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The Effects of Maternal Energy Restriction During Mid-Gestation on Growth Performance, Immune Function, and Gene Expression in the Resultant Beef Offspring

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THE EFFECTS OF MATERNAL ENERGRY RESTRICTION DURING MID-GESTATION ON GROWTH PERFORMANCE, IMMUNE FUNCTION, AND GENE EXPRESSION IN THE RESULTANT BEEF OFFSPRING

BY

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

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ABSTRACT

THE EFFECTS OF MATERNAL ENERGRY RESTRICTION DURING MID-GESTATION ON GROWTH PERFORMANCE, IMMUNE FUNCTION, AND GENE EXPRESSION IN THE RESULTANT BEEF OFFSPRING

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Fetal or developmental programming evaluates the effects of maternal alterations on the developing fetus and the potential consequences later in life. To understand the effects of mid-gestation energy restriction on beef cows and their calves the objectives of this dissertation were to determine the effects of dietary energy restriction on measurements associated with cow energy status, and the effects on growth performance, the humoral immune response, and subcutaneous adipose tissue gene expression in the resultant beef offspring.

Pregnant beef cows were allotted into 2 treatment groups during mid-gestation: 1) fed at maintenance (Positive Energy Status (PES)); or 2) fed just below maintenance (Negative Energy Status (NES)). Cows were evaluated for parameters reflective of cow energy status. Positive energy status cows maintained or gained condition where the NES cows lost condition during the mid-gestation treatment period, indicating the intended treatment was met.

Progeny were evaluated for growth performance characteristics from birth through the finishing phase. Birth weight was decreased in NES heifer calves ($P<0.05$). At weaning heifers from NES cows had a tendency ($P<0.10$) to be lighter than heifers

from PES cows. There was a tendency ($P<0.10$) for PES cows to wean heavier calves compared to NES cows. These results suggest NES during mid-gestation may affect birth weight and weaning weight. These differences in weight are overcome during the feeding phase.

Progeny were vaccinated against a novel antigen to measure the humoral immune response. There was a difference ($P<0.05$) over time with calves from PES cows having a greater antibody titer to the antigen than calves from NES cows. These results suggest cows in a NES during mid-gestation produce calves with a decreased ability to produce antibodies to a novel antigen and thus a decreased humoral immune response.

Progeny were selected to evaluate gene expression related to adipose tissue deposition. No differences in gene expression were detected in the weaning or final subsample ($P>0.05$). Two genes important in adipose differentiation had a tendency ($P<0.10$) to be different in the weaning subsample. These results suggest NES during mid-gestation may alter adipose differentiation around weaning, but not later in life.

CHAPTER I

Review of Literature

INTRODUCTION

A multitude of factors must converge to make each segment of the beef cattle industry successful and profitable. As we consider research to aid beef production practices a focus on the quantity and quality of the end product must be maintained. It is necessary to recognize areas where we can improve our current management practices to manipulate the end product and compete with other protein sources in the future. The ability to manipulate carcass composition could work to combat two major issues in beef production: inadequate marbling and excessive backfat. It is well documented that cattle with excessive fat and inadequate marbling are a costly expense to the beef industry. These deviations result in carcasses grading below Choice, and having USDA Yield Grades of 4 and 5. These high USDA Yield Grades and low USDA Quality Grades result in decreased carcass value, which does not include losses from feeding excessively fat cattle. Resolving these problems within the beef industry is necessary for continued growth and profitability for beef producers and improved product consistency for consumers.

The phenotype of any beef animal is a combination of its inherited genetic code and the environment in which it is raised. However, when considering the impact of environment on a beef animal, most research aimed at improving carcass composition has focused on postnatal strategies such as nutritional inputs and time on feed. While many of these strategies to influence adipose and muscle tissue development are successful

more efficiencies must be sought. Little effort has focused on the effects of the prenatal environment on the resultant carcass yield and quality of beef offspring. Recent findings may suggest that cow nutrition may influence the phenotype and ultimately efficiencies and composition of the beef offspring, which will be the focus of the remainder of this review.

