage intake, which consequently increased calf birth weights (Kennedy et al., 2016a).

We hypothesized that mid- to late-gestating beef cows receiving corn supplementation over winter will gain more BW and BCS, increase forage intake, indicate they are at a higher plane of nutrition with metabolic markers, and give birth to heavier, faster growing calves than cows not supplemented corn. The objectives of this study were to evaluate the effects of winter corn supplementation on gestating beef cow feed intake and behavior, BW and BCS, metabolic profiles, and neonatal growth.

Materials and methods

Experimental design, cows, and dietary treatments

All procedures were approved by the North Dakota State University (**NDSU**) Animal Care and Use Committee (A16010). Forty-seven multiparous, Angus-based beef cows carrying bull calves were assigned randomly to two dietary treatments. Treatments were control (**CON**; n = 23) receiving *ad libitum* access to a low-quality, forage-based TMR only (Table 1; 57.54%

TDN, 6.4% CP) and a treatment group (**SUP**; n = 24) receiving an additional corn at 0.2% of BW (94.5% TDN, 7.64% CP) in addition to *ad libitum* access to the basal TMR. Cows were stratified by BW and BCS across treatments. Cows weighed 661 ± 7.8 kg, had a BCS of 5.2 ± 0.1 (9-point scale), and were 7.5 ± 0.2 yr old at the start of the study.

The cows were housed in 4 adjacent pens (11 or 12 cows per pen), 2 for each treatment. To monitor feeding behavior and individual intake, cows were fitted with radio-frequency identification tags. After a 3-wk acclimation period, dietary treatments were applied and intake was monitored and controlled via electronically controlled feeders (RIC feeding system; Insentec B.V., Markenesse, Netherlands) beginning on d 110 of gestation for 22 wk. The RIC system is an automated system that identifies cows individually to control and monitor intake. The basal forage diet was provided *ad libitum* to each cow for the duration of the trial, but the corn was supplemented at a limited amount of 0.2% of BW (the system rejected the animal after allotment consumed).

Feed was provided three times daily at 0800, 1200, and 1600. If feeders were observed to be empty, additional basal diet was added. Biological and environmental variables were estimated for the beef cattle diet evaluation model (NRC, 2016). Gestation diet 1 consisted of a basal diet of 45% hay, 45% wheat straw, and 10% concentrated separator byproduct (**CSB**) on a DM basis; (56.45% TDN; 6.42% CP) was fed from d 110 of gestation until d 153 (due to the loss of a cow from an impacted abomasum). At d 154 of gestation, diets were adjusted to 60% hay, 30% wheat straw, and 10% CSB (Gestation diet 2; 58.63% TDN, 7.11% CP DM basis) until 2 weeks prior to calving (d 265 of pregnancy). Corn was supplemented to the SUP group at 0.2% (94.5% TDN, 7.64% CP on DM basis) of BW from d 110 until d 265. On d 265 of gestation, approximately two weeks before expected parturition, all cows were fed the same diets (45%

straw, 25% DDGS, 30% corn silage; 62.4% TDN and 11.6% CP on a DM basis) for *ad libitum* intake for a period of 5 wk; corn supplementation ceased. Feed intake and behavior during lactation were defined by the same parameters as the gestational intake and feeding behavior and averaged weekly for each cow. Data for all cows were still reported relative to their respective treatment groups during gestation. Additionally, no data were recorded for maternal feed intake during the 24 h period immediately following calving. Each pen contained 8 individual feeders with all 8 feeders in the 2 control pens containing the basal forage diet. The two supplemented pens each had 7 feeders with the basal forage diet and 1 feeder with the corn supplement. All cows had free access to water and a trace-mineralized salt block (955 to 985 g of NaCl/kg, 3.50 g of Zn/kg, 2.00 g of Fe/kg, 1.80 g of Mn/kg, 280 to 420 mg of Cu/kg, 100 mg of I/kg, and 60 mg of Co/kg; NRC, 2016).

Feeding behavior measurements with the Insentec feeder were characterized as described by Islas et al. (2014) and Kennedy et al. (2016a) and defined as events (number of meals daily), eating time (minutes per meal), intake rate (kg per min) and feed intake (kg per meal) averaged for each cow for 1 wk periods. Meals were characterized as a distinct eating period, which could include short breaks between visits separated by intervals no longer than 7 min (Forbes, 1995). Each feeding behavior parameter was measured and reported for forage (hay, wheat straw, and CSB TMR), corn, and total intake averaged each week for 22 wk of gestation.

Body weight, condition, and calf weights

Beginning at d 100 ± 6 d of gestation, cows were weighed mid-day every 4 wk until d 240 ± 6 of gestation. Body condition was scored using a 1 to 9 scale (with 1 = emaciated and 9 = obese; Wagner et al., 1988) every 4 wk from d 100 to d 240 of gestation by 3 technicians at each time point. Because the weight of the uterus and conceptus influence maternal BW during

gestation, the equation outlined in Silvey and Haydock (1978) was utilized to model gravid uterine weight gains over gestation so real maternal BW at d 110 and d 240 could be estimated, as well as ADG. The pairs were weighed immediately after parturition, 24 h postpartum and at 21 days of lactation when the cattle turned out for the summer grazing. Calves were also weighed at weaning (168 ± 16 d). Gestation length was calculated for each cow.

Feed analysis

The basal forage diet during both gestation and lactation were sampled weekly and analyzed (Table 2.1). Samples were dried in a 55ºC oven for at least 48 h and ground to pass a 1-mm screen. Samples were analyzed for DM, ash, N (Kjeldahl method), Ca, P, and ether extract by standard procedures (AOAC, 1990) and for NDF (using heat stable amylase and sodium sulfite and expressed inclusive of residual ash) and ADF (expressed inclusive of residual ash) concentrations sequentially by methods of Robertson and Van Soest (1981) using a fiber analyzer (Ankom Technology Corp., Fairport, NY). TDN was calculated as 97.6 – (0.974 x ADF). Crude protein was calculated as N concentration x 6.25.

Metabolite analysis

Non-esterified fatty acid concentration in plasma samples was measured using an in vitro enzymatic colorimetric assay (HR Series NEFA-HR, Wako Chemicals USA, Richmond, VA). Glucose concentrations were measured using a hexokinase based assay (Infinity Glucose Hexokinase; Thermo Trace, Louisville, CO). Urea concentration in plasma samples was measured by absorption of a urea-specific chromogen at 520 nm (QuantiChrom Urea Assay, BioAssay Systems, Hayward, CA) according to the manufacturer's specifications.

Table 2.1. Analyzed composition of cow diets derived from hay, straw, and concentrated separator by-product (CSB) during gestation, and straw, silage, and dried distiller's grains (DDGS) during early lactation

	Ingredient %					Nutrient[2] %				
Item	Hay	Straw	CSB[3]	Silage	DDGS	CP[5]	NDF	ADF	Ca	P
Gestation diets										
d 110 to 153	45	45	10	--	--	6.4	67.0	41.3	0.28	0.12
d 154 to 265	60	30	10	--	--	7.1	64.5	38.9	0.37	0.26
Corn[1]						7.6	14.1	3.2	0.01	0.54
Lactation diet										
d 266 to wk 3 lactation	--	45	--	30	25	11.6	61.9	36.1	0.48	0.37

[1] SUP cows received corn at 0.20% body weight, CON animals received no corn.

[2] Nutrient analysis of TMR of each diet fed during gestation and lactation and of individual feedstuffs.

[3] Concentrated separator by-product.

[4]was calculated as 97.6 – (0.974 x ADF).

[5]Crude protein was calculated as N concentration x 6.25.

Statistical analysis

All cow gestational feed intake and behavior data were analyzed with repeated measures by weekly averages of daily observations using generalized least squares (MIXED procedure; SAS Institute Inc., Cary, NC). Gestational metabolite data were also analyzed with the repeated measures by day of gestation statement using generalized least squares. Birth and lactation data were also analyzed with the generalized least squares to account for the random effect of sire. Model statements included cow, maternal diet (CON vs. SUP), day of gestation, and a diet by day of gestation interaction. Sire (n = 4) was treated as a random variable. For 0 and 24-h weight measurements, gestation length was analyzed as a covariate. For 3-wk calf weight measurements, age of the calf was used as a covariate. For 3-wk maternal metabolite and weight data, day of lactation was used as a covariate. All meaningful interactions were considered and in

the absence of interactions (P > 0.10), main effects were discussed. Various covariance structures were assessed; those structures with the lowest fit statistics were utilized (Wang and Goonewardene, 2004). Means were separated using least significant difference (PDIFF option of LSMEANS). Solutions for linear, quadratic, and cubic relationships were requested where applicable. Four cows were excluded from the lactational data analysis due to mortality (n = 3) and morbidity (n = 1).

Results

Body weight and condition measurements

At d 110 of pregnancy, after a three-week acclimation period, cows were scored with an average BCS of 5.2 ± 0.1. By the last BCS measurement at d 240 of pregnancy, a day by treatment interaction ($P = 0.03$; Figure 2.1) was observed as SUP dams gained BCS at a faster rate than CON dams which maintained BCS over pregnancy. Furthermore, a day by treatment interaction ($P < 0.01$; Figure 2.2) was observed for BW with both SUP and CON dams increasing over time ($P < 0.01$). The average BW at the start of the trial was 640 ± 19 kg and SUP cows gained more weight during gestation than CON cows. Not surprisingly, SUP cows also had a greater ($P < 0.001$) ADG than CON cows (0.68 vs. 0.46 ± 0.08 kg/day).

After accounting for weight of the gravid uterus, the analysis revealed that a day by treatment interaction for maternal BW was detected ($P = 0.01$) and SUP cows gained more body weight as gestation advanced than CON dams. Furthermore, SUP cows tended ($P = 0.06$) to consume more TDN daily (8.86 vs. 8.36 ± 0.18 kg/d) and NEm daily ($P < 0.0001$; 13.74 vs. 11.54 ± 0.31 Mcal/d) compared to CON cows. Crude protein consumption between the treatments did not differ ($P = 0.81$; 0.99 vs. 0.99 ± 0.02 kg/d). While CP was not altered by diet, NRC (2016) calculations of MP protein of the diet showed the SUP cows to meet only 55% of

their MP requirements for pregnancy compared to CON which met 91% MP requirements for pregnancy (Table 2.2). While the SUP dams met their daily NRC requirements for ME (102% NRC), the CON dams also met and exceeded their daily NRC requirements (110% NRC).

At parturition (0 hr postpartum), dam BW from SUP dams were similar ($P > 0.5$; 713.5 vs. 702.5 ± 13.4 kg) to CON regardless of gestational dietary treatment. Additionally, at 24 h postpartum, dam BW was unaltered ($P = 0.56$) by corn supplementation as SUP and CON dams weighed similarly (702.2 vs. 690.8 ± 14.0 kg). Furthermore, dam BCS was also unaltered ($P = 0.22$) by gestational dietary treatments as SUP and CON dams (5.6 vs. 5.4 ± 0.1) were not affected by gestational corn supplementation.

At 3 wk postpartum, corn fed dams weighed similar ($P = 0.98$) to hay fed dams (725.6 vs. 725.2 ± 13.4 kg). Furthermore, body condition scores did not differ between dietary treatments ($P = 0.12$) as SUP and CON dams scored similarly (5.50 vs. 5.25 ± 0.1). Additionally, SUP cows tended to have a reduced ($P = 0.07$) gestation length compared to CON cows (277.7 vs. 279.6 ± 1 d) while birth weight was not affected ($P > 0.90$) by treatment. This suggests that the calves from supplemented cows tended to mature faster than their non-supplemented counterparts.

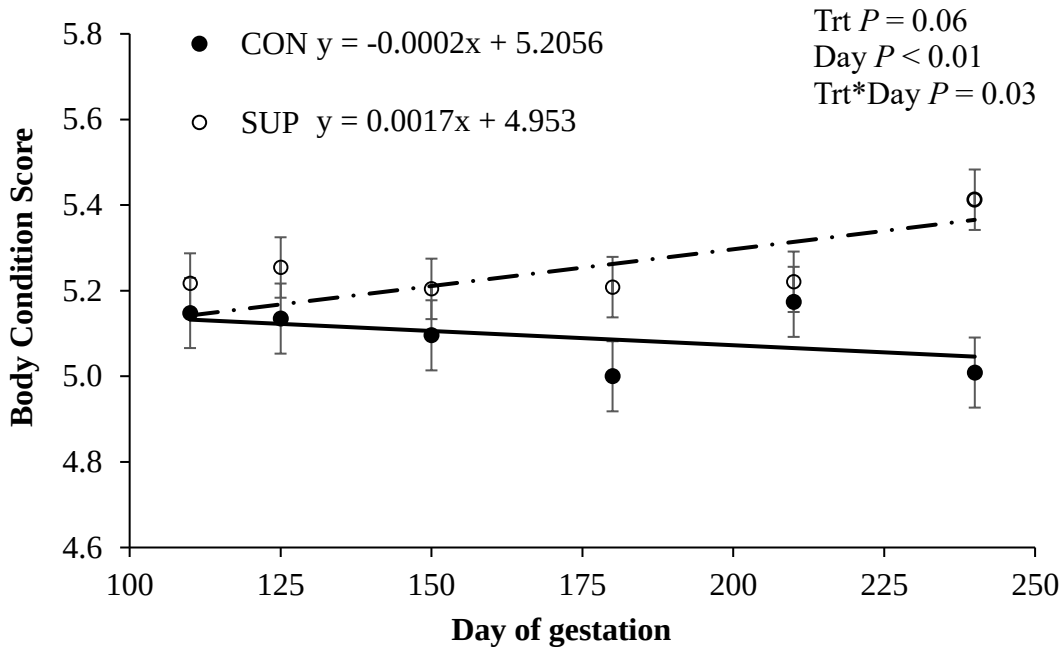

Figure 2.1. Gestational body condition score of beef cows fed the control and supplemented from d 100 to d 240 of gestation.

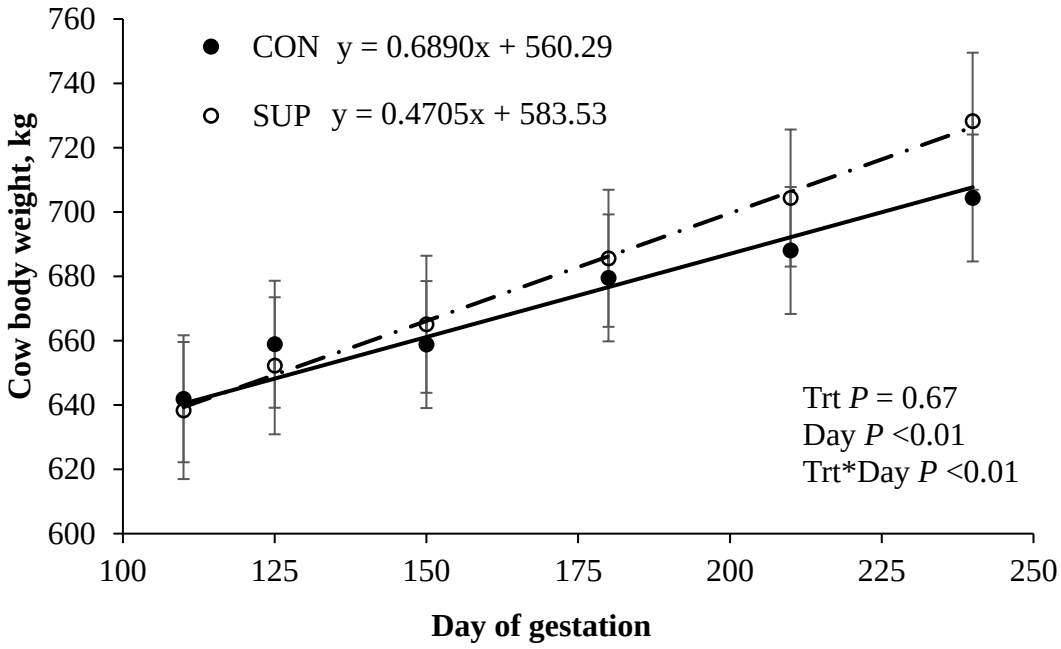

Figure 2.2. Gestational body weight changes of beef cows fed the control and supplemented from d 100 to d 240 of gestation.

Table 2.2. Nutrient requirements based on NRC model

	Requirements[1]	CON	SUP
Energy			
ME (diet), Mcal/kg	22.1	24.2	22.6
% NRC met		110%	102%
Protein			
MP, kg/d	0.54	0.54	0.52
% MP NRC met	---	100%	96%
Ruminal N balance, kg/d	---	0.03	0.02

[1]All calculations were made using the 2016 nutrient requirements of beef cattle.

Gestational dietary intake and behavior

A week by treatment interaction was observed ($P < 0.001$; Figure 2.5 and 2.6) for forage dry matter intake with cows receiving the corn supplement having suppressed ($P < 0.001$) intakes during gestation compared to the hay-fed dams (12.8 vs 14.4 ± 2.5 kg/d). Forage intake was altered by week ($P < 0.0001$) but did not increase as gestation advanced. Dry matter intake of corn supplement averaged 1.47 ± 0.13 kg/d from mid- to late-gestation and was influenced by week ($P < 0.0001$), increasing as gestation advanced. While corn supplementation suppressed roughage intake, it did not alter total dietary intake (roughage and corn) as SUP and CON dams consumed similar ($P = 0.75$) quantities of total intake (14.31 vs. 14.45 ± 0.30 kg/d) which fluctuated weekly ($P < 0.001$).

The time spent consuming roughage was also affected by a week by treatment interaction ($P < 0.001$; Figure 2.7) but the interaction was not biologically relevant as the CON dams spent more ($P < 0.001$) time consuming roughage than SUP dams (182.1 vs 220.7 ± 7.8 min per d) all but the week of 240-247 of gestation. Moreover, time spent consuming corn increased ($P < 0.001$) as gestation progressed. A week by treatment interaction ($P < 0.001$) was detected for

roughage intake rate over gestation but not altered by diet with both SUP and CON dams consuming roughage at similar rates ($P = 0.13$; 68.6 vs. 74.8 ± 2.8 g/min). There was a week by treatment interaction ($P < 0.001$) with variation early in the feeding period but flattened out as gestation progressed. We also observed an effect of day ($P < 0.001$) but this data was variable and inconsistent across gestation. Visits were not altered ($P = 0.61$) between SUP and CON (85.1 vs. 81.7 ± 5.5 visits daily). Additionally, corn bunk visits decreased ($P < 0.001$) over gestation by supplemented cows.

For roughage meals consumed, a week by treatment interaction ($P < 0.01$; Table 2.8) was observed but we do not think this is biologically relevant as corn supplementation increased ($P = 0.04$) the roughage meals per day in the SUP vs. CON dams (7.6 vs. 6.6 ± 0.3) most of gestation. Furthermore, meals were altered by week ($P < 0.001$) but meal number was highly variable and inconsistent week to week. Dietary corn supplementation did alter the number of forage meals consumed as SUP cows ate more ($P = 0.04$) roughage meals per day than CON cows. The cows receiving the corn supplement decreased ($P < 0.001$) the number of corn meals daily over gestation. Roughage intake consumed per visit was also altered ($P < 0.01$) by a week by treatment interaction but did not increase as gestation advanced. Additionally, no treatment effect of corn supplementation was detected on roughage intake per visit (0.20 vs. 0.19 ± 0.01 kg per visit) as SUP and CON dams consumed similar quantities per visit. As gestation advanced, the intake of corn per visit increased ($P < 0.0001$).

Roughage meal size was also detected to be influenced by a week by treatment interaction ($P < 0.001$; Figure 2.9) with cows consuming similar meal sizes over gestation but CON cows consuming larger ($P < 0.001$) roughage meals than SUP cows (2.40 vs 1.80 ± 0.10 kg/meal). Cows consuming corn increased ($P < 0.001$) their meal size as gestation advanced.

Total meal intake was altered by a week by treatment interaction ($P < 0.001$) however remained similar across gestation. Conversely, meal size was influenced by treatment as SUP cows consumed smaller ($P < 0.001$) meals than CON dams (1.20 vs. 2.40 ± 0.11 kg/meal).

No week by treatment interaction ($P = 0.52$; Figure 2.10) was observed for time spent consuming roughage meals but the SUP dams spent less ($P > 0.001$) time at the bunk per meal (3.71 vs. 5.11 ± 0.26 min/meal). Additionally, time spent consuming forage increased ($P < 0.001$) in both treatments and corn in SUP treatment as gestation progressed. A day by treatment interaction ($P < 0.01$) was detected for time spent consuming forage daily, however we are unsure of its biological relevance as the CON dams consistently spent more ($P < 0.0001$) time consuming roughage than SUP (4.32 vs. 10.19 ± 0.44 min per meal). Time spent consuming roughage weekly increased ($P < 0.0001$) as gestation progressed. There was no effect ($P = 0.83$) of corn supplementation on time spent per visit as SUP and CON cows (1.4 vs. 1.2 ± 0.2 min/visit) spent similar time at the bunk per visit.

Additionally, intake rate was influenced by week of gestation ($P < 0.001$; Figure 2.11) decreasing as gestation progressed in both CON and SUP cows. A week by treatment interaction ($P < 0.001$) was observed for total TMR intake rate with SUP dams eating feed faster than CON cows (42.0 vs. 34.0 ± 1.0 g/min). Like roughage intake, total TMR intake rate decreased ($P < 0.001$) with gestation.

