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Influence of Maternal Protein Restriction in Primiparous Heifers During Mid- And/Or Late Gestation on Dam Performance and Progeny Growth, Carcass Characteristics, and Gene Expression

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INFLUENCE OF MATERNAL PROTEIN RESTRICTION IN PRIMIPAROUS HEIFERS DURING MID- AND/OR LATE GESTATION ON DAM PERFORMANCE AND PROGENY GROWTH, CARCASS CHARACTERISTICS, AND GENE EXPRESSION

BY

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy in Animal Science degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidates are necessarily the conclusions of the major department.

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Head, Department of Animal Science Date

Dean, Graduate School Date

This dissertation is dedicated:

To my parents, Kevin and Beth

To my grandparents, Wade and Eleanor, Howard and Judy

For instilling in me a love of agriculture and
always encouraging me to chase my dreams

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Effect of nutrient status in gestating beef cows has been shown to impact dam performance and may affect developmental processes in the fetus that could influence offspring throughout their lives. One hundred eight Angus × Simmental heifers were utilized in a randomized complete block design with control (CON = slightly exceeding MP requirements) and restricted (R = approximately 80% of MP requirements) treatments applied during mid- and/or late gestation. Diets were formulated to be isocaloric and meet net energy requirements.

Dam performance measures were collected at the beginning and end of each gestation period. In a mid-gestation treatment × time interaction, R heifers lost BW and LM area ($P < 0.05$), and % IMF tended ($P < 0.10$) to decrease compared to CON heifers. Heifers restricted in late gestation gained less BW and lost BCS and LM area compared to CON heifers ($P < 0.05$). Dietary treatment did not affect milk production or subsequent reproductive performance ($P > 0.05$).

Progeny were evaluated for growth performance from birth through harvest. Gene expression in *longissimus dorsi* muscle was evaluated at birth and before harvest for a subset of calves. There were no differences due to maternal nutritional treatments

for calf birth, weaning, feedlot entry, or harvest BW ($P > 0.10$). There were no differences in DMI, ADG, or the majority of carcass characteristics ($P > 0.10$); however, LM area was increased in progeny from dams restricted in late gestation ($P = 0.04$). This was not significant when adjusted using HCW as a covariate ($P > 0.10$).

Maternal MP restriction throughout mid- and late gestation (R-R) or in late gestation only (CON-R) down-regulated ($P < 0.05$) genes involved in muscle tissue development compared to CON-CON progeny at birth. Prior to harvest, progeny restricted in late gestation only (CON-R) had decreased expression ($P < 0.05$) of genes related to muscle development compared to progeny restricted in mid-gestation (R-CON) or throughout gestation (R-R).

Despite differences in dam performance and gene expression of progeny, it appeared that offspring were able to recover from moderate MP restriction imposed during gestation.

CHAPTER I

Review of Literature

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INTRODUCTION

Feeding strategies that optimize livestock production while maintaining efficiency and minimizing input costs are necessary for a profitable and sustainable ranching operation. Feed costs (purchased feed, harvested forage, and grazing) account for approximately two-thirds of the total operating costs of a cow-calf operation (USDA, ERS, 2010), indicating that profitability is largely dependent on managing these inputs. In addition, the relationship between nutrition and reproduction in beef females has been studied for decades, with reproductive failure estimated to cost the beef industry between $400 to $500 million per year through decreased production, delayed reproduction, and treatment costs (Bellows et al., 2002). Reports in the literature indicate that insufficient prepartum nutrition results not only in reduced pregnancy rates (Wiltbank et al., 1964; Selk et al., 1988) and increased interval from calving to first estrus (Bellows and Short, 1978; Dunn and Kaltenbach, 1980), but also in reduced calf birth BW (Spitzer et al., 1995; Houghton et al., 1990), decreased calf survival (Corah et al., 1975), and decreased weaning BW (Stalker et al., 2006).

Recently, an increasing amount of interest has been directed toward controlled nutrition studies investigating the impacts of maternal nutrition on long-term physiological impacts on offspring. The ‘fetal origins’ hypothesis was developed using

epidemiological data from humans showing that conditions encountered in utero resulted in altered metabolism, long-term growth and development, and susceptibility to disease (Barker et al., 1993). Subsequent studies using animals have focused on determining a variety of long-term implications for health, productivity, and profitability of livestock, in addition to utilizing animals as models for human biomedical research (Bell, 2006). A number of factors can influence impacts of maternal nutrition on subsequent production, including environment, duration and severity of nutrient restriction, and ability of the dam to buffer negative effects to the fetus (Robinson et al., 2013). Efficient growth, development, and reproduction are key components of profitability in the livestock industry. Research aimed at understanding the mechanisms involved in response of offspring to maternal nutrient status is critically important for developing management strategies that will increase efficiency and ultimately profitability.

Nutrition in the Gestating Cow

The majority of cow-calf producers in the Northern Great Plains are dependent on grazed or harvested forage; however, research indicates that forage quality and/or quantity may be limiting factors in meeting nutrient requirements of gestating beef cattle (DelCurto et al., 2000). There are several factors that contribute to poor nutritional status during pregnancy in most forage-based production settings. Calving in many parts of the country typically occurs in winter or early spring, when rangeland forages are dormant and protein and energy concentrations are low. Beef cows are typically unable to consume adequate energy from forage to meet requirements for maintenance, gestation, or milk production (NRC, 2000). Energy and crude protein requirements in the third trimester are increased by approximately 20% and 14%, respectively, as compared to the

second trimester, resulting in a mismatch between cow requirements and nutrients available in forage (Adams et al., 1996). This situation results in the potential for nutrient shortages to occur in many production situations. Meeting nutrient requirements is important for proper growth and development of the fetus in addition to ensuring the dam has adequate BCS to calve successfully, produce adequate milk, and rebreed within 80 days of calving (NRC, 2000).

Nutrients are partitioned for various body functions including maintenance, growth, pregnancy, and lactation, and reserves can be repartitioned depending on demands (Short and Adams, 1988). The relative order of priority for nutrient partitioning is: 1) maintenance; 2) activity; 3) growth; 4) energy reserves; 5) pregnancy; 6) lactation; 7) additional energy reserves; 8) estrous cycles and initiation of pregnancy; and 9) excess reserves; although priorities may change depending on physiological stage (Short et al., 1990). Age can play an important role in the response of the animal to nutrient deficiency. In general, nutrients are partitioned to maternal tissues according to their metabolic rate, with greater priority toward maternal tissue growth and fat deposition in adolescent pregnancy compared to adults where the gravid uterus is second only to the brain and central nervous system (Redmer et al., 2004). Heifers bred to calve at two years of age have increased nutrient requirements for growth in addition to those for fetal development, which may lead to a nutrient deficiency when grazing low-quality pastures (Ciccioli et al., 2003). Increased susceptibility to nutrient deficiencies can result in delayed puberty and extended postpartum periods in young cows (Hawkins et al., 2000). Wiltbank (1970) reported that young cows nursing their first calves required 15 to 25

days longer to return to estrus than older cows, which could have negative impacts on reproductive performance the following year.

Although impacts of nutrient deficiency may be more pronounced in young cows, there is a strong relationship between adequate nutrition and reproductive performance for all cows in the herd, regardless of age (Short et al., 1990; Randel, 1990). Dunn and Kaltenbach (1980) developed several regression equations that described relationships between energy status, BW change, and reproductive performance. They found that if no BW loss occurred during gestation, 91% of multiparous and 64% of primiparous cows would return to estrus by 60 d postpartum. Since cows must conceive within 80 days postpartum in order to maintain a 365-day calving interval (Dunn and Kaltenbach, 1980), reducing the number of days of postpartum anestrus is a critical factor in determining reproductive efficiency and profitability.

An effective system to help producers in estimating energy reserves and nutrient status of cows is through the use of body condition scoring (BCS) as described by Wagner et al. (1988) where 1 = emaciated and 9 = obese. It is common for dam BW to fluctuate throughout the year depending on nutrient availability and management strategy; however, there are critical time points in the production cycle that may impact response to loss of condition. Morrison et al. (1999) demonstrated the ability to successfully manage a herd with a wide range of BCS during mid-gestation to calve at a BCS of 5 to 6. In a review of factors affecting reproduction in beef cattle, Dzuik and Bellows (1983) suggested that increasing BCS through higher quality forages and supplement is more cost-effective and efficient during gestation than after calving. Research indicates that BCS at calving is a useful indicator of postpartum reproductive

performance factors including reduced postpartum interval (Richards et al., 1986) and subsequent pregnancy rate (Selk et al., 1988). A BCS of 5 or greater at calving has been suggested to be the critical level for ensuring acceptable postpartum reproduction (Dziuk and Bellows, 1983; Richards et al., 1986; DeRouen et al., 1994).

Supplementation Strategies

Alternative grazing strategies and supplemental feeding are commonly used to meet increased nutrient requirements of cattle during biologically important times of the year, such as pre-calving, lactation, and pre-breeding. Both protein and energy supplements can result in positive responses to reproduction through higher energy intake, maintenance of or slight increases in BCS, and increased reproductive performance (DelCurto et al., 2000). Factors that contribute to variation in supplement intake and response to supplementation include supplement type and formation, delivery method, frequency of supplementation, and cow age and nutritional status (Bowman and Sowell, 1997).

Protein supplementation is a common strategy during times of low forage quality and/or quantity, and cow performance responses to various formulations, amounts, and feeding strategies have been well documented. However, impacts of protein supplementation at various stages of gestation on subsequent calf birth BW and performance are somewhat variable. Vanzant and Cochran (1994) provided supplemental alfalfa at 0.48%, 0.72%, or 0.96% of BW to pregnant cows grazing tallgrass prairie for approximately 90 days pre-calving. Although all cows lost BCS across the supplementation period, cows receiving the highest level of alfalfa lost the least amount of BW. Overall conception rate was not affected by treatment; however,

postpartum interval was reduced and calf weaning BW was increased in dams receiving greater levels of alfalfa.

Short et al. (1996) conducted a 4-year experiment to determine effects of feeding 3 kg of 34% protein supplementation to dams during fall grazing. Supplementation increased ADG of calves and increased cow BW gain, but results were highly dependent on forage quality and environmental conditions. Responses in terms of calf weaning BW or pregnancy rates from one year to the next were variable, indicating that supplement effects did not carry over into the subsequent production year. Stalker et al. (2006) found that supplemental protein fed to cows grazing dormant upland range during the third trimester resulted in improvements in BCS prior to calving, and increased the percentage of live calves at weaning. Calf birth BW was similar between treatments but progeny from protein-supplemented dams had 14 kg greater weaning BW compared to calves from unsupplemented dams. However, supplementation did not affect subsequent pregnancy rates or feedlot dry matter intake, average daily gain, or carcass weight of steer progeny.

Fike et al. (1995) reported no differences in calf birth or weaning BW in cows fed no supplement or 2 kg/d of low (12% CP), moderate (20.1% CP), or high (31.7% CP) protein supplement. Weight gain and BCS was greatest in cows receiving the high protein supplement, although average calving dates, percentage of cows cycling at the beginning of the breeding season, and overall conception rates were similar among treatments. A similar study conducted by Weder et al. (1999) also resulted in greater dam BW gains and heavier calf birth BW in cows fed high (18.8% CP) or low (15.2% CP) quality alfalfa compared to unsupplemented cows, but again, there were no effects

on reproductive characteristics. Zehnder et al. (2010) provided soybean meal (SBM) or alfalfa meal (ALM) to heifers receiving hay-based diets for 100 days prior to calving at either 100% or 112.5% of NRC (1996) recommendations for CP intake. Body condition score was not affected by protein source or amount, although heifers fed the higher level of supplement had greater ADG. There was no effect of treatments on calving ease, calf birth BW, or calf vigor.

Nutrient source and site of digestion may be one factor influencing response to protein supplementation. Rusche et al. (1993) utilized soybean meal (low ruminal escape protein) or corn gluten meal/blood meal (high in ruminally undegraded protein (RUP)) fed at 100% or 150% of NRC (1984) recommendations for CP intake to primiparous cows. Feeding a higher level of ruminal escape protein increased calf ADG; however, source and amount of protein did not affect overall conception rates. Mulliniks et al. (2012) reported that calves from dams that received a self-fed supplement in late gestation that contained 50% animal protein sources and 50% mineral had a lower incidence of sickness compared to calves from dams fed cottonseed meal supplement on a routine or intermittent basis. Authors speculated that results may be due to composition and quality of the metabolizable amino acid component in the self-fed supplement, which consisted of a high level of ruminally undegraded protein (RUP).

Energy supplements are typically utilized to meet nutrient deficiencies during times of low forage quality and/or quality, although responses to energy supplementation in the literature are mixed. Many researchers have reported no effect on production or just slight reductions in BW and BCS loss, and there are few reports of improvements in reproductive performance due to energy supplementation (Caton and Dhuyvetter, 1997).

However, potential negative consequences of energy deficiencies in the gestating cow and her fetus have been well documented, including impacts on cyclicity (Houghton et al., 1990; Wiley et al., 1991), decreased maternal BW, lighter calf birth BW (Tudor, 1972), and calf morbidity and mortality (Corah et al., 1975).

Energy supplements typically include feedstuffs that contain either structural (highly digestible fiber sources such as soyhulls, wheat middlings, or corn gluten feed) or nonstructural carbohydrates (NSC; high-starch feedstuffs such as corn, wheat, and barley). In general, supplementation with NSC may result in decreased forage intake and digestibility, which could influence response to supplementation. Bowman et al. (2004) reported no effect on calf birth BW, weaning BW, pregnancy rate, or calving interval due to supplementation with low (0.32 kg), intermediate (0.64 kg), and high (0.96 kg) levels of non-structural carbohydrate (NSC). Cows on all levels of NSC supplementation lost BW compared to the unsupplemented control during the first year of the study, but BW loss in the second year increased linearly with higher levels of NSC supplementation. This was likely due to the negative impact of NSC on forage intake and digestibility.

Radunz et al. (2012) investigated the effects of three types of energy sources consisting of grass hay, corn, or dried corn distillers grains fed to mature beef cattle at day 160 of gestation through parturition. Calf birth BW was greater for progeny from cows fed corn or distillers grains than those fed hay, and weaning BW tended to be less in calves from cows fed hay versus corn. As stated by Radunz et al. (2012), high-concentrate diets may allow more energy to be partitioned to the fetus, which could help explain increased fetal growth in calves from dams fed corn or distillers grains. Cows in this study were fed to meet or exceed nutrient requirements; therefore, no nutrient

restriction was imposed. There were no differences in ADG, hot carcass weight, or yield grade in finished steer calves; however, calves from corn-fed dams had the lowest marbling scores and intramuscular fat content. This response disagrees with most feedlot trials in the literature that report increased marbling in high-starch vs. low starch diets, suggesting that energy composition of maternal diet may affect fetal adipose tissue development and alter responses to diets fed postnatally.

Few studies have directly compared responses to protein and energy supplements. In a 4-year grazing study conducted by Huston et al. (1993), supplement was formulated to provide equal amounts of CP and 10%, 20%, or 40% of required dietary energy (DE). Supplementation resulted in reduced dam BW and BCS loss and increased calf weaning BW compared to progeny from unsupplemented dams. Marston et al. (1995) fed supplemental protein or energy for approximately 120 days prior to calving. Cows fed energy supplement had slightly greater BW gains and 11% greater pregnancy rates over protein-supplemented cows. Calf weaning BW was not affected by supplementation. Authors reported that cow BW and BCS changes were relatively low, indicating that influences on reproduction may occur even without major changes in other measurements of performance.

Sanson et al. (1990) fed ear corn alone, ear corn with a protein supplement, and protein supplement alone to pregnant cows grazing Sandhills winter range or receiving grass hay. Prior to calving, cows fed ear corn alone lost 10% of their BW; however, during calving, cows fed either treatment of ear corn gained BW, and protein-supplement fed alone resulted in BW loss. There was no effect of treatment on conception rate, calving date, or calf birth or weaning BW. These results are in agreement with those

reported by Beck et al. (1992), who fed pregnant cows diets of ammoniated wheat straw with supplement treatments of 1) no supplement (control); 2) LSG (1.36 kg of sorghum grain); 3) HSG (2.72 kg of sorghum grain; or 4) SG + SBM (1.02 kg of sorghum grain and 0.34 kg of soybean meal). All supplements increased cow BW gain over the control treatment, although there was no effect of treatment on calf birth BW, calf gain, or dam reproductive measures.

Nutrient availability and intake vary widely in production situations and can be affected by factors such as diet quality and quantity and environmental conditions. Although positive responses to supplementation in terms of reproductive performance and calf growth are not consistent across studies, negative impacts on production measures due to deficient prepartum CP and energy levels have been well documented (Rasby and Funston, 2016).

Concept of Developmental or Fetal Programming

Developmental Programming Hypothesis

The 'fetal origins hypothesis' refers to the concept that negative influences occurring during fetal development can result in permanent changes in physiology and metabolism in offspring (Barker, 1995). Dr. David Barker, a human physician and researcher, and his colleagues at the University of Southampton, suggested evidence of a relationship between impaired growth and development during gestation and incidence of several major diseases later in life, including coronary heart disease, high blood pressure, and Type 2 diabetes (Godfrey and Barker, 2000). Based on this hypothesis, the response to fetal undernutrition is thought to vary with the trimester of pregnancy in humans (Wilson, 1999). Birth BW is typically reduced due to nutritional restriction during the

first and second trimester, with increased blood pressure later in life associated with first trimester restriction and hypertension and diabetes associated with second trimester restriction. In the third trimester, nutrient restriction usually does not affect birth weight, but postnatal effects include hypertension, cholesterol, and increased potential for heart disease (Wilson, 1999).

One of the most studied cases related to the fetal origins hypothesis occurred during World War II in the German-occupied Netherlands. An interruption in rail transport of supplies by the Germans caused a severe famine, often referred to as the "Dutch Hunger Winter" (Lumey et al., 2007). The famine lasted approximately seven months, with many people forced to survive on less than 1,000 kcal per day. Food supplies were restored immediately after liberation in May of 1945. The famine was well-defined in terms of length of nutrient restriction and the population affected was typically well-fed before and after the famine, which created a 'natural experiment' used to examine potential impacts on offspring due to maternal undernutrition during specific times throughout gestation. The famine affected fertility and weight gain during pregnancy, maternal blood pressure, infant size at birth, and central nervous system development. There were also indications of postnatal metabolic issues and potential for increased risk of disease (Lumey et al., 2007).

Godfrey and Barker (2000) proposed that the effects of nutrition are not only related directly to reduced availability of nutrients, but also to a secondary response of hormonal effects that alter the development of fetal tissues during sensitive periods of human development. During normal pregnancy, hormones including glucocorticoids, insulin-like growth factors (IGF's), and leptin play important regulatory roles in fetal

development (Belkacemi et al., 2010). Maternal nutrient restriction changes level and exposure of the fetus to these hormones, contributing to effects on fetal growth, neural and cognitive deficits, muscle hypertrophy, and links to obesity later in life (Belkacemi et al., 2010). In addition to metabolic and physiological adaptations of the fetus, it has been suggested that epigenetic modifications in the fetus can result from a variety of internal and external stimuli. These epigenetic changes are heritable and allow gene expression in the fetus to respond to its maternal environment (Funston and Summers, 2013).

Although mechanisms that define the fetal programming hypothesis have not been fully determined, research in livestock has shown that compromised fetal growth may result in increased neonatal morbidity and mortality, reductions in feed efficiency and performance, negative impacts on body composition and meat quality, and even lifetime production (Wu et al., 2006). Recently, there has been increased focus by animal scientists to determine the extent to which nutritional status of the dam during pregnancy impacts postnatal productivity factors in beef cattle, including growth, feed intake and efficiency, reproductive performance, muscle development and meat quality. In addition to determining impacts of developmental programming on livestock production, animals are frequently utilized as a model to determine the mechanisms and long-term outcomes of maternal environment in humans.

Influence of Maternal Nutrition on Placental and Fetal Development, Birth BW, and Growth

Because of the potential for sheep to serve as a model for human fetal development, a great deal of research has focused on consequences of maternal nutrition in this species. Luther et al. (2005) reported that undernutrition in mid-gestation in sheep

resulted in variable impacts on placental and fetal growth; however, prolonged nutrition restriction through late gestation can compromise fetal growth. In sheep, placental weight ceases to increase after d 90 of gestation, while placental weight of cattle increases exponentially throughout gestation (Reynolds and Redmer, 1995). Although several studies in cattle have indicated that fetal growth may be altered, results are not consistent. It appears that sheep may be more susceptible to maternal nutrient restriction than cattle, perhaps due to differences in the timing, level, and length of nutrient restriction, as well as patterns of fetal growth (Caton and Hess, 2010). Additionally, it can be difficult to separate environmental influences such as milk production on postnatal growth and performance from maternal influences experienced during gestation (Robinson et al., 2013). Regardless of mixed responses, it is clear that maternal nutrient status during gestation has the potential to impact pre- and post-natal growth in offspring (Funston et al., 2010).

The normal length of gestation in cattle is 283 days or approximately 9 months. The gestation period is commonly divided into the first, second, and third trimester, with each trimester consisting of approximately three months. A great deal of research has focused on response of gestating cows to increased plane of nutrition during the third trimester since most of the fetal growth is occurring at this time; however, there is increasing evidence that adequate nutrition is important throughout all stages of gestation (Funston et al., 2010). Although impacts of nutrition may be more easily observed in the cow herd in terms of BW and reproductive measures, maternal nutrition and hormonal state can influence the growth rate of the placenta and fetus as early as several days after fertilization (Robinson et al., 1995). The influence of maternal environment on the

placenta is very important because it serves as the sole source of nutrients for the fetus (Vonnahme et al., 2013).