Gestating Cow Requirements

Beef cows are expected to produce a viable calf every year of their productive lives. In addition, producers also want to maximize the pounds of calf weaned from each cow in the most economical way possible (Stokes et al., 1986). In order to fulfill these two goals producers need to understand the changing requirements of beef cows throughout the year in their specific environment. It is also necessary to understand the requirements of a gestating bovine female to ensure proper fetal development, maintain adequate body condition for the dam to calve and produce milk, and preserve the ability to rebreed according to the Nutrient Requirements of Beef Cattle (NRC). This concept is even more significant in heifers or young cows as continued growth of the dam must be accounted for in addition to fetal development when calculating requirements for maintenance (NRC, 2000). An understanding of bovine requirements and utilization of available feedstuffs can improve profitability of an operation.

It is common to raise beef cows on native range in order to utilize non-tillable ground for production purposes. However, solely grazing native range pasture does not always meet nutrient requirements. Forage quality and quantity are dependent on plant maturity, precipitation, climate of a given region, snow cover, stocking rate, and forage

management (Vavra and Raleigh, 1976). According to the NRC (2000) when forage availability is decreased beef cows will use body reserves to compensate for the disparity between nutrients available and nutrients consumed in order to match their requirements at that time. This can often lead to loss of body condition as it becomes challenging for producers to match the changing nutrient requirements of the cow with her nutrient intake. It is also important to note that these requirements are cyclical dependent on the phase of her reproductive cycle, which again does not always match the nutrients available when grazing native range (Thomas, 1992). Cline et al. (2010) noted that as the grazing season progresses forage intake and quality decreases as plants mature making it critical to understand the nutrient requirements of beef cattle throughout their reproductive cycle.

The most common nutrients of concern for a beef cow are protein and energy (Thomas, 1992), and are dependent on the frame size of the cow, stage of production, cow condition, and other environmental factors like the weather (Jurgens, 2002). Beef cows will eat anywhere from 1.5% to 3% of their body weight everyday depending on the stage of production and feedstuff they are consuming (Jurgens, 2002; Thomas, 1992). Cattle are fed based on nutrient requirements calculated from their weight. Therefore, in order to ensure replacement heifers are receiving adequate nutrition the producer must estimate what the heifers' mature weight will be and feed accordingly for heifer and fetal growth. Both cows and heifers will increase intake closer to their calving date (Jurgens, 2002; NRC, 2000). A greater percentage of Total Digestible Nutrients (TDN) will be required, as well as, Net Energy for Maintenance (NE_m), Dry Matter Intake (DMI), and

Crude Protein (CP) increase independent of the mature weight as the cow or heifer approaches her calving date (Jurgens, 2002; NRC, 2000). However, cows and heifers with a heavier mature weight will have higher DMI than their lighter mature weight counterparts (Jurgens, 2002; NRC, 2000).

When discussing beef cow nutrient requirements frame size and the amount of milk she can produce become the important factors when evaluating the diet. Mature cows with smaller mature body weights will require more TDN, NE_m, and CP as a percentage compared to their larger framed counter parts (Jurgens, 2002; NRC, 2000). However, independent of cow mature size as peak milk production increases TDN, NE_m, and CP needed by the beef cow is also increased (Jurgens, 2002; NRC, 2000). As gestation length increases so do requirements for the cow & heifer, with the greatest requirements occurring during lactation (Jurgens, 2002). Variations for TDN, NE_m, and CP occur during gestation and lactation in order to account for stage of gestation, frame size, and milk production (Jurgens, 2002). Because of these differences in nutrient requirements within cows and throughout the year a producer must implement a calving program that will utilize feedstuffs to their benefit.