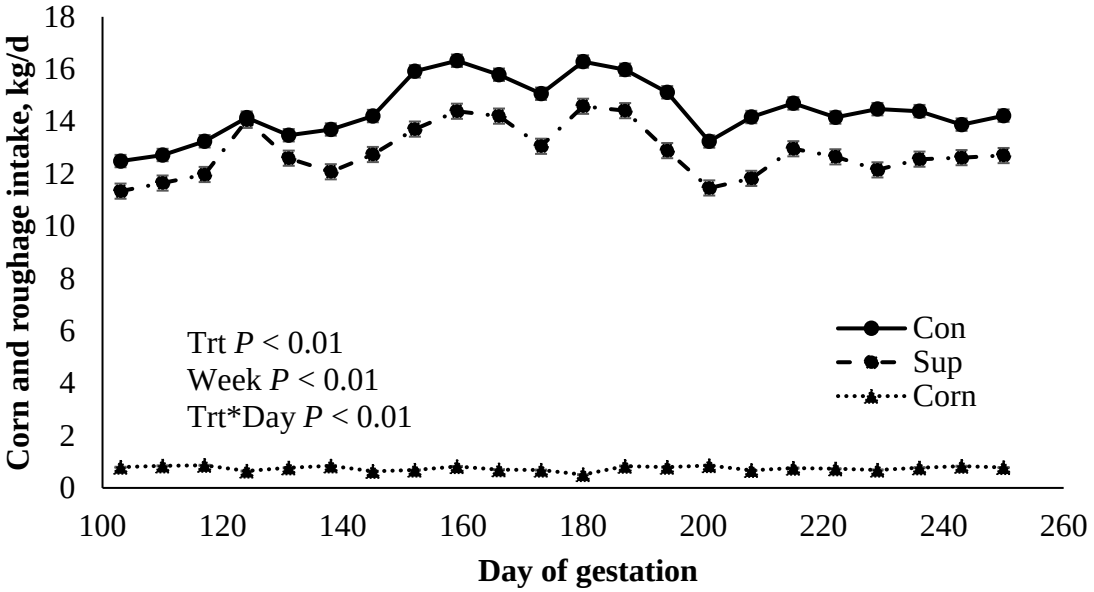

Figure 2.3. Gestational intakes of different diet components of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

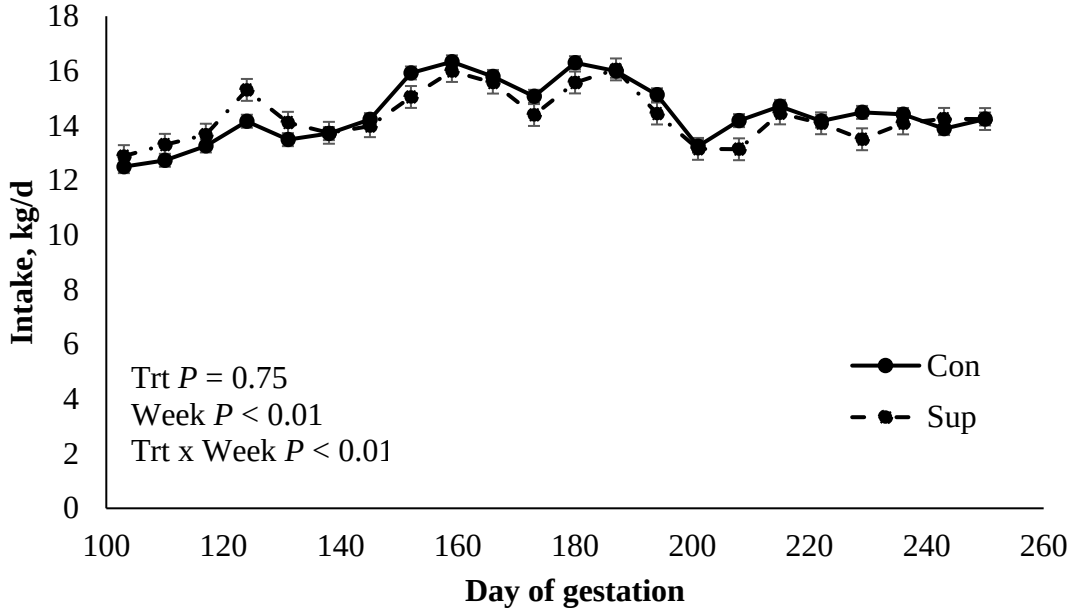

Figure 2.4. Gestational total intake (kg/d) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

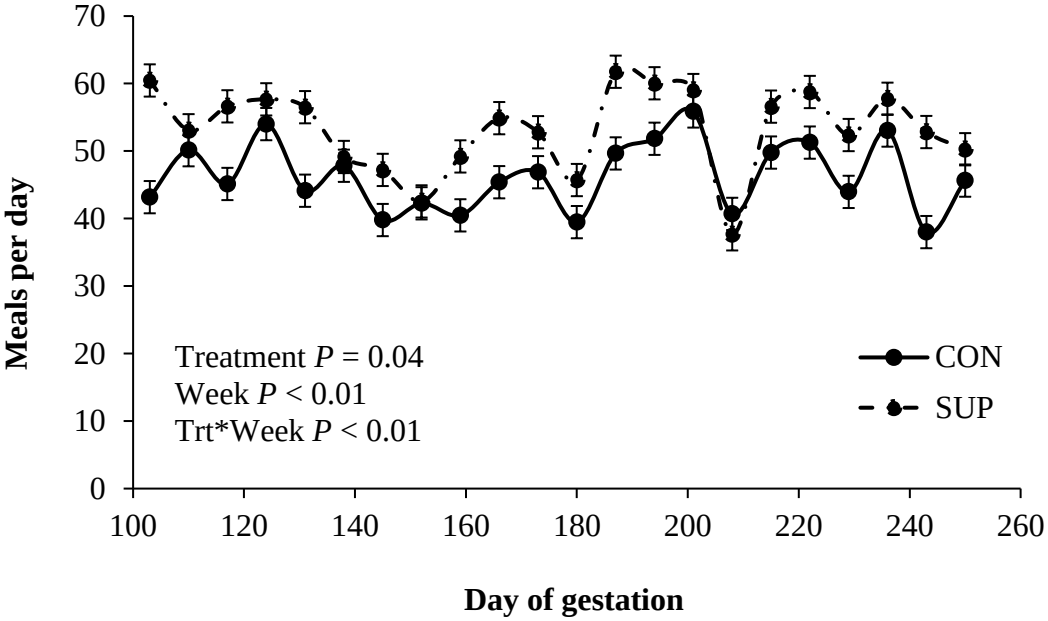

Figure 2.5. Gestational roughage meals of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

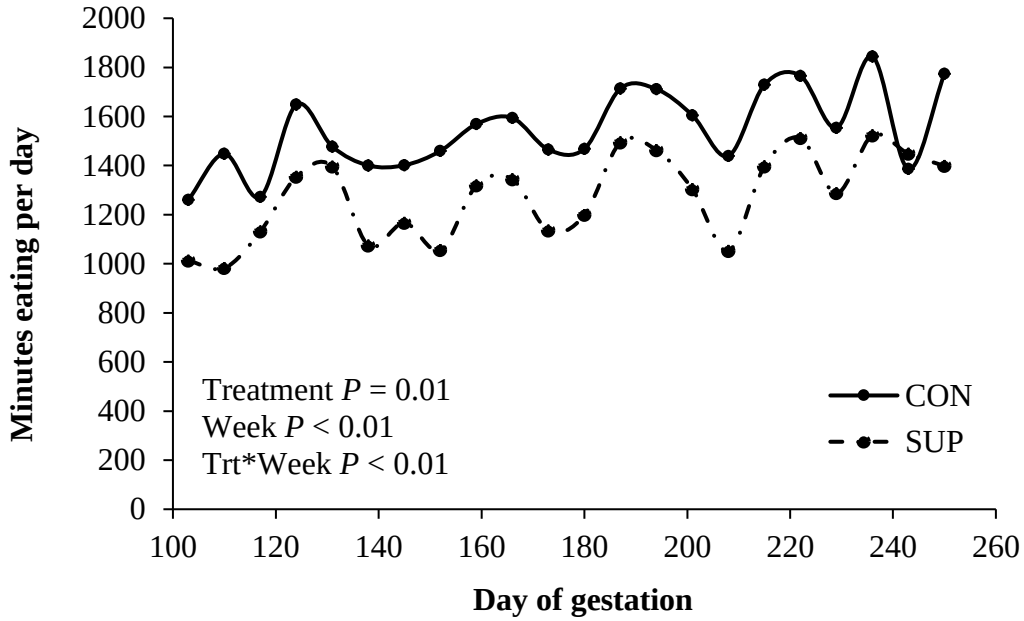

Figure 2.6. Gestational roughage intake time (min/d) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

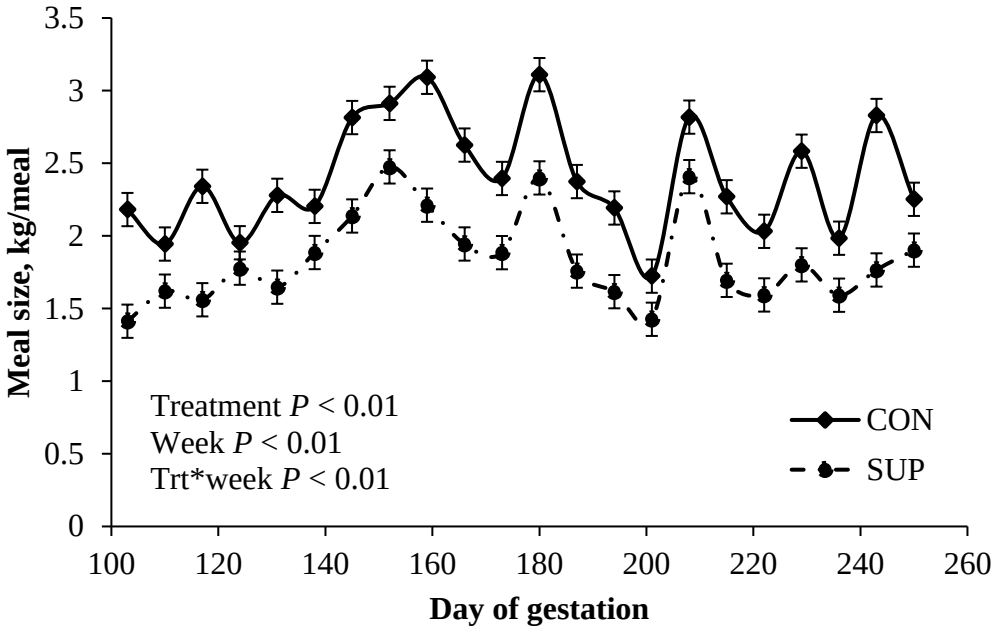

Figure 2.7. Gestational roughage meal size (kg/meal) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

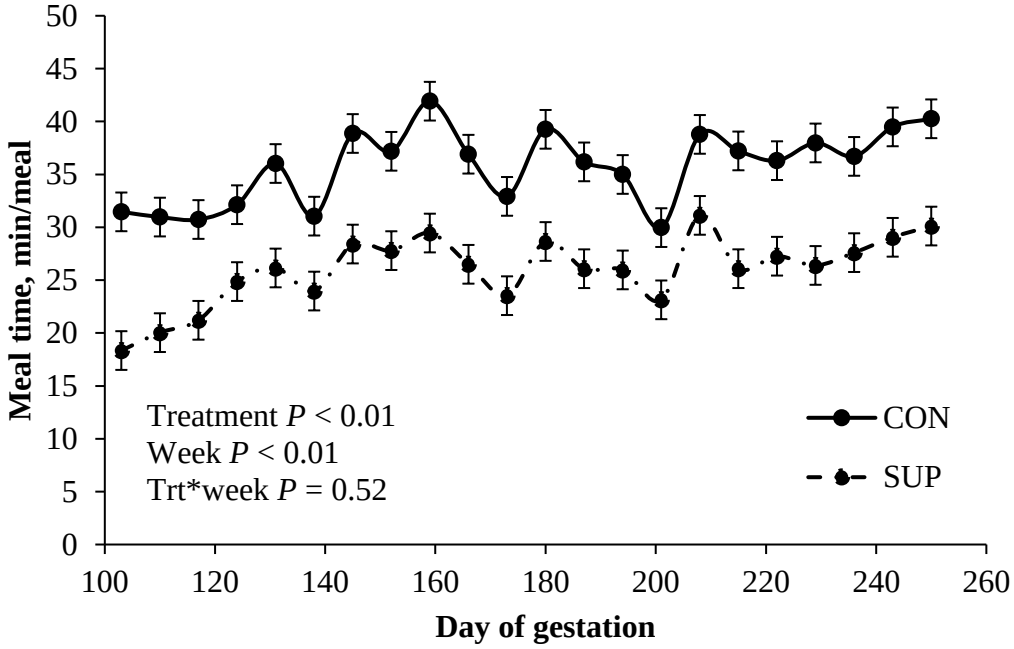

Figure 2.8. Gestational roughage meal time (min/meal) of beef cows fed the control and supplemented group from d 100 to d 240 of

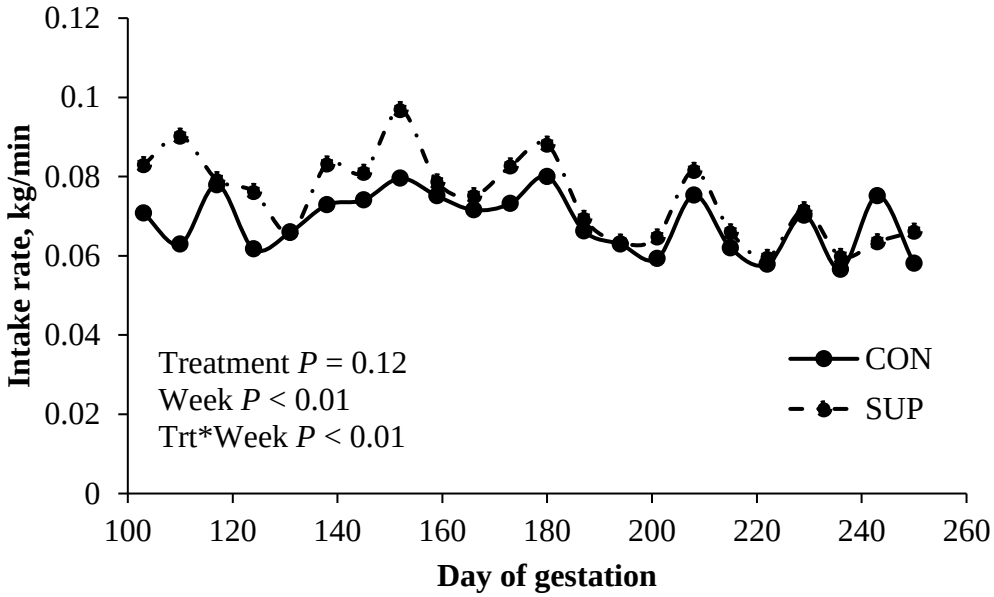

Figure 2.9. Gestational roughage intake rate (kg/min) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

Early-lactation intake and behavior

At d 265, all cows were transitioned to the same lactation diet and corn supplementation ceased. The twelve cows in one of the supplemented pens were relocated to the other 3 pens (4 additional per pen; n = 15 or 16 per pen) to make room for calving. Throughout the last few weeks of gestation and the first 3 weeks of lactation, intake was not altered by a week by treatment interaction ($P = 0.80$) but was influenced by week of lactation ($P < 0.001$). Furthermore, no treatment differences were detected ($P = 0.18$) as gestational corn supplemented dams consumed similar amounts of lactation ration (12.6 vs. 13.1 ± 0.3 kg/d; SUP vs. CON) as the CON dams.

Visit number was also not influenced by a week by treatment interaction ($P = 0.51$). Additionally, no treatment effects ($P = 0.63$) were observed for bunk visits (83.9 vs. 81.4 ± 3.7 visits per day) between SUP and CON dams, although they were altered ($P < 0.0001$) by week. Meal number was also not altered ($P = 0.62$) by a week by treatment interaction but was influenced ($P < 0.001$) by week of lactation. Additionally, meals consumed daily were not influenced by gestational dietary treatment ($P = 0.22$) with SUP and CON dams consuming similar meal numbers (8.1 vs. 7.6 ± 0.3). A treatment by week interaction ($P = 0.02$) was observed for time spent at the bunks which was variable and inconsistent so difficult to interpret and was not influenced by dietary treatment ($P = 0.98$; 145.4 vs 145.5 ± 4.9 min per d SUP vs. CON). Intake per week was also fluctuated frequently but changed ($P < 0.001$) weekly throughout lactation. However, cow gestation diet did not alter time A day by treatment interaction ($P = 0.04$) was also observed for intake rate which was altered ($P < 0.001$) by week but not by gestational dietary treatments ($P = 0.49$) with SUP and CON dams consuming the lactation ration at similar amounts (0.77 vs. 0.80 ± 0.02 kg/min).

In contrast, no week by treatment interaction ($P = 0.32$) was detected for lactation meal size, however, gestational dietary treatment did alter meal size as SUP dams had decreased ($P = 0.02$) meal sizes (5.4 vs. 5.6 ± 0.1 kg/meal) compared to CON dams. Week of lactation also influenced ($P = 0.02$) meal size. No week by treatment interaction ($P = 0.51$) was observed although intake rate was influenced ($P < 0.0001$) by week. Furthermore, no treatment effect ($P = 0.61$) was observed for lactation ration intake per visit (160 vs. 170 ± 1 g/visit) with SUP and CON dams consuming lactation rations at similar rates. Lactation meal time was also not influenced by a week by treatment interaction ($P = 0.33$) but was influenced by week ($P < 0.0001$). Meal size was also not influenced ($P = 0.14$) by dietary treatment as SUP and CON

dams consumed meals for a similar number of minutes (0.34 vs. 0.31 ± 0.001 min/meal). Lastly, a week by treatment interaction ($P = 0.02$) was detected for visit time but because of the inconsistency and lack of treatment differences ($P = 0.86$; 0.03 vs. 0.03 ±0.001 minutes per visit SUP vs. CON) is difficult to interpret. Minutes per visit also was highly variable but did change ($P = 0.03$) depending on week of lactation.

Cow blood metabolites

There was no day by treatment interaction ($P = 0.23$; Figure 2.10), nor main effects of treatment ($P = 0.12$) on glucose. There was a main effect of day of gestation ($P < 0.001$) on circulating plasma glucose which dropped in both treatments from d 100 to d 125 then remained throughout the rest of gestation. While no day by treatment interaction ($P = 0.14$; Figure 2.11) was detected for gestational NEFA, there was a main effect of treatment where SUP cows had decreased ($P < 0.001$) circulating levels of NEFA compared to CON cows. Additionally, a tendency ($P = 0.07$) for day was detected for NEFA which maintain consistent levels across gestation until d 240. Furthermore, while no day by treatment interaction ($P = 0.28$; Figure 2.12) was detected for urea during gestation, a treatment effect ($P = 0.02$) was observed as the SUP cows had decreased circulating urea than SUP throughout gestation. Additionally, day altered ($P < 0.001$) circulating urea levels which remained consistent across gestation aside from d 180.

Measurements of glucose (6.24 vs. 6.28 ± 0.44 m*M*), urea (10.92 vs. 9.51 ± 0.73 m*M*), and NEFA (545.65 vs. 620.07 ± 44.34 μ*M*) were similar ($P \geq 0.17$) between treatments for SUP vs CON cows at birth. By 24 h postpartum, there was no treatment effect ($P \geq 0.21$) on glucose (3.69 vs. 3.94 ± 0.14 m*M*) or urea (12.90 vs. 12.67 ± 0.68 m*M*) between SUP and CON dams, but SUP cows tended ($P = 0.06$) to have greater circulating NEFAs at 24 h postpartum (331.72 vs. 448.34 ± 43.07 μ*M*) compared to CON. By the third week post-calving, there was no effect

($P \geq 0.24$) of treatment on glucose (4.11 vs. 4.11± 0.08 m*M*), urea (8.46 vs. 8.85 ± 1.21 m*M*), or NEFA (596.06 vs. 702.18 ± 85.12 μ*M*) in SUP vs CON cows.

Calf growth

Calf birth weights were unaffected ($P = 0.87$) by maternal corn supplementation (39.6 vs. 39.8 ±1 kg; SUP vs. CON), as well as 24 h BW ($P = 0.68$; 40.0 vs. 40.5 ± 1.8 kg). At an average calf age of 3 wk postpartum, calves from SUP dams were heavier ($P = 0.05$) than calves from CON dams (72.5 vs 68.9 ± 3.5 kg). However, this disappeared ($P = 0.64$) by weaning (291.5 vs. 281.4 ± 8.1 kg for SUP vs. CON) at an average calf age of 168 d postpartum. Steers from corn-fed dams tended ($P = 0.06$) to have a greater ADG than steers from CON dams (1.48 vs. 1.35 ± 0.10 kg per day) until 3 wk postpartum when the pairs were transported to summer grazing pastures. This treatment effect of maternal diet disappeared ($P = 0.84$) by weaning (1.37 vs. 1.36 ± 0.10 kg per day).

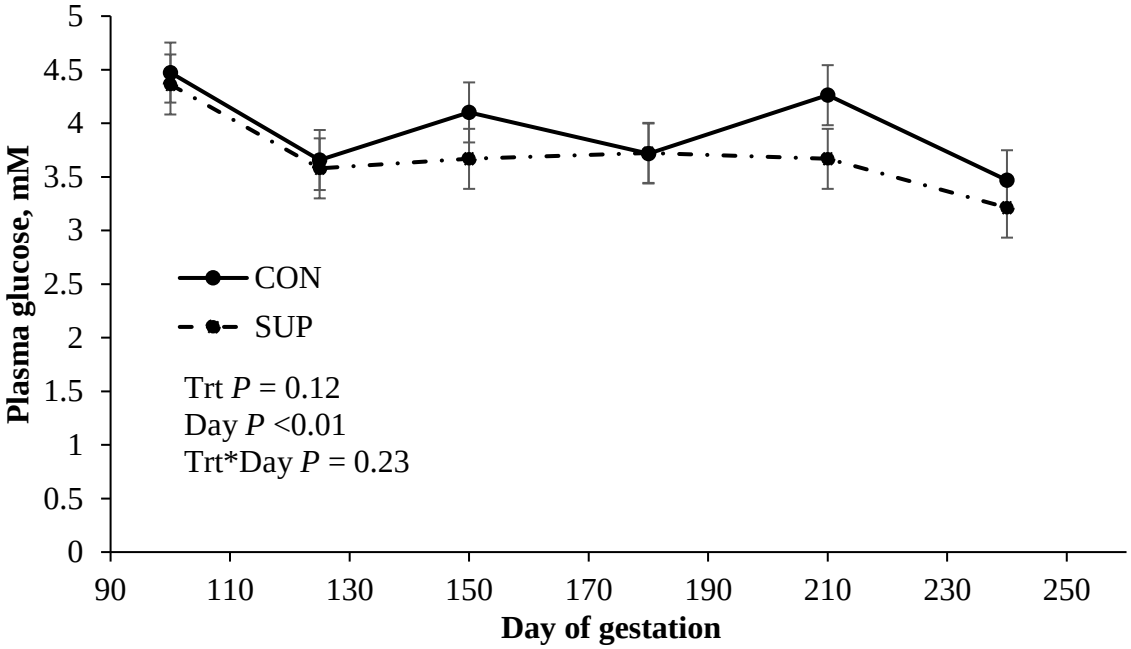

Figure 2.10. Gestational plasma glucose (m*M*) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