Formation and development of the placenta, known as placentation, occurs early in gestation and is critical in meeting the metabolic demands of the fetus (Reynolds and Redmer, 1995). In ruminants, knob-like structures called caruncles on the uterine wall serve as attachment sites for the fetal placenta through villi referred to as cotyledons. The caruncules and cotyledons form a unit called a placentome, which is the primary functional area of all respiratory, nutrient, and waste product exchange between the mother and fetus (Vonnahme, 2012). Uterine and placental blood flow is critical in delivering nutrients to the developing fetus, and will typically increase throughout gestation in a normal pregnancy (Reynolds et al., 2010). The majority of placental growth occurs in the first half of gestation; however, vascular growth continues to increase throughout gestation, along with increased transport capacity to support fetal growth and metabolism (Reynolds and Redmer, 1995). Inadequate placental environment leads to reduced nutrient availability in plasma, decreased oxygen carrying capacity of the umbilical vein, and decreased expression of growth factors necessary for development of new blood vessels, thereby reducing the ability of the fetus to uptake nutrients and potentially affecting postnatal growth and performance (Vonnahme, 2012).

Impaired growth and development of the embryo/fetus or its organs during pregnancy is known as intrauterine growth retardation (IUGR), and can cause significant fetal losses during all stages of gestation (Wu et al., 2006). Insufficient uterine capacity and inadequate nutrient absorption are two major factors that can contribute to embryonic/fetal loss, in addition to genetic factors, environmental temperatures, stress,

and management (Wu et al., 2006). A model developed in rats by Anderson et al. (2005) demonstrated reduced uteroplacental blood flow as a result of IUGR that not only impaired fetal growth but also resulted in persistent hypertension in following generations. Long et al. (2009) fed thirty multiparous beef cows to meet NRC (1996) recommendations (control, n = 15) or to provide 68.1% of NE_m and 86.7% of MP requirements (nutrient-restricted, n = 15) from d 30 to d 125 of gestation. At d 125 of gestation, necropsies were conducted in 10 cows from each treatment. Remaining nutrient-restricted cows were realimented to achieve similar BW and BCS of control cows, and both groups were necropsied on d 245 of gestation. Long et al. (2009) observed reduced cotyledonary weights, increased fetal brain weight, and tendencies for increased fetal heart weight and reduced placentome surface area in over half of nutrient-restricted cows affected by IUGR at d 125 of gestation. Reduced cotyledonary weights in nutrient-restricted cows were also observed at d 245 of gestation; however, fetal weights and caruncle weights were similar between treatments. In contrast, increased growth in the cotyledon and chorioallantois (vascular placental membrane) was reported in cows fed to achieve a thin BCS vs. those fed to achieve a moderate BCS when evaluated at d 259 of gestation (Rasby et al., 1990). Although impacts on placental development due to nutrient restriction were inconsistent in these two studies, it appears that compensatory mechanisms may reduce the influence of nutrient intake and BCS of the dam on fetal development in late gestation. In the study by Long et al. (2009), nutrient-restricted cows that appeared to be affected by IUGR were younger than restricted cows that were unaffected (3.5 vs. 5 years), which could indicate that younger cows are more susceptible to the effects of nutrient deficiency than older cows (Long et al., 2009). This concept is

supported by earlier work by Bellows and Short (1978), who found that calves from heifers had consistently lower birth BW than calves from mature cows.

Vonnahme et al. (2007) imposed a nutrient restriction (NR) on multiparous beef cows from days 30 to 125 of gestation, at which point half the cows were harvested. They noted a reduction in caruncular, cotyledonary, and total placentome weight in NR cows compared to control cows. The suppression in total placentome weight was still evident at day 250 in the remaining NR cattle, even after they were realimented to reach BCS similar to control cows. Fetal weight was not different between NR and control cows at d 125 or d 250; however, authors suggested that this could have been a result of the placenta compensating for reduced nutrient availability by increasing blood flow through the tissue (Vonnahme et al., 2007).

Fetal organogenesis occurs simultaneously with placental development in cattle (Funston et al., 2010). Organ development in the bovine fetus begins with the heart as early as d 21 of pregnancy, followed by limb and other organ development as early as day 25. Development of sex organs begins by day 45 in males and day 50 to 60 in females (Funston et al., 2010). Primordial follicle assembly in females begins around day 80 and occurs through day 150, and these follicles represent the oocyte supply available to a female after puberty (Funston and Summers, 2013). Maternal nutrient restriction during the first third of gestation can result in female offspring with smaller ovarian reserves, resulting in potential suboptimal fertility (Mossa et al., 2013).

Meyer et al. (2010) evaluated the effects of maternal nutrient restriction and stage of gestation on maternal and fetal visceral organ mass. Cows were fed native grass hay to meet NRC (2000) requirements for BW gain during early gestation (Control) or fed a

nutrient-restricted (NR) diet of millet straw that provided 68.1% of NE_m and 86.7% of MP requirements to lose BW (restricted) from d 30 to d 125 of gestation. At that point, some cows were slaughtered and necropsied to measure vascular density and fetal and maternal placental tissues. Remaining restricted cows were fed to achieve BCS equal to control cows and then slaughtered and necropsied on day 245. In this study, nutrient restriction from early to mid-gestation had little effect on fetal weight or organ growth, although NR fetuses had greater reticular mass at d 245. This suggests that compensatory gain may have occurred in fetal organs as cows were realimented. Nutrient restriction did reduce stomach complex, liver, and pancreas mass of dams, indicating that the maintenance of fetal tissue could have been at the expense of maternal tissue.

Houghton et al. (1990) found that low-energy diets after day 190 of gestation caused reductions in calf birth BW and 105-d BW compared to cows fed at maintenance. This agrees with results reported by Corah et al. (1975), who determined that restricted energy intake for 100 days prepartum in heifers (65% of NRC (1970) energy levels) and in cows (50% of NRC (1970) energy levels) caused a reduction in birth and weaning BW of calves. Additionally, calving death loss was 7% greater in heifers on the restricted diet compared to control heifers. The percentage of calves from restricted cows that were alive at weaning was 71%, compared to 100% in control cows. Prepartum nutrition level did not affect the level of milk production in heifers, indicating that weaning BW differences could have been a carryover effect of reduced fetal growth. Nutrient uptake by the fetus is greatest during the last trimester (Reynolds et al., 2010), which supports the conclusion by many researchers that energy restrictions in the dam during that time can have lasting impacts on fetal development.

Fetal growth restriction resulting in lower birth BW may limit the ability of cattle to experience compensatory growth (Greenwood et al., 2005). A summary of studies by Greenwood et al. (2010) provides evidence of this relationship. Authors reported that fetal growth restriction resulting in a decrease of 26% or 10.2 kg of birth BW limited the ability of cattle to exhibit compensatory gain. Cattle whose dams were severely nutrient restricted from d 80 of gestation until parturition remained smaller up to 30 months of age compared to counterparts whose dams were not nutrient restricted. Freetly et al. (2000) fed cows to: 1) maintain BCS from the second trimester until the subsequent breeding season (H-H-H); 2) lose BCS during the second trimester and regain during the third to be similar to maintenance cows at breeding (L-H-H); and 3) lose BCS during the second trimester and regain after 28 days of lactation to be equal to the other two treatments at breeding (L-L-H). Calf birth BW did not differ between the H-H-H and L-H-H groups, but were lowest in calves from L-L-H cows. Pregnancy diagnosis at weaning did not differ among treatments. The feed restriction in this study was moderate and was followed by additional feed in the third trimester; however, fetal growth was affected in cows restricted throughout late pregnancy (L-L-H treatment). These results differ from those of Morrison et al. (1999), who reported no differences in calf birth BW or calf 205-d BW in three groups of cows managed to maintain 1) BCS of less than or equal to four; 2) BCS of five or six; or 3) BCS of seven or greater. However, cows in the study conducted by Morrison et al. (1999) were placed on a higher plane of nutrition about 90 days prior to calving so that all cows would calve at a BCS of 5 to 6, which may have impacted results.

Influence of Maternal Nutrition on Growth, Feed Efficiency, and Carcass Characteristics

Nutritional deficiency during gestation is a common occurrence in many livestock production situations (Caton and Hess, 2010). One of the major principles of developmental programming as outlined by Nathanliesz (2006) is that there are critical periods of vulnerability during development wherein specific tissues and organ systems may be affected. Correlation between timing of nutrient restriction and stage of development appears to be a critical factor in determining mechanisms that influence postnatal performance and ultimately meat quality and yield. Development of economically important tissues such as skeletal muscle and fat begins in the ruminant within the first two months of conception. It is important to note that development of fat cells (adipocytes), structural cells (fibroblasts), and muscle cells (myocytes) occurs simultaneously, primarily through differentiation of mesenchymal stem cells (MSC; Du et al., 2010a). Alterations to the nutrient supply to the fetus can impact signaling pathways that dictate differentiation of cells, potentially altering composition of tissues (Zhu et al., 2004; Du et al., 2010a). Prenatal skeletal muscle development can be separated into an embryonic stage (primary myogenesis) and a fetal stage (secondary myogenesis; Du et al., 2010b). The majority of skeletal muscle fibers are formed between two and eight months of gestation, and there is no further net increase of formation after birth (Du et al., 2013). Skeletal muscle has a lower priority in nutrient partitioning when compared to other organs such as the brain, heart, and liver, and insufficient maternal nutrition in early gestation has been shown to reduce the number and size of myofibers in skeletal muscle (Zhu et al., 2004; Zhu et al., 2006; Du et al.,

2010a). This reduction in the formation of fibers may result in irreversible long-term effects on growth, performance, and meat quality (Du et al., 2010a).

The fetal stage may be the most important in terms of nutritional impacts of the dam on marbling of offspring. Adequate maternal nutrition increases the number of cells that are committed to adipogenesis, which will increase the number of intramuscular fat cells and therefore affect marbling (Du et al., 2010a). Accumulation of fat cells intramuscularly occurs mainly during late gestation, while adipose tissue accumulation in visceral, subcutaneous, or intermuscular tissue sites occurs during mid- to late-gestation (Du et al., 2013). Because adipocyte formation occurs sequentially, there is an opportunity to enhance marbling while not increasing overall fatness through strategic supplementation, although the mechanisms for this have not been defined (Du et al., 2013). When skeletal muscle development is impaired as a result of maternal nutrient restriction, activity of enzymes controlling fatty acid oxidation are reduced, contributing to undesirable increases in intramuscular triglyceride and visceral fat contents in skeletal muscle of lambs (Zhu et al., 2006). These results indicate a complex relationship between maternal nutrition levels and fat and muscle cell formation in the fetus. While undernutrition can negatively impact muscle development and increase fat deposition, overnutrition may also lead to increased fat deposition (Du et al., 2010a). Bispham et al. (2003) reported increased adipose tissue in fetuses that were on a low plane of nutrition during gestation, supporting research in humans that has found that increased circulation of hormones such as insulin-like growth factors (IGF's) and leptin can contribute to obesity later in life (Belkacemi et al., 2010).

In general, muscle fiber formation in ruminant species is stopped during late gestation, and muscles begin to increase in size and length (Du et al., 2013). However, differing responses in terms of fetal growth, development, and postnatal performance between cattle and sheep have been noted. Fahey et al. (2005) reported that restricting ewes to 50% of requirements prior to fetal muscle fiber formation (d 30 through 70 of gestation) resulted in fewer muscle fibers, although there was no effect on the number of muscle fibers when restriction occurred during (d 55 to 95) or after (d 85 to 115) fiber formation. However, restriction late in gestation (d 85 to 115) reduced muscle weight (Fahey et al., 2005). This data supports the hypothesis that restriction early in gestation during myogenesis can impact development of new muscle cells (hyperplasia), while restriction late in gestation may have more of an impact on growth (hypertrophy) of muscle cells. Nordby et al. (1987) found no detrimental effects on muscle fiber characteristics of lambs at slaughter that were born to ewes fed at 70% of NRC (1975) requirements during the first 100 days of gestation and then fed 70% of the amount of alfalfa hay fed to non-restricted ewes. Authors did note that differences may have been observed if measured at birth rather than at market weight, after elongation and overlapping of muscle fibers had occurred.

Another study utilizing a 50% restriction of TDN according to NRC (1985) requirements in early to mid-gestation resulted in reduced muscle fiber numbers of progeny in sheep (Zhu et al., 2004). In a study conducted by Ford et al. (2007), lambs from ewes fed 50% of NRC (1985) requirements from d 28 through d 78 of gestation followed by realimentation through lambing had increased slaughter weights, increased back fat and KPH fat, and tended to have reduced muscle mass as a percentage of HCW.

This suggests that restriction during mid-gestation resulted in reductions in skeletal muscle growth and increases in adipose tissue development that led to differences in BW gain. Similar to differential responses in birth BW between cattle and sheep due to nutrient restriction, it appears that muscle and adipose development in sheep may be more susceptible to restriction. Differences between cattle and sheep in the way that they respond to maternal nutrition could be due to a variety of factors, one of which may simply be differences in gestation length and developmental timelines.

Larson et al. (2009) conducted a three-year trial measuring steer growth performance from dams grazing either winter range (WR) or corn stalk residue (CR) and receiving no supplement (NS) or a protein supplement (PS). Cows grazing CR had increased calf birth BW compared to cows grazing WR. Weaning BW was least for calves from cows receiving NS and grazing WR; however, only 62% of cows on that treatment calved in the first 21 days, indicating that this result could partially be due to calf age. This supports other reports in the literature of increased postpartum interval in cows grazing low quality native range and receiving no supplementation prior to calving. There was no impact due to winter grazing system or protein supplement on external fat thickness or yield grade; however, steers from protein-supplemented dams had increased marbling scores and a greater proportion which graded Choice or higher. It is important to note that calves from this study were grazed on sub-irrigated meadows and fed a protein supplement for eight weeks following weaning, which may have influenced the lack of differences observed for carcass traits. Authors suggested that the increased marbling scores in calves from protein supplemented dams are potentially due to changes in the site of nutrient deposition and intramuscular fat deposition from late gestation

supplementation (Larson et al., 2009). These results are in partial agreement with Summers et al. (2011), who found no differences in 12th rib fat thickness, LM area, or yield grade in steers born to dams receiving high (HN; 0.95 kg/d) and low (LN; 0.37 kg/d) levels of protein supplement; however, increased marbling scores, final BW, and hot carcass weight were increased in steers from HN dams. Mulliniks et al. (2012) fed supplements consisting of 1) 36% CP cottonseed meal (CSM); 2) self-fed supplement containing 50% animal protein sources and 50% mineral (SMP); and 3) brief and intermittent supplementation of cottonseed meal (VAR) to cows in late gestation. Across four years of data collection, no treatment differences were reported for calf weaning, initial and final BW, or carcass characteristics (hot carcass weight, dressing percentage, marbling score, 12th rib fat thickness, LM area, and yield grade).

Data from Underwood et al. (2010) indicated that mid-gestation responses to maternal nutrition may include adipose tissue development and subsequent tenderness in beef steaks. In this study, cows were placed on improved pasture (IP) or native range (NR) during mid-gestation. Treatment had no impact on calf birth BW; however, increased weaning BW was observed in steer progeny from cows on IP, which could have been partially due to increased forage quality. Steers from dams grazing NR had lower ADG and tended to finish at a lighter final BW. Steaks from steers whose dams grazed IP had a lower shear force value, which is an indication of increased tenderness. Fat thickness was greater for IP carcasses; however, marbling score was similar between treatments. It should be noted that a small number of animals were used in this study (IP: n=12; NR: n=14) and that differences in carcass characteristics may have been observed if a greater number of animals were utilized.

Results from an unpublished study described by Du et al. (2011) demonstrated the impact of maternal nutrition and protein supplementation during gestation on muscle and adipose tissue development in steer offspring of beef cows. Thirty-six crossbred beef cows were randomly placed on a control diet receiving 100% (control) or 70% (NR) of NRC requirements or a nutrient-restricted diet with protein supplement (NRP). The NRP diet was designed to provide a similar amount of AA's to the small intestine as the control diet from d 45 to 185 of gestation. Steer offspring of NR dams had less fat thickness, although there were no significant differences between NR and NRP treatments. Steers from dams on the NRP treatment had reduced kidney, pelvic, and heart fat percentages and tended to have larger semitendinosus muscles. Adipocyte diameter tended to be greater in NR steers, although NRP steers tended to have a higher number of adipocytes.

In contrast to results reported above, there are other studies indicating few differences in carcass characteristics between progeny from dams on restricted or adequate levels of nutrition. Long et al. (2010) reported that steer calves from crossbred heifers fed a low-nutrition diet (LN; 55% of NRC (1996) requirements) or moderate nutrition diet (MN; 100% of NRC (1996) requirements) during early gestation were not different in terms of calf birth BW, gain from birth to weaning, or weaning BW. However, heifers fed in this study were commingled after day 115 and fed a diet that exceeded NRC (1996) requirements, which may have allowed fetuses to overcome potential effects of the nutrient deficiency experienced in early gestation, as demonstrated by Freetly et al. (2008). There were also no differences in hot carcass weight, fat thickness, dressing percentage, yield grade, or marbling score of steer progeny born to

LN or MN heifers. However, researchers did note increased area of individual muscle fibers and increased concentrations of DNA in the complexus muscle for steer progeny from dams receiving the LN diet, and speculated that this result could affect meat quality and tenderness. Greenwood et al. (2006) evaluated carcass characteristics of Piedmontese- or Wagyu-sired cattle with low (28.6 kg) and high (38.8 kg) birth BW and slow (554 g/day) or rapid (875 g/day) growth to weaning. Low birth BW calves weighed 56 kg less at 30 months of age, and carcasses and amount of retail beef product were 32 and 18 kg lighter, respectively. There were no differences in any other carcass quality measurements, indicating that reduction in birth BW of animals does not necessarily translate to impacts on carcass traits. Relationships among muscle fiber characteristics, growth, and meat quality have not been well defined (Lefaucheur, 2010), and factors controlling growth and development of skeletal muscle are of great interest to researchers. Also, the competitive relationship between muscle and fat development offers the opportunity to formulate maternal diets that could increase adipose development and potentially impact marbling without increasing subcutaneous fat. Increased understanding of the pathways affecting these processes is necessary to develop management strategies that allow for manipulation of tissue growth and lead to improved production efficiency and meat quality.

Influence of Maternal Nutrition on Gene Expression

Research indicates that nutrition during various stages of pregnancy can result in significant changes in structure, metabolism, and physiology of offspring. In the 'thrifty phenotype' hypothesis described by Hales and Barker (1992), it was suggested that metabolic adaptations are made during fetal life based on nutrient supply from the mother

with the expectation that a similar environment will be encountered at birth. Based on epidemiological evidence in humans, these adaptations can be detrimental to offspring if nutrient availability in the pre- and post-natal environment differ, resulting in alterations in cardiovascular and metabolic homeostasis and growth and body composition (Godfrey et al., 2007). Knowledge of mechanisms underlying responses is critical for preventing negative consequences on health and productivity of livestock. Fetal growth is a complex series of events influenced by factors such as size and capacity of the placenta, uteroplacental blood flow, nutrient transfer, and metabolic pathways (Wu et al., 2006). Long-term impacts on offspring as a result of nutrient restriction during gestation are likely due to both structural defects such as alterations in tissue and organ structure and functional defects such as changes in gene expression that can alter tissue function (Reynolds and Caton, 2012).

The term ‘epigenetics’ refers to biochemical modifications of the genome that impact gene function without changing DNA sequence. Increasing evidence indicates that maternal nutritional status can result in permanent alterations in gene expression and modifications in the fetal genome that alter the epigenetic state of the fetus (Wu et al., 2006). Epigenetic change is a normal physiological process that contributes to regulation of gene expression in an organism (Nilsson and Skinner, 2015). However, there are windows of time representing critical periods of development in which certain environmental conditions such as nutritional insults can interfere with normal epigenetic processes and result in changes that could be considered epimutations (Nilsson and Skinner, 2015). There are several mechanisms believed to regulate gene expression. The two most studied mechanisms include DNA methylation and histone modification.

Methylation of DNA involves the addition of methyl groups to the cytosine bases of DNA. This is regarded as an inhibitory mechanism since it causes condensation of the chromatin which determines structure of DNA, thereby silencing gene expression (Doherty et al., 2014). Histones are the primary protein components of chromatin, which determines the coiling and looping structure of DNA. Post-translational modifications to histone proteins that occur through enzymatic activity such as methylation and acetylation affect function of gene regulation (Doherty et al., 2014). An additional mechanism that has demonstrated a function in gene regulation but is less understood is the role of microRNAs or miRNAs. These are a family of non-coding RNAs that can negatively regulate gene expression through modification of sequences, structure, and expression of mRNA during and following transcription (He and Hannon, 2004). The processes outlined above often affect the way that an organism reacts to the environment, thereby influencing changes in gene expression.

Research has shown that maternal nutrition in beef cattle can alter placental growth and function, impact uterine blood flow and nutrient transfer to the fetus, and affect organ development and differentiation of various tissues such as fat and muscle (Funston and Summers, 2013). However, overall effects of developmental programming remain focused on phenotypic responses in offspring, with the underlying mechanisms responsible for these differences rarely investigated and consequently not well understood. Some of the challenges facing livestock scientists are widely varying nutrient concentrations in various feedstuffs and differences in bioavailability and metabolism of nutrients based on genetic diversity among individuals (Neibergs and Johnson, 2012). However, recent availability of the bovine genome sequence has

allowed for additional understanding of genes and mechanisms that play a role in regulation of developmental programming. Several recent experiments have analyzed differential gene expression in muscle and fat, both of which are economically important tissues in the beef industry.

A relationship between maternal nutrition and muscle, fat, and connective tissue development in offspring has been established (Du et al., 2015); however, mechanisms are not well understood. Reed et al. (2014) measured muscle fiber characteristics of lambs born to ewes fed 60%, 100%, or 140% of NRC (2007) requirements beginning on d 31 of gestation. At birth, lambs were allowed to nurse their mothers for up to 24 h. After euthanizing a portion of the lambs for muscle characteristics on d 1, remaining lambs were fed milk replacer until 60 d of age and then fed water, creep fed, and hay for 3 months. Compared with the control treatment, muscle fiber cross-sections were similar between ewes fed at 60% and 140% of NRC (2007) requirements in lambs at 1 d and 3 months of age. Interestingly, muscle lipid content was increased in offspring from overfed and restricted ewes compared to the control treatment at 1 d of age; however, muscle lipid content was increased in overfed ewes and decreased in restricted ewes compared to the control treatment at 3 months. Differential responses for muscle growth and fat deposition within muscle indicate that mechanisms for these responses could vary and may be highly specific depending on the timing, length, and severity of nutrient restriction, in addition to postnatal management strategies. Despite similar phenotypic responses in muscle tissue between overfed and restricted treatments, global gene analysis of offspring indicated that nutrient restriction affected genes involved in muscle growth and signal transduction, while overfeeding caused changes in expression of genes

that regulate muscle protein synthesis (Hoffman et al., 2016). Additional research to determine gene expression differences at various stages of fetal development could provide increased understanding of how these mechanisms may change depending on a specific nutrient restriction.