Generally in the upper Midwest many producers maintain a spring calving cow herd in order to utilize lush summer pasture at a time of greatest nutritional need because of lactation (Thomas, 1992). This period of time during the summer is also generally a time of weight gain prior to rebreeding, breeding season, and the first trimester of pregnancy (Thomas, 1992). Summer grazing pastures will supply most of the nutrients necessary to beef cattle with the exception of a mineral supplementation program

(Jurgens, 2002; Thomas, 1992). However, if pasture is inadequate from lack of precipitation, overgrazing, or snow cover, energy will need to be supplemented in some form (Jurgens, 2002). During the fall and early winter the second trimester of gestation or mid-gestation occurs for spring calving herds. During this period the nutrient requirements of a beef cow are the lowest and producers can take this opportunity to feed poor quality forages, like dormant native range or crop residue (Thomas, 1992). The last trimester of beef cattle gestation involves rapid fetal growth resulting in increased nutrient requirements of beef cows (Thomas, 1992). This period of gestation requires extra management to ensure proper nutrition to the dam through the use of supplementing protein and energy to ensure calf viability and prevent cow metabolic diseases (NRC, 2000; Thomas, 1992). The last trimester of gestation is an ideal time to utilize alternative feedstuffs for increased protein and energy in the diet. However, when using alternative feedstuffs it is necessary to know the quality and feed analysis of the feedstuff in order to prevent over- or under-feeding cattle (Thomas, 1992). High or average quality forages are likely adequate to meet the requirements of gestating beef cattle if fed at the appropriate amount, but poor quality roughages will likely need to be supplemented with protein and possibly energy (Jurgens, 2002; Thomas, 1992). Because of the changing nutrient requirements throughout the year due to the reproductive cycle of a beef cow the option of using different feedstuffs of varying qualities is applicable to producers.

Not meeting cow nutritional requirements during this period of time has severe economic repercussions in the form of lighter calves at weaning for the producer (Corah et al., 1975). Conversely, many producers try to save money earlier during mid-gestation by

utilizing poor quality forages and possibly not supplementing beef cows to save on feed expenses. While current research has shown that this practice will not negatively affect birth weight, it is important to provide protein supplementation to those cows in late gestation in order to recover body condition, ensure milk production, support fetal growth, and improve breed back rates (Funston et al., 2010; Long et al., 2012; Underwood et al., 2010). To this point most research evaluating supplementation during gestation is driven by the cow sector where it has been proven to help improve breed back rates (Houghton et al., 1990). Research has also evaluated protein supplementation during the last trimester for economical traits like weaning weight, where no protein supplementation can result in decreased weaning weight (Larson et al., 2009; Stalker et al., 2006). Most research evaluating cow nutrition during gestation has looked at the effects of prepartum nutrition on calf weight and subsequent reproductive performance of the cow. Little research exists on cow nutrition during gestation and the consequences on postnatal growth and performance. Before discussing these factors an understanding of embryonic and fetal development is needed.

Embryonic & Fetal Development

Placentation

The placenta plays a large role in fetal development in most mammalian species. Prior to the formation of the placenta fertilization, early embryogenesis, maternal recognition of pregnancy, and implantation or attachment of the embryo must occur in eutherian mammals (Senger, 1997). The necessity for placentation has long been noted and studied by many researchers who categorize the placenta's 3 key aspects: 1) the

placenta involves intimate contact between fetal and maternal membranes, 2) this contact is between the fetal membranes that make up the chorioallantois and the maternal uterine mucosa known as the endometrium, and 3) physiological exchange between fetal and maternal tissues is the primary role for the placenta (Reynolds and Redmer, 1995; Wildman et al., 2006). Through these intimate contacts, known as placentomes, exchange of gases, nutrients, and wastes occurs between the dam and fetus (Ramsey and Crosby, 1982). The placentomes allow for exchange of substrates from nutrients ingested by the dam to the fetus which are required for fetal growth (Reynolds and Redmer, 1995; Senger, 1997). The efficiency with which nutrients are exchanged and the support for growth in the last half of gestation are largely attributed to the growth of uteroplacental vascular beds during the first few months of gestation (Reynolds and Redmer, 1995). As previously stated this uteroplacental growth is important for normal fetal growth to occur.

Development

Along with placental size and nutrient transfer, the parity, age of the dam, frame size of the dam, genotype from both the maternal and paternal side, thermal environment, and maternal nutrition all impact fetal growth and development (Robinson et al., 2012). Because nutrient alterations to the dam can change the environment in which the fetus is developing, it is important to understand the growth of the fetus *in utero*. This growth can be broken down into three distinct phases from conception to birth: 1) ovum, 2) embryonic, and 3) fetal (Aberle, 2001). The ovum phase is the shortest phase lasting up to 14 days, but includes a very important developmental stage when the primary cell layers are developed into the endoderm, mesoderm, and ectoderm (Yang et al., 2011).