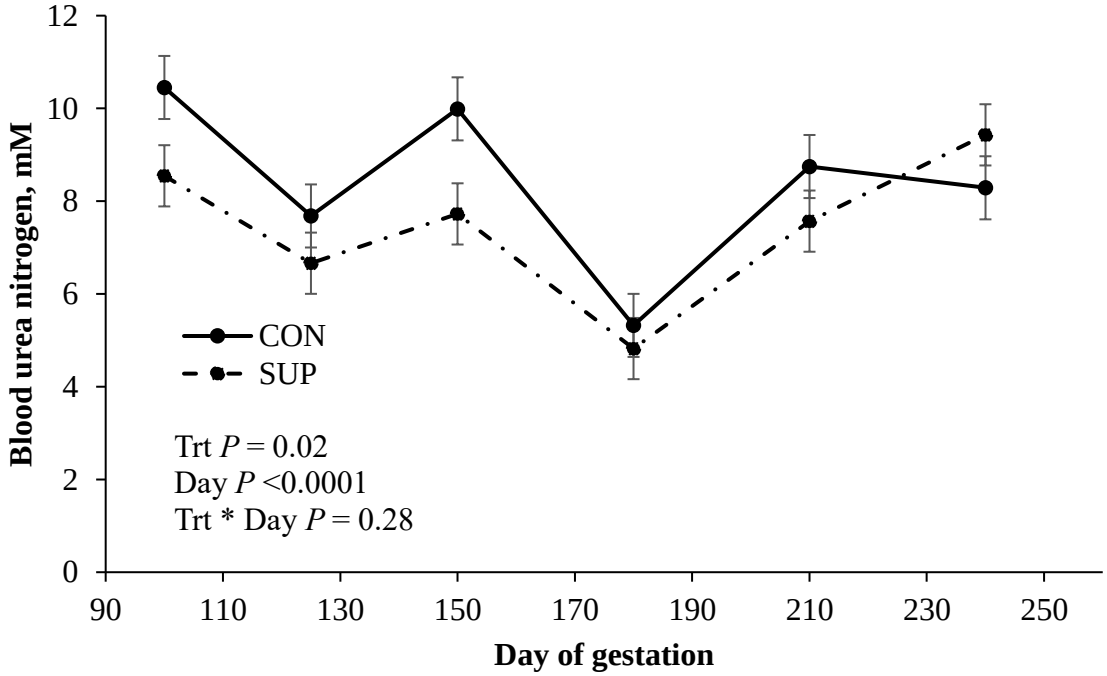

Figure 2.11. Gestational plasma urea concentration (m*M*) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

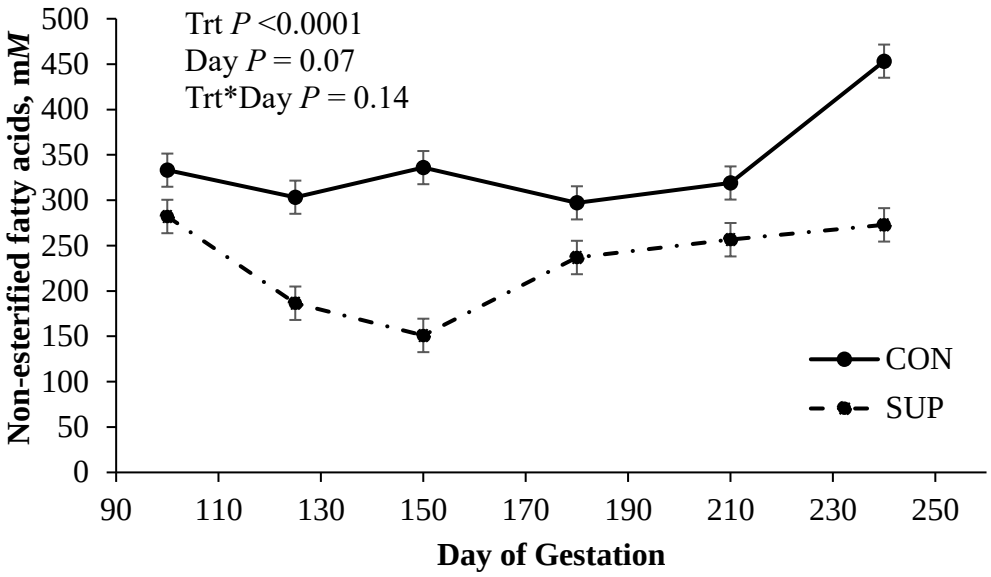

Figure 2.12. Gestational plasma non-esterified fatty acids (μ*M*) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

Discussion

We reject our hypothesis that corn supplementation will increase roughage consumption and, thereby, increase calf birth weights and growth. While TDN and NE_m intake were increased in SUP cows compared to CON dams, intake of roughage was suppressed in SUP cows. This negative effect of corn supplementation on forage intake has been well demonstrated in beef cattle fed forage-based basal diets (Mould et al., 1983; Firkins et al., 1984; Lusby and Wagner, 1986; Chase and Hibberd, 1987; Sanson et al., 1990; Pordomingo et al., 1991; Loy et al., 2007; Leupp et al., 2009) This effect of reductions in forage intake resulting from energy supplementation is referred to as substitution (Caton and Dhuyvetter, 1997). Additionally, as reviewed in Caton and Dhuyvetter (1997), several factors could be responsible for this effect including decreased ruminal pH, carbohydrate effect, and altered forage digestibility.

Previous studies have suggested that corn supplementation less than 0.25% of BW does not decrease forage utilization (Pordomingo et al., 1991; Matejovsky and Sanson, 1995) which disagrees with what we observed. It should be recognized that the variation in reductions of forage intake because of energy supplementation could be due differing basal forage quality and sources. While studies reviewed by Caton and Dhuyvetter (1997) varied greatly in their forage digestibility responses to energy supplementation, this could be in part due to dietary protein concentration. In fact, a study by Sanson et al. (1990) suggest that when CP is limiting, energy supplementation alone might worsen the CP deficiency.

This might explain how the increased calculated caloric intake per unit of metabolic body weight in our SUP dams did not account for changes in BW and BCS (data not shown). This could be potentially explained by the likely shift in ruminal microbial populations with probable lower ruminal pH as energy supplementation drives bacteria towards greater amylolytic and lower cellulolytic populations (Caton and Dhuyvetter, 1997). This diet driven shift has been documented through RT-qPCR analysis in cannulated dairy steers that were first fed roughage based diets then transitioned to a ration with higher starch concentrations (Tajima et al., 2001).

To navigate around this substitution effect on fiber digestion and still positively influence the energy status of beef cows fed a low-quality forage diet, alternative energy sources with readily degradable fiber such as wheat midds and corn gluten have been shown to not decrease forage intake as much as high concentrate supplements (Carey et al., 1993). Furthermore, other studies that have fed corn to gestating beef cows have navigated around this effect by supplementing an additional protein supplement and urea to try to prevent a subsequent protein restriction (Loerch et al., 1996; Radunz et al., 2013). While a day by treatment interaction was observed for dam BW and BCS in cows receiving a corn supplemented, the relationship when

accounting for calories consumed per unit of metabolic body weight (data not shown). This is could be due to the substitution effect of corn on forage intake.

While corn supplementation suppressed maternal forage intake throughout the trial, total intake was unaffected by 0.2% of BW corn supplementation. Comparable results were observed in cattle fed a high concentrate barley supplement as a reduction in forage intake was observed but total feed intake was unaltered (Westvig, 1992; reviewed in Caton and Duhyvetter, 1997). The decrease in roughage resulting from the substitutionary effect of corn can be explained physiologically through chemostatic or metabolic feedback that regulate intake to attempt to meet energetic and protein requirements of the ruminant (Illius and Jessop, 1996). Balance of protein and energy is essential to maintain because the animal integrates multiple feedbacks to regulate feed intake and an imbalance can have detrimental impacts on intake (Illius and Jessop, 1996; Fisher, 1996). When dietary imbalances between protein and energy exist, voluntary DMI of high energy diets can be reduced because of the metabolic limitations to processing energy (Fisher, 2002). This could be the reason we observed not only suppressed roughage intake when corn was supplemented but also documented decreased time spent consuming forage (min/d), decreased intake rates (kg/min), smaller meals (kg/meal), less roughage consumed per visit (kg/visit), as well as decreased visit and meal time.

The aforementioned intake constraints as a result of protein to energy ratio imbalance become even more exacerbated when protein is limiting; however, diets containing more protein than needed may not be as detrimental as the animal can use the surplus protein as an energy source (Fisher, 2002). This would agree with previous work from our lab provided late gestating beef cows (d 180 to d 246) with *ad libitum* access to forage and limit fed dry corn distiller's grains with solubles (DDGS) supplement at 0.3% BW (Kennedy et al., 2016a). Through this

supplementation strategy, maternal roughage intake and calf birth weights were both improved (Kennedy et al., 2016b). Additionally, the DDGS supplemented animals spent more time consuming roughage, and had increased meal sizes and intake rates suggesting the positive impact that DDGS supplementation had on maintaining the protein to energy ratio and positively influencing intake and feeding behavior (Kennedy et al., 2016a).

However, this increase in calf birth weights are not always increased in studies using DDGS supplementation to pregnant beef cows. A study that supplied beef cows with DDGS at 0.4% of BW with *ad libitum* access to grass hay also noticed an increase in total DMI consumption, although roughage intake and calf birth weights were not improved (Klein et al., 2014). However, when DDGS are supplemented up from 0.5 to 1.0% of BW, intake and corresponding intake behavior were decreased as DDGS supplement increased linearly in growing calves (Islas et al., 2014). This would agree with the idea that cattle are sensitive to protein: energy ratios and even protein supplementation at elevated levels can have negative impacts on forage intake but this is not currently fully understood in gestating beef cows. Our trial suggests that maintaining the proper protein to energy ratio, not just increasing dietary calories is necessary for positive impacts on calf growth as observed in Kennedy et al. (2016). This would also be supported by Mordhorst et al. (2016) that reversed the protein to energy ratio by increasing dietary protein without allowing increased forage intake also did not improve calf birth weights.

Additionally, because cows were returned to pens with Insentec feeders 24 h after calving in order of calving, original pen compositions were altered. The authors recognize that this as well as the pre-calving redistribution of one supplemented could potentially mask some effects

of gestational treatment as a new social hierarchy could have been established and influenced feeding behavior.

Often, the circulating concentrations of NEFA generally increase as caloric intake decreases and can be used to indicate mobilization of lipid stores (Lucy et al., 1991). In the current study, CON dams had greater concentrations of circulating NEFA throughout the gestation, which agrees with Radunz et al. (2013) that observed that hay–fed dams tended to have higher NEFA when compared to corn-fed dams during gestation. However, dietary treatment differences in plasma NEFA are not necessarily indicative of different energetic status where researchers (Althen et al., 1990; Rusche et al. 1993, Sullivan et al., 2009; Islaset al. 2014) observed no differences in circulating NEFA while other metabolic parameters were modified. In the current study, NEFA concentrations were greater in CON dams prior to beginning dietary treatments and remain elevated during gestation, so it is difficult to determine if this is due to energetic status or other factors. Additionally, circulating concentrations of NEFA on this project were less than half the value those reported in Klein et al. (2014) and Mordhorst et al. (2016) for cows that were nutrient restricted due to being fed low quality forage. Combined with the NRC (2016) estimation of both SUP and CON dams receiving 100 to 110% of their daily needs, respectively, perhaps the difference detected between treatments are not strictly an indicator of metabolic status and just a by-product of fatty acid metabolism. Additionally, NEFA tended to be greater in SUP than CON dams at 24 h postpartum but not at parturition or at 3 weeks of lactation.

Serum urea can be an indicator of protein supplied in the diet, protein degradation in the rumen, and N utilization in the rumen (Reed et al., 2007). Although circulating urea could be from many sources including the diet (Hammond, 1983), the MP restriction brought about corn

supplementation likely explains the SUP cows having decreased urea compared to CON dams in the current study. This would agree with Swanson et al. (2017) noting a decrease in urea in MP restricted (60% of MP requirements) ewes compared to control ewes (100% of MP requirements). Klein et al. (2014) also observed decreased concentrations of urea in the blood of cows with lower concentration of protein in their diet. Furthermore, cattle that were supplemented with dietary protein also had higher circulating urea in their blood (Radunz et al., 2013; Klein et al., 2014; Islas et al., 2014; Mordhorst et al., 2016) The authors acknowledge that circulating concentrations of urea began lower in SUP cows prior to beginning dietary treatments on d 110 of gestation and it is difficult to explain those differences.

The principal source of glucose in the ruminant is from gluconeogenesis, driven by the production of volatile fatty acids (propionate) by ruminal microbes (Young, 1977). It is not surprising that no differences were observed in plasma glucose concentrations as both SUP and CON dams met their daily NRC requirements. Radunz et al. (2013) also observed that there were no dietary differences in circulating glucose because of corn supplementation to late gestating beef cows compared to a hay diet. Sheep limit-fed high grain diets had greater maternal circulating plasma glucose (Susin et al., 1995) which improved lamb birth weights (Stevens et al., 1990). In the current study as well as other studies when corn supplement was provided to gestating beef cows (Radunz et al., 2013) limited impacts on circulating plasma glucose was observed. This variability could be due to decreased acetate: propionate ratio that has been documented in wet distillers grain fed steers when compared to steers fed a higher concentrate diet (Vander Pol et al., 2009). Neither of our treatments were receiving supplemental protein, it is not surprising their glucose was unaltered.

Calf birth weights were not altered as result of maternal dietary corn supplementation which agrees with data from Loerch et al. (1996) who also did not observe heavier birth weights from calves from corn fed dams. The effects of supplementation on calf birth weight are highly variable and dependent on many factors but many studies supplementing energy or protein did not observe improved calf weights until weaning or at all (Stalker et al., 2006; Underwood et al., 2010; Klein et al., 2014; Shoup et al., 2015; Wilson et al., 2015; Mordhorst et al., 2016;). Additionally, birth and 24 h weights were also not improved by maternal corn supplementation. Interestingly, steers from corn-fed dams at 21 days postpartum were heavier than calves from hay fed dams but this effect disappeared at weaning. While maternal intake did not differ during the first 3 wk of lactation, perhaps the access to higher quality lactation ration with DDGS enabled the SUP cows to produce either more milk or higher quality milk for their calves. In fact, steer ADG until shipping at 21 days was greater in steers from dams receiving the supplement. One possible explanation is that the CON calves were beginning to show signs of the "thrifty hypothesis" and were more efficient with their nutrients when in an extensively managed environment. Calves from dams that were either supplemented with additional protein, adequate protein or high protein also did not have increased weaning weights (Schoonmaker et al., 2003; Summers et al., 2015). There is much variation in the literature about whether supplementation improved weaning weights. One likely explanation is the variability of forage quality in the various trials. Another potentially reason could be a ruminal response due to the supplemental dietary protein providing with the low-quality forage, correcting for a ruminal microbial nitrogen deficiency and improving rumen degradable protein

Implications

While supplementation of additional dietary energy did increase TDN and NEm consumed by the corn-supplemented dams, a likely dietary imbalance between protein to energy ratio was established and voluntary dry matter intake of roughage was reduced. This is most likely because of the metabolic limitations of processing energy, likely ruminal, and as well as the shift in ruminal microbial populations that result in decreased forage fermentation efficiency. While this feeding strategy was not detrimental to calf birth weight and did maintain cow BW and BCS, high-starch energy supplementation seems to have limited benefits unless provided with supplemental protein. In fact, elevated levels of high-starch supplementation could even have detrimental impacts on maternal metabolism and fetal growth if a severe protein restriction was created. A more effective feeding strategy could involve the supplementation of high energy sources with readily degraded fiber that does not have an inhibitory effect on forage intake. However, because the current feeding strategy of 0.2% corn supplementation has no impact on fetal growth and birth weights, corn appears to be a good substitute for hay if the primary goal is to maintain BCS and prevent fetal growth restriction. In fact, this feeding strategy could be advantageous economically for the producer depending on the cost of feed inputs or availability of resources.

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CHAPTER 3. USING CORN SUPPLEMENTATION FOR OVERWINTERED BEEF COWS DURING MID- TO LATE-GESTATION DECREASES PLACENTAL VASCULARITY BUT DOES NOT ALTER UTERINE BLOOD FLOW OR NEONATAL PERFORMANCE

Abstract

Gestating cows fed low-quality forage diets in extensively managed operations are often at risk of nutrient restriction. Thus, the objective of this study was to evaluate the effects of supplementing corn with low-quality forage to gestating beef cows by tracking uterine hemodynamics and neonatal performance. We hypothesized that mid- to late-gestating beef cows receiving corn supplementation would have greater uterine hemodynamics, increased placental vascularity, and give birth to faster growing calves. Multiparous Angus-based cows (n = 47) carrying bull calves were assigned randomly to treatments receiving corn supplementation at 0.02% of BW (SUP; n = 24) or no supplement (CON; n = 23). All cows were fed the same basal diet (60% hay, 30% wheat straw, and 10% CSB). Intake was monitored individually with Insentec feeders from d 100 of gestation through calving. Uterine hemodynamics were monitored using Doppler ultrasonography every 28 days from the start of supplementation until d 240 of pregnancy. At birth, pair weights, colostrum samples, and placental tissues were collected. Calf weights were recorded at 3 weeks postpartum and weaning (d 168). Representative cotyledons were excised from fetal membranes and histological analysis of placental vascularity with hematoxylin and eosin was conducted. All measurements and data collected were analyzed with the MIXED procedure of SAS and means were separated using the LSMEANS statement in SAS. Uterine hemodynamics, placental weight, and colostrum variables were not altered by supplementation ($P \geq 0.13$). Placental vascular surface density was

suppressed ($P < 0.01$) by maternal corn supplementation. While calf birth weights were not altered by maternal corn supplementation ($P > 0.50$), calves from SUP dams were heavier at 3 weeks postpartum ($P = 0.05$) but not at weaning ($P > 0.60$). While corn did decrease maternal roughage intake, it does appear to be a good substitute for hay as it does not have negative effects on birth or weaning weights.

Introduction

Inadequate maternal nutrition during pregnancy can lead to a decrease in carcass quality of the offspring, including altered fat deposition, muscle fiber type, and reduced meat quality (Wu et al., 2006). In fact, offspring of cows that did not receive protein supplement in late-pregnancy had reduced postnatal gain and intramuscular fat accretion (Stalker et al., 2006; Funston et al., 2010). Unfortunately, nutrient restriction is still common in extensively managed, overwintered, gestating beef cows due to limited access to high quality forage. Nutrient restriction can be resolved by improving the forage quality of beef cows during late gestation, but if that feed resource is not available, an alternate feeding strategy should be employed.

One way to estimate nutrient supply to the gravid uterus is by measuring uterine arterial blood flow (Ferrell, 1991). Doppler ultrasonography is a non-invasive, repeatable way to measure changes in uterine hemodynamics to detect the consequences of poor maternal nutrition and target when therapeutic interventions should be applied (Vonnahme and Lemley, 2011). We previously demonstrated that the supplementation of dried distiller's grains plus solubles (**DDGS**) with *ad libitum* access to low-quality forage to beef cows in late gestation increased dry matter intake and uterine blood flow to the gravid uterus thereby improving calf birth weights (Kennedy et al., 2016b). Thus, it is logical to target winter nutritional management strategies

during gestation to increase uterine blood flow for improved fetal development and carcass characteristics.

We hypothesized that corn supplementation of beef cows during mid- to late-gestation would result in increased feed intake, greater total uterine blood flow, and birth heavier, faster growing calves. The objectives of this study were to evaluate the effects of corn supplementation in gestating beef cow on uterine and mammary hemodynamics, endocrine profiles, placental morphology, colostrum production, and neonatal performance and growth.

Materials and methods

Experimental design, cows, and dietary treatments

All procedures were approved by the North Dakota State University (**NDSU**) Animal Care and Use Committee (IACUC #A16010). Treatments applied to the cows have been previously described (Tanner et al., 2017). Briefly, 47 multiparous, Angus-based beef cows carrying bull calves (confirmed via ultrasonography at d 70 of gestation) from artificially inseminated by 4 different sires from the Central Grasslands Research and Extension Center in Streeter, ND were transported to the NDSU Beef Cattle Research Complex in Fargo, ND. The cows were assigned randomly to a control treatment group (**CON**; n = 23) and a treatment group (**SUP;** n = 24). Both treatments received a basal diet of (30% straw, 60% hay, and 10% concentrated separator by-product; **CSB**) with the SUP group receiving an additional corn supplement at 0.2% of BW in addition to the *ad libitum* TMR. After a 3-wk acclimation and training period, dietary treatments were applied and intake was monitored and controlled via roughage intake control feeders (Insentec B.V., Markenesse, The Netherlands) beginning on d 100 of gestation for 22 wk. At d 265 of pregnancy, all cattle were fed a common lactation diet (45 % straw, 30% corn silage, and 25% DDGS).

Upon beginning the trial at d 100 of gestation until d 240, cows were weighed and body condition scored every 28 d and the effects of maternal dietary treatment on cow weight gain and BCS have been previously reported (Chapter 2). On each weigh day, blood samples were collected via jugular venipuncture and each sample was centrifuged for 20 min at 1,380 x g at 4 °C to separate each serum and plasma samples for respective analyses. Additionally, weights and jugular blood samples were collected on each cow-calf pair at parturition, 24 h postpartum, and 3 wk postpartum. All samples were stored at -20°C until analysis for concentrations of cortisol, triiodothyronine (**T3**), thyroxine (**T4**), progesterone (**P4**), and estradiol 17-β (**E2**).

Uterine and mammary hemodynamics

Uterine hemodynamic measurements were taken in accordance with Kennedy et al. (2016b) by using color Doppler ultrasonography (model SSD-3500; Aloka ProSound Alpha 6) equipped with a 7.5 MHz linear finger-probe transducer (Aloka UST-672; Aloka, Wallingford, CT, USA) at d 100, d 125, d 150, d 210, and d 240 of pregnancy. Briefly, ipsilateral and contralateral uterine arterial blood flow was measured by inserting a 7.5 MHz finger probe into the rectum and tracing the descending aorta to the bifurcation of the internal and external iliac arteries. The internal iliac artery was identified then traced caudally to locate the descending uterine artery. Probe placement was confirmed by its Doppler coloration, fremitus, and maneuverability. Measurements were taken immediately posterior to the first branch of the uterine artery for consistency.

Mammary hemodynamics measurements were taken in accordance with Mordhorst et al. (2016) at d 240 of pregnancy. Briefly, ipsilateral and contralateral external pudendal (mammary) blood flow were measured by locating the descending aorta was identified and tracing to the bifurcation of the external and internal iliac arteries. The external iliac artery was traced until the

external pudendal artery could be visualized. The external pudendal artery is considered representative of blood supply to the mammary gland (Götze et al., 2010; Potapow et al., 2010; Mordhorst et al., 2016). Doppler mode was utilized to confirm measurement of the artery and not surrounding vasculature.

For each arterial blood flow measurement, a minimum of three similar cardiac waveforms from three separate ultrasonography evaluations were assessed per side to calculate average pulsatility index (**PI**), resistance index (**RI**), and uterine arterial blood flow. The Doppler ultrasound instrument was programmed to automatically calculate PI [9\(peak systolic velocity - end diastolic velocity)/ mean velocity]; RI [(peak systolic velocity – end diastolic velocity) / peak systolic velocity]; and blood flow [(mL/min) = mean velocity (cm/s) x (π/4) x CSA (cm^2) x 60 s]. Maternal heart rate (**HR**) was reported as a two-beat average for each series of three waveforms and averaged across sides on each day. Ipsilateral horn was identified as the side of the animal with the greatest blood flow.

Parturition

Calving was monitored continuously on a 24 h basis from d 265 of gestation until all calves were born. Upon observation of the water breaking, the time and environmental conditions were recorded until the calf was on the ground. The pairs were brought immediately into the barn for monitoring and health analysis following the procedures as described (Kennedy et al., 2016b). Briefly, in addition to calf BW and sex, crown-rump length and heart girth measurements were also recorded at birth. Additionally, all calves were knife-castrated with aseptic techniques after 2 mL of a 2% (v/v) lidocaine (MWI/VetOne, Boise, ID) at 12 h post-partum.