Impacts of maternal undernutrition and protein supplementation during early- and mid-gestation on growth, adipocyte size, and expression of nutrient transporters and transcription factors in offspring was investigated by Long et al. (2012). Cows received native grass hay containing 6.2% CP (DM basis) and one of three supplement treatments including control (CON; soybean meal-based supplement), nutrient-restricted (NR; 70% of NE_m and CP supplied by CON diet), and nutrient-restricted + protein-supplemented (NRP; 70% of NE_m supplied by the CON diet plus a RUP supplement formulated to provide duodenal essential AA flow equivalent to the CON diet). Average adipocyte diameter in perirenal, subcutaneous, mesenteric, and omental adipose tissue was increased in offspring from NR dams compared with CON, with NRP offspring either intermediate or similar to CON offspring. Concentrations of DNA in adipose tissue depots were reduced and mRNA expression of fatty acid transporter 1 was increased in subcutaneous adipose tissue in NR offspring.

Micke et al. (2011a) fed heifers 240% or 70% of CP recommendations during the first and second trimesters of gestation, resulting in a 2 × 2 factorial treatment structure (high or low protein during the first or second trimester). Skeletal muscle fibers and key regulators of adipogenesis (IFG1, IGF2, and their receptors) in skeletal muscle were measured in offspring at 680 d of age. Cross-sectional areas of longissimus dorsi (LD) and semitendinosus (ST) muscles measured via ultrasound were greater for male

offspring born to dams fed low protein diets in the first trimester compared to dams fed high protein diets; however, there were no differences in muscle size of female offspring during either trimester due to maternal treatment. Additionally, mRNA expression of IFG1, IGF2, and the IGF2 receptor was increased in ST muscle of male offspring born to heifers that were on a protein-restricted diet during the first trimester. Authors suggested that changes in maternal metabolic and placental hormones in response to maternal dietary treatment may have signaled mRNA expression, with the majority of differences occurring during the first trimester of gestation. They speculated that there may be an interaction between fetal sex steroid and maternal nutrient intake that resulted in sex-specific effects on fetal muscle development. Micke et al. (2011b) also evaluated expression of leptin (LEP) in addition to IGF1, IGF2, and their receptors in various depots (subcutaneous, perirenal, and omental) of adipose tissue in offspring. High protein diets in the first trimester increased LEP and IGF1 in perirenal fat depots of all progeny. High protein diets in the second trimester increased IGF1 in perirenal and omental fat depots of both steers and heifers, with increased LEP in perirenal depots of male progeny only. It appears that various depots of adipose tissue may have different responses to maternal nutrient status.

Increased concentrations of DNA were reported in complexus muscle and adipose tissue of finished steers whose dams were fed a low-nutrition diet (55% of NRC (1996) requirements) compared to steers from dams fed at 100% of NRC (1996) requirements in early gestation (Long et al., 2010). These results indicate that there were a greater number of nuclei in skeletal muscle and a greater number of adipocytes in offspring restricted during gestation. Also, abundance of mRNA for genes involved in fat

metabolism and glucose uptake were decreased in perirenal adipose tissue of steers from dams on the low-nutrition diet, but similar in subcutaneous adipose tissue between treatments.

A study investigating differing maternal energy supply during mid-gestation and effects on muscle histology and genes regulating fetal adipose and muscle development was conducted by Jennings et al. (2016). Heifers were assigned to dietary treatments providing 146% (HIGH), 87% (INT), or 72% (LOW) of energy requirements from d 85 to d 180 of gestation, with fetuses removed at d 180 of gestation via cesarean section. There were no differences in fetal growth or muscle histology characteristics of LM or semitendinosus muscles among treatments. Gene expression of subcutaneous fat samples did not differ among treatments; however, there were differences in gene expression of fetal LM. This could be due to differences in timing of development of fat and muscle tissues, with fetuses harvested before appreciable amounts of adipose tissue had an opportunity to develop. Two prominent transcription factors involved in differentiation of adipocytes (preadipocyte factor-1; PREF-1, and CCAAT/enhancer-binding protein-β; C/EBP-β) were differentially expressed in LM muscle. Expression of PREF-1 was upregulated in fetal LM of HIGH fetuses compared to INT; however, expression was similar between LOW and HIGH treatments. Expression of C/EBP-β was upregulated in LOW fetuses as compared to INT. These responses indicated that both over- and under-feeding can activate changes in differentiation and development of adipocytes within the LM. There were no differences in gene expression of transcription factors necessary for myoblast proliferation (myogenic factor 5; Myf5, myoblast determination protein 1; MyoD) among treatments in fetal LM samples. Myogenin is thought to play a role in

terminal differentiation of muscle fibers, and expression was upregulated in LOW fetuses. This could indicate that the overall number of myofibers might be reduced due to energy restriction; however, lack of differences in muscle fiber number among treatments did not support this. Similarly, upregulation of μ-calpain (important for protein turnover) in HIGH compared to INT fetuses could suggest increased migration, fusion, and proliferation of myoblasts due to overfeeding; however, this was not supported by differences in number or size of muscle fibers.

The combination of the genome and the epigenome determines the response of the animal to its environment. The concept of epigenetics and its role in gene expression has been well-established; however, the ability to differentiate between epigenetic changes integral to development and those due to environmental changes is limited. Results of production studies evaluating subcutaneous fat deposition of offspring due to nutrient restriction have resulted in highly variable responses, including no differences (Greenwood et al., 2006; Larson et al., 2009; Summers et al., 2011, Micke et al., 2011b), reductions (Greenwood et al., 2010), and increases (Ford et al., 2007) in adipose tissue between progeny from restricted vs. control dams. Similar conflicting responses have been observed for impacts of maternal nutrition on skeletal muscle development, with results highly variable depending on timing and severity of the nutritional insult as well as the species being investigated (Zhu et al., 2006; Hoffman et al., 2016; Jennings et al., 2016).

Despite lack of phenotypic differences in many of the studies described above, measurable differences in gene expression suggest that potential fetal adaptations to nutrient restriction may have taken place in preparation for a nutrient-poor environment

at birth. Although knowledge of processes affecting DNA modification is increasing, it is unclear how these patterns change in response to maternal nutrition and the postnatal environment. Additional research to determine how these processes may change in relation to each other will improve the ability to select animals based on genetic potential and develop targeted nutritional management strategies to improve animal health and production.

CONCLUSION

Forage-based livestock production systems often result in reduced quality or overall quantity of nutrients necessary to meet requirements at critical periods in gestation. A majority of research is conducted in late gestation, yet organ development, fetal skeletal muscle growth, and adipogenesis begin earlier in gestation. More information is needed to determine the specific relationship between maternal nutrition and differentiation of stem cells in muscle, which could affect overall carcass quality. Since most research is focused on production responses, there is need for a greater understanding of the mechanisms that influence response. Although several researchers have examined effects on placental growth and development, knowledge of the mechanisms that control nutrient flow to the fetus and how these mechanisms may shift in times of nutrient deficiencies is somewhat limited. It is well understood that nutrient requirements are increased as a result of fetal growth, particularly in the third trimester; however, mechanisms utilized by the dam in meeting these requirements are not well understood.

The consequences of prenatal nutrition on economically important traits such as feed efficiency, gain, and carcass quality are varied. Though there are often differences

in weaning BW attributed to fetal programming, factors such as postnatal nutrition and subsequent milk production appear to provide potential for confounding effects. Further studies in a controlled research environment are needed to separate pre- and postnatal responses to maternal nutrition and develop a more thorough understanding of the mechanisms that influence the relationship between prenatal development and postnatal performance, growth, and carcass characteristics.

The timing and severity of nutrient restriction may affect performance in the dam and her offspring. In addition, diet composition, various protein and energy sources, and nutrient profiles may influence nutrient availability and uptake by the fetus. Additional research is needed to explore the effects of how energy and protein source may influence fetal development when total nutrient intake is limited. To date, most research has investigated impacts of nutrient restrictions during the first and third trimester on phenotypic responses of offspring in terms of growth and carcass characteristics. To our knowledge, there is not another beef cattle study focused on MP restriction during mid- and/or late gestation to evaluate potential differences in timing of the restriction on progeny performance.

Therefore, the objectives of this dissertation were to:

1. determine the effects of dietary MP restriction in mid- and/or late gestation on measurements associated with cow BCS, BW, and metabolic indicators of protein and energy status and postnatal calf performance to weaning;
2. determine the effects of dietary MP restriction in mid- and/or late gestation on growth performance, feed efficiency, and carcass characteristics of progeny; and

3. determine the effects of dietary MP restriction in mid- and/or late gestation on differential gene expression in skeletal muscle of progeny at birth and prior to harvest

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CHAPTER II

Influence of maternal protein restriction in primiparous heifers during mid- and/or late gestation on dam and suckling calf performance through weaning

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ABSTRACT

Effects of nutrient status in gestating beef cows has been shown to impact performance of the dam and offspring; however, most research is focused on energy or a total diet restriction and a single period of gestation. The objective of this study was to evaluate the effects of maternal metabolizable protein (MP) restriction in primiparous heifers during mid- and/or late gestation on dam and suckling calf performance through weaning. One hundred eight two-year-old Angus × Simmental heifers were allocated to a randomized complete block design with a 2 × 2 treatment structure. Pens within each block were randomly assigned to either CON (slightly exceeding MP requirements) or R (approximately 80% of MP requirements) treatments. Diets were formulated to be isocaloric and meet net energy requirements. Half of the pens on the CON treatment were reassigned to the R treatment at the end of mid-gestation and vice versa in a crossover design. Heifer BW, BCS, ultrasound body composition, blood metabolites, milk production and composition, calving data, and calf weaning weights were measured. There was an interaction for mid-gestation treatment × time for change in BW during mid-gestation, with heifers on the R treatment losing BW while CON heifers maintained BW ($P = 0.002$). In a late gestation treatment × time interaction, restricted heifers gained

approximately half as much BW and lost BCS compared to CON heifers ($P < 0.05$). There was a mid-gestation treatment × time interaction for LM area change, with R heifers losing over twice as much LM area as CON heifers ($P = 0.04$). A mid-gestation × time interaction ($P = 0.03$) indicated a tendency ($P < 0.10$) for increased IMF loss in heifers on the R treatment at the end of mid-gestation. In a late gestation treatment × time interaction, MP restriction in late gestation increased loss of LM area by 4-fold ($P = 0.03$). There were no changes in 12th rib subcutaneous fat thickness ($P > 0.05$) across treatments or time. Concentration of β-hydroxybutyrate was reduced ($P = 0.02$) and NEFA concentration tended ($P = 0.06$) to be decreased for R vs. CON heifers in mid-gestation. Dietary treatment did not affect calf birth BW, milk production, milk composition, calf weaning BW, or subsequent reproductive performance ($P > 0.05$). Decreased available MP appeared to result in mobilization of maternal body reserves during the restriction; however, it did not impact calf birth BW or growth to weaning.

INTRODUCTION

Nutrient status of gestating beef cows can have various long-term implications on growth, feed intake and efficiency, and performance of offspring (Funston et al., 2012). Research indicates that developmental status of the fetus at the time of a maternal nutrient deficiency plays a role in postnatal responses of the offspring (Freetly et al., 2000; Morrison, et al., 1999; Wiley et al., 1991). Inadequate nutrition from early to mid-gestation has been shown to impact organ development, muscle fiber formation, weaning weights, carcass weights, and meat tenderness (Du et al., 2010; Long et al., 2012; Underwood et al., 2010; Zhu et al., 2004). Additionally, supplementation provided during late gestation in beef cattle has resulted in improved calf weaning weights, improved feed efficiency, and increases in marbling scores and quality grades (Martin et al., 2007b; Stalker et al., 2006; Larson et al., 2009). However, much of the available research has been limited to a single period of development (e.g. early or late gestation) and has evaluated the effects of an energy restriction or a reduction of total dietary dry matter intake.

Metabolizable protein is defined as true protein absorbed in the intestine, consisting of microbial protein and ruminally undegraded protein (RUP; National Academies of Sciences, Engineering, and Medicine, 2016). Reports are available in the literature that describe performance responses of cattle due to various levels of ruminally degraded protein (RDP) and ruminally undegraded protein (RUP) (Martin et al., 2007a; Engel et al., 2008; Larson et al., 2009). However, limited data is available on the effect of a protein restriction during gestation and the subsequent developmental programming effect, particularly utilizing the metabolizable protein (MP) system. Evidence in rodent

models indicates that maternal protein restriction reduced birth weight, elevated blood pressure, and resulted in metabolic changes that led to insulin resistance in offspring (Langley-Evans et al., 1996; Zeng et al., 2012). However, results of these studies cannot be directly applied to ruminants due to the complexities of the ruminant digestive tract and differences in placental and fetal development among species. To our knowledge, there are no studies that have investigated the effects of an individual nutrient deficiency such as metabolizable protein across multiple stages of gestation in beef cattle.

While the primary goal of research examining maternal nutrient levels during gestation is to evaluate lifetime performance of offspring, particularly post-weaning, it is also important to characterize the impact of a gestational nutrient restriction on performance of the dam and early postnatal life of the calf. Therefore, the objective of this study was to evaluate the effect of maternal protein restriction from mid- to late gestation in first-calf heifers on dam nutrient status and performance and suckling calf performance through weaning.

MATERIALS AND METHODS

Animals and Experimental Design

The South Dakota State University Institutional Animal Care and Use Committee approved all procedures involving animals. One hundred eight two-year-old Angus x Simmental heifers were pen-fed at the SDSU Cottonwood Range and Livestock Field Station near Philip, SD during the time gestational nutrient restrictions were imposed. Prior to the beginning of the study, yearling heifers were synchronized and time-bred to a single Angus sire on June 7, 2013. Following AI, all heifers were exposed naturally to Angus bulls for 60 days. Rectal ultrasonography was conducted in mid-September to detect pregnancy and fetuses were sexed and aged.

Treatments were arranged in a 2 × 2 factorial structure with 2 levels of dietary metabolizable protein (MP) provided during two stages of gestation (mid and late). Dietary MP levels included: control (CON**;** slightly exceeding MP requirements) and restricted (R**;** approximately 80% of MP requirements supplied based on Level 2 of NRC (2000). Heifers were blocked by BW as well as age and sex of the fetus, resulting in 3 blocks with 4 pens per block. At the end of the mid-gestation treatment period, half of the pens on the CON treatment were reassigned to the R treatment and half of the pens on the R treatment were reassigned to the CON treatment, resulting in four treatment combinations (CON-CON, CON-R, R-CON, and R-R). Each treatment combination was randomly assigned to one pen per block for a total of 3 pen replicates per treatment combination.

Diets were based on calcium hydroxide treated wheat straw, crude glycerin, and concentrates (Table 2.1). Wheat straw was ground through a 12.7 cm screen using a tub

grinder and treated with $Ca(OH)_2$ using the SecondCrop™ process (ADM Animal Nutrition, Quincy, IL) to increase energy value. Targeted $Ca(OH)_2$ was 6.6% DM basis and water was added to achieve approximately 50% moisture. Treated straw was packed into production-scale Ag-Bags (Ag-Bag, St. Nazianz, WI). Both CON and R concentrate formulations contained ground corn, ground corn cobs, a rumen-protected fat product (Energy Booster 100®, Milk Specialties Global, Eden Prairie, MN), urea, and crude glycerin. Most ingredients were chosen to be sources of energy so diets were isocaloric and met NE_m and NE_g requirements as predicted by NRC (2000). Urea was utilized to meet bacterial N requirements and ensure that fermentation capacity would not limit energy value of the diet. The CON concentrate also contained porcine bloodmeal to slightly exceed the MP requirement. Diets were formulated to be isocaloric and meet predicted NRC (2000) requirements for NE_m and NE_g in both CON and R treatments.

Dry supplement was mixed with crude glycerin on a daily basis immediately before feeding using two batch mixers to avoid cross-contamination between CON and R diets. Supplement was offered at around 1000 hours daily and was completely consumed in approximately 1 hr. Following supplement consumption, heifers were offered access to calcium hydroxide treated wheat straw. Dietary straw samples were collected on a daily basis and composited. Bunks were cleaned and orts were weighed and sampled once weekly. Straw and ort DM was determined by drying for 24 h at 60°C in a forced air oven. Diet formulations and amount of feed offered were adjusted throughout gestation to account for increased energy needs for the growing heifer and the developing fetus. Despite balancing diets to achieve predicted MP and energy concentrations in the diets, consumption of wheat straw by heifers was less than predicted. Although desired

MP and energy levels were not consistently achieved for each diet formulation based on calculated intakes, percentage of MP requirements supplied was 101% for CON heifers and 81% for R heifers when averaged across the study (Table 2.1).

Calf Management

Heifers were removed from their respective pens and dietary treatments immediately before or following calving. Within 24 h of birth, calves were weighed and tagged, and male calves were banded using a premium castration ring plier (Neogen Corp., Lansing, MI). Calving information, cow BCS at calving, and Beef Improvement Federation (BIF) scores for calving difficulty (1= No difficulty, no assistance; 2 = Minor difficulty, some assistance (easy pull); 3 = Major difficulty, often mechanical assistance (hard pull); 4 = Caesarian section or other surgery; 5 = Abnormal presentation (i.e., breech)) and calf vigor (1= Nursed immediately, healthy; 2= Nursed on own but took time; 3= Required assistance to suckle; 4= Died shortly after birth; 5= Dead on arrival) were recorded. Pairs were managed as a common group on native pastures through weaning, with no further nutritional restrictions imposed on dams or their offspring.

Heifer Performance Measurements

Heifer performance data were collected at the initiation of the trial, at the time of treatment crossover, and approximately 3 weeks prior to calving. Individual heifer BW was recorded, and body condition score (BCS) was determined using a 9-point scale (1 = extremely emaciated, 9 = extremely obese; Wagner et al., 1988) with observations from three trained, independent observers. Ultrasound images were recorded and analyzed to determine 12^{th} rib subcutaneous fat thickness, percent intramuscular fat (% IMF), and longissimus muscle area (LMA) for each heifer using an Aloka 500V (Aloka,

Wallingford, CT). Blood samples were collected via coccygeal or jugular venipuncture using 10 mL evacuated serum tubes (BD Vacutainer, Becton, Dickinson, and Company, Franklin Lakes, NJ). Blood samples were immediately placed on ice, allowed to clot, and then centrifuged at 1,500 × g for 10 minutes. Serum was decanted into 12 × 75 mm plastic tubes, capped, and immediately frozen (-20˚ C). Serum samples were subsequently shipped to the South Dakota State University Animal Disease Research and Diagnostic Laboratory and analyzed for metabolites to indicate protein and energy status, including β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), bovine serum albumin (BSA), blood urea nitrogen (BUN), total protein (TP), and glucose (GLC). All samples were analyzed using standardized reagents (β-Hydroxybutyrate Reagent Set, Point Scientific, Canton, MI; HR Series NEFA-HR (2), Wako Diagnostics, Richmond, VA; ACE Albumin, BUN/UREA, Total Protein, and Glucose Reagent kits, Alfa Wasserman Diagnostic Technologies, Inc., West Caldwell, NJ) on an automated chemistry analyzer (VetACE Clinical Chemistry System, Alfa Wasserman Diagnostic Technologies, Inc., West Caldwell, NJ).

Milk Production and Composition

A sub-set of 34 AI-bred heifers representing each treatment combination (n = 8 in CON-CON and R-CON treatments; n = 9 in CON-R and R-R treatments) was randomly selected to measure milk production on d 62 ± 5 of lactation. Heifers were gathered from pasture and separated from their calves. Heifers received 1 mL of oxytocin via IM injection 5 min prior to milking. A portable milking machine (Porta-Milker, The Coburn Company, Inc., Whitewater, WI) was used, followed by hand stripping until dry. Time was recorded when milking was complete and milk was discarded. Heifers remained

separated from calves and the milking process was repeated approximately 4 h later. After the second milking, milk was weighed to determine production, and samples were collected and shipped to the Heart of America Dairy Herd Improvement Association laboratory (Manhattan, KS) for analysis of fat, protein, somatic cell count, lactose, total solids, and milk urea nitrogen. All analyses were conducted via near-infrared spectroscopy using a Bentley FTS/FCM (Bentley Instruments, Inc., Chaska, MN). This instrument exceeds the IDF 148A standard and ICAR requirements for somatic cell counting and IDF 141C:2000 and ICAR requirements for component measurement using AOAC approved methodology. Twenty-four hour milk yield was calculated using the following equation:

$$\text{24 h yield} = (\text{Total wt. of } 2^{nd} \text{ milking} \div \text{time between end of milkings}) \times 24$$

Subsequent Heifer Reproductive Response

To determine potential carryover effects of MP restriction on return to cyclicity, blood samples were collected and processed using sampling procedures described above on d -10 and 0 relative to initiation of an estrus synchronization protocol. Circulating concentrations of progesterone were analyzed in serum samples by RIA using methodology described by Engel et al. (2008). Intra- and interassay coefficients of variation were 3.6% and 6.7%, respectively, and assay sensitivity was 0.4 ng/mL. Heifers were considered to be cycling if serum progesterone concentrations were > 1 ng/ml in either sample. Heifers were synchronized utilizing a modified 7-day controlled internal drug release (CIDR) CO-Synch protocol, followed by artificial insemination on July 2 and 3, 2014. Heifers were exposed to a bull for an additional 60 d. Conception to

AI was determined via ultrasound 41 d post-AI, and final pregnancy rates were determined via ultrasound 117 d post-AI.

Statistical Analysis

Data were analyzed as a 2 × 2 factorial treatment structure in a randomized complete block design using the MIXED procedure of SAS (SAS Institute, Cary, NC) with pen as the experimental unit. Denominator degrees of freedom were approximated using the Kenward-Roger option in the model statement (Kenward and Roger, 1997) for all analyses.