Minimizing the risk of improper development is necessary as these primary cell layers will develop into tissues and organs. Specifically, the endoderm will develop into digestive and endocrine systems, the mesoderm will develop into muscle, skeletal, and cardiovascular tissues, and the ectoderm will develop into the nervous system and skin (Senger, 1997). The embryonic phase is marked by the development and differentiation of tissues and organs, but does not include substantial growth. The embryonic phase lasts between 25 to 45 days of gestation depending on the species (Aberle, 2001). This time period includes the ovum phase, but specifically in beef cattle occurs during the first 2 months of gestation and includes some primary myogenesis (Aberle, 2001; Du et al., 2010a). The fetal phase of growth makes up the remainder of development and growth prior to birth (Aberle, 2001). The fetal phase in cattle begins at approximately the second month of gestation and continues until birth (Du et al., 2010a). This phase is defined by growth of tissues and organs through both hypertrophy and hyperplasia. During this phase some primary myogenesis occurs as well as secondary myogenesis, and the initiation of adipogenesis in the bovine fetus (Aberle, 2001; Du et al., 2010a). About 75 percent of whole body fetal growth in a ruminant occurs during the last 2 months of gestation when hypertrophy of tissues is occurring, including muscle fiber hypertrophy (Du et al., 2010a; Robinson, 1977). After birth, whole body growth continues in a sigmoidal-type curve over time, with the growth of bone and muscle tissue occurring rapidly, and eventually gradual accretion of fat. Specifically postnatal growth is slow directly following birth, but is followed by a rapid rate of growth for bone and muscle tissue (Aberle, 2001; Hafez, 1963). Eventually growth of muscle and bone will cease

once mature size is reached. Prior to cessation of bone and muscle growth fat accretion will increase, and will continue through the life span of the animal if enough energy is present (Aberle, 2001; Bell et al., 1987). The amount of postnatal growth that can occur naturally is affected by genetics and factors that influence mesenchymal multipotent cell differentiation during gestation (Du et al., 2011).

Since many tissues come from the same mesenchymal multipotent cells during fetal development an understanding of embryology is necessary prior to discussing the differentiation of the mesoderm (Du et al., 2011; Senger, 1997). Specifically, muscle development involves myogenesis or the formation of muscle, adipogenesis or the formation of adipose, and fibrogenesis or the formation of connective tissue (Du et al., 2011). Skeletal muscle is derived from somites, which are a part of the mesoderm (Buckingham et al., 2003). Primary myogenesis approximately begins after the first month of gestation in beef cattle, followed by secondary myogenesis from about 2 ½ months to 7 ½ months of gestation (Du et al., 2010a). This is followed by muscle fiber hypertrophy which rapidly increases from around 5 ½ months of gestation into postnatal development (Du et al., 2010a). Adipose tissue is also derived from somites, and these somites differentiate from mesenchymal stem cells. A third type of cells, fibroblasts, also come from mesenchymal stem cells illustrating 3 different types of tissues are all derived from the same pool of cells for growth of different types of tissues (Du et al., 2010a). When nutrient supply to the fetus is altered, the signaling pathway which will commit the mesenchymal stem cells to differentiate into muscle fibers, adipocytes, or fibroblasts is

potentially altered resulting in changes in composition of the fetus (Du et al., 2010a; Zhu et al., 2004).