Cows were weighed and samples for metabolic profiles were collected via jugular venipuncture and the caudal-right teat was milked completely right after parturition for colostrum weight and component analysis. In accordance with Kennedy et al. (2016b), the time to placental expulsion was recorded and each placenta was collected immediately. After placental weights were recorded, cotyledonary and intercotyledonary tissues were dissected and weighed. After weighing, representative cotyledons were processed and fixed in formalin for histological preparation. Furthermore, the smallest and largest cotyledons were weighed and total cotyledon number was quantified. After 24 h, cow-calf pairs were returned outdoors after monitoring in individual indoor pens.

Placental vascularity

Fixed cotyledons were embedded in paraffin and sectioned on a microtome at 5 μm. Sections were stained with hematoxylin and eosin. Four representative images were taken of each sample at 20x magnification with a Axio Imager.M2 microscope equipped with an AXioCamHR3 color camera and AxioVision 4.8 software (Carl Zeiss International, Jena, Germany). Image analysis was performed using the Image-Pro Premier 9.2 software package (Media Cybernetics, Inc., Rockville, MD). At least three randomly chosen polygons were drawn in each image to determine vascular area density (**VAD,** % = vascular area/unit tissue area), indicator of blood flow and vascular surface densities (**VSD**, μm^2/unit tissue volume in μm^3), indicator of nutrient exchange (Vonnahme et al., 2015) as described by Borowicz et al. (2007). Total vascular volume in ml was calculated as VAD × total cotyledonary weight (g), assuming VAD is equivalent to vascular volume density (Borowicz et al., 2007).

Cortisol analysis

Cow and calf serum cortisol concentrations were determined via solid-phase, competitive chemiluminescent enzyme immunoassay kits (stored at 4ºC) and evaluated according to manufacturer's instructions (IMMULITE® 1000; Siemens, Los Angeles, CA, USA). Within each assay, low-, medium-, and high-cortisol pools were assayed in duplicate (26.35 ± 1.11, 193.75 ± 5.95, and 320.63 ± 18.12 ng/mL respectively). Serum samples were brought to room temperature (23ºC) and 200 µL of serum was added to barcode-labeled sample collection cups for analysis. The average intra-assay and inter-assay coefficients of variation for cortisol were 6.5% and 4.3% respectively.

Thyroid hormone analysis

Cow and calf serum T3 and T4 concentrations were determined via IMMULITE® 1000 (Siemens, Los Angeles, CA, USA) solid-phase, competitive chemiluminescent enzyme immunoassay kits (stored at 4ºC) and evaluated according to manufacturer's instructions. Within each assay, low-, medium-, and high-T3 (0.73 ± 0.08, 2.04 ± 0.51, and 3.48 ± 0.15 ng/mL respectively) and T4 pools (39.5 ± 3.8, 115.2 ± 9.53, and 140.1 ± 7.4 ng/mL respectively) were assayed in duplicate. Serum samples were brought to room temperature (23ºC) and 200 µL of serum was added to barcode-labeled sample collection cups for analysis. Each sample was run in duplicate. The average intra-assay and inter-assay coefficients of variation for T3 were 13.5%, and 6.5% respectively. The average intra-assay and inter-assay coefficients of variation for T4 were 7.7% and 5.4% respectively.

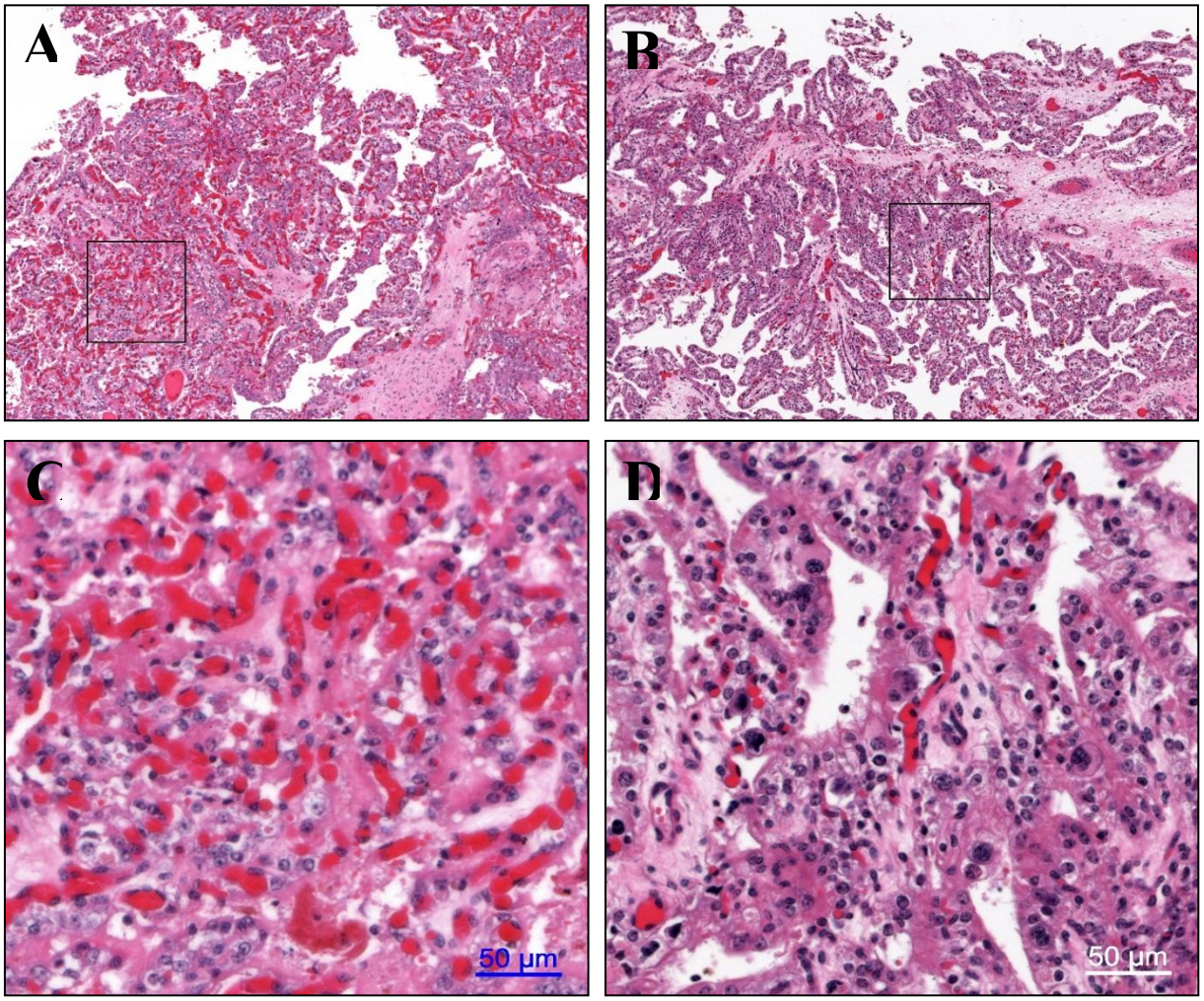

Figure 3.1. Hemotoxylin & eosin staining of cows fed the control (A &C) and supplemented (B & D) group from d 100 to d 240 of gestation. C and D are magnified sections of A & B (identified squares).

Estradiol-17β and progesterone analyses

Plasma E2 concentrations were determined by RIA using methods described by Perry and Perry, 2008. The average intra-assay and inter-assay CV for E2 was < 15%. Plasma P4 concentrations were determined by RIA. The average intra-assay and inter-assay CV for P4 was 4.5% and 9.6% respectively.

DHIA colostrum analysis

Colostrum samples were collected from dams within 30 minutes of expulsion of the calf. One cc of oxytocin (VetOne, Boise, Idaho) was administered intramuscularly to encourage milk letdown prior to colostrum collection. The caudal- right teat was milked completely with an automated milk-collection device (InterPuls, Albinea, Italy), which was sanitized between cows. The samples were placed into DHIA (Dairy Herd Improvement Association) vials and stored at 4 °C until analysis (Dairy Lab Services, Inc., Dubuque, IA).

Statistical analysis

All cow gestational blood flow data were analyzed with repeated measures by weekly averages of daily observations using generalized least squares (MIXED procedure; SAS Institute Inc., Cary, NC). Gestational hormone data were also analyzed with the repeated measures by day of gestation statement using generalized least squares. Birth and lactation data were also analyzed with the generalized least squares to account for the random effect of sire. Model statements included cow, maternal diet (CON vs. SUP), day of gestation, and a diet by day of gestation interaction. Sire (n = 4) was treated as a random variable. For 0 and 24-h weight measurements, gestation length was analyzed as a covariate. For 3-wk calf weight measurements, age of the calf was used as a covariate. For 3-wk maternal and calf hormone and weight data, day of lactation was used as a covariate. All meaningful interactions were

considered and in the absence of interactions (P > 0.10), main effects were discussed. Various covariance structures were assessed; those structures with the lowest fit statistics were utilized (Wang and Goonewardene, 2004). Means were separated using least significant difference (PDIFF option of LSMEANS). Solutions for linear, quadratic, and cubic relationships were requested where applicable. Four cows were excluded from the lactational data analysis due to mortality (n = 3) and morbidity (n = 1).

Results

As previously reported (Chapter 2) roughage intake was reduced ($P < 0.001$) in SUP vs. CON cows (12.8 vs. 14.4 ± 0.29 kg/d DM basis). However, kg of TDN consumed tended to be greater in SUP cows ($P = 0.06$) because of the substitutionary effect of corn intake (1.47 ± 0.1 kg/d DM basis). Additionally, day by treatment interaction were observed for cow BW ($P < 0.01$) and BCS ($P \leq 0.05$) as SUP cows gained more BW and BCS as gestation advanced.

Uterine blood flow and hemodynamics

No day by treatment interactions ($P \geq 0.13$) were detected for any hemodynamic measurements. While maternal treatment did not impact ($P \geq 0.27$) uterine blood flows (Figures 3.2 A, B and 3.3 C) there was a day effect where blood flow increased ($P < 0.0001$) as gestation advanced in ipsilateral, contralateral and total blood flow (Figures 3.2 A, B and 3.3 C). Maternal heart rate was not affected ($P = 0.27$; Figure 3.3 D) by treatment, but increased as gestation advanced (Figure 2D). Area under the curve analyses were not affected by treatment ($P > 0.20$; data not shown).

While PI was not altered ($P > 0.10$) by treatment, PI decreased ($P > 0.0001$) in both the ipsilateral and contralateral uterine arteries as gestation advanced (Figure 3.4 A, B). The RI measurement in the contralateral uterine artery was not affected ($P > 0.20$) by treatment and

decreased ($P < 0.0001$) as gestation advanced (Figure 3.5 D). Ipsilateral uterine artery RI tended ($P = 0.06$) to be increased in SUP cows compared to CON cows (Figure 3.5 C) and the area under the curve also was increased in SUP vs CON cows (76.23 vs. 74.69 ± 1.44 arbitrary units). Interestingly, blood hematocrit was altered by maternal diet ($P < 0.001$) with SUP cows having lower hematocrit than CON (37.4 vs. 35.0 ± 0.5%; Figure 3.6).

Mammary blood flow

At d 240 of pregnancy, there was no effect ($P \geq 0.12$) of treatment on PI, RI, total mammary gland blood flow, or mammary blood flow ipsilateral to the pregnant uterine horn (Table 3.2). The mammary gland blood flow contralateral to the pregnant uterine horn was decreased ($P = 0.05$) in SUP vs CON cows (Table 3.1).

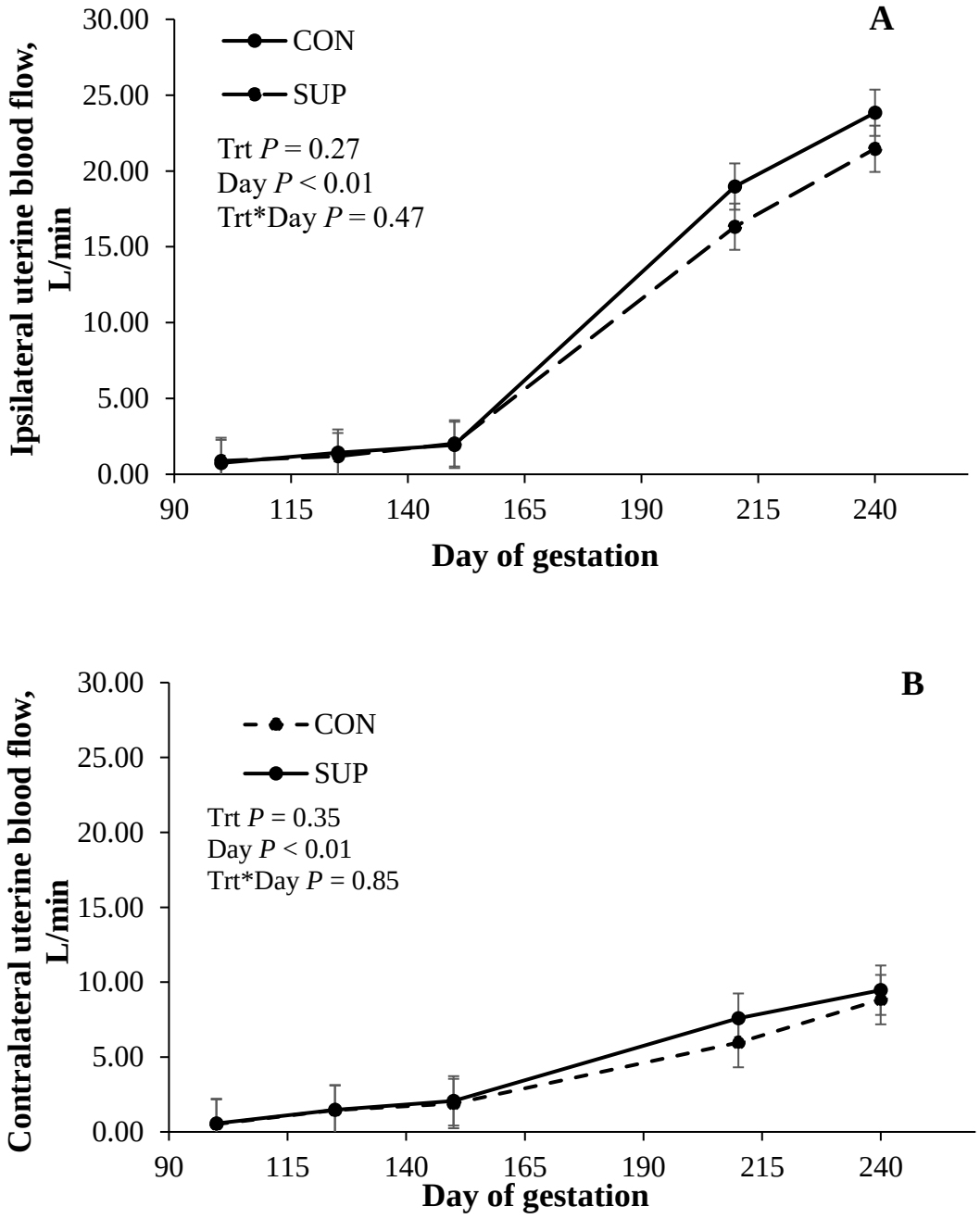

Figure 3.2. Ipsilateral uterine artery blood flow (A) and contralateral uterine blood flow (B) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

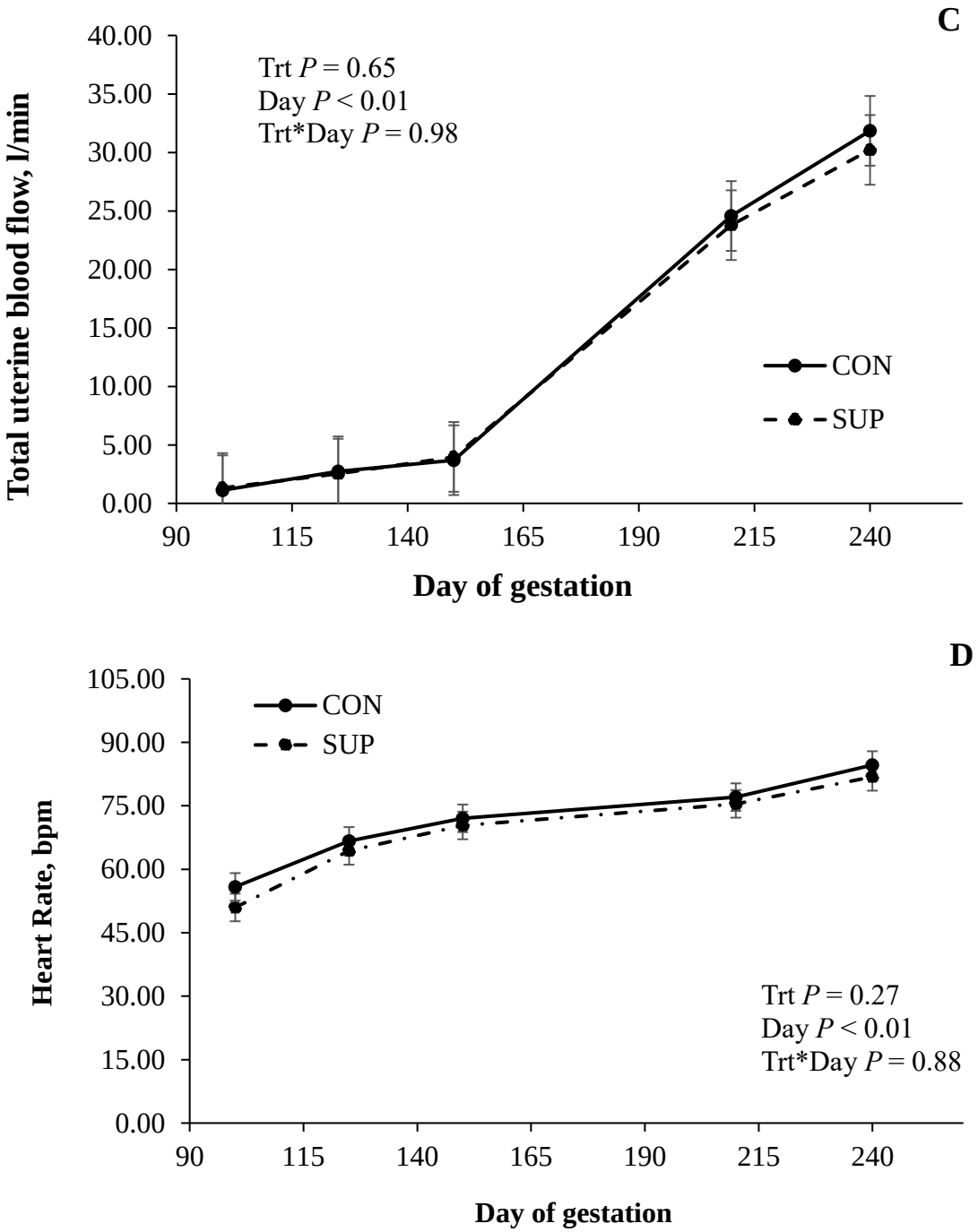

Figure 3.3. Total uterine artery blood flow (C) and maternal heart rate (D) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

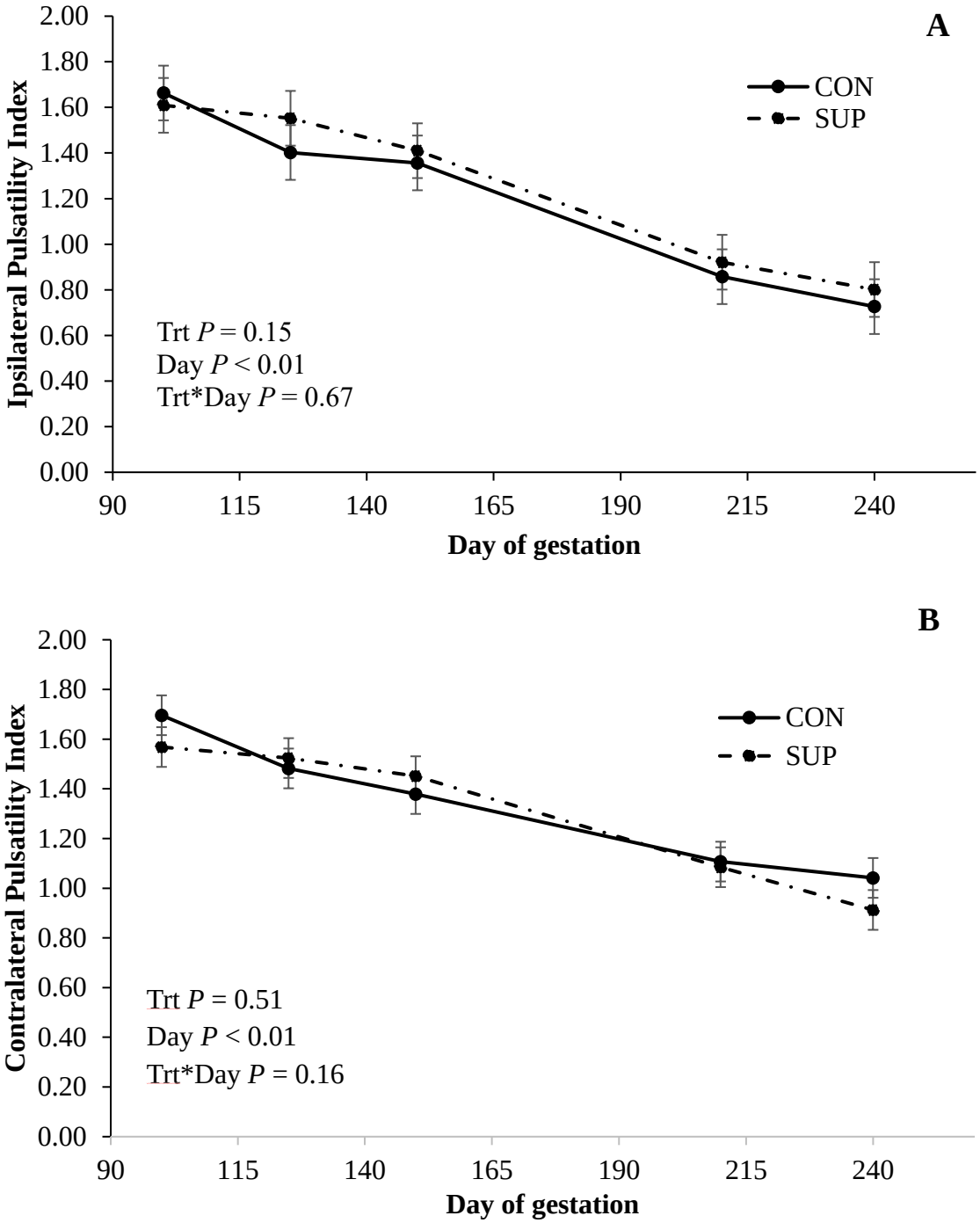

Figure 3.4. Ipsilateral pulsatility index (A) and contralateral pulsatility index (B) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