Measures repeated over time (BW, BCS, ultrasound measurements, and blood collection) included time and its interactions with maternal nutritional treatment as fixed effects. For these variables, initial BW, BCS, ultrasound measurements, and blood metabolite levels were included in the model as covariates. Covariance structures were evaluated for each variable in the repeated measures analysis, and were selected based on the best model structure fitting the data based on Schwarz's Bayesian Information Criteria (BIC). Repeated measure variables were also analyzed as change in each variable during each period of gestation (e.g. final BW – initial BW). Calf data were analyzed using the same model with calf sex included as a fixed effect.

Milk production and composition data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) to determine the effects of mid- and late gestation treatment and their interaction with individual animal as the experimental unit. Means were separated using the PDIFF option of the LSMEANS statement of SAS and were considered significant at $P < 0.05$, with tendencies considered at $0.05 \leq P < 0.10$.

The influence of maternal nutritional treatments on subsequent reproductive response was analyzed using a binary distribution in the GLIMMIX procedure of SAS. Mid- and late gestation treatments and their interaction were included as fixed effects with pen as the experimental unit.

RESULTS AND DISCUSSION

Heifer Body Weight and BCS

Body weights, BCS, ultrasound measurements, and blood samples were collected from all heifers in each treatment group at the beginning and end of each treatment period in order to determine effects of MP restriction on heifer performance in mid- and late gestation. Inadequate nutrition during gestation is common in many range-based livestock production systems due to increased nutrient requirements and reduced forage quality and quantity (DelCurto et al., 2000). Cows may experience a negative energy balance when energy expenditures for physiological functions such as maintenance and reproduction exceed intake of energy or protein (Dunn and Moss, 1992). Although specific mechanisms have not been determined, it appears that decreases in dam body condition due to nutrient deficiency can result in negative impacts on placental development and vascularization as well as organ and tissue development in progeny, potentially impacting long-term growth and performance (Funston et al., 2010).

By design, there were no differences ($P > 0.20$) in initial BW or BCS between treatments (mean 437 kg ± 17.2 and 5.25 ± 0.147, respectively). A mid-gestation treatment (CON vs. R) × time (treatment crossover and end of study) interaction was observed for change in heifer BW and BCS ($P < 0.05$; Table 2.2). However, when means were separated for these variables, only BW change from the beginning to the midpoint of the study was different between treatments. Heifers on the R treatment lost 19 kg while heifers on the CON treatment maintained BW during mid-gestation ($P = 0.002$), despite diets being balanced to slightly exceed energy requirements. There was a tendency ($P < 0.10$) for increased BCS loss in heifers on the R treatment during mid-

gestation. Similarity among means during late gestation indicated there was no carryover effect of mid-gestation treatment into the late gestation period.

A late-gestation treatment (CON vs. R) × time interaction was also observed for change in heifer BW and BCS ($P < 0.05$; Table 2.2). When means were separated for these variables, there was a tendency ($P < 0.10$) for increased BCS and reduced BW and BCS loss for R heifers during mid-gestation as a result of late gestation treatment. Because late gestation treatments had not yet been applied in mid-gestation, these results were anomalous. Although all heifers gained BW during late gestation, the MP restriction resulted in lower BW gains ($P = 0.001$) compared to the CON treatment. In addition, restricted heifers lost BCS in late gestation whereas heifers on the CON treatment maintained BCS ($P = 0.007$).

There was a tendency ($P = 0.058$) for an interaction between mid- and late gestation treatments for BW change, wherein heifers on the R diet during one or both periods of gestation (CON-R, R-CON, and R-R) gained less weight than heifers on the CON diet throughout gestation (CON-CON; mean 3 vs. 12 kg ± 5.7, respectively). Despite equal and adequate levels of NEm and NEg based on NRC (2000) across treatments, MP restriction reduced the ability of restricted heifers to maintain BW and BCS. Insufficient intake of either protein or energy can result in a negative energy balance, which is often accompanied by loss of BW and body condition (Dunn and Moss, 1992). Research indicates that pregnant dams encountering a nutrient restriction may compensate for the fetus by catabolizing fat stores and lean body tissue to maintain pregnancy and normal body function (Freetly et al., 2008). Body weight change responses are in agreement with Sasser et al. (1988), who assigned gestating heifers to

two dietary groups at 150 d prepartum designed to provide 100% of NRC energy requirements and either adequate or deficient CP. Animals on the protein-deficient diet consistently weighed less at 112 and 56 d prepartum, at calving, and at 40 d postpartum (Sasser et al., 1988). Carstens et al. (1987) also observed reduced maternal BW gain during the last trimester of gestation due to a protein restriction.

Van Emon et al. (2014) conducted a 2-year study in which treatments with similar energy content but differing levels of MP (100%, 60%, or 40% of NRC requirements in yr 1; 60%, 100%, or 140% of NRC requirements in yr 2) to pregnant ewes in late gestation (~ d 100 of gestation through lambing). Across both years of the study, ewe BW change increased linearly throughout gestation as MP in the diet increased; however, linear increases in BCS change due to increasing dietary MP were only observed in year 1. Similar to results observed in the present study, all ewes gained BW during the last trimester of gestation; however, lower intakes of MP reduced BW gains (Van Emon et al., 2014). It appeared mobilization of body tissues occurred as a result of the MP restriction in mid-gestation. Earlier research by Reeves et al. (1972) reported bovine fetuses increased in weight from 3.6 kg at d 163 of gestation to 30 kg at d 281 of gestation. Therefore, it is reasonable to assume the majority of BW change in the last trimester may have been due to fetal growth. Although heifers on both treatments in late gestation gained weight, loss of BCS observed for heifers on the R treatment indicated heifers appeared to be mobilizing body tissue stores to compensate for fetal demands, while CON heifers were able to maintain BCS.

Heifer Ultrasound Measurements

A mid- gestation treatment (CON vs. R) × time interaction ($P = 0.042$) was observed for changes in LM area as determined by ultrasound measurements (Table 2.3). From the beginning of the study to treatment crossover at the end of mid-gestation, MP-restricted heifers lost over twice as much LM area as heifers on the CON treatment ($P = 0.042$). When means were separated for LM area change, there was no carryover effect of mid-gestation treatment on change from treatment crossover to the end of the study ($P > 0.10$).

There was a significant interaction ($P = 0.006$) for late gestation treatment (CON vs. R) × time (treatment crossover vs. end of study; Table 2.3) for LM area; however, there were no differences ($P > 0.10$) between treatments for either time point when means were separated for this variable. A late gestation treatment × time interaction was also observed ($P = 0.031$) for LM area change. From treatment crossover to the end of the study, restricted heifers lost over 4 fold the amount of LM area as CON heifers. These results suggested muscle tissue was being catabolized to mobilize tissue protein in compensation for the dietary MP restriction.

There were no differences ($P > 0.05$) in 12th rib fat thickness or change in fat thickness due to main effect of treatment or any treatment × period of gestation interactions. Despite significant interactions between mid-gestation treatment × time for percentage of IMF ($P = 0.005$), there were no differences between treatments for either midpoint or final percentage of IMF when means were separated for this variable ($P > 0.10$). There was a mid-gestation treatment × time interaction for IMF change ($P = 0.026$); however, means only tended ($P = 0.096$) to be different between treatments, with

increased IMF loss for restricted heifers at the mid-point of the study. There were no differences ($P > 0.10$) between treatments for this interaction at the end of the study.

A mid-gestation treatment × late gestation treatment × time interaction was observed for IMF ($P = 0.013$; Fig. 2.1). For the ultrasound conducted at treatment crossover, percentage of IMF tended ($P < 0.10$) to be greatest for the CON-CON treatment and least for R-CON and R-R, with CON-R intermediate and not different from any other treatment combination. For the ultrasound conducted at the end of the study, percentage of IMF was similar ($P > 0.10$) for heifers from CON-CON, R-CON, and R-R treatments. Heifers on the CON-R treatment had the lowest amount of IMF, and tended to be different ($P < 0.10$) from the CON-CON treatment and similar to other treatments. It appeared the greatest impact on IMF resulted from an MP restriction imposed only in late gestation (CON-R). The increase in percentage of IMF observed in the R-R treatment could be attributed to loss of LM area for heifers restricted in late gestation (i.e., IMF as a proportion of LM area increased as the total LM area decreased).

There was also a tendency ($P = 0.081$) for an interaction between mid- and late gestation treatments for change in IMF; however, there were no differences ($P > 0.14$) among any treatment combinations (CON-CON, CON-R, R-CON, and R-R) when means were separated. Additionally, least square means for heifers on the CON-CON and R-R treatments were not different from zero ($P > 0.63$), indicating % IMF did not change for these treatment × period of gestation combinations. Least square means for the CON-R treatment was less than zero ($P = 0.041$), and the least square means for the R-CON treatment tended ($P = 0.064$) to be less than zero, indicating heifers restricted during both mid- and late gestation lost IMF during the time that MP was restricted in their diet.

Despite minor differences in percentage of IMF, there was not strong evidence to support impacts on heifer IM fat depots as a result of MP restriction.

Mobilization of body tissues in response to nutrient deficiency is not uncommon. Taylor et al. (2016) measured change in BW, BCS, 12th rib fat thickness, and LM area from the beginning to the end of mid-gestation for cows fed to maintain BCS of 5 to 5.5 (positive energy status) vs. cows fed at 80% of maintenance energy requirements. Inducing a negative energy status resulted in reduced final BW and BCS, smaller LM area, and decreased 12th rib fat thickness compared to cows in a positive energy status (Taylor et al., 2016). Observed reductions in BW, BCS, and LM area in the current study, without substantial impacts on subcutaneous or IM fat, indicate MP restriction resulted in catabolism of maternal muscle tissue with minimal impacts on adipose tissue.

Heifer Blood Metabolite Concentrations

Blood metabolite analyses were conducted at multiple time points throughout the trial to provide a more comprehensive view of the metabolic status of heifers, assuming MP restriction would result in changes in concentrations of various metabolites. However, there were no differences ($P > 0.10$) due to main effects of treatment or any mid- × late gestation treatment interactions for the majority of blood metabolites (Table 2.4). Additionally, there were no differences for changes in blood metabolites due to main effect of treatment or any treatment × time interactions ($P > 0.05$). Concentration of BHB was reduced ($P = 0.018$) for heifers on the R treatment in mid-gestation, and there was also a tendency ($P = 0.061$) for reduced concentrations of NEFA for R heifers during mid-gestation. Little research is available on plasma metabolite concentrations in gestating beef cows; however, elevated NEFA and BHB and reduced glucose

concentrations are often used as indicators of negative energy balance in dairy cows (Adewuyi et al., 2005). Reduced concentrations of BHB and NEFA for restricted heifers in the current study were not expected since these metabolites are typically produced as a result of lipolysis when other sources of energy such as glucose and amino acids are not available. However, other reports in the literature examining the effect of diet on metabolic status have also yielded mixed responses.

Rusche et al. (1993) provided 100% or 150% of NRC recommendations for CP to primiparous heifers based on low (L; soybean meal) or high (H; corn gluten meal/bloodmeal) sources of RUP, and reported no differences for NEFA or insulin concentrations. However, higher levels of CP in the diet increased plasma urea N and tended to increase plasma glucose (Rusche et al., 1993). In addition, feeding high levels of RUP decreased plasma glucose and urea N. Anthony et al. (1986) fed isocaloric diets containing low CP (LP; 81% of NRC requirements) or high CP (HP; 141% of NRC requirements) in late gestation. At 10 d prepartum, daily blood samples were collected via jugular vein cannulae, which showed reduced BUN and glucose concentrations for heifers receiving the LP diet.

Sletmoen-Olson et al. (2000) provided no supplement or one of three supplements providing low, medium or high levels of RUP (53, 223, or 412 g RUP/kg supplement DM, respectively) to cows in late gestation and early lactation. Plasma glucose of the low RUP treatment group was consistently higher than cows fed the medium or high RUP supplement during late gestation. Plasma insulin concentration was also increased by supplementation compared to the control treatment, while plasma NEFA levels were reduced in supplemented vs. control cows. Sletmoen-Olson et al. (2000) concluded that

various levels of RUP can alter plasma concentrations of metabolites in gestating and lactating beef cows.

Rhind et al. (1991) reported differences in NEFA profiles for lactating sheep with different rates of milk production; however, feed intake and glucose profiles were similar among groups. Consequently, Rhind (2004) suggested while circulating levels of nutrients and metabolites are good indicators of animal well-being, they are often dependent on pool size and entry rates and not clearly or consistently related to protein or energy intake. Minimal differences in metabolite concentrations in our study, despite differences in cow performance, indicate additional research is warranted to further characterize the relationship between blood metabolite levels and MP restriction.

Calf Birth BW and Performance

There were no interactions ($P > 0.05$) of mid- by late-treatment for any calf variables; therefore, only main effect means are presented. Late gestation treatment influenced cow BCS at calving, with 0.2 greater BCS in CON vs. R heifers ($P = 0.042$; Table 2.5). However, nutritional treatments experienced by heifers during mid- and/or late gestation did not affect calving difficulty, calf vigor, or calf birth BW ($P > 0.05$; Table 2.5). Moreover, calf weaning BW was not affected ($P > 0.05$) by mid- or late-gestation treatment. As expected, sex was a significant factor in the model, with bull calves heavier than heifer calves at birth (31 vs. 28 ± 1.79 kg respectively; $P = 0.0005$) and weaning (212 vs. 204 ± 8.71 kg; $P = 0.018$). In beef cattle, severe nutrient restriction from the last half to one-third of pregnancy appears to be required to reduce fetal growth (Greenwood et al., 2005). Although our study did encompass the majority of the second and all of the third trimester of pregnancy, lack of birth weight response agrees with

previous research indicating energy available to the dam may have a greater influence on birth weight than protein (Holland and Odde, 1992). Since treatments utilized in the current study were formulated to be isocaloric, it is possible MP-restricted dams were able to overcome a protein deficiency by mobilizing body stores (particularly LM area; Table 2.3), thereby reducing potential impacts on offspring.

Several studies have reported differences in calf birth weight due to energy restrictions (Corah et al., 1975; Bellows and Short, 1978; Dunn et al., 1969), while supplying various levels of protein during gestation have shown mixed results. Sasser et al. (1988) reported calf birth BW and calving difficulty scores were not affected by CP deficiency, similar to results in the current study. However, Sasser et al. (1988) found calves from dams fed adequate protein tended to be heavier than calves from protein-deficient dams at 2 months and 105 d of age. In the previously described study by Van Emon et al. (2014), there were no differences in lamb BW at birth due to differing levels of MP in yr 1 of the study; however, there was a tendency for ADG to weaning and lamb weaning BW to increase as maternal MP intake increased. In yr 2, there were no significant effects of MP on lamb birth BW, ADG, or weaning weight. In the current study, it appeared that MP restriction had no impact on suckling calf performance from birth to weaning.

Heifer Milk Production and Composition

An estimate of peak milk production was conducted due to the potential for prepartum nutrition to influence milk production and postnatal growth of calves (Corah et al., 1975; Houghton et al., 1990). In the current study, no differences ($P > 0.05$) were observed for peak milk production due to maternal dietary treatment (Table 2.5). This is

in contrast to results reported by Bartle et al. (1984), who conducted a study in which dams received 150% vs. 85% of NRC crude protein requirements for the last 60 d of gestation followed by reallocation to an adequate (100%) or low (80%) energy diet. Milk production did not differ due to postpartum dietary treatment; however, milk production for dams on the high protein diet prepartum was increased by 0.8 kg/d vs. those on the low protein diet (Bartle et al., 1984).

There were no differences in our study ($P > 0.05$) for milk composition in terms of somatic cell count, fat, or total solids (mean 110 ± 65, 3.28% ± 0.108, and 9.30% ± 0.076, respectively). Heifers on the R treatment in mid-gestation had reduced milk urea nitrogen compared to control heifers ($P = 0.021$; 14.05% ± 0.496 vs. 15.77% ± 0.478, respectively). There was also a tendency ($P = 0.090$) for slightly reduced milk protein for heifers on the R treatment in late gestation vs. CON heifers (mean 3.20 vs. 3.40% ± 0.081, respectively). Heifers on the R treatment in late gestation tended ($P = 0.060$) to have slightly increased lactose content in their milk compared to the CON treatment (mean 4.92% ± 0.092 vs. 5.16% ± 0.083, respectively); however, this result would not likely be considered to have biological significance. Despite these responses, it has been reported that dietary protein has minimal effects on milk fat or protein concentrations due to complex interactions among ruminal degradation of nutrients, hormonal influences, and biochemical pathways necessary for synthesis and secretion of milk solids (Sutton, 1989). Therefore, it is possible that additional factors outside of maternal dietary treatments may have influenced results. Lack of differences in milk production and minimal differences in milk composition indicate these were not confounding factors in

the ability to measure postnatal growth and performance of offspring due to maternal nutritional treatment in the current study.

Subsequent Heifer Reproductive Response

Proportion of heifers returning to cyclicity by the beginning of the breeding season following calving was not influenced by MP restriction during mid- or late gestation or their interaction ($P > 0.05$; Table 2.5). Heifer BW and BCS were similar ($P > 0.10$) at breeding (Table 2.5). In addition, there were no differences ($P > 0.10$) between nutritional treatments in regard to proportion of heifers that conceived to AI. Mulliniks et al. (2013) grazed heifers post-weaning on dormant forage and provided supplement containing 36% CP with either 36% RUP (36RUP) or 50% RUP (50RUP). Heifers that received the 50% RUP supplement had 13% greater pregnancy rates than those that received 36% RUP, despite lack of differences in BW from initiation of supplementation until pregnancy diagnosis. Although heifers were not protein-restricted and were provided MP at a different phase in physiological development, these results still indicated reduced RUP may result in less than optimal effects on reproductive response. It is possible restricted heifers in the current study were able to overcome any potential negative impacts on reproduction that may have been expected due to gestational dietary treatments between calving and breeding.

It should be noted that a study investigating time of AI (36 vs. 60 h) and fresh vs. frozen semen was imposed on heifers in the current study at the time of AI. While there were no interactions among our treatments and treatments in the superimposed study ($P > 0.5$); semen type in itself was highly significant ($P = 0.02$), as was time of AI ($P = 0.001$). Additionally, heifers from our study were not equally stratified across treatments

from the superimposed study; therefore, percentage of heifers that conceived from AI cannot be attributed directly to nutritional treatments they received during gestation. Data for overall pregnancy rates could not be statistically analyzed because all except 2 cows became pregnant. The GLIMMIX model would not converge when all treatments had virtually 100% overall pregnancy.

It is well-documented that BCS at calving is an important factor in length of postpartum interval and subsequent pregnancy rates (Selk et al., 1988; Richards et al., 1986). Morrison et al. (1999) fed a group of cows to achieve BCS ranging from 3 to 8 up to the last trimester of pregnancy, and then managed each group to achieve BCS of 5 at calving. There were no differences in pregnancy rates, date of conception, or calf birth weight reported in that study (Morrison et al., 1999). Although heifers did respond to MP restriction in terms of losses in BW, BCS, LM area, and slight changes in % IMF, it is possible that subsequent reproductive performance differences were not observed in the current study because BCS losses were not severe and heifers across our treatments calved at BCS near 5 (Table 2.5).

IMPLICATIONS

In summary, this study provided evidence that MP-restricted heifers mobilized body reserves at the time that the restriction was imposed based on responses observed for changes in BW, BCS, ultrasound LM area and % IMF. However, MP restriction in mid- and late gestation did not impact calf birth or weaning BW. In addition, MP restriction did not impact heifer blood metabolite levels, 12th rib subcutaneous fat thickness, milk quantity or composition, or subsequent reproductive performance. These results imply the MP restriction in this study may have been moderate enough for dams to make metabolic adaptations that allowed sufficient nutrients to be supplied to the developing conceptus. Further investigations on maternal metabolic changes and effects of nutrient restriction are necessary to understand mechanisms of responses. Gestational MP restriction may influence offspring performance post-weaning, but elicited minor responses in offspring growth from birth to weaning in this study.

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Table 2.1. Dietary components and nutrients consumed by heifers receiving a control (CON = slightly exceeding MP requirement) or restricted (R = approximately 80% of MP requirement supplied) diet during mid- and/or late gestation based on NRC (2000) calculations[1]

	Diet formulation 1[2]		Diet formulation 2[2]		Diet formulation 3[2]	
	CON	R	CON	R	CON	R
Item	---- % DM basis ----					
Wheat straw[3]	59.81	59.62	54.14	53.65	51.22	51.28
Crude glycerin[4]	15.66	17.97	13.27	15.27	14.52	14.54
Dry supplement[5]						
Ground corn	-	-	10.27	10.02	10.79	11.03
Ground corn cobs	16.77	16.56	11.33	11.43	11.84	12.51
Energy Booster 100®[6]	3.42	3.06	7.38	7.46	7.74	8.20
Porcine bloodmeal	1.62	-	1.65	-	1.54	-
Sodium phosphate (XP 40)	1.57	1.56	1.39	1.43	1.73	1.62
Urea, 46%	1.08	1.18	0.51	0.67	0.54	0.75
Magnesium oxide, 54%	0.032	0.034	0.032	0.031	0.034	0.034
TM Green[7]	0.015	0.014	0.020	0.019	0.010	0.010
Selenium, 0.06% yellow	0.009	0.012	0.011	0.013	0.013	0.015
Vitamin AD 10:1	0.004	0.004	0.004	0.004	0.005	0.005
	---- Nutrient composition of diet predicted by NRC (2000) based on actual intake ----					
Diet CP, %	7.0	5.3	5.7	4.6	5.7	4.9
Bacterial N balance, g/d	11	11	-1	-1	2	2
MP, %	108.7	88.4	101.4	78.3	93.2	77.2
NE_m, Mcal/kg	1.24	1.17	1.37	1.40	1.44	1.44
NE_g, Mcal/kg	0.67	0.61	0.79	0.82	0.85	0.85

[1] Diets formulated based on NRC (2000) predictions for MP, NE_m, and NE_g requirements for heifers throughout gestation

[2] Diet formulation 1 fed from 11/2/13 – 12/14/13, diet formulation 2 fed from 12/15/14 – 1/18/14, and diet formulation 3 fed from 1/19/14 – calving. Amounts of supplement using each formulation were adjusted throughout gestation.