The flow of nutrients from the dam to the fetus is required for fetal growth and development. In general females try to maintain the flow of nutrients to the fetus at the expense of body condition to a certain point where she can no longer maintain her normal body functions (Koong et al., 1982). When nutrient flow is altered signaling pathways change which pathway will be "turned on" or "turned off" and can lead to nutrient partitioning, which can alter growth and composition of growth (Godfrey and Barker, 2000). Nutrient partitioning refers to how the body directs the flow of nutrients or calories consumed towards traits such as milk production, lean tissue growth, fetal growth, or towards adipose development in the dam (Bray, 1991). However, the fetus will also partition nutrients if necessary in order to maintain survival. Primarily the fetus will first direct nutrients towards vital organ development, possibly overcompensating toward vital organs (Long et al., 2009). Because 3 different types of cells, myoblasts, adipoblasts, and fibroblasts, come from the same pool of cells it is important to understand the differentiation and development of these cell lineages. Since the fetus can partition nutrients towards increased organ mass in response to changes in nutrient flow it is possible that the fetus can also partition nutrients among these three different types of cells. Therefore, understanding how changes in nutrient flow to the fetus could potentially affect tissue development, specifically within these 3 tissue types, is of interest to researchers. More importantly, since bovine skeletal muscle is a protein source for humans it is of interest as to whether or not proper dam nutrition during

gestation could positively or negatively affect the amount of muscle produced from the resultant offspring.

Organogenesis

Although organogenesis, or the formation of organs, usually occurs early through mid-gestation, much of the growth of these tissues and organs occurs in the last trimester (Fowden et al., 2006). It has been noted that most organs are well formed by the end of the embryonic stage with only minor differentiation and substantial growth occurring during the last trimester (Winters et al., 1942). Organogenesis, similar to myogenesis, adipogenesis, and fibrogenesis, can be affected by dam nutrient alterations during gestation. Research on normal fetal development from many studies are varied in their findings and are likely dependent on species, timing of the nutrient alteration, and the type of nutrient altered (Meyer et al., 2010). Most tissues necessary for survival like the brain and heart develop first *in utero* and are thought to have priority over available nutrients, possibly resulting in altered development of other tissues and organs (Hafez, 1963; Long et al., 2009). One specific project evaluated fetuses from dams that were fed at 68% NE_m and 86.7% of metabolizable protein for the first 125 days of gestation. Meyer et al. (2010) discovered an increase in total intestinal vascularity within the fetus when evaluated at 245 days of gestation suggesting increased intestinal efficiency for absorption of nutrients later in life. It also suggests that an early gestational nutrient restriction followed by realimentation to meet the nutrient requirements could possibly increase gastrointestinal growth and vascularity (Meyer et al., 2010). Usually gastrointestinal tissues are thought to undergo growth in the period of time right before

birth or shortly thereafter unlike other organ growth, which occurs in late gestation, but this research suggests development can be affected earlier in gestation (Hafez, 1963; Meyer et al., 2010). In this same group of cattle some fetuses underwent intaruterine growth restriction (IUGR) as a result of nutrient restriction causing a decrease in fetal weight, fetal empty carcass weight, abdominal circumference, brain weight, heart weight, liver weight, and total lung weight compared to nutrient restricted and control fetuses (Long et al., 2009). However, fetal organ weight as a percentage of fetal weight was greatest in nutrient restricted IUGR fetuses for brain, and heart weight compared to control and only nutrient restricted fetuses (Long et al., 2009). This suggests the fetus is able to compensate for decreased nutrients, but might be overcompensating and altering growth of other tissues which occur during the same time. Similarly, in fetuses from ewes fed at 70% of their requirements throughout gestation a decrease in the weight of the brain, thymus, pancreas and kidney were reported (Osgerby et al., 2002). Also in pigs that incurred IUGR, kidney function was altered (Bauer et al., 2002). Altered kidney function in offspring from nutrient altered mothers has implications in health problems later in life such as hypertension (Bassan et al., 2000; Bauer et al., 2002; Long et al., 2009).

In addition to altered adipogenesis, fibrogenesis, myogenesis and organogenesis, fetal size has been shown to be affected by dam nutrient intake prior to parturition, specifically within the last 30 days (Bellows and Short, 1978; Laster, 1974). This decrease in size is usually related to a decrease in nutrient intake prior to parturition in order to decrease calving difficulty (Bellows and Short, 1978; Corah et al., 1975). The

change in weight is likely caused by a decrease in hypertrophy, not hyperplasia. However, to date limited research exists investigating if altered nutrition during mid-gestation would alter growth of the resultant fetuses in beef cattle, and in what tissue or tissues would growth be altered.