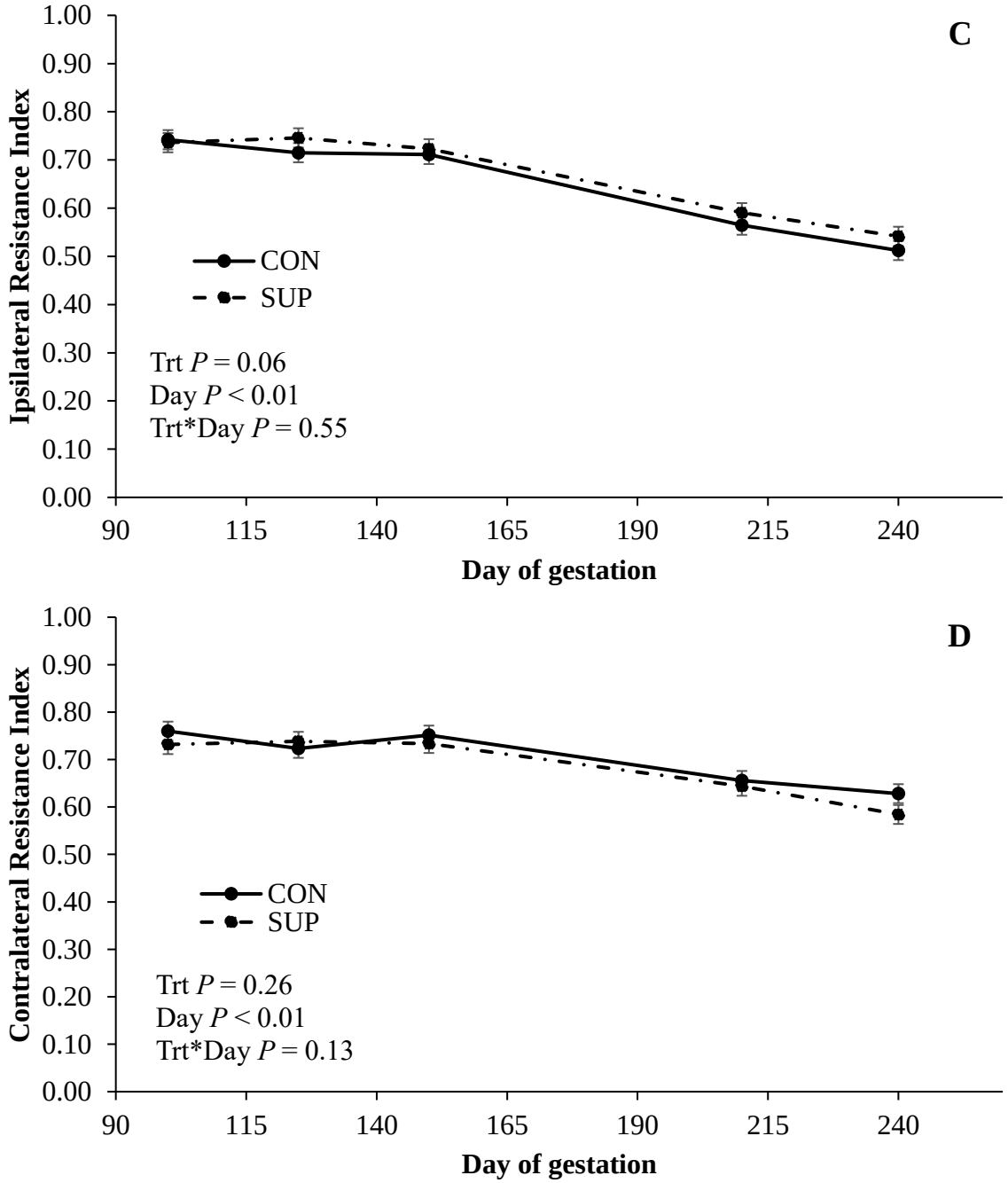

Figure 3.5. Ipsilateral resistance index (C) and contralateral resistance index (D) of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

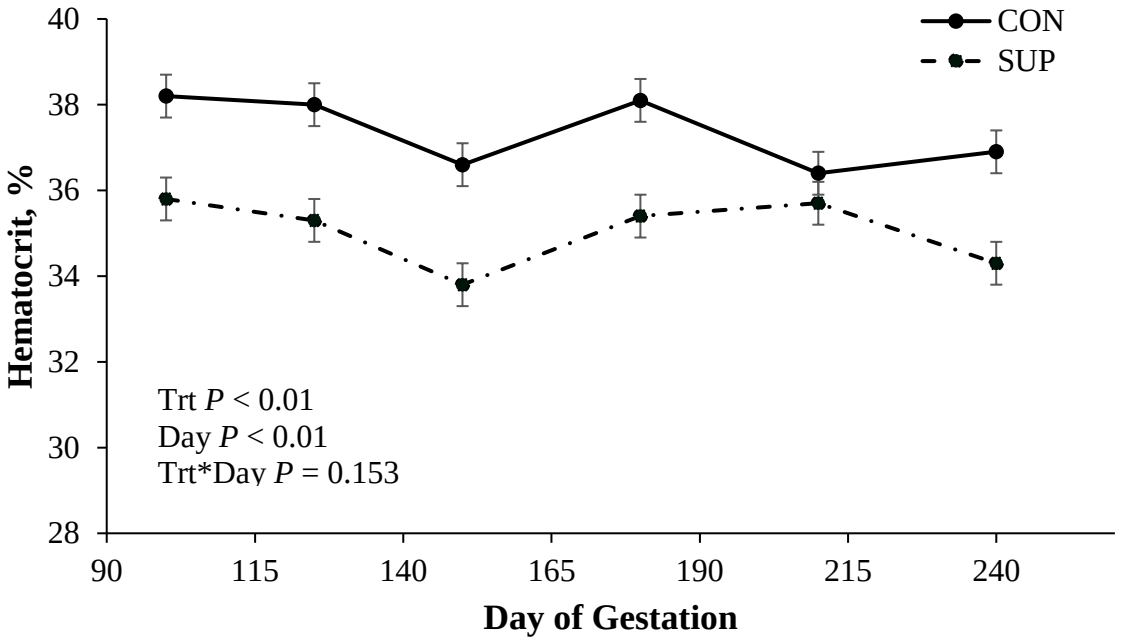

Figure 3.6. Gestational hematocrit of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

Table 3.1. Cow mammary blood flow measurements at d 240 of gestation

Trait	CON	SUP	SEM	*P*-Value
Mammary Blood Flow				
Pregnant side, L/min	2.71	2.36	0.27	0.36
Non-pregnant side, L/min	2.80	2.11	0.24	0.05
Total, L/min	5.51	4.47	0.47	0.12
Pulsatility Index				
Pregnant side	1.28	1.40	0.08	0.30
Non-pregnant side	1.31	1.34	0.07	0.78
Resistance Index				
Pregnant side	0.68	0.70	0.02	0.59
Non-pregnant side	0.69	0.70	0.02	0.62

Maternal endocrine profiles

No day by treatment interactions were observed for serum cortisol, T3, T4, T3:T4 or progesterone ($P > 0.20$). While gestational T3, T3:T4 and cortisol decreased as gestation advanced ($P < 0.01$), T4 increased ($P < 0.0001$) and progesterone concentration remained similar ($P = 0.74$) throughout the days monitored.

The SUP cows had decreased ($P = 0.05$) serum cortisol and tended to have reduced ($P = 0.06$) serum T4 compared to CON cows (Figure 3.7 and Figure 3.8). Maternal diet did not influence circulating serum T3 ($P > 0.5$; Figure 3.9) T3:T4 (P > 0.7; Figure 3.10) or plasma progesterone ($P = 0.74$; Figure 3.11) as SUP and CON dams had similar concentrations throughout gestation.

At calving, maternal serum cortisol (29.3 vs. 30.4 ± 2.5 ng/mL; SUP vs CON), T3 (0.94 vs. 0.97 ± 0.05 ng/mL; SUP vs. CON), and progesterone (1.73 vs. 0.96 ± 0.49 ng/mL) were not altered ($P > 0.25$) by dietary treatment. However, serum T4 at parturition was reduced in SUP cows ($P = 0.04$; 37.0 vs. 32.1 ± 1.6 ng/mL) compared to CON cows. Furthermore, plasma E2 at birth was increased ($P = 0.02$) in SUP vs CON cows (148. 28 vs. 116.44 ± 9.69 pg/mL).

At 24 h post-calving, cortisol was similar between CON and SUP dams ($P = 0.83$; 15.83 vs. 15.32 ± 2.45 ng/mL). However, T3 and T4 were decreased ($P \leq 0.04$) in SUP vs CON cows (T3: 1.05 vs. 1.19 ± 0.04 ng/mL; T4: 48.9 vs. 61.3 ± 2.6 ng/mL). While E2 was similar ($P = 0.17$) between SUP vs CON cows (15.97 vs. 7.27 ± 4.38 pg/mL), P4 in SUP cows was decreased ($P = 0.02$) compared to CON (0.48 vs. 1.86 ± 0.40 ng/mL) 24 h after calving.

At 21 d postpartum, maternal cortisol was reduced ($P = 0.04$) in SUP vs CON dams (21.50 vs. 28.38 ± 3.30 ng/mL). While treatment effect did not affect ($P = 0.72$) circulating T3

(0.85 vs. 0.83 ± 0.04 ng/mL; SUP vs CON) T4 tended to be reduced ($P = 0.10$) in SUP vs CON cows (32.15 vs. 35.31 ± 1.3 ng/mL).

Parturition

Gestation length tended ($P = 0.07$) to be shorter in SUP vs CON cows with no difference in incidence of dystocia ($P > 0.5$) compared to CON cows (Table 3.2). At parturition, dam BW ($P > 0.50$; 713.5 vs. 702 ± 13.4 kg) and BCS ($P > 0.20$; 5.6 vs. 5.6 ± 0.1) were similar for CON and SUP, respectively. As reported in Chapter 2, calf birth weights (39.6 vs. 39.8 ±1 kg) were also unaffected ($P > 0.50$) by maternal corn supplementation. Additionally, calf heart girth (Table 3.2; $P > 0.40$) and crown-rump length (Table 3.2; $P > 0.20$) at birth were also not influenced by maternal diet.

Placental measurements collected at birth were similar across treatments ($P > 0.2$), except for the smallest cotyledonary weight which was increased in SUP vs CON cows (Table 3.2). Corn supplementation did not influence colostrum production ($P = 0.64$; 633 vs. 707 ± 110 g) as SUP vs. CON dams produced similar quantities of colostrum from their caudal-right quarter. Colostrum fat (4.14 vs. 3.67 ± 0.63%), protein (9.90 vs. 9.90 ± 3.32%), lactose (1.68 vs. 2.00 ± 0.22%), and urea (2.32 vs. 2.02 ± 0.30 mM) were not altered between dietary treatments as SUP vs. CON dams had similar colostrum composition.

Placental vascularity

The SUP cows tended to have decreased ($P = 0.10$) cotyledonary VAD (Table 3) compared to CON cows. Additionally, SUP cows had decreased ($P < 0.01$) cotyledonary VSD (Table 3.3) compared to CON cows. However, total vascular volume was not altered by dietary treatment ($P > 0.20$; Table 3).

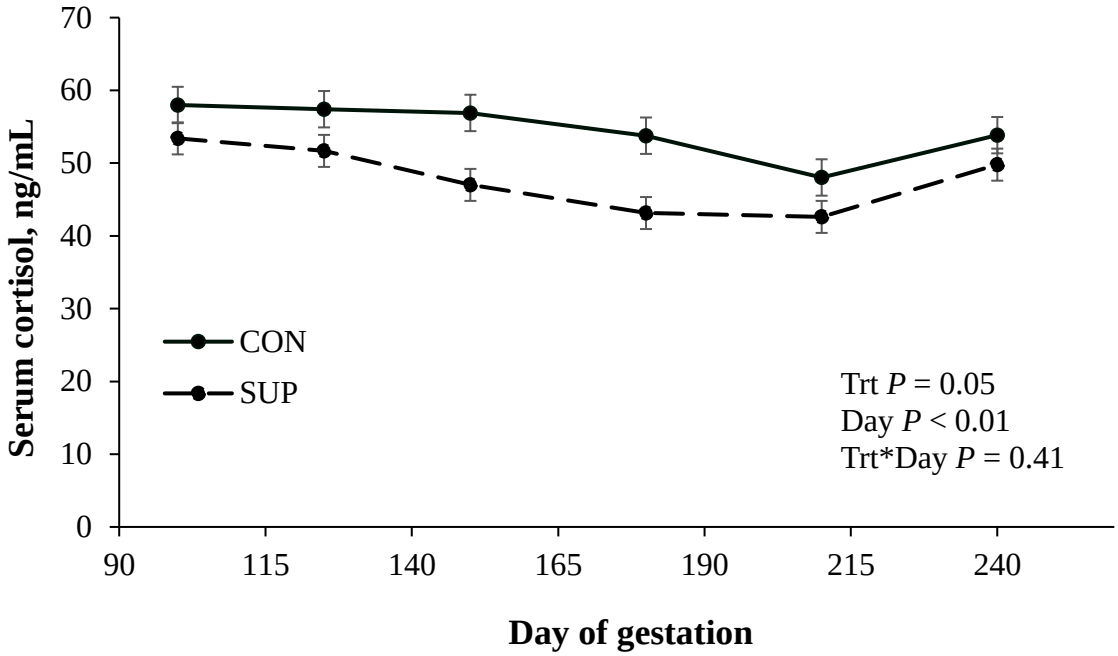

Figure 3.7. Gestational serum cortisol of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

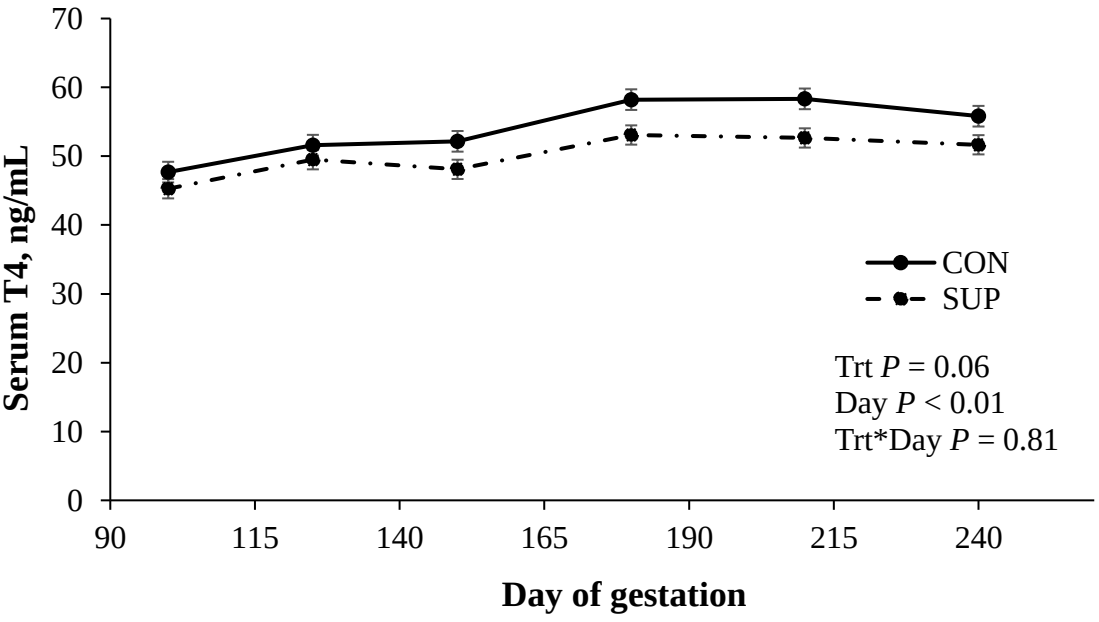

Figure 3.8. Gestational serum thyroxine of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

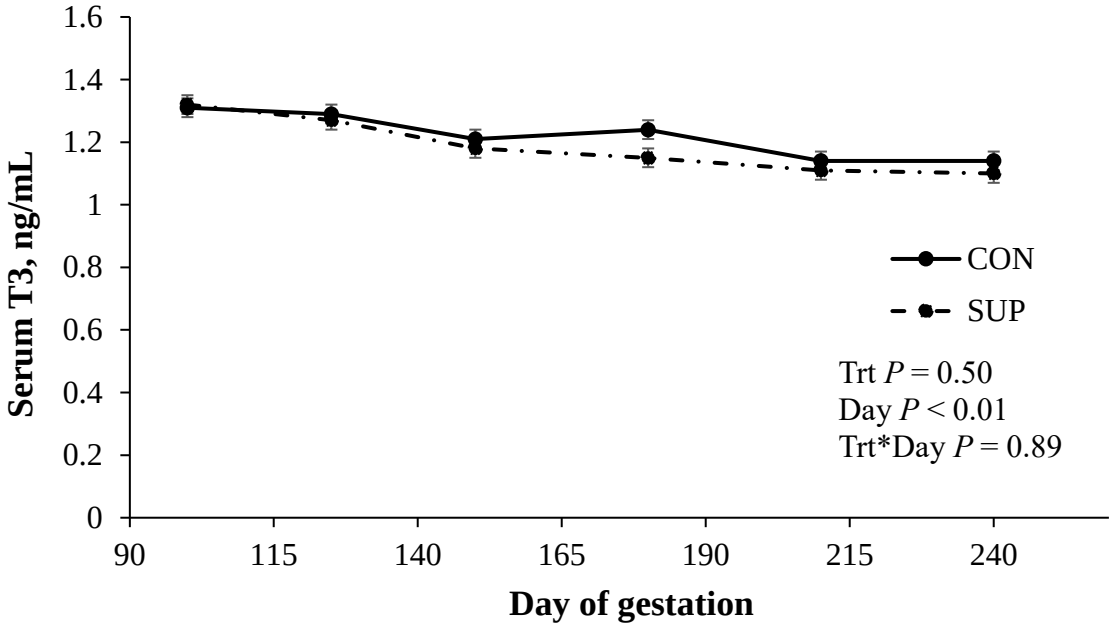

Figure 3.9. Gestational serum triiodothryonine of beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

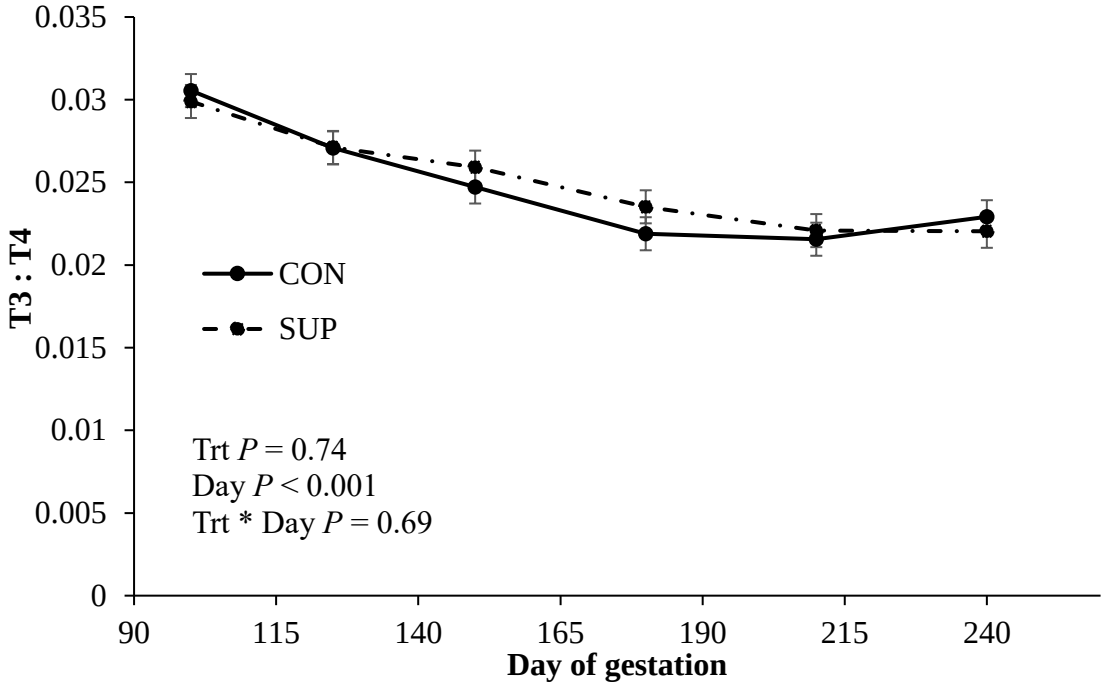

Figure 3.10. Gestational T3 to T4 ratios of cows fed the control and supplemented group from d 100 to d 240 of gestation.

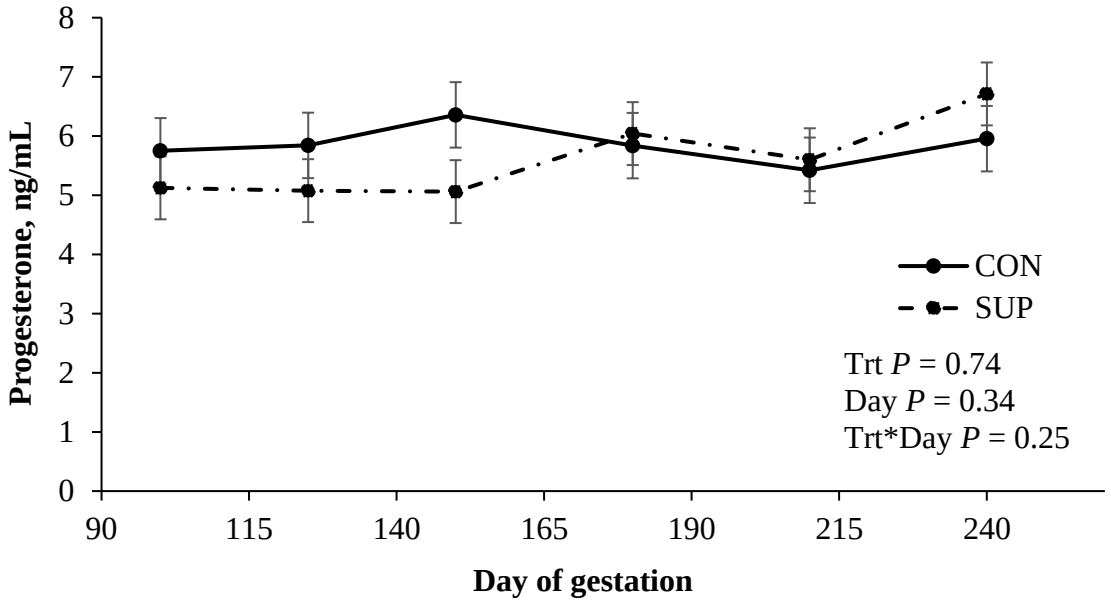

Figure 3.11. Gestational plasma progesterone of cows fed the control and supplemented group from d 100 to d 240 of gestation.