[3] Nutrient composition of wheat straw: 49.39% DM; 4.75% CP; 57.48% ADF; 66.78% NDF; 49.75% TDN; 0.95 Mcal/kg NE_m; 0.40 Mcal/kg NE_g

Table 2.1 continued…

[4] Crude glycerin contained 82.3% glycerol, 9.5% water, 0.56% CP, 0.04% methanol, 8.07% ash, and 0.90% MONG (matter organic non-glycerol; defined as 100 – glycerol content (%) + water content (%) + ash content (%)). Crude glycerin sourced from Minnesota Soybean Processors, Brewster, MN

[5] Dry supplement formulated and mixed by Hubbard Feeds Inc., Mankato, MN

[6] Milk Specialties Global, Eden Prairie, MN

[7] TM Green mineral mix contained 15.2% S; 330 ppm Co; 33,000 ppm Cu; 1,650 ppm I; 132,000 ppm Mn; 99,000 ppm Zn, 3,300 ppm CuCl; 1,856 ppm EDDI; 132,000 ppm $MnSO_4$; and 99,000 ppm $ZnSO_4$

Table 2.2. Least square means for mid- and late gestation treatments (CON = slightly exceeding MP requirements; R = approximately 80% of MP requirements) × time (treatment crossover and end of study) interactions for heifer BW, BW change, body condition score (BCS), and BCS change[1]

	Treatment crossover		End of study			
Item	CON	R	CON	R	SEM	*P*-value
	--- Mid-gestation treatment × time ---					
BW, kg	431	418	453	443	8.61	0.295
BW change, kg	-5[a]	-19[b]	21	26	5.74	**0.002**
BCS	4.92	4.82	4.74	4.78	0.046	0.106
BCS change	-0.30[c]	-0.46[d]	-0.18	-0.04	0.081	**0.027**
	--- Late gestation treatment × time ---					
BW, kg	421	428	451	445	8.60	**0.011**
BW change, kg	-16[c]	-9[d]	30[a]	17[b]	5.73	**0.001**
BCS	4.81[c]	4.92[d]	4.81	4.71	0.046	**0.022**
BCS change	-0.46[c]	-0.30[d]	0.00[a]	-0.22[b]	0.081	**0.007**

[1] Statistical analysis was not conducted for initial BW and BCS because these values were utilized as covariates for analysis of midpoint and final BW and BCS

[a,b] Within gestation period, means lacking a common superscript differ ($P < 0.05$)

[c,d] Within gestation period, means lacking a common superscript tend to differ ($P < 0.10$)

Table 2.3. Least square means for mid- and late gestation treatments (CON = slightly exceeding MP requirements; R = approximately 80% of MP requirements) × time (treatment crossover and end of study) interactions for heifer ultrasound measurements[1]

	Treatment crossover		End of study			
Item	CON	R	CON	R	SEM	*P*-value
	--- Mid-gestation treatment × time ---					
LM area, cm^2	48.21	47.29	47.30	46.72	0.348	0.208
LM area change, cm	-0.70[a]	-1.59[b]	-0.89	-0.58	0.273	**0.042**
12th rib fat thickness, cm	0.54	0.51	0.46	0.45	0.020	0.477
12th rib fat thickness change, cm	0.00	-0.03	-0.08	-0.06	0.017	0.235
IMF, %	5.84	5.70	5.77	5.77	0.075	**0.005**
IMF change, %	-0.06[c]	-0.20[d]	-0.07	0.06	0.054	**0.026**
	--- Late gestation treatment × time ---					
LM area, cm^2	47.57	47.92	47.29	46.73	0.348	**0.006**
LM area change, cm	-1.33	-0.96	-0.27[a]	-1.20[b]	0.273	**0.031**
12th rib fat thickness, cm	0.53	0.51	0.46	0.45	0.020	0.903
12th rib fat thickness change, cm	-0.01	-0.01	-0.06	-0.08	0.017	0.538
IMF, %	5.82	5.73	5.79	5.75	0.075	0.146
IMF change, %	-0.09	-0.18	-0.03	0.03	0.058	0.184

[1] Statistical analysis was not conducted for initial ultrasound measurements because these values were utilized as covariates for analysis of midpoint and final ultrasound measurements

[a,b] Within gestation period, means lacking a common superscript differ ($P < 0.05$)

[c,d] Within gestation period, means lacking a common superscript tend to differ ($P < 0.10$)

Table 2.4. Least square means for main effects of mid- and late gestation treatments (CON = slightly exceeding MP requirements; R = approximately 80% of MP requirements) for concentrations of heifer blood metabolites[1]

	Mid-gestation		Late gestation			Mid	Late
Item	CON	R	CON	R	SEM	*P*-value	*P*-value
Albumin (BSA), g/dL	3.11	3.07	3.11	3.07	0.043	0.494	0.475
Glucose (GLU), mg/dL	75.26	73.81	74.92	74.15	1.218	0.415	0.659
Blood urea nitrogen (BUN), mg/dL	4.52	4.35	4.13	4.73	0.452	0.798	0.376
Total protein (TP), g/dL	6.37	6.25	6.36	6.26	0.120	0.419	0.450
β-hydroxybutyrate (BHB), mg/dL	1.98[a]	1.66[b]	1.76	1.88	0.072	**0.018**	0.255
Non-esterified fatty acids (NEFA), mmol/L	0.33[c]	0.28[d]	0.30	0.31	0.014	**0.061**	0.778

[1] Initial blood metabolite levels utilized as covariates for analysis of repeated measures

[a,b] Within gestation period, means lacking a common superscript differ ($P < 0.05$)

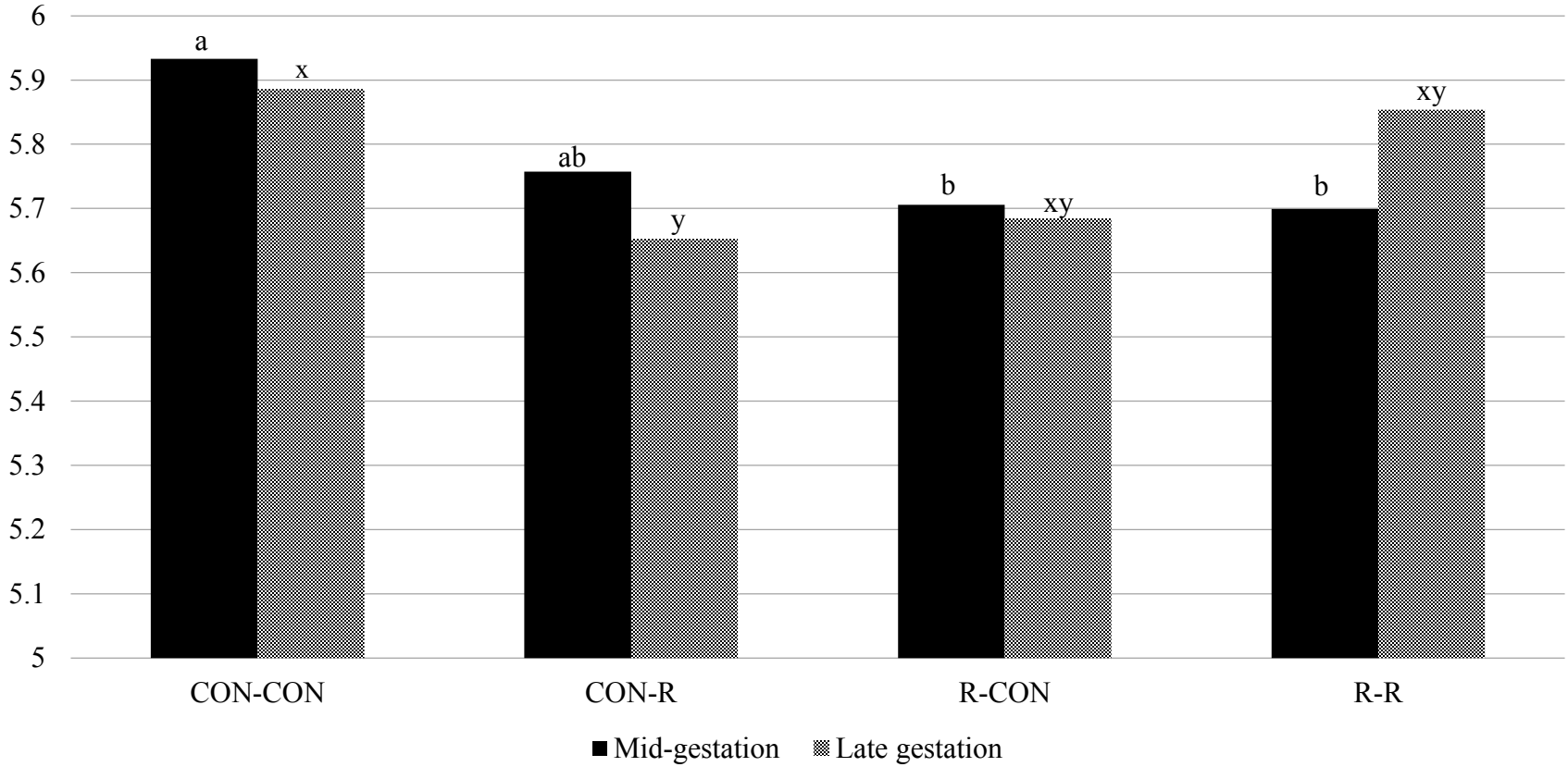
[c,d] Within gestation period, means lacking a common superscript tend to differ ($P < 0.10$)

Table 2.5 Least square means for main effects of mid- and late gestation treatments (CON = slightly exceeding MP requirements; R = approximately 80% of MP requirements) on calf performance from birth to weaning and subsequent heifer reproductive response[1]

	Mid-gestation		Late gestation			Mid	Late
Item	CON	R	CON	R	SEM	*P*-value	*P*-value
Heifer BCS at calving	4.9	4.8	4.9[a]	4.7[b]	0.112	0.568	**0.042**
Calving difficulty score	1.07	1.07	1.13	1.00	0.060	1.000	0.132
Calf vigor score	1.05	1.13	1.16	1.02	0.062	0.347	0.127
Calf birth BW, kg	30	29	29	30	1.82	0.282	0.247
Peak milk production, kg	9.05	9.27	9.12	9.20	0.540	0.763	0.916
Calf weaning BW, kg	211	204	207	208	8.96	0.221	0.926
Return to cyclicity, %	91	87	89	89	5.33	0.523	0.945
Heifer BW at breeding	444	442	445	441	16.0	0.646	0.489
Heifer BCS at breeding	4.7	4.7	4.8	4.7	0.072	0.893	0.248
Pregnant to AI, %	44.2	62.9	50.2	57.1	7.17	0.110	0.521
Overall pregnancy rate[1]	96	100	98	98	-	-	-

[1] Data shown are means for each treatment group. Overall pregnancy rates did not converge because only 2 heifers failed to become pregnant

Figure 2.1. Least square means for mid-gestation treatment × late gestation treatment × time interaction for % IMF based on ultrasound measurements for heifers receiving a control (CON; slightly exceeding MP requirement) or restricted (R; approximately 80% of MP requirement supplied) diet during mid- and/or late gestation



[a,b] In mid-gestation, means lacking a common superscript tend to differ ($P < 0.10$)
[x,y] In late gestation, means lacking a common superscript tend to differ ($P < 0.10$)

CHAPTER III

Influence of maternal protein restriction in primiparous heifers during mid- and/or late gestation on progeny feedlot performance and carcass characteristics

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ABSTRACT

Maternal nutrient restriction in beef cows impacts developmental processes in the fetus that may influence lifetime performance. This study investigated impacts of MP restriction in primiparous heifers during mid- and/or late-gestation on progeny feedlot performance and carcass characteristics. One hundred eight Angus × Simmental heifers were blocked by BW, method of conception (AI or natural service, based on fetal age at ultrasound), and calf sex and allocated to 12 pens in a randomized complete block design with a 2 × 2 factorial treatment structure including 2 stages of gestation (mid- and late) and 2 levels of dietary protein (control [CON]; slightly exceeding MP requirements and restricted [R]; approximately 80% of MP requirements). Pens were randomly assigned to CON or R treatments within blocks during mid- and/or late gestation. Heifers were removed from treatments after calving and pairs were managed as a common group. Following weaning, calves were backgrounded for two weeks then finished in a GrowSafe feeding system on a typical feedlot diet. Individual carcass measurements were collected. No differences were observed for initial or final calf BW, DMI, or ADG due to maternal nutritional treatments throughout the feeding period ($P > 0.10$). There was a tendency ($P < 0.10$) for improved G:F for progeny from dams restricted in late

gestation. Hot carcass weight, adjusted 12th rib fat thickness, KPH, USDA Yield Grade, marbling score, and proportion of carcasses in each USDA Quality Grade were not influenced ($P > 0.10$) by maternal diet. Progeny of dams on the R treatment in late gestation had greater LM area ($P = 0.04$) vs. progeny from CON dams, but not when adjusted on a HCW basis ($P > 0.10$). Proportion of progeny receiving USDA Yield Grade 3 designation was least from dams restricted only in late gestation (CON-R), and greatest from dams restricted throughout gestation (R-R; $P < 0.05$). Minimal differences in animal performance and carcass characteristics suggest MP restriction during mid- and late gestation did not have a significant developmental programming effect.

INTRODUCTION

The fetal origins hypothesis suggests that exposing the fetus to an adverse environment *in utero* leads to permanent programming of tissue function and increased risk of disease (Drake and Walker, 2004). Nutrient restriction during gestation in livestock may result in unfavorable fetal and postnatal growth, nutrient utilization, and health as well as changes in body composition and meat quality (Wu et al., 2006).

Metabolizable protein (MP) is defined as true protein absorbed in the intestine, consisting of microbial protein and ruminally undegraded protein sources (RUP; National Academies of Sciences, Engineering, and Medicine, 2016). Because MP represents the supply of amino acids available for absorption, it should be utilized as an indicator of how protein intake during gestation can affect offspring performance. Research in sheep has indicated a reduction in availability of amino acids to the fetus due to nutrient restriction (Lekatz et al., 2011; Lemley et al., 2013); however, little research has been conducted investigating the effects of maternal MP restriction on progeny growth, feed efficiency, and carcass characteristics of beef cattle.

Most of the literature referencing fetal programming in livestock has focused on fetal development and performance of offspring; however, nutrients available to the fetus can impact numerous tissues relevant to meat production. Skeletal development begins at an early stage of embryonic development, with primary muscle fibers in cattle estimated to begin forming at less than 47 d of fetal life and secondary muscle fibers around 90 d of fetal life (Brameld et al., 2010). Skeletal muscle is particularly susceptible to maternal nutrient deficiency due to its reduced priority in nutrient partitioning compared with other organs during development and the fact that muscle fiber numbers do not increase after

birth in ruminants (Du et al., 2013, Zhu et al., 2006). Myocytes, adipocytes, and fibroblasts are all derived from mesenchymal stem cells, and evidence suggests factors shifting cell differentiation away from myogenesis can result in replacement of muscle fibers with fat cells (Du et al., 2010b).

Maternal under-nutrition at various stages of development can alter tissue development in the offspring and influence postnatal performance and feed efficiency during the finishing phase (Funston et al., 2010b). The "thrifty phenotype" hypothesis as described by Hales and Barker (2001) suggests inadequate nutrition during fetal life "programs" offspring to develop a postnatal metabolism adapted to survive in a nutrient-poor environment, which can result in obesity later in life if adequate nutrition is provided.

Mature mass and body composition can be altered by starvation or protein deficiency early in fetal life (Owens et al., 1993), potentially leading to performance and production differences regardless of whether early measures such as calf birth weight are affected (Funston et al., 2012). In growing animals, muscle is energetically more efficient than fat (Wu et al., 2006). Thus, it would be reasonable to assume progeny born to dams that were nutritionally restricted during gestation would have reduced skeletal muscle development and efficiency of nutrient utilization. Our hypothesis was that MP restriction in mid- and late gestation would result in reductions in postnatal growth and skeletal muscle, increased carcass adiposity, and reduced feed efficiency. Therefore, the objectives of this study were to investigate the impacts of MP restriction in mid- and late gestation on feedlot performance and carcass characteristics of progeny.

MATERIALS AND METHODS

Animals and Experimental Design

The South Dakota State University Institutional Animal Care and Use Committee approved all procedures involving animals. One hundred eight Angus × Simmental heifers were pen-fed at the SDSU Cottonwood Range and Livestock Field Station near Philip, SD during the time gestational nutrient restrictions were imposed. Prior to the beginning of the study, yearling heifers were synchronized and time-bred to a single Angus sire on June 7th, 2013. Following AI, all heifers were exposed naturally to Angus bulls for 60 days. Rectal ultrasonography was conducted in mid-September to detect pregnancy and fetuses were sexed and aged.

Treatments were arranged in a 2 × 2 factorial structure with 2 levels of dietary metabolizable protein provided during two stages of gestation (mid and late). Dietary MP levels included: control (CON**;** slightly exceeding MP requirements) and restricted (R**;** approximately 80% of MP requirements supplied based on Level 2 of NRC (2000). Heifers were blocked by BW as well as age and sex of the fetus, resulting in 3 blocks with 4 pens per block. At the end of the mid-gestation period, half of the pens on the CON treatment were reassigned to the R treatment and half of the pens on the R treatment were reassigned to the CON treatment, resulting in four treatment combinations (CON-CON, CON-R, R-CON, and R-R). Each treatment combination was randomly assigned to one pen per block for a total of 3 pen replicates per treatment combination.

Diets were based on calcium hydroxide treated wheat straw and concentrates, and were adjusted throughout gestation to maintain MP balance across treatments and account for increased nutrient and energy requirements for the growing heifer and the

developing fetus (NRC, 2000; Table 3.1). Concentrate formulations between treatments were similar, except that porcine bloodmeal was added to the CON formulation to slightly exceed the MP requirement. Diets were formulated to be isocaloric and meet predicted NRC (2000) requirements for NE_m and NE_g in both CON and R treatments. Despite balancing diets to achieve predicted MP and energy concentrations in the diets, consumption of wheat straw by heifers was less than predicted. Although desired MP and energy levels were not consistently achieved for each diet formulation based on calculated intakes, percentage of MP requirements supplied was 101% for CON heifers and 81% for R heifers when averaged across the study (Table 3.1). Immediately after calving, heifers were removed from treatments and pairs were managed as a common group through weaning. There were no further treatments applied to dams or progeny beyond gestational treatments of the dam.

Progeny Weaning and Feedlot Management

Five calves were removed from the study prior to weaning due to death or issues with their dam that inhibited study protocols and objectives. Two calves died due to weather-related stressors shortly after birth. One calf was removed from the study and sold when its dam died of complications due to post-calving vagal nerve paralysis. One calf was early-weaned by its dam, and both cow and calf were removed from the study and sold. One calf was injured when pairs were grazing summer pasture and was humanely euthanized prior to weaning. The remaining 103 steer and heifer calves received pre-weaning vaccinations on September 2, 2014, including killed vaccines for clostridial diseases (Vision 7 Somnus with SPUR, Merck Animal Health, Madison, NJ) and Pasteurellosis (Mannheimia Haemolytica, Colorado Serum Company, Denver, CO),

plus a modified live vaccine for respiratory viruses (Vista 5 SQ, Merck Animal Health, Madison, NJ). Calves were weaned, dewormed (Dectomax Pour On, Pfizer, New York, NY), and received booster vaccines on October 6, 2014. They were backgrounded on high quality grass hay and dried distillers grains for two weeks at the SDSU Cottonwood Range and Livestock Field Station before being shipped approximately 430 km to the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE.

Calves were allocated to four feedlot pens based on sex and method of conception (AI or clean-up through natural service) and adapted to a final finishing diet over 110 d using 4 step-up diets (Table 3.2). Calves remained within these four groups and were placed in a GrowSafe feeding system (GrowSafe Systems Ltd., Airdrie, AB Canada) to collect individual feed intake data beginning November 22 for AI-bred calves and December 13 for bull-bred calves due to space limitations in the facility. All calves received the same diet whether they were being fed in standard feedlot pens or in the GrowSafe system, as the only treatment applied to calves in this study was maternal dietary treatment. Initial feedlot weights were collected on November 20 and 21, 2014 for AI-bred calves, and on December 11 and 12, 2014 for bull-bred calves, following 10 d of adaptation in the GrowSafe feeding system. All steers received an initial feedlot implant of Revalor-IS (80 mg trenbolone acetate and 16 mg estradiol), and heifers received Revalor-IH (80 mg trenbolone acetate and 8 mg estradiol; Merck Animal Health, Madison, NJ) on November 20, 2014. Cattle were re-implanted with Revalor-200 (200 mg trenbolone acetate and 20 mg estradiol; Merck Animal Health, Madison,

NJ) and dewormed with Agrimectin (Agri Laboratories Ltd., St. Joseph, MO) on March 3, 2015.

Progeny Harvest and Carcass Evaluation

Cattle were fed and managed to maintain health and achieve an industry average endpoint of approximately 1.3 cm of backfat at harvest. The AI-bred steers and heifers were shipped approximately 100 km to Tyson Fresh Meats in Lexington, NE on May 13, 2015, and bull-bred steers and heifers were shipped to the same processing facility on June 3, 2015. Individual carcass measurements included HCW, LM area, 12th rib fat thickness, and estimated percentage of KPH. Yield Grade was calculated according to USDA guidelines, and marbling score and carcass maturity were recorded and used to determine USDA Quality Grade. Cattle were not weighed prior to shipping to the processing facility to reduce incidence of bruising and injury; therefore, final live BW was determined as HCW divided by 0.625 (assumed dressing percentage).

Statistical Analysis

All progeny feedlot performance and carcass data were analyzed using original dam pen assignments as the experimental unit. Initial and final BW, feedlot performance measures (DMI, ADG, and G:F), and carcass characteristics (HCW, LM area, 12th rib fat thickness, KPH, USDA Yield Grade, and marbling score) were analyzed using the MIXED procedure of SAS to determine differences due to the fixed effects of maternal nutritional treatment during mid- and late-gestation and their interaction. Fixed effect of calf sex was also included in the model for all analyses. Denominator degrees of freedom were approximated using the Kenward-Roger option in the model statement (Kenward and Roger, 1997) for all analyses. Least squares means and SEM were estimated and

separated by LSD (i.e. the PDIFF option) and were considered significant at $P \leq 0.05$, with tendencies considered at $P < 0.10$.