Fetal Programming

Growth and development of the bovine fetus are impacted by a number of factors including genetics, maternal maturity, and the maternal environment in which a fetus develops. These factors influence the carrying capacity of the placenta, uteroplacental transfer of nutrients from the dam to the fetus, and overall nutrient availability to the fetus (Reynolds et al., 2010b; Wu et al., 2006). When any of these factors are not optimized or are impaired, normal development of the fetus will be affected. Fetal programming or developmental programming is an area of research that evaluates the effects of maternal nutrient alterations or health changes and the consequential effects on the developing fetus. Fetal programming may be the result of a stimulus or an insult to the dam during a critical period of development that has lasting effects on metabolism, physiology, and structure of the offspring (Godfrey and Barker, 2000). Human neonates that have undergone gestational events leading to reduced birth weight or otherwise developmentally compromised have an increased risk for lifelong health complications (Reynolds et al., 2010b; Wu et al., 2006). Livestock have been shown to have similar problems related to negative gestational environments including increased morbidity and mortality, altered postnatal growth such as reduced average daily gain and weaning weights, altered body composition such as a decrease in muscle and an increase in

adipose deposition, metabolic disorders, cardiovascular disease, and dysfunction of other important organs like the liver (Wu et al., 2006). The most significant work has evaluated the lack of nutrients to the dam and fetus during gestation, as this situation is common and has more relevance, especially in the livestock industry (Barker, 2002; Godfrey and Barker, 2001). More recently in humans, the effects of maternal over nutrition on the fetus have been of interest to researchers and the potential implications it has on offspring health later in life (Castro and Avina, 2002; Ramsay et al., 2002). Both forms of altered nutrition and the resultant effects on the fetus are important and relate to health and productivity.

Maternal Nutrient Restriction

Limited nutrient availability for the dam during gestation is most common in a livestock production setting. As previously discussed, many production systems utilize a spring calving system resulting in cows gestating during the winter months. In the upper Midwest forage availability and quality may be altered, especially during the winter months when snow cover is present and pasture is in a dormant state (Vavra and Raleigh, 1976). Often this results in a period of inadequate nutrition to gestating cows at some point in their pregnancy. Therefore, it is important to understand the effects of altered maternal nutrition on the development, growth and subsequent composition of the offspring. Fetal programming as a result of maternal under-nutrition was first elaborately discussed in relation to human epidemiology (Barker, 1995; Godfrey et al., 1994). Specifically in humans, low birth weights were more highly correlated with coronary heart disease, insulin resistance, and hypertension later in life than babies born with

average birth weights (Barker, 1995). Along with many diseases that manifest later in life, altered body composition in adult life has also been noted in babies with low birth weights. In states of nutrient deficiencies it is hypothesized that fetuses will shift stem cells towards adipogenesis in order to create a "thrifty" phenotype in preparation for being born into a nutrient sparse environment (Hales and Barker, 1992). This phenomenon or metabolic syndrome combines several different factors that will likely result in obesity. The potential decrease in β-cell mass or islet function resulting in the development of non-insulin dependent diabetes (Type 2), other organ malfunction like the liver, and hypertension combined with excess calories manifests into metabolic syndrome and obesity (Hales and Barker, 1992; Hales and Barker, 2001). This shift towards adiposity likely results from the potential to decrease muscle as stem cells shift towards adipogenesis and away from myogenesis (Du et al., 2010a). Other human epidemiological data shows fetal under-nutrition during the first trimester of pregnancy is associated with smaller birth weight, smaller body proportions, elevated blood pressure, and an increased risk for having a stroke in adult life (Barker, 1995; Barker and Clark, 1997; Godfrey and Barker, 2000). Under-nutrition during the second trimester is associated with infants having low birth weights, and as adults are more prone to insulin resistance or deficient in production of insulin, have elevated blood pressure, develop Type 2 diabetes, and develop coronary heart disease (Barker, 1995; Godfrey and Barker, 2000; Hales and Barker, 2001). During the third trimester of gestation in humans, nutrient restricted fetuses have increased incidences of resistance to growth hormone or deficient in the production of growth hormone, have elevated blood pressure, elevated