Table 3.2. Calving and placental measurements

Trait	CON	SUP	SEM	*P*-Value
Gestation length, d	279.6	277.7	1.0	0.07
Calf birth BW, kg	39.6	39.8	1.0	0.87
Heart girth, cm	80.1	80.9	0.8	0.46
Crown-rump length, cm	86.9	84.0	4.3	0.21
Calf 24 h BW, kg	40.5	40.0	1.8	0.68
Placenta weight, kg	4.5	4.3	0.3	0.57
Cotyledons, n	90.8	80.0	17.8	0.28
Cotyledon weight, kg	1.7	1.6	0.1	0.45
Intercotyledonary weight, kg	2.3	2.1	159.1	0.59
Largest cotyledon weight, g	85.4	83.3	7.0	0.69
Smallest cotyledon weight, g	0.4	0.9	0.4	0.04
Fetal BW: Placental weight, g/g	9.7	9.8	0.6	0.89
Fetal BW: Cotyledonary weight, g/g	17.9	27.1	16.0	0.33
Cotyledon wt: Placental weight g/g	0.40	0.4	0.0	0.48
Vascular area density, %	10.8	8.9	1.0	0.10
Vascular surface density, $\mu m^2/\mu m^3$	1024.0	626.8	97.0	<0.01
Total vascular volume, mL	190.3	165.6	24.3	0.24

Neonatal endocrine profiles

Neonatal calf cortisol (111.31 vs. 123.2 ± 5.2 ng/mL), T3 (3.19 vs. 3.84 ± 0.29 ng/mL), T3: T4 (0.0039 vs. 0.0038 ± 0.003) profiles were not altered ($P > 0.10$) by maternal corn supplementation as offspring from SUP and CON dams had similar circulating concentrations. However, maternal corn supplementation tended ($P = 0.08$) to lower calf circulating T4 (95.76 vs. 111.1 ± 8.4 ng/mL) when compared to calves from hay fed dams.

At 24 h postpartum, neonatal cortisol (34.35 vs. 42.78 ± 4.0 ng/mL), T3 (6.72 vs. 6.92 ± 0.24 ng/mL), T4 (142.97 vs. 143.46 ± 7.5 ng/mL), T3: T4 (0.051 vs. 0.051 ± 0.005) and glucose (6.50 vs. 6.89 ± 0.61 mM) were not altered ($P > 0.15$) between calves from SUP vs. CON dams.

At 3 weeks postpartum, calf cortisol (8.51 vs. 7.22 ± 1.2 ng/mL), T3 (3.01 vs. 3.16 ± 0.12 ng/mL), T4 (51.19 vs. 52.63 ± 3.3 ng/mL), T3: T4 (0.062 vs. 0.060 ± 0.003) and glucose (5.80 vs. 5.81 ± 0.26 mM) were not altered ($P > 0.40$) by gestational maternal diet as SUP vs. CON calves had similar circulating hormone and metabolite concentrations.

Neonatal performance

As reported in Chapter 2 calf birth weights (39.6 vs. 39.8 ±1 kg) and 24 h weights (40.0 vs. 40.5 ± 1.8 kg) were unaffected ($P > 0.60$) by maternal corn supplementation. At 3 wk post-partum, calves from SUP dams were heavier ($P = 0.05$) than calves from CON dams (72.5 vs 68.9 ± 3.5 kg). However, this effect disappeared ($P = 0.64$) by weaning (d 168) as the offspring from SUP and CON dams were similar in weight (291.5 vs. 281.4 ± 8.1 kg). Steers from corn-fed dams had higher ($P = 0.06$) ADG than steers from hay-fed dams (1.48 vs. 1.35 ± 0.1 kg per day) until 3 wk postpartum when the pairs were transported to CGREC for summer grazing. This treatment effect of maternal diet disappeared after the pairs were moved to an extensively

managed environment as steer ADG was unaffected (P = 0.84) calves from SUP gained weight at a similar rate to calves from CON dams (1.37 vs. 1.36 ± 0.1 kg per day).

Discussion

We reject our hypothesis that corn supplementation from mid- to late-gestation would increase uterine blood flow and increase neonatal growth performance. While TDN and NEm intake were increased in SUP cows, this increased dietary energy intake did not alter uterine blood flow. This is likely due to the substitutionary effect of corn on roughage intake as discussed in Chapter 2 which has also been well demonstrated in beef cattle fed forage-based basal diets (reviewed in Caton and Dhuyvetter, 1997).

Uterine and mammary hemodynamics

Placental nutrient transport efficiency has been demonstrated to be directly related to uteroplacental blood flow and consequently, fetal growth restriction is highly correlated to the growth and development of the uteroplacental unit (Reynolds and Redmer, 1995). Maternal nutritional plane has been implicated in altering hemodynamics to the uteroplacental unit (Lekatz et al., 2011; Lemley et al., 2012; Kennedy et al., 2016) so proper maternal nutrition can be considered as a potential therapeutic strategy to improve IUGR pregnancy outcomes. In fact, ewes that were nutrient restricted to 60% of their NRC requirements from mid- to late-gestation experienced a decrease in both umbilical and uterine blood flows (Lemley et al., 2012). Interestingly, when beef cows were nutrient restricted to 60% of their daily NRC requirements during early (d 30 to 140) pregnancy, uterine blood flow was unchanged (Camacho et al., 2014). Perhaps this is due to differences in stage of pregnancy or inherent species differences.

In the current study, although the corn supplemented dams had decreased maternal MP supply, the restriction did not appear severe enough to influence overall total uterine blood flow

and thus, calf birth weights were unaffected. Similarly, isocalorically-fed ewes that were MP restricted to 80% of their daily requirements observed reductions in uterine blood flows and fetal weights (Lekatz et al., 2015). However, when MP was restricted to 60% of their requirements, ewes experienced greater uterine blood flow but a reduction of fetal weights, thus suggesting that MP deficiency does alter hemodynamics in an unsuccessful attempt to rescue fetal growth (Lekatz et al., 2015). Conversely, the supplementation of protein to beef cows in late gestation (d 180 to 246) also increased uterine hemodynamics to the uteroplacental unit and positively benefits fetal growth as calf birth weights were improved (Kennedy et al., 2016). Cows were provided *ad libitum* access to forage and limit fed a DDGS protein supplement (0.3% BW), dry matter intake was increased (Kennedy et al., 2016a) and uterine arterial blood flows and calf birth weights were both improved (Kennedy et al., 2016b).

These studies along with the current study suggest that perhaps it is dietary metabolizable protein that is driving these changes in uterine blood flow rather than total energy intake. Not only does protein supplementation have a positive impact on birth weight, but other studies report that maternal dietary protein level during gestation impacts calf growth and subsequent carcass quality. For example, Shoup et al. (2015b) examined the effects of maternal dietary protein supplementation on offspring carcass quality and found that dams receiving additional protein during late gestation produced calves with increased marbling and backfat at slaughter.

However, it also appears that protein supplementation alone is not solely driving blood flow and increased fetal growth. When beef cows were limit-fed forage while being fed a DDGS protein supplement in late gestation (d 190 to d 240), a decrease in uterine blood flow occurred in supplemented cows (Mordhorst et al., 2016). Surprisingly, calf birth weight was unaffected despite the decrease in uterine blood flow (Mordhorst et al., 2016). This observation suggests

that providing a dam with a dietary protein supplement (DDGS) is only effective when complemented by *ad libitum* access to low quality forage as seen in Kennedy et al. (2016). This idea is supported by Lemley et al. (2014) who found that higher dry-matter intakes are linked to higher insulin-like growth factor-1 and, perhaps, could increase glucose and amino acid uptake in both fetal and maternal tissues. Further studies should be conducted to better understand the relationship between nutrient type (energy, MP) and stage of gestation corresponding uterine blood flow changes.

Limited research has been completed to study the effects of maternal nutrition and dietary supplementation on mammary blood flow during late gestation in beef cows. Mordhorst et al. (2016) supplemented limit-fed DDGS and forage and observed no impacts on mammary blood flow. While ipsilateral mammary blood flow was not altered in the current study, we observed a reduction in mammary blood flow contralateral to the calf in SUP dams and a potential reduction in overall mammary blood flow in corn-fed dams. Perhaps this is due to carbohydrate effect documented in the corn-fed dams, reducing circulating plasma urea (Chapter 2) and thus, reducing the availability of circulating urea available for *de novo* amino acid synthesis (reviewed in Reynolds and Kristensen, 2008). Unlike Berger et al. (2016) that have observed correlations between milk yield and mammary blood flow we did not observe this relationship at birth as both treatments produced similar amounts of colostrum at parturition. Furthermore, our laboratory has not detected any differences in beef cattle mammary gland development or vascularity (Vonnahme et al., 2015) due to maternal nutrition. Thus, further studies should be conducted to elucidate maternal nutritional factors resulting in altered mammary hemodynamics, milk production and milk nutrient transport to the calf.

Placental vascularity

In the bovine placenta, cotyledonary growth increases throughout gestation (Vonnahme et al., 2007) and, thus, factors like vascularity could be impacted throughout gestation. While most measurements of uterine blood flow as well as placental weights and efficiency were unaltered in the current study by corn supplementation, placental vascularity was suppressed in the cotyledons from SUP dams. Perhaps, this reduced vascularity accounts for some of the increase in the arterial resistance index ipsilateral to the conceptus of the SUP treatment group when compared to the greater vascularity of the CON cotyledons.

When beef cows were nutrient restricted (60% of NRC) in early pregnancy (until d 120) but later realimented, decreased cotyledonary VAD and VSD were observed at d 250 when compared to CON (100% of NRC) cows (Vonnahme et al., 2007; Vonnahme et al., 2015). Perhaps, when both treatments in the current study were placed onto a lactational diet 2 to 4 weeks before parturition which consequently produced more MP, a similar realimentation effect was observed in the SUP dams who were slightly MP restricted during gestation. This is supported by a similar decrease in cotyledonary VAD and VSD in the cotyledonary tissue collected at parturition. The lack of phenotypic differences in the calves up-to weaning is also supported by the lack of birth weight differences, suggesting that perhaps this vascularity change occurred very late in pregnancy after most fetal growth had occurred.

Regarding how MP could potentially influence cotyledonary vascular responses, sheep who were fed similar caloric-level diets with various levels of MP supplementation (60%, 100%, and 140%) had altered cotyledonary responsiveness to bradykinin induced vasodilation (Lekatz et al., 2010). In both high (140%) and low (60%) MP treatments, enhanced responsiveness to bradykinin was detected (Lekatz et al., 2012). While placental arteries from cows that were

nutrient restricted during pregnancy were more sensitive to bradykinin induced vasodialation than control fed dams (Vonnahme et al., 2011), more work should be done to evaluate the effects of MP restriction with adequate ME requirements (100% NRC) in late pregnancy on bradykinin responsiveness in the cotyledonary arteries.

Endocrine profiles

Cortisol is well recognized for its regulatory effects of energy metabolism. It works in concert with other hormones, helping maintain blood glucose by enhancing gluconeogenesis through the covalent activation of phosphenolcarboxykinase (Ryan, 1975). In high concentrations, it has also been demonstrated to have catabolic effects on body protein reserves (Ryan, 1975). In the current study, while cortisol was increased in hay fed dams, it is difficult give the current parameters of the study to determine whether this is due to social or behavioral stress or as a metabolic pro-gluconeogenic hormone. As reviewed in Chang and Zhang (2008), maternal plasma cortisol has been demonstrated to double over gestation, suggesting its physiological relevance in fetal growth.

Notably, circulating cortisol decreased over gestation in both treatments which could suggest a potential acclimation to being handled throughout the trial progressed which is well documented in beef cattle (Cooke, 2014). This effect was also observed in gestating ewes, independent of maternal nutritional plane (Lemley et al., 2014). Another well documented metabolic effect of cortisol is the liberation of lipids into circulation (Ryan, 1975). Our study would agree with this as NEFA and cortisol were greater in CON dams. Surprisingly, serum cortisol at parturition was less than circulating cortisol during gestation in the dam. Conversely, sheep experience a spike in serum cortisol at parturition (Lemley et al., 2014) which would suggest that sheep and cattle production of cortisol diverges at parturition.

Cortisol has also been suggested to play a role in mediating the uterine arterial blood flow increase during late gestation, as adrenalectomized ewes entering late gestation failed to increase uterine blood flow compared to normal and high cortisol ewes (Jensen et al., 2005). In fact, abnormal levels of circulating cortisol are often indicative of IUGR and often results in increased morbidity and mortality (Jensen et al., 2005). Cortisol has been suggested to illicit responses locally and systemically to alter uteroplacental perfusion by altering maternal plasma volume during late gestation in the ewe (Jensen et al., 2002; Jensen et al., 2005). We observed the opposite phenomenon in our gestating beef cows as gestational hematocrit was elevated in our control cows which had greater gestational cortisol.

Conversely, glucocorticoids well recognized for their role in growth restriction pregnancies as they are well documented potentiators of angiotensin II, vasopressin, and norepinephrine, induce vasconstriction (Xia et al., 2002; Yagil and Krakoff, 1988). Glucocorticoids also decrease uterine arterial endothelial nitric oxide synthase (eNOS) mRNA and protein expression and subsequent NO release in both pregnant and non-pregnant animals, although this effect is still not well understood (Malek et al., 1999; Li et al., 2007). Interestingly, evidence that these mechanisms are attenuated in pregnant animals compared to non-pregnant animals as suggested in the sheep by the pregnant state altering ligand-receptor affinity and 11-beta-hydroxysteroid dehydrogenase 1 activity (Xiao et al., 2002). Although cortisol in the current study was elevated in the control group, total uterine blood flow was not altered so it appears that circulating cortisol levels in this experiment were not enough to induce vasoconstriction in the control cows or alter fetal growth as calves of both treatments weighed similar amounts. In fact, the control cows which had higher levels of cortisol also had greater cotyledonary vascularity. Additionally, there were no treatment differences in fetal cortisol

because of maternal gestational diet but levels do decrease after birth which agree with Osorio et al. (2013) which also found that maternal nutrition (restricted vs. control) did not impact calf cortisol but calf cortisol did decrease after birth in both treatments.

The biologically critical thyroid hormones T3 and T4 play many diverse roles in regulating adult protein and energy metabolism (Cassar-Malek et al., 2006). While they are critical for mobilizing maternal body reserves to supply nutrients to the developing conceptus, they cannot cross the placenta (Cappoen, 1989). In the human, maternal total T3 and T4 are elevated during weeks 17 through 36 of gestation in the human while all fetal thyroid hormones (total T4, free T4, total T3, thyroxine-binding globulin; TBG, and thyroid-stimulating hormone; TSH) increased as gestation progressed and reverse T3 decreased (Radunovic et al., 1991). Additionally, maternal total T3 was correlated with fetal T3 and T4 in the human (Radunovic et al., 1991). While few studies have examined the role of T3 and T4 in the pregnant bovine, more is understood about the role of T3 and T4 in the bovine fetus. The fetal thyroid gland differentiates between d 75 to 90 of gestation and has been shown to play a role in regulating the development and differentiation of the fetal liver, heart and skeletal muscles as well as fetal adipose development (Cassar-Malek et al., 2006). This is because of the activation of the hypothalamic-pituitary- thyroid axis which is regulated by leptin (Micke et al., 2015).

Maternal diet can also play a sex-specific role by controlling the fetal nutritional plane as male progeny exposed to a maternal protein restriction in-utero resulted in greater free T4 at birth (Micke et al., 2015). This is not terribly surprising as nutrient restricted calves had been previously shown to have greater gluconeogenic hormones such as cortisol and T3 at birth (Hough et al., 1990). Interestingly, we observed the opposite effect in our study where male

progeny born to dams who received adequate MP tended to be greater at birth but this effect disappeared by 24 h postpartum.

In the current study, although maternal total T3 did not differ according to dietary treatment it did decrease over gestation which is supported by what is observed in pregnant ewes (Lemley et al., 2014) but opposite to what is recorded in the human (Radunovic et al., 1991). Interestingly, CON dams had higher circulating levels of T4 when compared SUP dams. Although it is surprising that only T4 and not T3 levels were elevated in control dams, this elevation is expected as maternal intake is a one of the key factors influencing thyroid activity (Lemley et al., 2014). The ratios of thyroid hormones and activities are affected by type-1 5'-deiodinase activity (Cassar-Malek et al, 2006) so perhaps, deiodinase activity could have been altered by diet. In fact, total T3:T4 ratios were greater in heifer calves exposed to high protein diets during gestation (Micke et al., 2015). Additionally, the inverse relationship observed between T3 and T4 as gestation advanced could be due to the increasing in body weight consistent with advancing pregnancy and positive energy balance.

Aside from its critical biological role in sustaining pregnancy, progesterone has demonstrated to contribute to uterine blood flow regulation including maintaining phasic contractility of the uterine arterial smooth muscle by regulating the alpha1-adrenergic/ calmodulin system (Ford, 1995; Ford et al., 1982). However, in the present study, neither circulating progesterone nor uterine blood flow were altered by our corn supplementation strategy.

Additionally, circulating progesterone levels have been documented to be altered by increased feed intake in beef cattle (Kennedy et al., 2016b) as the DDGS supplemented consumed more roughage than their control fed counterparts. This effect was not observed in the

present study as the CON treatment which consumed more roughage had similar levels of circulating progesterone over gestation. Perhaps, this is because although roughage intake was decreased in the supplemented group due to the substitutionary effect of corn, total intake remained similar (Tanner et al., 2017) so progesterone was unaltered. This would also agree with Mordhorst et al. (2016) as beef cow progesterone concentrations were also not altered likely because forage was limit-fed, not *ad libitum* which did not allow the cows to increase consumption and likely alter their steroid hormone profiles.

Neonatal performance

As previously reported (Tanner et al., 2017) calf birth weights were not altered as result of maternal dietary corn supplementation which agrees with data from Loerch et al. (1996) but contradicts Radunz et al., 2010. This apparent variability could be explained by the greater inclusion of corn in the overall diet (60% vs. 10%) in the Radunz study compared to our diets. However, the effects of supplementation (either protein, energy or improved pasture) on calf birth weight are highly variable across all studies and dependent on many factors. In fact, it is common for birth weight differences in beef cattle not to be detected until weaning (Klein et al., 2014; Shoup et al., 2015; Stalker et al., 2006; Underwood et al., 2010; Wilson et al., 2015). Like the current study, Mordhorst et al. (2016) also did not observe differences in birth weight, crown-rump length (CRL) or heart-girth (HG) measurements. Additionally, while Kennedy et al. (2016) documented increased birth weights with DDGS supplementation with *ad libitum* forage, they also did not record differences in CRL and HG measurements. This would suggest fetal proportional growth was not altered by supplementation, even with increased birth weights. Furthermore, like the current study, calves from dams that were supplemented also did not document improved weaning weights (Schoonmaker et al., 2003; Summers et al., 2015) because

of maternal gestational dietary treatment. Despite the lack of birth, weaning, and HCW differences, Summers et al. (2015) did observe differences in carcass composition as 12th rib fat thickness and longissimus tenderness was reduced in gestationally nutrient restricted animals. Growth and carcass traits for calves from the current project are yet to be assessed.

Implications

While corn supplementation in cows seemed to alter cotyledonary placental capillary vascularity, it appears to be composition of the diet that ultimately drives uterine blood flow and subsequent calf growth. As evidenced by the lack of uterine blood flow and calf birth weight changes, energy supplementation without ample protein seems to have limited impacts on prenatal growth. In fact, dietary supplemental protein combined with *ad libitum* access to forage seems be much more effective in improving fetal growth by increasing uterine arterial blood flow. This suggests that care should be taken to supply dams with adequate protein during gestation to prevent altered hemodynamics and reduced fetal growth. However, because corn supplementation does not negatively impact fetal growth and birth weights, corn does appear to be a good substitute for hay. In fact, this feeding strategy could be economically advantageous to the producer especially depending on the cost of feed inputs or availability of resources.

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CHAPTER 4. GENERAL DISCUSSION AND FUTURE DIRECTIONS

General discussion

While the current study examines the limitations of starch supplementation on uterine hemodynamics and neonatal performance, the true mechanisms of increasing uterine blood flow remains to be elucidated. Protein supplementation with limit-fed forage decreases uterine blood flow but did not impact calf birth weights (Mordhorst et al., 2016). However, protein supplementation with *ad libitum* access to forage increases uterine blood flow and improves calf birth weight (Kennedy et al., 2016). This increase in uterine blood flow is confounded by the simultaneous increase in caloric intake as well as alterations in the dietary protein to energy ratio. However, both Camacho et al. (2014) and the current study (A.3 and A.10) support that either increasing or decreasing calories does not impact uterine blood flow. Perhaps an increase in metabolizable protein is driving these changes in uterine blood flow rather than total energy intake although. Alternatively, this increase in uterine blood flow could be driven by a protein to energy ratio.

Future directions

To better understand the mechanisms controlling uterine hemodynamics and create helpful winter supplementation strategy for beef producers with limited access to forage, further work should be done examining various levels of MP intake and energy to protein ratios. Uterine hemodynamics, steroid hormone concentrations, liver steroid catabolism through liver biopsies, and postnatal growth and performance should all be measured. Furthermore, the role of uteroplacental blood flow on postnatal performance in the offspring should also be examined.

However, one feasible way to examine the role of corn supplementation on postnatal calf performance is to examine the role of maternal nutrition during gestation on epigenetic

developmental programming of offspring muscle development and carcass traits in beef cattle. Because muscle biopsies have already been collected on the neonatal calves and will be collected for nucleic acid work at slaughter, this project will be well within the scope of the current study.

Background

Previous research has demonstrated that cow nutrition during pregnancy can alter calf muscle growth and carcass composition. We wish to investigate how dietary supplementation with corn during mid to late pregnancy affects calf muscle growth and carcass composition and to understand the epigenetic mechanisms causing these effects. The long-term goal is to develop recommendations for pregnant cow nutrition to improve calf carcass traits.

It is well established that improper maternal nutrition during pregnancy can alter offspring health and performance in livestock (Wu et al., 2004). Improper maternal nutrition has been associated with a decrease in carcass quality including altered fat deposition, muscle fiber type and reduced meat quality (Wu et al., 2006). Nutrient restricting beef cows during gestation resulted in offspring with reduced carcass weights (Greenwood et al., 2004). Funston et al. (2010) demonstrated that poor maternal nutrition negatively affects offspring intramuscular fat and postnatal growth. These effects extend across species as lambs born to nutrient restricted mothers also had altered muscle formation as well as increased connective tissue and intramuscular adiposity (Zhu et al., 2006).