The influence of maternal nutritional treatments on proportion of cattle assigned to each USDA Yield and Quality Grade were analyzed using a binary distribution in the GLIMMIX procedure of SAS. Fixed effects of mid- and late gestation treatments and their interaction were included in the model. Least squares means and SEM of the proportions were estimated using the ILINK option and separated as described above.

RESULTS AND DISCUSSION

There were no interactions ($P > 0.10$) observed for mid- or late gestation treatment for feedlot performance measures or carcass characteristics of progeny; therefore, only overall main effect means are presented.

Progeny Feedlot Performance

There were no differences ($P > 0.10$) in initial or final BW, DMI, or ADG of progeny due to maternal nutritional treatment during the backgrounding and finishing phase; however, there was a tendency ($P = 0.084$) for slightly improved G:F for progeny whose dams were on the R treatment in late gestation (Table 3.3). Small differences in G:F were inconsistent with similar treatment means for DMI and ADG, therefore would not be considered biologically relevant.

Lack of BW differences during the finishing period were consistent with lack of differences among treatments for BW at birth and weaning ($P > 0.20$; mean 30 ± 1.8 kg and 208 ± 9.0 kg, respectively). Greenwood and Cafe (2007) reported severe growth restriction of cattle early in life resulted in reduced growth potential throughout the production cycle, although BW equivalent to normally grown cattle could be obtained given more time on feed. Therefore, it seems reasonable to assume differences in progeny BW due to maternal dietary treatment would not appear during the finishing period given the lack of influence on birth and weaning weights in the current study.

Stalker et al. (2006) conducted a study in which mixed-age cows were provided either no supplement or a 42% CP supplement at 0.45 kg/d while grazing dormant native range forage during the last trimester of gestation. Although a nutrient restriction was not technically imposed, it would be reasonable to assume cows on the control treatment

would be deficient in protein in late gestation. Stalker et al. (2006) did not observe differences for feedlot ADG, DMI, or feed efficiency for steer progeny due to maternal dietary treatment. Three follow-up studies with slight variations in treatment arrangements resulted in no influence of maternal nutrition on heifer progeny ADG or G:F (Martin et al., 2007); a tendency for increased ADG and feed intake for steer progeny from protein-supplemented cows, but no overall difference among treatments for overall BW gain efficiency (Larson et al., 2009); and similar DMI and RFI for heifer progeny from control and supplemented dams (Funston et al., 2010a).

Summers et al. (2011) conducted a two-year study wherein spring-calving cows grazed dormant forage in late gestation, with cows at one location receiving 0.95 kg/d of 31.6% CP supplement (HN) and cows at a second location receiving 0.37 kg/d of the same supplement delivered 3 times per week (LN). Although there were differences between years, final feedlot BW, ADG, DMI, and G:F were not different among progeny due to maternal nutritional treatment. Another protein supplementation study by Banta et al. (2006) provided evidence of a similar lack of response for feedlot performance of progeny from dams fed soybean meal, soybean hull-based supplement, or whole sunflower seeds for 76 d in mid- to late gestation.

Results from these studies indicate little, if any, influence of protein supplementation during mid- to late gestation on subsequent feedlot performance of offspring. In contrast, progeny from cows grazing native range vs. improved pasture from mid- to late gestation had reduced ADG, less total BW gain and a tendency for decreased final BW despite similar initial weights upon entering the feedlot (Underwood et al., 2010). Variable performance responses observed in supplementation studies in

grazing livestock are inherent due to differences in formulation, amount, and timing of supplementation, in addition to environmental differences affecting forage quality. When comparing the results of the current study to available literature, it is important to note few studies have evaluated protein requirements and responses to supplementation on the basis of MP rather than CP. In addition, there is a paucity of data from researchers who have isolated the influence of protein alone by ensuring diets are balanced to provide similar amounts of energy.

In a study designed to evaluate the effects of energy restriction in mid-gestation on growth performance of offspring, Taylor et al. (2016) fed cows at a level to achieve or maintain BCS of 5 to 5.5 (positive energy status) or at 80% of energy requirements for BW maintenance (negative energy status). Dam energy status had no effect on birth BW, weaning weight, or feedlot performance measures. Taylor et al. (2016) only applied dietary treatments during mid-gestation, after which point cows were realimented and fed a common diet through weaning. While cow weight and BCS were not evaluated prior to calving, it is possible that cows experienced compensatory growth from the end of the treatment period to parturition. Although the current study was focused on the effects of MP restriction rather than energy and the duration of the nutritional restriction was increased to encompass mid- and late gestation, the level of restriction compared with Taylor et al. (2016) was similar. It appears gestating cows may be able to compensate for reduced nutrient intake at this level and that a more severe restriction would be required to observe effects on birth weight and postnatal growth of progeny.

Long et al. (2010) fed low (55% of NRC requirements for NE_m and 50% for CP) or moderate (100% of NRC requirements) nutrition diets to cows beginning on d 32 of

gestation through d 115 of gestation, at which point cows were commingled and fed in excess of requirements to calving. There were no differences in ADG of progeny from low or moderate nutrition dams. However, steers from restricted dams were heavier at the beginning of the finishing period and tended to have greater slaughter weights compared with steers from dams on the moderate nutrition treatment, indicating prenatal nutrition in early pregnancy had a moderate developmental programming effect on postweaning growth. A greater degree of variation in response might be expected given the severity of the restriction.

Summers et al. (2015) compared the effects of meadow hay fed during late gestation with no supplement (CON) vs. two supplements providing 28% CP but with differing levels of ruminally undegraded protein (59% RUP; HI or 34% RUP; LO). There were no differences in ADG, reimplant or final BW, or G:F; however, RFI was improved for calves born to supplemented dams (HI and LO) vs. CON dams. In contrast, Underwood (2007) induced a global nutrient restriction in gestating cows by providing 68% of energy and 87% of MP requirements from d 31 through d 125 of gestation followed by realimentation to achieve similar BCS to control cows by d 220 of gestation. Steers from nutrient-restricted dams had slightly increased ADG and feed efficiency compared to steers from dams on the control treatment. In addition, the proportion of lean tissue in 9-10-11 rib sections was increased for progeny restricted in gestation, indicating a potential compensatory or “thrifty phenotype” response.

Robinson et al. (2013) conducted a stepwise regression analysis to determine the influence of maternal nutritional status during pregnancy on production characteristics up to 30 months of age. Despite large numbers of experimental units used in a multi-year

study, no specific influences of chronic severe nutritional restriction from mid-gestation through calving on feed use efficiency were detected. These results suggest environment and other factors affecting postnatal calf growth still play a large role in lifetime performance of beef cattle, perhaps in conjunction with or in addition to the specific nutritional environment encountered during gestation.

Progeny Carcass Characteristics

There was no influence ($P > 0.10$) of maternal diet during gestation for progeny HCW, adjusted 12th rib fat thickness, KPH, USDA Yield Grade, marbling score, or proportion of carcasses in each USDA Quality Grade (Table 3.4). Longissimus muscle area for calves whose dams were restricted in late gestation was greater ($P = 0.039$) compared with those from dams on the control treatment; however, there was no difference among treatment groups ($P = 0.231$) when LM area was analyzed using HCW as a covariate (Table 3.4). Although it may have appeared MP restriction during late gestation resulted in increased LM area of progeny, similar treatment means between groups with the HCW adjustment indicated this response was primarily a function of body size.

There was a mid- × late gestation treatment interaction ($P = 0.049$) for proportion of progeny in the USDA Yield Grade 3 category (Figure 3.1). Progeny from dams restricted throughout gestation (R-R) had the greatest proportion of USDA Yield Grade 3 designations, while progeny from dams restricted only in late gestation (CON-R) had the least (72.1% ± 10.02 vs. 37.6% ± 10.84, respectively). Progeny from CON-CON and R-CON treatments were intermediate (63.1% ± 10.61 and 55.9% ± 11.33, respectively) and similar to other treatments ($P > 0.05$). This response is difficult to interpret since there

were no significant main effects or interactions observed for any other USDA Yield Grade category. In addition, mean USDA Yield Grade and all carcass characteristics included in yield grade calculations (HCW, KPH, fat thickness) were similar ($P > 0.10$) among treatments. The USDA Yield Grade 3 category is considered acceptable by industry standards (i.e. would not be discounted), and it is unlikely that differences among treatment groups can be directly attributed to developmental programming.

Impacts of maternal nutrient restriction on muscle fiber development and ultimately meat quality are evident based on available literature; however, most reports have utilized sheep as the experimental unit. Studies conducted in pregnant ewes restricted to 50% of nutrient requirements from d 28 to 78 of gestation resulted in down-regulation of protein synthesis in fetal muscle, reduction of secondary myofibers, and an increase in intramuscular triglyceride content, which is known to predispose insulin resistance in skeletal muscle (Zhu et al., 2004; 2006). Fahey et al. (2005) found changes in muscle characteristics of lambs born to ewes restricted to 50% of nutrient requirements from d 30 to 70 of gestation, while restriction late in gestation (d 85 to 115) reduced weight of LM, semitendinosus, and vastus lateralis muscles of offspring. Lambs were harvested in order to determine muscle characteristics; therefore, longer-term impacts on muscle growth, performance, and carcass quality were not measured.

Ford et al. (2007) fed multiparous ewes at 100% or 50% of nutrient requirements between d 28 and 78 of gestation, then fed all ewes at 100% of requirements from d 79 of gestation through lambing. Lambs from nutrient-restricted ewes had increased finish weights, greater amounts of KPH fat, and tended to have reduced LM and semitendinosis muscle weights as a percentage of HCW. The results of the above studies support the

hypothesis that maternal undernutrition dur ing early to mid-gestation will result in increased BW and fat deposition and impact skeletal muscle development in sheep. However, severe restrictions implemented in many of these examples may not be applicable across a wide variety of practical production situations. In addition, responses appear to be less consistent for beef cattle. Greenwood et al. (2005) reported significant differences in BW and growth characteristics at all stages of life (birth, weaning, backgrounding, feedlot entry, feedlot ADG, and final end BW) for cattle severely nutrient-restricted from d 80 to 90 of gestation until birth; however, there were no differences in carcass composition at similar carcass weights. It is important to carefully consider mechanisms by which maternal nutrition can impact various developmental processes such as morphology and metabolism of fetal tissues and how responses may vary depending on the timing, level, and duration of dietary restriction.

Primary myogenesis occurs early in gestation and forms muscle cell templates for secondary myogenesis, which occurs up to around d 180 of gestation and forms the majority of muscle fibers in beef cattle (Du et al., 2013). Research indicates the number of primary myofibers in beef cattle is determined genetically, while the number of secondary fibers is more likely to be impacted by factors such as maternal nutrition, with the majority of differentiation occurring in the last third of gestation (Picard et al., 2002). Adipogenesis, or the process of fat cell development, begins during mid-gestation in ruminants, which overlaps with the period of secondary myogenesis (Du et al., 2010a). Because myogenesis and adipogenesis are competitive processes, there is potential for both muscle and fat to be impacted by maternal nutrition. Nutrient restriction earlier in development appeared to affect muscle hyperplasia or cell number, while late restriction

had more impact on hypertrophy or growth of muscle cells (Fahey et al., 2005). Additionally, nutrient restriction in mid gestation has been shown to enhance fat deposition during late gestation (Symonds et al., 2003).

Treatments for the current study were initiated during mid-gestation, with heifers either remaining on their original treatment or changing to the alternative treatment in late gestation in order to elucidate the effects of timing of nutrient restriction on progeny muscle and adipose development and ultimately feedlot performance and carcass characteristics. It was hypothesized that a restriction of MP in mid-gestation would reduce nutrient supply available to muscle cells, resulting in reduced protein synthesis and skeletal muscle growth. An MP restriction in late gestation was expected to result in increased fatness of progeny as more cells would be expected to differentiate into adipocytes rather than muscle fibers. However, progeny from dams restricted in late gestation had increased LM area compared with progeny from dams on the control treatment, which was unexpected. Nonetheless, this response appeared to be primarily a function of HCW, and no differences were observed for fat thickness or marbling. Similarly, Micke et al. (2010) reported LM area of both steer and heifer carcasses was increased in progeny from dams receiving low (70% of CP requirements) vs. high (240% of CP requirements) nutrition diet during mid-gestation, with significant effects removed when LM area was corrected for HCW. In contrast, Underwood et al. (2010) also found no differences in LM area of steers whose dams were placed on improved pasture (IP) or native range (NR) in mid- to late-gestation; however, heavier HCW and increased 12^{th} rib fat thickness were observed in progeny from IP dams. In another study, there were no

differences in HCW, fat thickness, dressing %, Yield Grade, marbling score, or LM area for progeny from dams fed 100% vs. 55% of nutrient requirements (Long et al., 2010).

Restricting dams to 80% of NE_m in mid- gestation did not influence progeny HCW, LM area, KPH, or marbling score; however, tendencies for decreased 12^{th} rib backfat and lower final USDA Yield Grade were observed in progeny from dams in a negative energy status due to the restriction (Mohrhauser et al., 2015). In the study described previously by Summers et al. (2011), marbling scores were greater for steer progeny from HN dams compared with progeny from LN dams; however, there were no differences in 12^{th} rib fat thickness, LM area, or USDA Yield Grade and Quality Grade. Minimal differences in performance and carcass characteristics were likely due to the fact there was not a true nutrient restriction imposed in their study; however, it appears increased supplementation during late gestation had an effect on intramuscular fat development. This response appeared to support a hypothesis presented by Du et al. (2010a) stating that adequate maternal nutrition can influence marbling by enhancing adipogenesis in fetal skeletal muscle.

Other researchers have reported improvements in carcass quality of offspring due to maternal protein supplementation; however, results have not been consistent. Steer progeny from cows receiving 0.45 kg/d of 28% CP supplement during the last trimester of gestation had greater marbling scores and a greater proportion of carcasses grading USDA Choice than progeny from dams that did not receive a protein supplement, with no effect on Yield Grade (Larson et al., 2009). These results were in contrast to those of Stalker et al. (2006), who found no effect of protein supplementation on carcass characteristics of steer progeny. Further conflicting responses were reported by Long et

al. (2012), as Yield Grade and adipocyte diameter were increased in progeny from dams fed a nutrient-restricted diet (NR; 70% of Con) vs. progeny from dams fed a control (Con; 100% of NRC recommendations) or nutrient-restricted + protein supplement (NRP; 70% of Con + essential AA supply to small intestine equal to Con) diet from d 45 to d 185 of gestation. These authors attributed the increase in adipocyte size to a reduction in skeletal muscle in NR offspring as verified by a tendency for reduced semitendinosus muscle as a percentage of HCW. Reductions in skeletal muscle mass due to nutrient restriction would be expected to enhance fat accumulation as excess energy is diverted to adipose development (Du et al., 2015). Additional amino acids supplied by a protein supplement should result in the opposite effect by enhancing protein synthesis and muscle growth and reducing fat accumulation; however, differences in supplement composition may have contributed to this response.

The majority of phenotypic responses for feedlot performance and carcass characteristics were non-significant or were inconsistent with expected results; therefore, the hypothesis that MP restriction would result in reductions in postnatal growth and skeletal muscle, increased carcass adiposity, and reduced feed efficiency was rejected. Skeletal muscle reaches maturity at around d 105 of gestation in sheep and d 210 of gestation in beef cattle, and nutrient restriction after that point had little impact on muscle fiber number (Du et al., 2010a). Although differences in muscle fiber development in offspring were not directly measured in the current study, lack of differences in feedlot performance and carcass characteristics indicate maternal dietary treatment had minimal, if any, effect on muscle fiber development. The mid-gestation treatment was initiated around d 150 of gestation, which would be near the end of secondary myogenesis based

on the fetal development timeline developed by Du et al. (2010a). Although there was potential for MP restriction to affect adipogenesis and potentially muscle fiber hypertrophy, it is possible the treatment was applied too late to impact the critical window of muscle fiber development. Additionally, the late gestation treatment did not begin until after skeletal muscle is estimated to have reached maturity, which also could have impacted observed responses. Although researchers have attributed responses in lifetime performance of livestock to the maternal nutritional environment, inconsistent results suggest the need to continue to elucidate mechanisms of response and separate postnatal factors such as environment and milk production from factors thought to be programmed during gestation.

IMPLICATIONS

Metabolizable protein restriction of heifers in mid- and late gestation did not substantially influence efficiency, feedlot performance, or carcass characteristics. Although results of this study do not agree with reports in the literature of long-term effects on performance and carcass quality of beef cattle offspring, the concept of developmental programming merits further investigation to elucidate complex relationships of maternal nutrition, fetal development, and postnatal response. Inconsistency in developmental programming research results may be due to timing, intensity, and duration of nutrient restriction, influence of specific dietary restriction, and a host of environmental factors. Results indicate offspring may be able to recover from moderate MP restriction during development when exposed to an unrestricted nutritional environment postnatally. Future investigation is warranted to determine specific impacts of maternal nutrient restriction on metabolic changes and development of specific tissues in the fetus that can impact lifetime performance and production of beef cattle.

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Table 3.1. Dietary components and nutrients consumed by heifers receiving a control (CON = slightly exceeding MP requirement) or restricted (R = approximately 80% of MP requirement supplied) diet during mid- and/or late gestation based on NRC (2000) calculations[1]

	Diet formulation 1[2]		Diet formulation 2		Diet formulation 3	
	CON	R	CON	R	CON	R
Item	---- % DM basis ----					
Wheat straw[3]	59.81	59.62	54.14	53.65	51.22	51.28
Crude glycerin[4]	15.66	17.97	13.27	15.27	14.52	14.54
Dry supplement[5]						
Ground corn	-	-	10.27	10.02	10.79	11.03
Ground corn cobs	16.77	16.56	11.33	11.43	11.84	12.51
Energy Booster 100®[6]	3.42	3.06	7.38	7.46	7.74	8.20
Porcine bloodmeal	1.62	-	1.65	-	1.54	-
Sodium phosphate (XP 40)	1.57	1.56	1.39	1.43	1.73	1.62
Urea, 46%	1.08	1.18	0.51	0.67	0.54	0.75
Magnesium oxide, 54%	0.032	0.034	0.032	0.031	0.034	0.034
TM Green[7]	0.015	0.014	0.020	0.019	0.010	0.010
Selenium, 0.06% yellow	0.009	0.012	0.011	0.013	0.013	0.015
Vitamin AD 10:1	0.004	0.004	0.004	0.004	0.005	0.005
	---- Nutrient composition of diet predicted by NRC (2000) based on actual intake ----					
Diet CP, %	7.0	5.3	5.7	4.6	5.7	4.9
Bacterial N balance, g/d	11	11	-1	-1	2	2
MP, %	108.7	88.4	101.4	78.3	93.2	77.2
NE_m, Mcal/kg	1.24	1.17	1.37	1.40	1.44	1.44
NE_g, Mcal/kg	0.67	0.61	0.79	0.82	0.85	0.85

[1] Diets formulated based on NRC (2000) predictions for MP, NE_m, and NE_g requirements for heifers throughout gestation

[2] Diet formulation 1 fed from 11/2/13 – 12/14/13, diet formulation 2 fed from 12/15/14 – 1/18/14, and diet formulation 3 fed from 1/19/14 – calving. Amounts of supplement for each formulation were adjusted throughout gestation.