concentrations of cholesterol, suffer from coronary heart disease, and increased problems with stroke throughout adult life (Barker, 1995; Godfrey and Barker, 2000). Low birth weights caused from maternal under-nutrition during gestation are associated with increased autoimmunity problems in adult life and poor immune system development (Phillips et al., 1993). Thorough reviews of human epidemiological data revealed these connections between maternal nutrient restriction during gestation and health problems later in life. However, livestock can frequently encounter periods of nutrient restriction during gestation as well, therefore it is important to understand the impact of gestational environment on the offspring.

Global Nutrient Restriction

During times of restriction, specific nutrients can be restricted depending on each production system. In ruminants it is often hard to separate energy and protein. If protein in the form of nitrogen is limited, microbial growth is also likely being limited and this will decrease digestion leading to a reduction in feed intake and energy intake (Church, 1993; Griswold et al., 2003). If energy is restricted the ruminant animal will catabolize protein for an energy source, which could result in a protein restriction (Church, 1993). Because of these interactions and associative effects many times protein and energy are altered together.

First Trimester Restriction

Glucose is one of the main substrates used in fetal development throughout all trimesters of development, whether it is from the dam or generated from fetal metabolism (Funston et al., 2010; Reynolds et al., 1990). Often nutrient requirements during early

gestation are overlooked because of the limited requirements during this stage of gestation (Funston et al., 2010). Even though most fetal growth occurs during the last 2 months of gestation in ruminants, many important events occur during the early stages of gestation such as cell differentiation, placental growth, vascularization, and fetal organogenesis, which are necessary for normal fetal development (Funston et al., 2010). Long et al. (2010a,b) evaluated the effects of feeding heifers at 55% of their NRC energy requirements and 50% of their CP NRC requirements from day 32 of gestation through day 83 of gestation (the first trimester) followed by commingling of the restricted and control group to be fed in excess of their daily requirements. The resultant offspring's birth weight and postnatal growth were not affected by early gestational nutrient restriction, but glucose clearance was increased in nutrient restricted calves compared to control calves (Long et al., 2010b). Feedlot performance and carcass characteristics were also not different between the two treatment groups, but lungs and trachea of the nutrient restricted group weighed less at slaughter (Long et al., 2010a). Furthermore, although no differences were observed in carcass traits, researchers did note an increase in muscle fiber area in nutrient restricted progeny (Long et al., 2010a). In a similar study, multiparous cows were allotted to one of three groups at day 45 of gestation: 1) control fed to 100% of NRC recommendations, 2) nutrient restricted group fed at 70% NE_m and CP of the control diet, and 3) nutrient restricted plus a protein supplement (ruminally undegradable protein (RUP)) provided through day 185 of gestation when they were commingled and fed like the control group (Long et al., 2012). Among these three treatment groups there were no effects on birth weight, weaning weight, or most carcass

characteristics (Long et al., 2012). However, the progeny that were nutrient restricted during gestation had higher yield grades compared with both the control group and the nutrient restricted treatment that was supplemented with protein (Long et al., 2012). In addition, adipocyte diameter was increased in subcutaneous, mesenteric and omental adipose tissue when compared to both the control treatment group and the nutrient restricted plus protein treatment group (Long et al., 2012). In sheep a similar experiment was performed where multiparous ewes were fed either 50% or 100% of NRC requirements from day 28 through day 78 then all fed at 100% of their requirements through lambing (Ford et al., 2007). Both groups of lambs exhibited similar birth weights and crown rump lengths, but at slaughter the energy restricted group was heavier, had more back fat and kidney pelvic heart fat, and tended to have reduced *longissimus* muscle and *semitendinosus* muscle as a percentage of the hot carcass weight (HCW) (Ford et al., 2007). These results suggest increased postnatal growth, but decreased skeletal muscle which is detrimental to the agricultural industry. In another sheep study using the previous treatments researchers observed a decrease in the number of myofibers in nutrient restricted lambs, and an increase in the intramuscular triglyceride content within skeletal muscle suggesting this time in ovine gestation is important for muscle and marbling development (Zhu et al., 2006). There is a disparity between these different ruminant studies where ovine muscle and adipose development appear to be affected more so than bovine muscle and adipose development. The differences in development observed between the sheep and cattle studies may be the result of species differences,

the severity of the restriction, the potential for IUGR imposed on sheep, and the timing of the restriction as cattle and sheep have different gestation lengths.