In the upper Midwest and Canada, extensively managed cattle operations are common which can lead to nutrient restriction via low quality forage diets in over-wintered gestating beef cows (Shepperd et al., 2015). Increasing forage quality fed to late gestating beef cows led to an increase steer hot carcass weight (Underwood et al., 2010). Furthermore, re-alimentation of cows that were previously nutrient restricted during early pregnancy led to an increase in muscle fiber

size and muscle progenitor cell numbers in their offspring (Gonzales et al. 2013). In addition, supplementation of protein to the dam increased calf birth weights and altered fetal muscle growth (Larson et al., 2009) as well as increased marbling and backfat of steer calves at slaughter (Shoup et al., 2015b). Moreover, Radunz et al. (2010) found that beef cows limit-fed corn during gestating produced calves that were heavier than those from hay-fed dams. These calves also had heavier weaning weights, faster glucose disappearance rate, and less intramuscular fat than calves from hay-fed dams (Radunz et al., 2012).

Maternal diet during pregnancy has also been suggested to impact carcass quality through epigenetic modifications such as DNA methylation (a mechanism through which traits are "programmed" by developmental insults such as undernutrition). Beef cows fed corn based diets during pregnancy had altered expression of imprinted genes and DNA methyltransferases in the skeletal muscle of their calves shortly after birth (Wang et al., 2015). In a similar study, sheep from dams fed diets high in starch (corn) during pregnancy had altered expression of imprinted genes in skeletal muscle and subcutaneous fat (Lan et al., 2013). In addition, differences in the DNA methylation pattern in skeletal muscle of offspring from ewes fed corn vs. hay diets during pregnancy was demonstrated by bisulfite sequencing (Lan et al., 2013).

The purpose of the study is to examine the effects of cow winter feeding strategies on the epigenetic mechanisms of developmental programming of calf carcass characteristics. The calves will be slaughtered at a commercial abattoir and full carcass data will be collected.

At 7 days of age (± 2 days) muscle biopsies were collected from the *longissimus dorsi* muscle from each calf. At slaughter, a second biopsy will be collected from the same muscle within 30 minutes of slaughter. Similar procedures have been conducted by collaborators and ample RNA was extracted for RNA-seq analysis. Both biopsies will be used for gene expression

and epigenetic analysis. Intramuscular adipocytes will be separated from skeletal muscle using laser-capture microscopy then processed for RNA-sequencing.

A sub-set of 40 samples (n=10 per treatment per time point) of both skeletal muscle and adipose would be analyzed by RNA sequencing (RNA-seq). This technique measures and quantifies the expression of all expressed genes, identifying all genes that are up or down-regulated in response to treatment. Expression of these genes will be validated and measured in the remaining samples using qPCR.

The genes of interest identified by RNA-seq will be further investigated for the epigenetic mechanism causing their change in expression. DNA methylation will be measured through bisulfite sequencing. Histone modification will be assayed by chromatin immunoprecipitation (ChIP) and PCR. These data will be correlated with the carcass data and uterine hemodynamics to provide insight regarding the mechanisms associated with maternal nutrition during pregnancy and muscle growth and development in the offspring. By generating this 'data map' of the transcriptome and potential epigenetic modifications, we could potentially identify and later target mechanisms that are being altered by winter feeding strategies. To our knowledge, this would be the first project to examine the effects of altered maternal nutrition during mid-to late-pregnancy on the transcriptome of skeletal muscle and adipose over time. This project could provide insight into how maternal diet in pregnant beef cows alters growth and metabolism of beef steers between early life and their terminal endpoint. Ideally, this project could provide us with the framework to eventually optimize maternal feeding strategies to influence value adding traits such as marbling *in utero*.

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APPENDIX

Table A.1. The effects of maternal nutrient restriction on placental parameters.

Parity and stage	Diet	Offspring	Δ dam BW	Fetal/ Birth weights	Placental weights	Placental vascularity	Study
N; d 183-term	CON= 100% NRC or RES 55% NRC	♂ + ♀	RES ↓	RES ↓	---	---	Corah et al., 1975
M; d 193-term	CON= 100% NRC or RES 57% NRC	♂ + ♀	RES ↓	NSE	---	---	Hough et al., 1990
M; Mid-gestation - rebreeding	Maintain BCS (HHH); ↓ BCS 2nd trimester then ↑ (LHH); or ↓ BCS until 28 d of lactation (LLH)	♂ + ♀	HHH > LLH at calving	LLH ↓	---	---	Freetly et al., 2000
M; d 30-125	100 vs. 50% NRC from d30-125 then 100% NRC to d 250	♂	---	NSE	NR Cot and Car ↓ @ d 125 and 250	NR ↑ Akt & ERK1/2 phosphorylation @ d 125	Zhu et al., 2006
M; d 30-125	100 vs. 50% NRC from d30-125 then 100% NRC to d 250	♀	RES ↓ then realimented BCS 5.75	NSE	---	Realimentation ↑ CAR CSD; ↓ COT CAD and CSD	Vonnahme et al., 2007
M; d 30-125	100 vs. 50% NRC from d30-125 then 100% NRC to d 250	♀	RES ↓ then realimented BCS 5.75	NR-IUGR ↓ d 125	NR-IUGR ↓ Cot wt	---	Long et al., 2009

M = multiparous
N= nulliparous
NSE = No significant effect

Table A.2. The effects of maternal nutrient restriction on uterine hemodynamics and birth weights.

Parity and stage	Diet	Offspring	Δ dam BW	Uterine blood flow	Placental weights	Fetal/ Birth weights	Study
M; early through late	CON= 100% NRC, RES = 60% NRC; d 85 some realimented RR, RC, CC; d 140 realimented RRC, RCC, CCC	♂ + ♀	d 85 = R ↑ BW Δ; d 140= BW ↓ RR; d 254 = RCC ↑	d 210- 240 Uterine BF= RRC ↑ UBF [3]	d 85 = NR ↑ placentome # and weight; d 140 = RR ↑ placentome # and ↓placental efficiency [3]	d 85 = C ↓; d 140= NSE; d 254= NSE[3]	Camacho et al., 2014a
M; early through mid	CON = 100% NRC; RES= 60% NRC until d 140	♂ + ♀	RES ↓ period 1	Period 2: NR ↓ Ipsi UBF	---	---	Camacho et al., 2014b
P; ER= d 0-60; LR= d 213-term	CON diet = 100% NRC; RES diet= 60% NRC either early (E) or late (L)	♂ + ♀	LR ↓	---	NSE	NSE	Spiegler et al., 2014
M; d 47-240	Cattle fed 100% NRC (CON) or 150% NRC (OVER)	♂ + ♀	OVER ↑	---	---	---	Duarte et al., 2014
P; Pre-breeding management until d 45	SPRING or FALL fed LOW input or CONventional	♂ + ♀	CON|S > CON|F > LOW|F > LOW|S	SPRING ↑UBF; LOW ↑ adjusted UBF	---	NSE = treatment; SPRING ↑	Cain et al., 2017

P = primiparous; M= multiparous

NSE = No significant effect

[1]Data from Prezotto et al., 2015, [2]Kennedy et al., 2017 (In-Press), [3]Camacho et al., 2013 (Thesis)

Table A.3. The effects of maternal nutrient supplementation on uterine hemodynamics, placental parameters, and neonatal performance.

Parity and stage	Diet	Offspring	Supplement	Offspring weights	Uterine/ umbilical blood flow	Weaning	Study
M; Diets fed: Nov-April	Exp. 1 & 2 (Sup= 5 kg shelled corn, Exp 3= 6.4 kg corn	♂ + ♀	1.2 kg pelletted supplement	Corn ↑ Exp 1 & 3	---	Corn↑ trial 1	Loerch, 1996
P; d 42-99 (Early); d 100-198 (Mid)	2 x 2 factorial; Diets of High (H; 14%) or Low (L; 7%) CP fed during 1st or 2nd trimester	♂ + ♀	---	NSE	---	---	Perry et al., 1999
M; Diets fed: Trial 1 (Nov-Feb); Trial 2 (Jan-April)	Corn= 5.8 kg corn, 1.2 kg orchardgrass hay, Pasture (21 - ha), or hay	♂ + ♀	1.1 kg pelleted supplement for corn fed	NSE	---	NSE	Schoonmaker et al., 2003
P; Early and mid-gestation; realimentated late gestation	High (H; High ME and CP) or Low (L; Low ME and CP)	♂ + ♀	---	---	Estrone sulfate and bovine placental lactogen ↓ High protein	---	Sullivan et al., 2009
P; Early and mid-gestation; realimentated late gestation	High (H; High ME and CP) or Low (L; Low ME and CP)	♂ + ♀	---	HH > LL	Umb diameter↑ high protein (first trimester); ↓high protein (2nd trimester)	---	Micke et al., 2010a

Table A.3. The effects of maternal nutrient supplementation on uterine hemodynamics, placental parameters, and neonatal performance (continued).

Parity and stage	Diet	Offspring	Supplement	Offspring weights	Uterine/ umbilical blood flow	Weaning	Study
M; d 177 - 13 d lactation	5 supplementation diets: DGSLow, DGSIntermediate, DGSHigh, positive status (POS) and negative (NEG)	♂ + ♀	DGSL (0.77kg/d); DGSI (1.54 kg/d); DGSH (2.31kd/d)	DGSH > NEG	---	NSE	Winterholler et al., 2012
P; PERI= d -60 to 23; POST = d 23 - d 98 then 12% CP	High (14%) or Low (7%) protein wither PERI-conceptually or POST-conceptually	♂ + ♀	---	LPRE ↑ ♂	♂ LPERI ↓ UBF d 150; LPERI ↓ PI at d 120; LPERI ↑ MUA at d 210	---	Hernandez-Medrano et al., 2015
M; d 200-270	CON = 90% corn stover, 10% corn silage basal diet; SUP = CON diet + 0.3% BW DDGS	♂ + ♀	DDGS 0.3% BW	SUP ↑	SUP ↑UBF and HR, ↓PI	SUP ↑ [2]	Kennedy et al., 2016
M; Exp 1. d 190-218; Exp 2. d 170-234	Hay at 2% BW (CON), DDGS+ hay (SUP)	♂ + ♀	DDGS at 1.7 g/kg BW	NSE	SUP ↓ UBF	NSE	Mordhorst et al. 2016

P = primiparous; M= multiparous
[1]Data from Prezotto et al., 2015
[2]Kennedy et al., 2017 (In-Press)
[3]Camacho et al., 2013 (Thesis)
NSE = No significant effect

Table A.4. The effects of maternal nutrient restriction during gestation on offspring postnatal performance and carcass parameters

Parity & Stage	Diet	Offspring	Fetal/ Birth weights	Weaning weights	Feed efficiency	HCW	USDA QG	USDA YG	12th rib fat	Study
M; d 80- term	High (H) or Low (L) plane of nutrition during gestation. Cross-over at birth	♂ + ♀	H ↑	NSE	H ↑ ADG and DMI	---	NSE [3]	NSE [3]	NSE [3]	Café et al., 2006 & 2009
P; d 32- d 115 then realimented	Low (55% NRC) or Moderate nutrition (100%)	♂ + ♀	NSE	NSE	NSE	---	NSE	NSE	NSE	Long et al., 2010 a & b
P; Early (EG) and mid (MG) gestation	High (H; High ME and CP) or Low (L; Low ME and CP)	♂ + ♀	↑HH and ↓ LL	♂ low CP EG ↑ BW; ♀ high CP EG ↑ BW	---	H CP females ↑ HCW	---	---	High CP ♂ ↑	Micke et al., 2010b
M; d 120- d 180	Native (NR; protein restricted) or improved pasture (IP)	♂	NSE	IP ↑	IP ↑ ADG	IP ↑ HCW	NSE	NSE	IP ↑	Underwood et al., 2010
M; d 84- 175	Dormant range = + Energy Status (PES) or 80% of NRC = - energy status (NES)	♂ + ♀	---	---	---	NSE	NSE	PES ↓	PES ↑	Mohrhauser et al., 2013

Table A.4. The effects of maternal nutrient restriction during gestation on offspring postnatal performance and carcass parameters (continued).

Parity & Stage	Diet	Offspring	Fetal/ Birth weights	Weaning weights	Feed efficiency	HCW	USDA QG	USDA YG	12th rib fat	Study
M; d 135- d 271	Low starch (LS; haylage) or high starch (HS; corn silage)	♂ + ♀	---	---	---	---	---	---	---	Wang et al., 2015
M; d 195- d 283	Corn coproducts (COP) or cool-season hay (HY)	♂ + ♀	COP ↑	NSE	NSE	NSE	NSE	NSE	NSE	Wilson et al., 2015a
P; d 85-180	High (146% NRC), Intermediate (87%), and Low (72%)	♂ + ♀	NSE	---	---	---	---	---	---	Jennings et al., 2016

P = primiparous; M= multiparous

NSE =No significant effect

[1]Data from Moisa et al., 2016

[2]Data from Greenwood et al., 2006

[3] Data are from carcass ultrasounds

Table A.5. The effects of maternal supplementation during gestation on offspring postnatal performance and carcass parameters.

Parity & Stage	Diet	Offspring	Supplement	WW[1]	Feed efficiency	HCW[2]	USDA QG	USDA YG	12th rib fat	Study
M; Late gestation - early lactation	M = meadow; H= hay	♂ + ♀	0.45 kg/d with 42% CP (PS) or no supplement (NS)	M-PS ↑	NSE	NSE	NSE	NSE	NSE	Stalker et al., 2006
M; Nov-Mar	Winter range (WR) or corn residue (CR)	♂ + ♀	Protein supplement (PS) or none (NS)	WR - PS > WR-NS; all CR =	NSE	WR-PS ↑	PS ↑	NSE	NSE	Larson et al., 2009
M; d 160 - d 275	Hay; or 4.1 kg DDGS; 5.3 kg corn	♂ + ♀	1 kg pelleted supplement for DDGS and Corn	Corn > hay	NSE	NSE	Corn ↓	NSE	NSE	Radunz et al., 2010 & 2012
M; d 45- d 185	CON diet = 100% NRC, NR= 70%: NRP= 70% + essential AA	♂ + ♀	NRP treatment only= essential AA supplement to equal CON	NSE	---	NSE	NR ↑ adipocyte	NR ↓	NSE	Long et al., 2012
M; d 205- d 265	Forage based diets	♂ + ♀	CSM or SMP supplement fed at 454g/d	NSE	NSE	NSE	NSE	NSE	NSE	Mulliniks et a., 2013

Table A.5. The effects of maternal supplementation during gestation on offspring postnatal performance and carcass parameters (continued).

Parity & Stage	Diet	Offspring	Supplement	WW[1]	Feed efficiency	HCW[2]	USDA QG	USDA YG	12th rib fat	Study
P; d 192-term	CON= meet NRC CP; or HP = exceed CP	♂ + ♀	DDGS	HP > CON	NSE	NSE	NSE	NSE	NSE	Gunn et al., 2014 & 2017
M; d 180-term; early	Pastures fed And normal or early wean	♂ + ♀	LS = 2.16 kg/d, HS= 8.61 kg/d or none (NS)	LS > NS at EW	LS-NW ↑	NSE	HS > NS	NSE	NSE	Shoup et al., 2015a & b
P; d 142-term	CON= hay; HI= hay + DDGS: LO= hay + corn-gluten	♂ + ♀	HI- DDGS (0.83 kg/d); LO- corn gluten (0.83 kg/d)	NSE	CON ↑DMI and RFI	NSE	CON ↑	CON < LO	CON > LO	Summers et al., 2015 a & b
M; d 193-283	Pasture grazing with DDGS (SUP) or none (CON)	♂ + ♀	2.1kg DM DDGS pelleted	NSE	NSE	NSE	NSE	NSE	CON ↑	Wilson et al., 2015b
M; d 180-term	M= meadow; H= hay	♂ + ♀	70% DDGS, LS = 2.16 kg/d, HS= 8.61 kg/d	---	---	---	---	---	---	Moise et al., 2015

P = primiparous; M= multiparous
NSE =No significant effect
[1]BW = birth weights; [2]WW = weaning weights

Table A.6. The effects of maternal supplementation on heifer fertility.

Parity & Stage	Diet	Offspring	Supplement	Birth weights	Cow re-breeding %	Weaning weights	Fertility	Feed efficiency	Study
M; Late gestation (LG) or early lactation (EL)	Dams fed subirregated meadow; H= dams fed cool-season grass hay	♀	Protein supplement 0.45 kg/d with 42% CP (PS) or no supplement (NS)	NSE	NSE	PS & M ↑	PS and H ↑ fertility rates	NS ↓ RFI and DMI	Martin et al., 2007
M; Nov-Mar	Winter range (WR) or corn residue (CR)	♀	Protein supplement (PS) or no supplement (NS)	NSE	---	WR -PS > WR-NS; all CR =	NSE	CR-PS ↓ ADG and G: F	Funston et al., 2010

M= multiparous
NSE =No significant effect

Table A.7. Non-nutritional models of IUGR in the bovine model.

Parity & Stage	Dam stressor	Offspring	Δ dam BW	Fetal/ Birth weights	Hemodynamic measurements	Organ weights	HCW	USDA QG	REA	Study
M & P; d 160-190 to term	Shade (S) or no shade (NS)	♂ + ♀	NS ↓	NS 8% ↓	NS ↑ HR; NSE hematocrit	---	---	---	---	Collier et al., 1982
M; d 100- 174	Heat stress; Non-heat stress	♂ + ♀	---	HS 18% ↓	Uterine BF= 34% ↓; Umbilical BF= 23% ↓	HS ↓ fetal liver, heart, and spleen	---	---	---	Reynolds et al., 1985
M; All of gestation	Singleton or twin pregnancy	♂ + ♀	---	Twin ↓	---	---	Singleton ↑	Twins ↑	Singleton ↑	Echternkamp and Gregory, 2002
M & P; All of gestation	Singleton or twin pregnancy	♂ + ♀	---	Twin ↓	---	---	---	---	---	Echternkamp et al, 2006
M; d 269-term	Either August or October	♂ + ♀	---	NSE	---	---	---	---	---	Wright et al., 2014

P = primiparous; M= multiparous
NSE= No significant effect

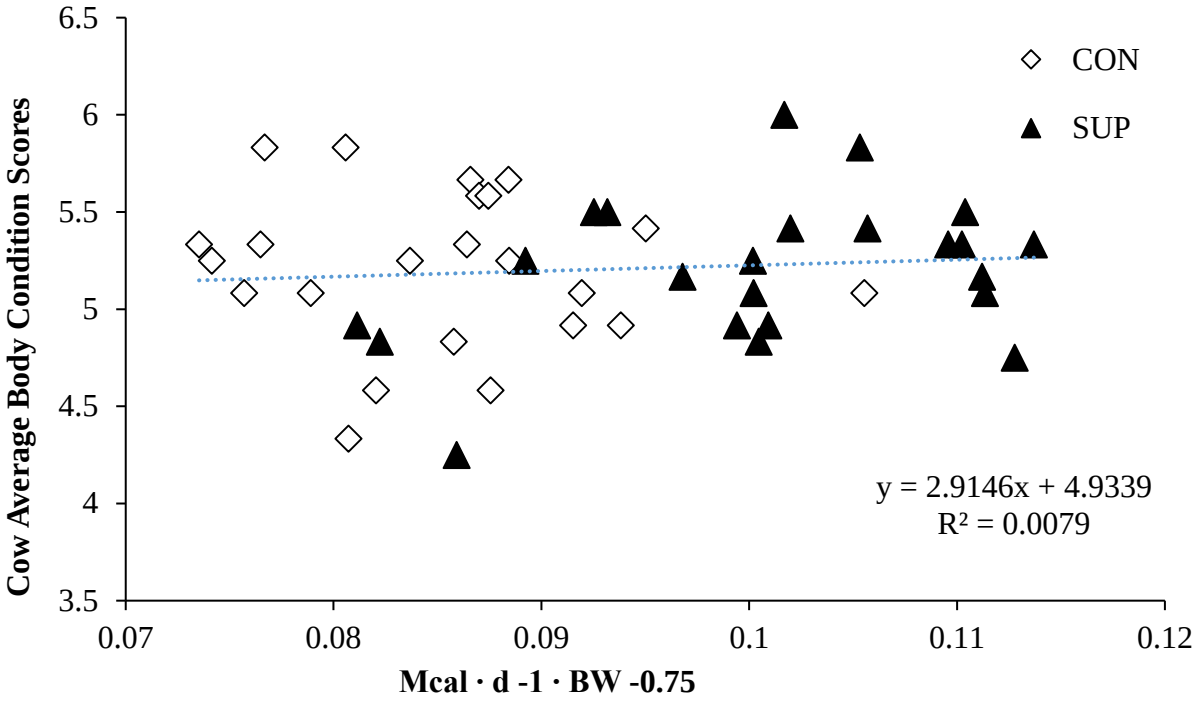

Figure A.1. Gestational change in body condition score change in beef cows relative to per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

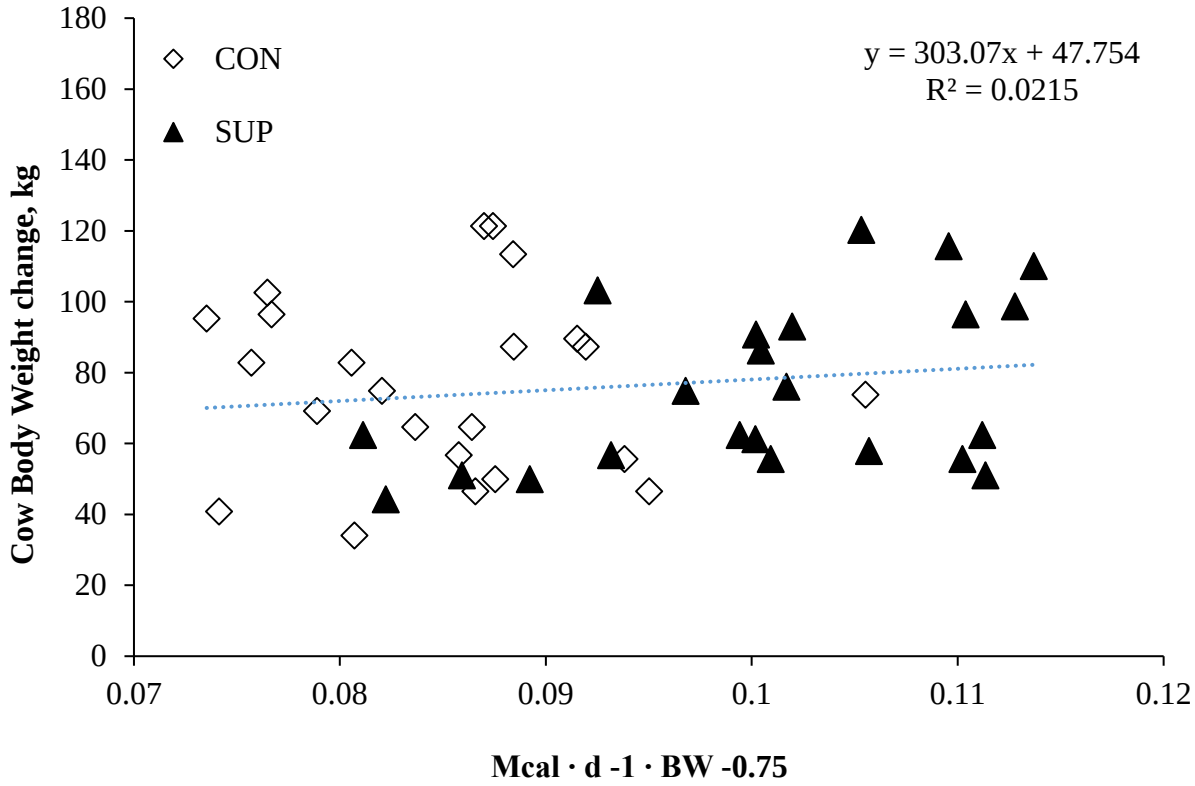

Figure A.2. Gestational change in body weight change in beef cows relative to intake per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