[3] Nutrient composition of wheat straw: 49.39% DM; 4.75% CP; 57.48% ADF; 66.78% NDF; 49.75% TDN; 0.95 Mcal/kg NE_m; 0.40 Mcal/kg NE_g

Table 3.1 continued…

[4] Crude glycerin contained 82.3% glycerol, 9.5% water, 0.56% CP, 0.04% methanol, 8.07% ash, and 0.90% MONG (matter organic non-glycerol; defined as 100 – glycerol content (%) + water content (%) + ash content (%)). Crude glycerin sourced from Minnesota Soybean Processors, Brewster, MN

[5] Dry supplement formulated and mixed by Hubbard Feeds Inc., Mankato, MN

[6] Milk Specialties Global, Eden Prairie, MN

[7] TM Green mineral mix contained 15.2% S; 330 ppm Co; 33,000 ppm Cu; 1,650 ppm I; 132,000 ppm Mn; 99,000 ppm Zn, 3,300 ppm CuCl; 1,856 ppm EDDI; 132,000 ppm $MnSO_4$; and 99,000 ppm $ZnSO_4$

Table 3.2. Diet composition (DM basis) of backgrounding and finishing rations for progeny of heifers fed a control (CON = slightly exceeding MP requirement) or restricted (R = approximately 80% of MP requirement supplied) diet during mid- and/or late gestation[1]

Item	Step-up rations 1-4				Finishing ration
Dates fed	10/20-10/27/14	10/27-11/2/14	11/3-12/19/14	12/20-2/8/15	2/9/15-Harvest
Dry rolled corn, %	20	30	41	48	48
Grass hay, %	35	25	14	7	7
Corn gluten feed, %	35	35	35	35	40
Grower supplement[2], %	10	10	10	10	-
Finisher supplement[3], %	-	-	-	-	5
Nutrient composition[4]					
DM, %	74.25	75.25	75.91	77.07	75.05
CP, %	12.91	12.99	11.48	13.13	11.47
NE_m, mcal/kg	1.54	1.63	1.77	1.80	1.79
NE_g, mcal/kg	1.33	1.41	1.53	1.56	1.61

[1] Dietary MP levels based on NRC (2000) predicted requirements; mid-gestation treatment applied mean d 148 through 216 of gestation; late gestation treatment applied mean d 217 of gestation through parturition

[2] Supplement formulated to provide minerals and vitamins to meet nutrient requirements (NRC, 2000) using dried distillers grains, limestone, iodized salt, ammonium chloride, trace mineral mix, Vitamins A, D, and E, monensin (Rumensin, Elanco Animal Health, Greenfield, IN), and tylosin phosphate (Tylan 40, Elanco Animal Health Greenfield, IN)

[3] Supplement formulated to provide minerals and vitamins to meet nutrient requirements (NRC, 2000) using ground corn, limestone, iodized salt, ammonium chloride, trace mineral mix, Vitamins A, D, and E, monensin (Rumensin, Elanco Animal Health, Greenfield, IN), and tylosin phosphate (Tylan 40, Elanco Animal Health Greenfield, IN)

[4] Nutrient composition for each ration based on wet chemistry analyses as reported by Ward Laboratories, Inc., Kearney, NE

Table 3.3. Main effect least square means for feedlot performance for progeny of heifers fed a control (CON = slightly exceeding MP requirement) or restricted (R = approximately 80% of MP requirement supplied) diet during mid- and/or late gestation[1]

	Mid-gestation		Late gestation			*P*-value	
Item	CON	R	CON	R	SEM	Mid	Late
Initial BW[2], kg	259	254	255	259	4.99	0.434	0.550
Final BW[3], kg	573	565	562	575	9.30	0.401	0.225
DMI, kg	10.06	10.06	10.06	10.06	0.143	0.984	0.972
ADG, kg	1.82	1.80	1.79	1.84	0.029	0.557	0.176
G:F	0.182	0.179	0.178	0.183	0.002	0.369	**0.084**

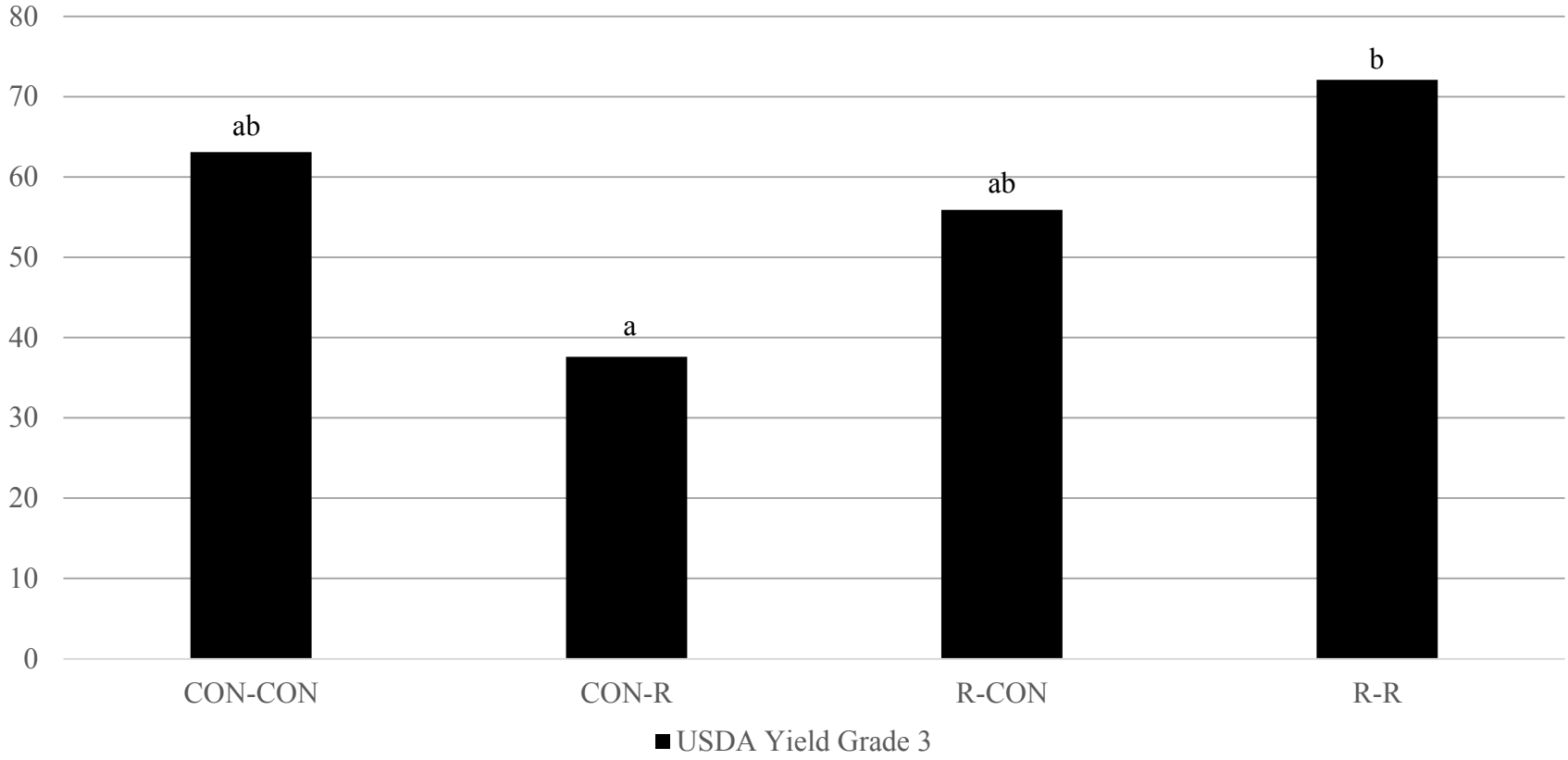
[1] Dietary MP levels based on NRC (2000) predicted requirements; mid-gestation treatment applied mean d 148 through 216 of gestation; late gestation treatment applied mean d 217 of gestation through parturition
[2] BW based on average of 2-day weights
[3] BW based on HCW/0.625 (assumed dressing percentage)

Table 3.4. Main effect least square means for carcass characteristics for progeny of heifers fed a control (CON = slightly exceeding MP requirement) or restricted (R = approximately 80% of MP requirement supplied) diet during mid- and/or late gestation[1]

	Mid-gestation		Late gestation			*P*-value	
Item	CON	R	CON	R	SEM	Mid	Late
HCW, kg	358	353	352	359	5.82	0.400	0.222
Adj. 12th rib FT[2], cm	1.59	1.54	1.63	1.50	0.073	0.661	0.248
LM area, cm^2	91.7	91.3	90.0[a]	92.9[b]	1.63	0.774	**0.039**
Adj. LM area[3], cm^2	91.3	91.7	90.6	92.3	1.88	0.756	0.231
KPH, %	2.24	2.13	2.14	2.23	0.085	0.230	0.342
Yield grade	2.76	2.67	2.79	2.65	0.135	0.597	0.443
Marbling score[4]	514	515	520	509	22.8	0.982	0.601
USDA Quality Grade[5]							
All Choice, %	81.0	86.1	80.7	86.4	5.82	0.622	0.588
Prime, %	19.0	13.9	19.3	13.6	5.82	0.622	0.588
USDA Yield Grade[6]							
Yield grade 2, %	20.5	15.6	15.0	21.1	6.18	0.650	0.581
Yield grade 3, %	50.4	64.4	59.6	55.5	9.18	0.181	0.695
Yield grade 4, %	19.3	16.0	21.6	14.2	10.33	0.671	0.341

[1] Dietary MP levels based on NRC (2000) predicted requirements; mid-gestation treatment applied mean d 148 through 216 of gestation; late gestation treatment applied mean d 217 of gestation through parturition
[2] Adj. 12th rib FT, cm = (Preliminary Yield Grade – 2) / 2.5) × 2.54
[3] Adj. LM area determined using HCW as a covariate in the model
[4] 400 = Small^{00}; 500 = Modest^{00}; 600 = Moderate^{0}
[5] No animals received a select Quality Grade
[6] GLIMMIX analysis failed to converge for USDA Yield Grade 1 (n = 1) or Yield Grade 5 (n = 2)
[a,b] Within gestation period, means lacking a common superscript differ ($P < 0.05$)

Figure 3.1. Least square means for mid-gestation treatment × late gestation treatment interaction for proportion of USDA Yield Grade 3 designations for progeny of heifers receiving a control (CON; slightly exceeding MP requirement) or restricted (R; approximately 80% of MP requirement supplied) diet during mid- and/or late gestation[1]



[1] $P = 0.049$

[a,b] Means lacking a common superscript differ ($P < 0.05$)

CHAPTER IV

Influence of maternal protein restriction in primiparous heifers during mid- and/or late gestation on progeny gene expression in *longissimus dorsi* muscle

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ABSTRACT

Maternal nutrient restriction has long-term consequences for postnatal performance of offspring due to impacts on metabolism and development of economically important tissues such as muscle and fat. This study investigated impacts of maternal MP restriction in mid- and late gestation on the transcriptome of beef cattle at birth and prior to harvest. One hundred eight Angus × Simmental heifers were blocked by BW, method of conception (AI or natural service, based on fetal age at ultrasound), and calf sex and allocated to 12 pens in a randomized complete block design with a 2 × 2 factorial treatment structure including 2 stages of gestation (mid- and late) and 2 levels of dietary protein (control [CON]; approximately 102% of MP requirements and restricted [R]; approximately 80% of MP requirements). Within 48 h of birth, biopsy samples were collected from longissimus muscle of a sub-set of 3 male calves per treatment combination. Pairs were managed as a common group from calving through weaning, and calves were finished in a GrowSafe feeding system on a typical feedlot diet. Additional biopsy samples were collected from the same sub-set of male calves approximately 3 weeks prior to harvest. Total RNA was extracted and gene expression analysis was conducted using RNA sequencing (RNA-Seq). Pairwise comparisons for

each treatment combination (CON-CON, CON-R, R-CON, and R-R) were conducted at the gene level, with differentially expressed genes analyzed for Gene Ontology (GO) terms and KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathways. Total number of differentially expressed genes was greatest for CON-CON vs. CON-R progeny in samples collected at birth and prior to harvest. Maternal MP restriction throughout mid- and late gestation (R-R) or in late gestation only (CON-R) down-regulated ($P < 0.05$) genes involved in muscle tissue development compared to CON-CON progeny at birth. Restriction during mid-gestation only (R-CON) down-regulated genes in triglyceride metabolic processes compared to CON-CON ($P < 0.05$). Prior to harvest, progeny restricted in late gestation only (CON-R) had decreased expression ($P < 0.05$) of genes related to muscle development compared to progeny restricted only in mid-gestation (R-CON) or throughout gestation (R-R). Key genes and pathways related to growth, development, and metabolism of skeletal muscle of beef cattle were impacted by MP restriction, with differential responses based on timing and duration of restriction.

INTRODUCTION

The 'developmental programming' hypothesis was originally developed based on epidemiological evidence in humans that showed a relationship between poor maternal nutrition and low birth weight, followed by increased incidence of metabolic diseases such as heart disease, stroke, diabetes, and hypertension (Barker et al., 2002). Hales and Barker (1992) described a 'thrifty phenotype' hypothesis, suggesting that the nutritional environment encountered by the fetus during development results in metabolic adaptations to prepare it for a similar environment at birth.

Based on epidemiological evidence in humans, it appears that the early developmental environment and the environment encountered later in life often result in a 'mismatch' that can result in increased risk of disease (Godfrey et al., 2007). There is increasing evidence indicating that the developmental programming hypothesis is relevant to livestock production, with compromised fetal growth linked to increased fatness, reduced muscle growth, and increased incidence of metabolic disorders (Reynolds and Caton, 2012). Research conducted in livestock over the past 50 years has shown that meeting nutrient requirements in late gestation is important in reducing postpartum anestrous interval, obtaining optimal pregnancy rates, and maximizing survival and health of offspring (Wiltbank et al., 1964; Corah et al., 1975; Bellows and Short, 1978). More recently, there has been increased focus on investigating the relationship between maternal environment and long-term physiological impacts in offspring that can impact livestock health, productivity, and profitability (Bell, 2006).

Muscle growth is the primary determinant of meat production in beef cattle, and the prenatal environment appears to have a great deal of influence on muscle

development. The majority of muscle fibers in beef cattle are formed during the second to eighth month of gestation, with no further net increase in muscle fiber number after birth (Du et al., 2010). Several studies have shown that nutritional restriction of dams during mid- to late gestation can influence muscle fiber development and ultimately affect growth and meat quality characteristics of offspring (Greenwood et al., 2009; Larson et al., 2009; Underwood et al., 2010). Although the precise mechanisms for these responses have not been fully elucidated, Reynolds and Caton (2012) stated that long-term effects on progeny due to insults encountered during fetal life likely include both irreversible changes in tissue and organ structure and epigenetic changes such as DNA methylation or histone modification that silence or activate gene expression without changing DNA sequence. Development of muscle and fat tissues in livestock may be impacted by inadequate maternal nutrition through reductions in muscle fiber number and through epigenetic modifications to genes involved in cell differentiation and tissue development (Du et al., 2011).

It is fundamentally understood that a given genotype can result in different phenotypes depending on environmental conditions (Gicqel, 2008). There is increasing interest in the role that nutritional and environmental factors play in determining regulation of genes that can influence livestock performance potential (Neibergs and Johnson, 2012). However, little research is available that documents global changes in the transcriptome of muscle tissue related to developmental programming effects. Therefore, the objective of this study was to characterize changes in gene expression related to muscle and adipose tissue development and metabolism in neonates and mature offspring as a result of maternal MP restriction experienced during development.

MATERIALS AND METHODS

Animals and Experimental Design

The South Dakota State University Institutional Animal Care and Use Committee approved all procedures involving animals. One hundred eight two-year-old Angus x Simmental heifers were pen-fed at the SDSU Cottonwood Range and Livestock Field Station near Philip, SD during the time gestational nutrient restrictions were imposed. Prior to the beginning of the study, yearling heifers were synchronized and time-bred to a single Angus sire on June 7, 2013. Following AI, all heifers were exposed naturally to Angus bulls for 60 days. Rectal ultrasonography was conducted in mid-September to detect pregnancy and fetuses were sexed and aged.

Treatments were arranged in a 2 × 2 factorial treatment structure with 2 levels of dietary protein: control (CON; slightly exceeding MP requirements) and restricted (R; approximately 80% of MP requirements) provided during 2 stages of gestation: mid- (mean d 148 through 216 of gestation) and late (mean d 217 through parturition). Heifers were blocked by BW as well as age and sex of the fetus, resulting in 3 blocks with 4 pens per block. At the end of the mid-gestation period, half of the pens on the CON treatment were reassigned to the R treatment and half of the pens on the R treatment were reassigned to the CON treatment, resulting in four treatment combinations (CON-CON, CON-R, R-CON, and R-R). Each treatment combination was randomly assigned to one pen per block for a total of 3 pen replicates per treatment combination.

Diets were based on calcium hydroxide treated wheat straw and concentrates, and were adjusted throughout gestation to maintain MP balance across treatments and account for increased nutrient requirements for the growing heifer and the developing fetus (NRC, 2000). Concentrate formulations between treatments were similar, except

that porcine bloodmeal was added to the CON formulation as a source of RUP to slightly exceed the MP requirement. The NRC (2000) requirements for NE_m and NE_g were met in both dietary treatments, and diets were formulated to be isocaloric (Table 4.1). Immediately after calving, heifers were removed from treatments and pairs were managed as a common group through weaning, with no further nutritional restrictions implemented in dams or progeny.

Progeny Weaning and Feedlot Management

Five calves were removed from the study prior to weaning due to death or issues with their dam that precluded study protocols and objectives. One hundred three steer and heifer calves were weaned on October 6, 2014, and backgrounded on high quality grass hay and dried distillers grains for two weeks at the SDSU Cottonwood Range and Livestock Field Station before being shipped approximately 430 km to the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE. Calves were allocated to four feedlot pens based on sex and method of conception (AI or clean-up through natural service) and received standard feedlot rations consisting of dry rolled corn, grass hay, corn gluten feed, and supplement. Individual feed intake data were collected beginning November 22 for AI-bred calves and December 13 for bull-bred calves following 10 d of adaptation in a GrowSafe feeding system (GrowSafe Systems Ltd., Airdrie, AB Canada). All calves received the same diet whether they were being fed in standard feedlot pens or in the GrowSafe system, as the only treatment applied to calves in this study was maternal dietary treatment.

Muscle Biopsy Sample Collection

Biopsy samples (approximately 40 mg) were collected from the *longissimus dorsi* muscle for analysis of gene expression on a subset of three male AI-bred calves from each treatment combination within 48 h of birth (BIRTH**)** and again approximately 3 weeks prior to harvest (PRE-HARVEST**)**. Calves were pre-selected by choosing dams representative of treatment means for BW and BCS at the beginning of the study and at treatment crossover. An area approximately 12.7 cm^2 was shaved using surgical precision blades and scrubbed with a povidone-iodine solution, followed by a 70% alcohol solution. A total of 5 mL of lidocaine was injected in a circle of beads around the planned incision site. After local anesthesia was established, a 10 mm incision was made using a sterile No. 11 scalpel, and a BARD Magnum Reusable Core Biopsy System with a 12 G × 10 cm needle was used to collect muscle tissue (C.R. Bard, Inc., Tempe, AZ). Tissue was immediately removed from the biopsy needle and snap frozen in liquid N before storage at −80°C. The injection site was sprayed with Vetericyn antimicrobial topical spray (Vetericyn, Rialto, CA) and calves were closely monitored until fully recovered. No ill effects were observed in any calves on either biopsy date.

Total RNA Extraction and Gene Expression Analysis

Total RNA was extracted from muscle samples using the miRNeasy Mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Purity of the RNA was evaluated by spectroscopy to ensure samples had an optical density 260:280 ≥ 2.0. All purified total RNA samples were stored at −80°C prior to analysis. A transcriptome library was created using 2 μg total RNA from each individual RNA sample and sequenced (2 sequencing lanes per sample) using the sample preparation protocol for the

Illumina HiSeq 2000 genome analyzer (Illumina Inc., San Diego, CA). Single-end reads of 50 nt for each sample were generated, and the 3' adaptor (TGGAATTCTCGGGTGCCAAGG) for sequencing was removed from the raw reads. After removing the 3' adaptor, reads with lengths less than 10 nt were excluded.

Raw individual RNA sequence reads were aligned to the reference beef cattle genome (Ensembl UMD3.1) using the software package Tophat (v2.0.12; available at: https://ccb.jhu.edu/software/tophat/index.shtml), with transcript abundance normalized and evaluated in Fragments Per Kilobase of transcript per Million mapped reads (FPKM) using the Cuffdiff module of Cufflinks (v2.2.1; available at: http://cole-trapnell-lab.github.io/cufflinks/). Transcriptome library preparation, sequencing, and expression analysis was completed by LC Sciences, LLC (Houston, TX).

Statistical Analysis

Pairwise comparisons were conducted at the gene level, with differentially expressed genes (DEG) analyzed for Gene Ontology (GO) terms and KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathways using GAGE (Generally Applicable Gene-set Enrichment for Pathway Analysis) v2.18 of Bioconductor (https://www.bioconductor.org/packages/release/bioc/html/gage.html) and DAVID (Database for Annotation, Visualization, and Integrated Discovery) v6.8 (https://david.ncifcrf.gov/conversion2.jsp). Significance for DEG was determined using a q-value of < 0.05, which is an adjusted *P*-value using an optimized false discovery rate (FDR) approach (Benjamini and Hochberg, 1995). Significance for GO terms and KEGG pathways was determined based on fold enrichment differences > 1.5 and adjusted *P*-values < 0.05.

RESULTS AND DISCUSSION

A summary of read alignments for birth and harvest samples is presented in Table 4.2. The total number of mapped reads in samples collected at birth ranged from 18 million to nearly 29 million, with a mapping percentage of approximately 99%. Mapped reads in samples collected at harvest were between 9 million and 14 million, with a mapping percentage of approximately 97%.

Across both sampling points, the greatest number of DEG were among progeny from dams restricted in late gestation (CON-R) compared to dams on the control diet throughout mid- and late gestation (CON-CON), with notably fewer DEG in other treatment comparisons involving the CON-CON treatment (Table 4.3).

In a study examining gene expression profiles in fetal *longissimus dorsi* muscle of beef cattle at various stages of myogenesis and muscle maturation, Lehnert et al. (2007) reported the most marked increase or decrease in gene expression was observed in late gestation. Therefore, it is possible that gene expression was more responsive to maternal nutritional treatments in our study during that time (i.e. gene expression differences being greatest in the CON-R treatment). It is also possible that progeny from the R-CON treatment may have been able to overcome potential consequences of MP restriction when realimented to the CON diet in late gestation. Based on the reduced number of differentially expressed genes between CON-CON and R-R progeny, it seems that some type of genetic adaptation may occur when restriction is implemented for a greater proportion of the gestational period. Timing of the restriction (mid- vs. late) resulted in a number of differentially expressed genes; however, there were minimal pathways that were significantly different among progeny from these treatments at either time point.

Pairwise Comparisons

CON-CON vs. CON-R: Birth

Inadequate maternal nutrition can negatively impact fetal skeletal muscle development, which involves multiple processes of cell differentiation and specialization, including myogenesis, adipogenesis, and fibrogenesis (Du et al., 2011). Genes involved in the GO pathway for muscle tissue development (GO:0060537; DEG including *ZFAND5*, *MYL3*, *HOMER1*, *PLN,* and *PPP3CA*) were down-regulated ($P = 0.032$) in progeny from dams on the CON-R treatment compared to CON-CON progeny. The MP restriction imposed on CON-R progeny would have occurred toward the end of secondary myogenesis, at a time when existing muscle fibers are undergoing hypertrophy (Du et al., 2010). Although the majority of muscle fibers should have already formed prior to MP restriction, gene expression differences between the restricted and control treatments in late gestation indicate that development of muscle tissue may still be impacted at that time.

Additional GO terms down-regulated ($P < 0.05$) in progeny from CON-R dams included energy reserve metabolic process (GO:0006112), protein catabolic process GO:0030163), and fatty acid metabolic process (GO:0006631). The KEGG pathway for insulin signaling was also down-regulated in the CON-R treatment ($P = 0.037$). Progeny of ewes restricted to 50% of nutrient requirements from early to mid-gestation had a decreased number of secondary myofibers and reduced protein synthesis in muscle (Zhu et al., 2004). Ford et al. (2007) also reported hyperglycemia and altered insulin secretion in male lambs from ewes restricted in early gestation. Although muscle fiber development and enzyme activity were not characterized in this study, differences in

gene expression for muscle tissue development, energy and protein metabolic processes, and insulin signaling indicate that MP restriction late in gestation had a negative impact on genes involved in skeletal muscle development and potentially predisposed offspring to insulin resistance.

In progeny from dams on the CON-R treatment, GO terms that were up-regulated ($P < 0.03$) included protein processing (GO:0016485), protein maturation (GO:0051604), and negative regulation of cell proliferation (GO:0008285). It appears that MP restriction in late gestation caused shifts in pathways that resulted in reductions in the rate of cell proliferation and specifically affected protein synthesis. Shang et al. (2007) reported that the Wnt signaling pathway enhanced myogenesis and inhibited adipogenesis in cultured mesenchymal stem cells. Progeny from dams in the CON-R treatment in the current study showed increased gene expression of the SFRP family, which can function as inhibitors of the Wnt signaling pathway (Logan and Nusse, 2004). These results indicate that MP restriction, particularly in late gestation, may have shifted gene expression in favor of adipogenosis rather than myogenesis.

CON-CON vs. CON-R: Pre-harvest

Decreases in gene expression for pathways related to muscle structure development (GO:0061061) and muscle tissue morphogenesis (GO:0060415) were observed among progeny from CON-R compared to CON-CON treatments prior to harvest ($P < 0.05$). Pre-harvest GO terms up-regulated ($P < 0.05$) in the CON-R compared to the CON-CON treatment included lipid transport (GO:0006869), fat cell differentiation (GO:0045444), and fatty acid metabolic process (GO:0006631). In general, expression of genes related to protein and muscle were decreased in the CON-R

treatment and genes related to adipose and metabolic adaptations were increased in the CON-R treatment in muscle samples collected at birth and at harvest.

CON-CON vs. R-CON: Birth

Expression of *DGAT2* and *LPL*, genes involved in the triglyceride metabolic process (GO:0006641), were decreased in R-CON vs. CON-CON progeny (fold enrichment -38.03; $P = 0.047$). Diacylglycerol acyltransferase (*DGAT2*) is a key enzyme in the rate-limiting step of triglyceride synthesis and has been shown to impact carcass traits in beef cattle. Li et al. (2009) investigated single nucleotide polymorphisms (SNP) in bovine *DGAT2* and reported associations with carcass traits such as hot carcass weight and KPH fat, although no associations were found between *DGAT2* polymorphisms and marbling score. Wang et al. (2012) reported that expression of *DGAT2* in adipose tissue was positively correlated with backfat thickness, and was increased in steers classified as having thick vs. thin backfat. It has been suggested that adequate maternal nutrition may increase fat deposition in offspring (Du et al., 2010), and gene expression results indicate that progeny from unrestricted dams compared to those restricted early in gestation may have increased potential for carcass fatness. This is somewhat contradictory to results observed in the pairwise comparison between CON-CON and CON-R progeny, indicating that MP restriction increased expression of genes in pathways related to lipid metabolism and fat cell development. However, adipogenesis occurs primarily in the last trimester of gestation (Du et al., 2010). It is plausible that a restriction early (R-CON) rather than late (CON-R) in gestation (R-CON) would have different influences on pathways related to adipose development and metabolism.

CON-CON vs. R-CON: Pre-harvest

Number of gene expression differences among progeny from CON-CON and R-CON were the least of the pairwise comparisons in our study (Table 4.3) at both birth and prior to harvest. Although there were no significant KEGG pathways or GO terms observed in the pre-harvest samples, there were some interesting gene expression differences. Growth hormone-releasing hormone was down-regulated (fold enrichment -3.96; $P = 0.044$) in R-CON progeny. Growth hormone (GH) is a lipolytic hormone that activates lipase, which mobilizes fat from adipose tissue (Heffernan et al., 2001). Uncoupling protein 3 (*UCP*) was also down-regulated in R-CON progeny (fold enrichment -1.51; FDR $P = 0.002$). Research results indicate that this gene is up-regulated in muscle when fatty acids are available as an energy source (Weigle et al., 1998). Reduced gene expression results for genes related to lipolytic processes in restricted progeny could indicate reduced availability of free fatty acids and metabolic signals that would preserve available adipose tissue rather than utilizing it as an energy source.

CON-CON vs. R-R: Birth

Similar to results with the CON-CON vs. CON-R pairwise comparison, we found that genes involved in muscle tissue and muscle organ development (*ZFAND5*, *MYL3*, *HOMER1*, and *PLN*) were down-regulated (fold enrichment -14.35; $P = 0.033$) in progeny from dams restricted throughout gestation (R-R) vs. those on the control treatment (CON-CON). Additionally, positive regulation of fat cell differentiation (GO:0045600) and triglyceride metabolic process (GO:0006641) were up-regulated in R-R progeny ($P < 0.03$). It appears that late gestation is a critical time for development of

muscle and fat since differences in gene expression that affect development of these tissues were not as pronounced when the restriction occurred in mid-gestation. It is also possible that R-CON progeny were able to compensate for the nutritional restriction after being placed on the control diet in late gestation, and that differential expression of genes would have been observed if fetuses had been harvested at the end of mid-gestation instead of being allowed to develop to term.

CON-CON vs. R-R: Pre-harvest

The mitogen-activated protein kinase (MAPK) pathway was down-regulated in the R-R treatment compared to CON-CON ($P = 0.01$), with differentially expressed genes including *MAX*, *CACNA2D4*, *GADD45A*, *GADD45G*, and *RPS6KA1*. The MAPK signaling pathway plays a role in proliferation, differentiation, development, transformation, and apoptosis of cells (Zhang and Liu, 2002). Gene ontology terms for triglyceride metabolic process (GO:0006641) and regulation of cell growth (GO:0001558) were up-regulated in R-R progeny ($P < 0.05$). These results indicate that MP restriction throughout mid- and late gestation may have negatively impacted gene expression for pathways related to cell growth and differentiation.

CON-R vs. R-CON: Birth

The CON-R treatment resulted in up-regulation (fold enrichment 2.42; $P = 0.025$) of genes involved in proteolysis (GO:0006508; DEG including *MMP2*, *CPXM1*, *PAMR1*, *MMP23B*, *C2*, *CISH*, *MMP14*, *ADAMTS2*, *CPZ*, and *ADAMTS4*). This supports research suggesting that maternal undernutrition may result in increased protein degradation and decreased protein synthesis in fetal skeletal muscle (Wu et al., 2006). Thus, it appears

that an MP restriction late in gestation resulted in increased activation of pathways that degrade proteins in muscle, possibly as a conservation mechanism.

Gene ontology pathways terms that were up-regulated ($P < 0.05$) in progeny from the R-CON treatment included protein transport (GO:0015031) and protein catabolic process (GO:0030163). Interestingly, the histone modification biological process (GO:0016570) showed greater expression in the R-CON treatment compared to CON-R (fold enrichment 4.12; $P = 0.021$). While epigenetic mechanisms pertaining to DNA modification were not measured in the current study, it appears that histone modification (a potential mechanism for epigenetic change) may have been affected by our treatments. Two KEGG pathways that were up-regulated in the R-CON treatment were the MAPK signaling pathway and the Wnt signaling pathway (FDR $P < 0.02$). Genes up-regulated in the R-CON treatment that play a role in the Wnt signaling pathway included *PPP3CA*, *PPP2CB*, *PPP3R1*, *MAPK8*, *CACYBP*, *MAP3K7*, *CSNK2A1*, *MAPK9*, *PPP3CB*, *PRKACB*, and *SIAH1*.

CON-R vs. R-CON: Pre-harvest

While there were a large number of genes that were differentially expressed among CON-R and R-CON, there were no significant GO terms or KEGG pathways for muscle, adipose tissue, protein or energy metabolism that were up-regulated in the CON-R treatment. In contrast, GO terms for muscle structure development (GO:0055001), muscle cell differentiation (GO:0042692) and muscle cell development (GO:005001) were up-regulated for the R-CON treatment ($P < 0.05$).

CON-R vs. R-R: Birth

There were a number of genes and GO terms up-regulated ($P < 0.05$) for progeny from the R-R treatment, including fatty acid metabolic process (GO:0006631; DEG including *EHHADH*, *ELOVL5*, *GHR*, *ELOVL6*, *GPAM*, *PRKAR2B*, *LPL*, *ACSM1*); lipid biosynthetic process (GO:0008610; DEG including *ABHD5*, *ELOVL5*, *DGAT2*, *ELOVL6*, *FDX1*, *GPAM*, *LPL*, *ACSM1*); and neutral lipid metabolic processes (GO:0006638; DEG including *DGAT2*, *GPAM*, *LPL*, *ABHD5*). Restriction of MP throughout gestation appeared to play a major role in processes related to a variety of lipid-related metabolic pathways compared to a restriction in mid-gestation only.

CON-R vs. R-R: Pre-harvest

Although there were a number of differentially expressed genes between CON-R and R-R progeny, differences in pathways related to fatty acid and lipids observed in muscle tissue samples collected at birth were not maintained in the samples taken prior to harvest.

R-CON vs. R-R: Birth

Genes in metabolic processes related to protein were up-regulated ($P < 0.01$) in the R-CON treatment, including GO:0009309~amine biosynthetic process, GO:0006563~L-serine metabolic process, and GO:0008652~cellular amino acid biosynthetic process (DEG including *ASNS*, *PHGDH*, *PSAT1*, *CBS*, and *PSPH*). The KEGG pathway for glycine, serine, and threonine metabolism (DEG including *PHGDH*, *PSAT1*, *CBS*, *ALAS2*, and *PSPH*) was also up-regulated ($P = 0.019$) in this treatment.

Similar to responses in other pairwise comparisons, pathways related to lipid metabolism were up-regulated in progeny from dams restricted throughout gestation (R-

R). The acylglycerol metabolic process (GO:0006639) and fatty acid metabolic process (GO:006631) were up-regulated ($P < 0.05$) in the R-R treatment. Two KEGG pathways up-regulated ($P < 0.05$) in R-R vs. R-CON were the PPAR signaling pathway (DEG including *PPARG*, *PLIN1*, *UCP1*, *ANGPTL4*, and *LPL*) and glycerolipid metabolism (DEG including *AGPAT2*, *DGAT2*, *GPAM*, and *LPL*).

R-CON vs. R-R: Pre-harvest

When comparing two treatment groups that both experienced an MP restriction during mid-gestation, it is interesting to note that muscle tissue development (GO:0060537) and muscle cell differentiation (GO:0042692) were up-regulated ($P < 0.05$) in the R-CON treatment compared to R-R. It is possible that increased duration of the MP restriction in the R-R treatment resulted in differential gene expression among progeny. There were no KEGG pathways up-regulated in R-CON, and no GO terms or KEGG pathways up-regulated in the R-R treatment.

SUMMARY

Overall, an increase in the number of DEG in pre-harvest samples compared to at-birth samples was observed. This agrees with a report by Sudre et al. (2003), who collected muscle samples from bovine fetuses at different stages of development (d 110, 180, 210, and 260 of gestation), and also from 15-month-old bulls of the same breed types to generate differential expression patterns over time. Although samples from mature animals in their study were not collected from the same experimental animals as was the case in our study, there were similarities in overall gene expression based on developmental stage of the animal. Small differences in gene expression were observed from d 110 to d 210 of gestation, with increased differences observed from d 210 of

gestation on (Sudre et al., 2003). Moreover, they found differences in gene expression were more pronounced at 15 months of age. In the current study, the proportion of DEG with fold change differences ≥ 1.5 were greater in samples collected at birth vs. those collected prior to harvest; however, this could be a function of the reduced number of total raw and mappable reads in the pre-harvest samples (Table 4.2).

Differentiation of mesenchymal stem cells to myogenic, adipogenic, or fibrogenic cells is a competitive process shaped by a number of regulatory factors (Yan et al., 2013). In the at-birth samples, genes in pathways associated with muscle tissue development (*ZFAND5*, *MYL3*, *HOMER1*, *PLN*) were down-regulated in calves whose dams were restricted throughout gestation (R-R) or only in late gestation (CON-R) compared to those on the control diet throughout gestation (CON-CON). In addition, genes related to positive regulation of fat cell development were upregulated in calves from dams on the R-R treatment compared with CON-CON. Gene expression differences between the CON-CON and CON-R in terms of muscle development vs. adipose development appeared to be maintained throughout the life of the animal.

Peñagaricano et al. (2014) provided diets containing various energy sources (alfalfa haylage (HY; fiber), corn (CN; starch), or dried distillers grains (DG; fiber, protein, and fat)) to ewes during mid- to late gestation. Fetal muscle and adipose tissue were collected on d 130 of gestation, and RNA was sequenced. Expression of genes associated with skeletal muscle development and skeletal muscle cell differentiation were decreased in fetal *longissimus dorsi* muscle in the CN diet compared to HY and DG diets. Although the primary focus was to investigate the impact of maternal energy source on the fetal transcriptome, it is interesting to note that the CN diet was lower in protein than

either DG or HY diets. Results from our study provide additional evidence that expression of genes related to muscle tissue development are affected by reduced maternal protein availability.

Genes involved in fatty acid, lipid, and triglyceride metabolic processes (*DGAT2*, *LPL*, *GPAM*, *LIPE*) were up-regulated in calves from the R-R treatment compared with the CON-R treatment at birth, providing evidence that MP restriction throughout mid- and late-gestation may stimulate lipogenesis. Zhu et al. (2006) restricted pregnant ewes at 50% of requirements from d 28-78 of gestation, and reported that intramuscular triglyceride content (IMTG) was increased in skeletal muscle of lambs from nutrient-restricted (NR) ewes. Additionally, reductions in insulin sensitivity and glucose utilization in skeletal muscle of NR progeny suggested that lambs would be predisposed to obesity and diabetes. In the current study, MP restriction in late gestation (CON-R) resulted in down-regulation of genes involved in the KEGG pathway for insulin signaling compared to the control treatment (CON-CON) in the at-birth samples.

Epigenetic change is defined as alterations in DNA function without alterations in DNA sequence that occurs through a variety of mechanisms such as DNA methylation, histone modification, and non-coding RNA (Scholtz et al., 2014). Although changes in gene expression are regulated by epigenetic modifications, we did not utilize experimental protocols that would allow us to determine the mechanisms responsible for the observed responses. Measurable differences in gene expression indicate that fetal adaptations to MP restriction may have occurred; however, these results conflict with measured phenotypic responses in offspring. Although MP restriction resulted in decreases in dam BW and BCS in addition to reductions in dam *longissimus dorsi* muscle

area based on ultrasound measurements, there were no differences ($P > 0.10$) among treatments for progeny birth weight, weaning weight, or feedlot performance, with minimal differences in carcass characteristics.

Epigenetic modifications of the genome allows for heritable changes in gene expression; however, epigenetic states are reversible and can be modified over time by environmental factors (Jaenisch and Bird, 2003). Following the gestational environment experienced by animals in this study, there were no further dietary restrictions and all animals were exposed to the same postnatal environment. Noticeable changes in gene expression over the lifetime of the animals could be due to a variety of external factors in addition to the original gestational environment.

IMPLICATIONS

Differentially expressed genes among all treatment combinations in our study provided evidence that maternal nutrient status in mid- and late gestation can impact programming of muscle and fat tissues and metabolic processes in beef cattle. However, inconsistency in expression of genetic pathways taken at two time points suggest that these processes are influenced by external factors such as environment. Asynchrony among gene expression differences, live animal performance, and carcass quality characteristics indicate that further research is needed to characterize the biological relevance of genetic pathways related to developmental processes in beef cattle.

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Table 4.1. Dietary components and nutrients consumed by heifers receiving a control (CON = slightly exceeding MP requirement) or restricted (R = approximately 80% of MP requirement supplied) diet in mid- or late gestation based on NRC (2000) calculations[1]

	Diet formulation 1[2]		Diet formulation 2[2]		Diet formulation 3[2]	
	CON	R	CON	R	CON	R
Item	---- % DM basis ----					
Wheat straw[3]	59.81	59.62	54.14	53.65	51.22	51.28
Crude glycerin[4]	15.66	17.97	13.27	15.27	14.52	14.54
Dry supplement[5]						
Ground corn	-	-	10.27	10.02	10.79	11.03
Ground corn cobs	16.77	16.56	11.33	11.43	11.84	12.51
Energy Booster 100®[6]	3.42	3.06	7.38	7.46	7.74	8.20
Porcine bloodmeal	1.62	-	1.65	-	1.54	-
Sodium phosphate (XP 40)	1.57	1.56	1.39	1.43	1.73	1.62
Urea, 46%	1.08	1.18	0.51	0.67	0.54	0.75
Magnesium oxide, 54%	0.032	0.034	0.032	0.031	0.034	0.034
TM Green[7]	0.015	0.014	0.020	0.019	0.010	0.010
Selenium, 0.06% yellow	0.009	0.012	0.011	0.013	0.013	0.015
Vitamin AD 10:1	0.004	0.004	0.004	0.004	0.005	0.005
	---- Nutrient composition of diet predicted by NRC (2000) based on actual intake ----					
Diet CP, %	7.0	5.3	5.7	4.6	5.7	4.9
Bacterial N balance, g/d	11	11	-1	-1	2	2
MP, %	108.7	88.4	101.4	78.3	93.2	77.2
NE_m, Mcal/kg	1.24	1.17	1.37	1.40	1.44	1.44
NE_g, Mcal/kg	0.67	0.61	0.79	0.82	0.85	0.85

[1] Diets formulated based on NRC (2000) predictions for MP, NE_m, and NE_g requirements for heifers throughout gestation

[2] Diet formulation 1 fed from 11/2/13 – 12/14/13, diet formulation 2 fed from 12/15/14 – 1/18/14, and diet formulation 3 fed from 1/19/14 – calving. Amounts of supplement using each formulation were adjusted throughout gestation.

[3] Nutrient composition of wheat straw: 49.39% DM; 4.75% CP; 57.48% ADF; 66.78% NDF; 49.75% TDN; 0.95 Mcal/kg NE_m; 0.40 Mcal/kg NE_g

Table 4.1 continued…

[4] Crude glycerin contained 82.3% glycerol, 9.5% water, 0.56% CP, 0.04% methanol, 8.07% ash, and 0.90% MONG (matter organic non-glycerol; defined as 100 – glycerol content (%) + water content (%) + ash content (%)). Crude glycerin sourced from Minnesota Soybean Processors, Brewster, MN

[5] Dry supplement formulated and mixed by Hubbard Feeds Inc., Mankato, MN

[6] Milk Specialties Global, Eden Prairie, MN

[7] TM Green mineral mix contained 15.2% S; 330 ppm Co; 33,000 ppm Cu; 1,650 ppm I; 132,000 ppm Mn; 99,000 ppm Zn, 3,300 ppm CuCl; 1,856 ppm EDDI; 132,000 ppm $MnSO_4$; and 99,000 ppm $ZnSO_4$

Table 4.2. Summary of sequencing read alignments to the reference genome for tissue samples collected at two time points (birth and harvest) from *longissimus dorsi* muscle of steer progeny from dams fed a control (CON = slightly exceeding MP requirement) or restricted (R = approximately 80% of MP requirement supplied) diet in mid- and late gestation[1]

	Treatment		Birth samples				Pre-harvest samples			
Steer	Mid[2]	Late[3]	Raw reads	Mappable reads[4]	% Mapped	Removed reads	Raw reads	Mappable reads	% Mapped	Removed reads
Z203	CON	CON	26,690,902	26,515,482	99.34	175,420	14,276,006	13,990,361	98.00	285,645
Z226	CON	CON	24,887,739	24,701,390	99.25	186,349	10,682,350	10,329,494	96.70	352,856
Z229	CON	CON	27,064,239	26,808,234	99.05	256,005	10,585,765	10,432,651	98.55	153,114
Z227	CON	R	28,718,632	28,298,679	98.54	419,953	13,617,536	12,942,078	95.04	675,458
Z242	CON	R	29,140,538	28,980,870	99.45	159,668	13,668,862	13,349,107	97.66	319,755
Z251	CON	R	22,730,624	22,573,795	99.31	156,829	9,937,893	9,724,247	97.85	213,646
Z202	R	CON	24,740,798	24,550,900	99.23	189,898	11,318,922	10,982,190	97.03	336,732
Z259	R	CON	20,825,399	20,651,418	99.16	173,981	10,987,327	10,835,861	98.62	151,466
Z266	R	CON	22,798,163	22,669,242	99.43	128,921	12,241,258	11,993,495	97.98	247,763
Z224	R	R	23,961,591	23,803,135	99.34	158,456	10,326,352	9,592,745	92.90	733,607
Z239	R	R	21,043,500	20,894,110	99.29	149,390	13,801,773	13,200,467	95.64	601,306
Z272	R	R	18,301,822	18,051,561	98.63	250,261	12,831,619	12,546,200	97.78	285,419

[1] Dietary MP levels based on NRC (2000) predicted requirements based on mean heifer BW and stage of gestation

[2] Mid-gestation treatment applied mean d 148 through 216 of gestation

[3] Late gestation treatment applied mean d 217 of gestation through parturition

[4] The 3' adaptor (TGGAATTCTCGGGTGCCAAGG) for sequencing was removed from the raw reads, with lengths less than 10 subsequently excluded. Remaining reads were mappable reads.

Table 4.3. Number of differentially expressed genes of sequencing read alignments to the reference genome for tissue samples collected at two time points (birth and harvest) from *longissimus dorsi* muscle of steer progeny from dams fed a control (CON = slightly exceeding MP requirement) or restricted (R = approximately 80% of MP requirement supplied) diet in mid- or late gestation[1]

	At-birth samples			Pre-harvest samples		
Pairwise comparisons[2]	DEG[3]	Up-regulated	Down-regulated	DEG[3]	Up-regulated	Down-regulated
CON-CON vs. CON-R	652	411	241	1,357	220	1,137
CON-CON vs. R-CON	81	40	41	103	44	59
CON-CON vs. R-R	191	64	127	216	77	139
CON-R vs. R-CON	642	120	522	1,169	1,034	135
CON-R vs. R-R	184	76	108	726	678	48
R-CON vs. R-R	168	72	96	129	53	76

[1] Dietary MP levels based on NRC (2000) predicted requirements; mid-gestation treatment applied mean d 148 through 216 of gestation; late gestation treatment applied mean d 217 of gestation through parturition

[2] Pairwise comparisons based on nutritional treatment combinations of dams throughout mid- and late gestation (i.e., CON-CON represents dams on the CON treatment in mid- and late gestation; CON-R represents dams on the CON treatment in mid-gestation and the R treatment in late gestation, etc.)

[3] DEG = differentially expressed genes; significance determined by using False Discovery Rate (FDR) Adjusted P-value ≤ 0.05