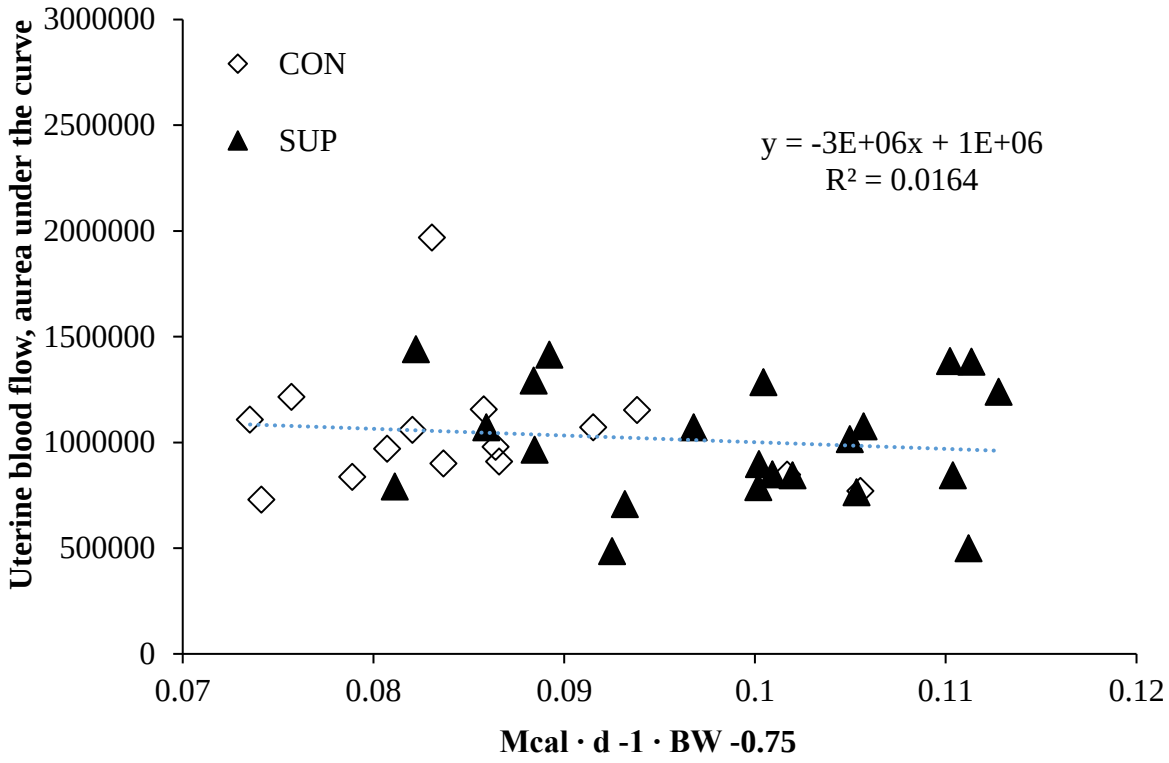

Figure A.3. Gestational uterine blood flow AUC in beef cows relative to intake per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

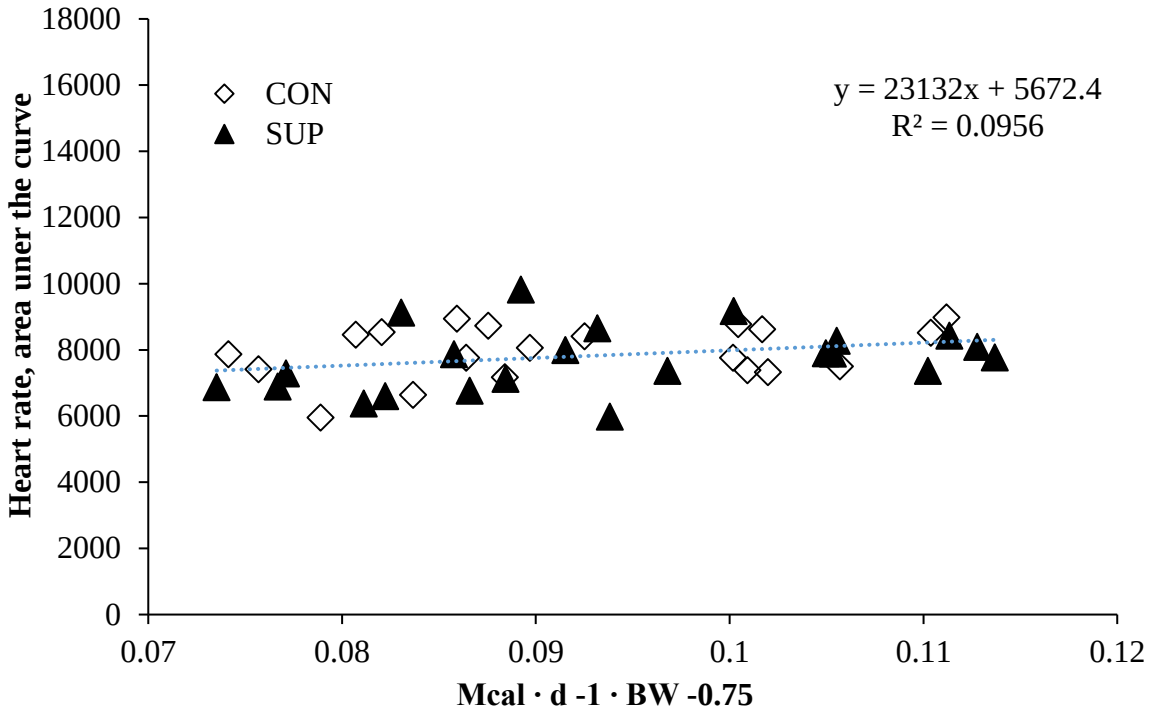

Figure A.4. Gestational maternal heart rate AUC in beef cows relative to intake per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

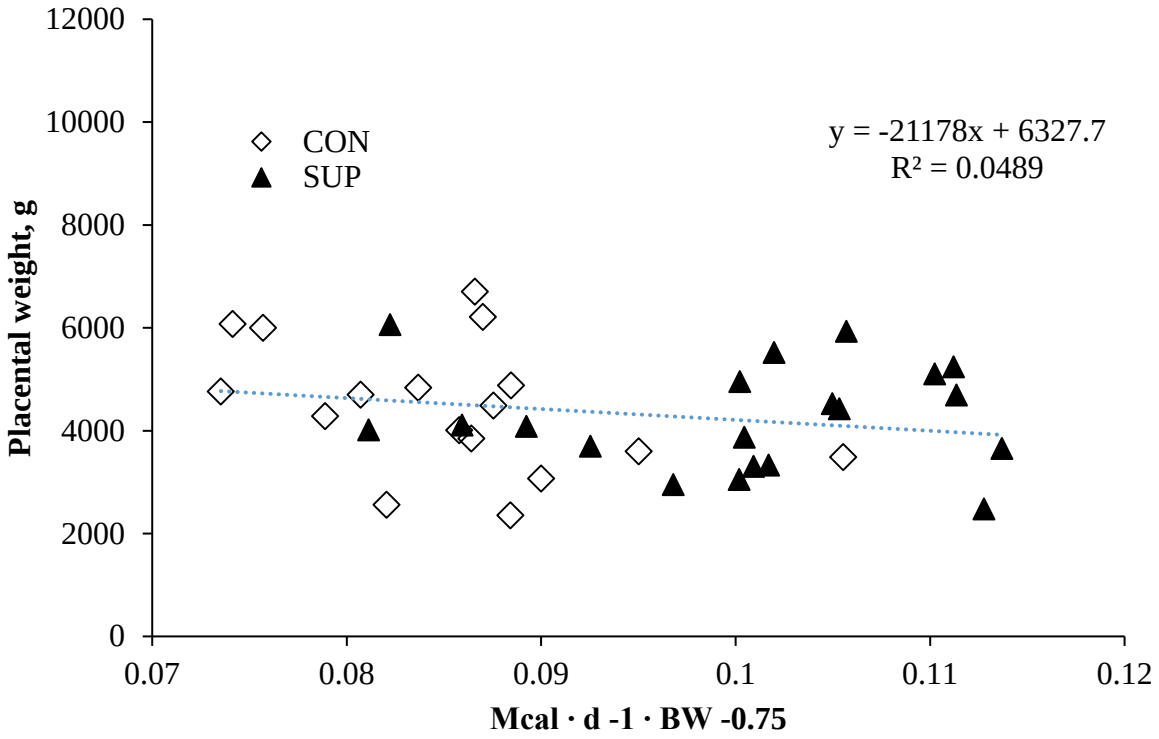

Figure A.5. Gestational placental weight (g) in beef cows relative to intake per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

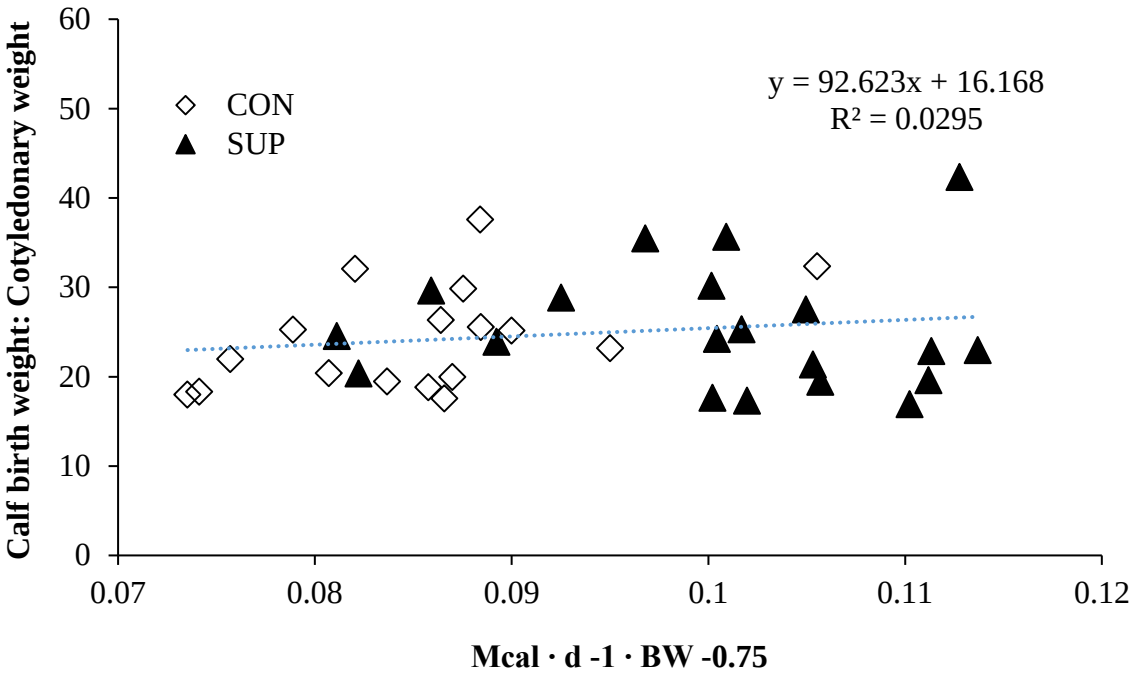

Figure A.6. Calf birth weight: cotyledonary weight in beef cows relative to intake per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

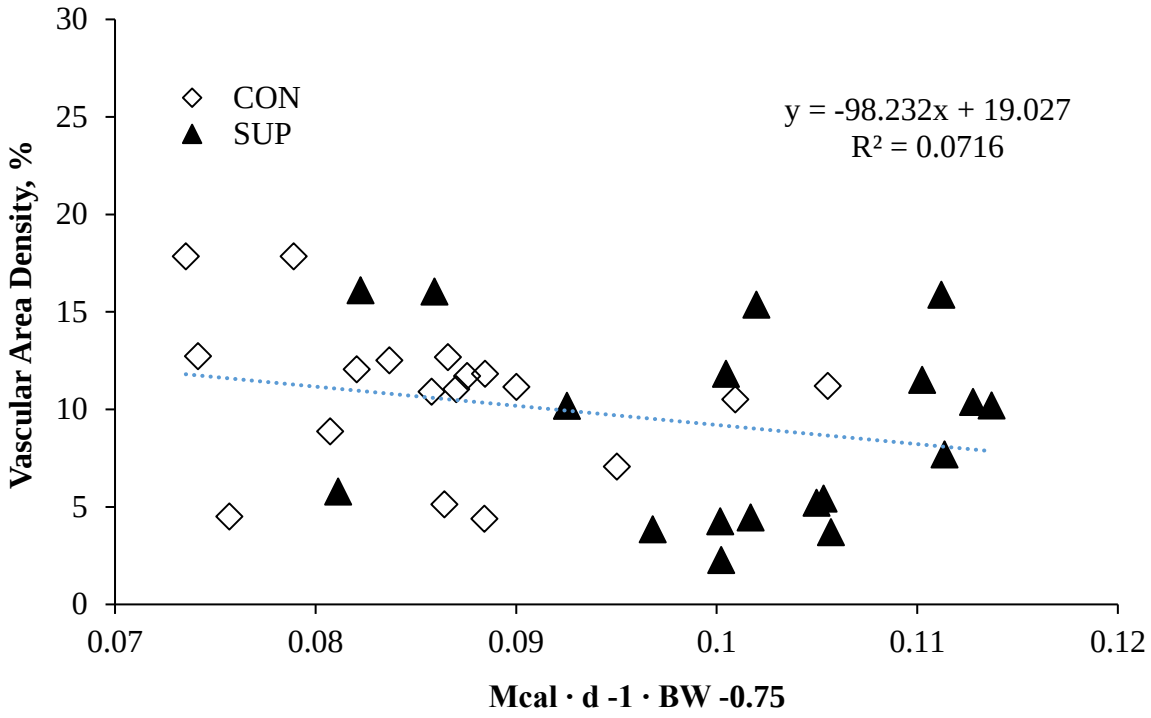

Figure A.7. Vascular area density (%) in beef cows relative to intake per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

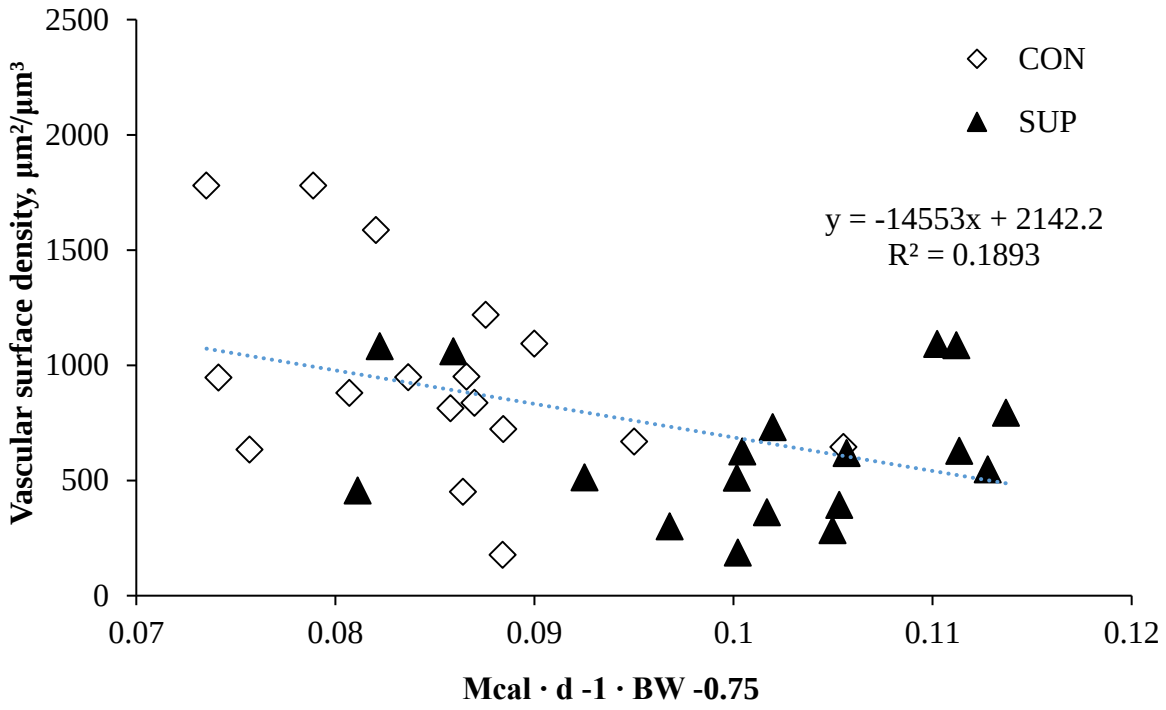

Figure A.8. Vascular surface density (µm²/µm³) in beef cows relative to intake per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

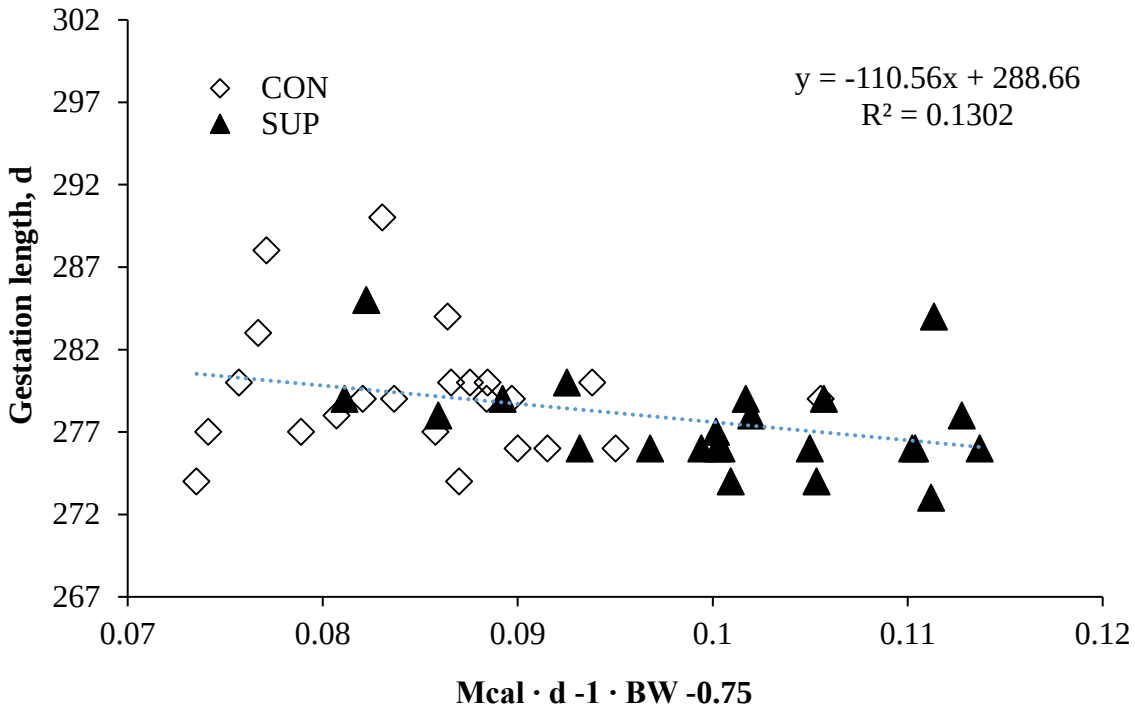

Figure A.9. Gestation length in beef cows relative to per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.

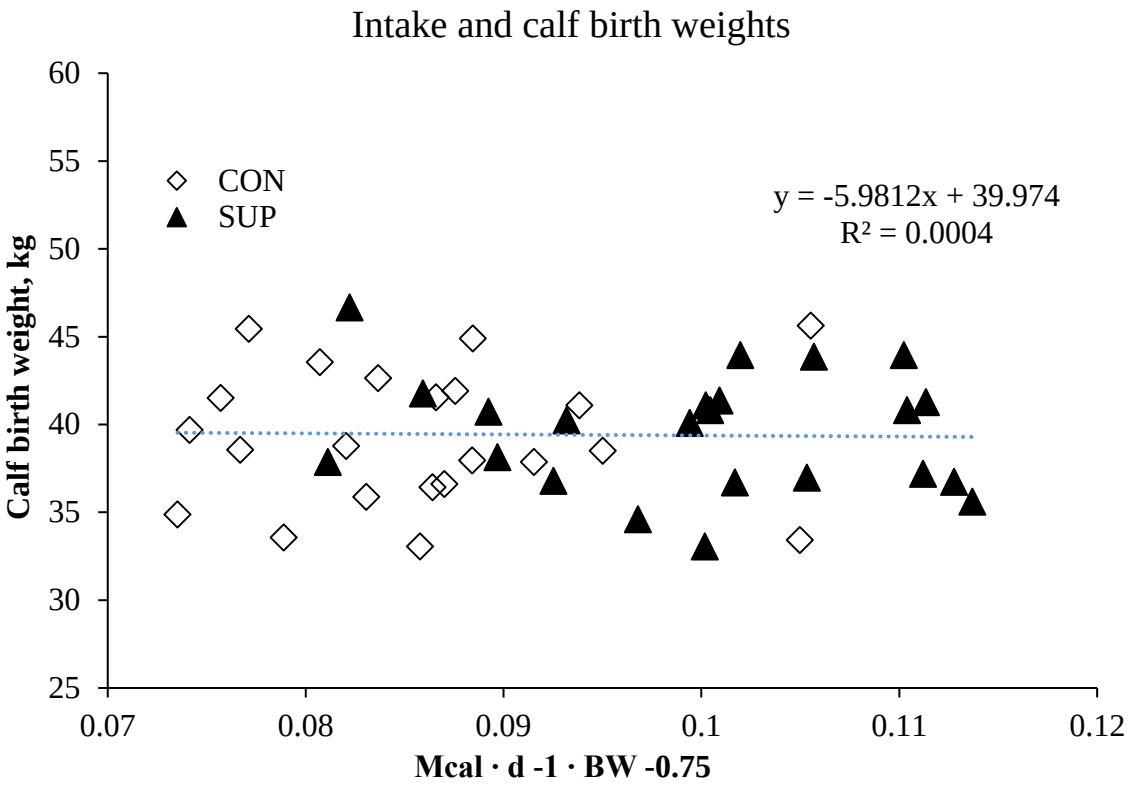

Figure A.10. Calf birth weights in beef cows relative to intake per unit of metabolic body weight in beef cows fed the control and supplemented group from d 100 to d 240 of gestation.