Second Trimester Restriction

Nutrient restriction during mid-gestation has also been overlooked for a similar reason to the first trimester: most of growth occurs during the last trimester of gestation and producers have been told this is an opportune time to save on feed costs by using low quality forages because of the low nutrient requirements of the dam (Thomas, 1992). Therefore there is little research that has evaluated only energy restriction during mid-gestation. One study fed heifers either 1% of their body weight at day 90 (restricted) through 60 days before calving when energy was reduced to 0.8% of body weight or 1.5% of body weight (non-restricted) from 90 days through parturition. Calf birth weight and gestation length was decreased in nutrient restricted heifers (Warrington et al., 1988). Unfortunately there were no performance or carcass data from the offspring on this study and it is difficult to determine if the change in birth weight is due to the restriction in mid-gestation, late gestation, or a combination of both. It is also difficult to determine if the decrease in birth weight is from decreased muscle fiber hyperplasia or muscle fiber hypertrophy as muscle cross sections were not collected. A study by Radunz et al. (2012) evaluated different energy sources from 160 days of gestation through parturition. Dams were fed grass hay, corn, or dried distillers grains (DDGS) to achieve similar energy intakes in order to determine the effects of energy source on long-term fetal growth. Calf birth weights were greater from the corn and DDGS treatment compared to the hay treatment. There was a tendency for weaning body weight to be lower in the hay

treatment versus the corn treatment, but that difference did not persist through feedlot performance or health measurements (Radunz et al., 2012). There were no differences in carcass characteristics except an increase in dressing percent in the hay versus the DDGS treatments and greater percent ether extract in *longissimus* muscle at the 12th rib in the hay group versus the corn treatment group (Radunz et al., 2012). Although this study was not solely focused on the second trimester, the data suggests the type of feedstuff the dam ingests during gestation can affect the fetus later in life. These differences are likely related to differences in substrates provided to the fetus during gestation. Underwood et al. (2010) investigated the difference between cows grazing a native range pasture limited in protein versus cows grazing an improved pasture during mid-gestation (ranging from 120 days to 210 days of gestation). Cows limited in protein from grazing native range pasture gave birth to progeny with a lower average daily gain (ADG), total body weight gain, live weight, HCW, and a smaller amount of 12th ribfat thickness than progeny from cows grazing improved pasture. There was also an increase in tenderness in the progeny from cows grazing improved range, but there were no changes in calpastatin content, troponin-T degradation, or collagen content (Underwood et al., 2010). Therefore the mechanism of this improvement in tenderness remains unclear. Because there is such a limited amount of data evaluating the effects of energy restriction during mid-gestation and the consequences on the resultant bovine fetus, more research is needed to validate potential implications on growth and development to provide producers with management strategies.

Third Trimester Restriction

More research has been conducted on nutrient restriction during the last trimester of gestation due to concerns with health consequences, postnatal calf growth, and implications for reproductive performance in cows and heifers (Corah et al., 1975; Dunn et al., 1969; Wiltbank et al., 1962). Corah et al. (1975) performed 2 experiments evaluating energy restriction during the last 100 days of gestation in heifers and second calf cows. In the heifer experiment the low group was fed 65% of NRC requirements where the cows were fed 50% of NRC requirements until 30 days prior to calving when they were fed 117% of their requirements. In both the heifers and the cows, calves from the low energy treatment groups had lighter birth weights, increased mortality, and decreased weaning weights compared to their control counterparts which were fed at 100% of maintenance throughout gestation (Corah et al., 1975). In the heifer treatment group weaning weight was not a function of milk production as there were no differences between the control and treatment group suggesting a change in composition or potentially stunting growth. However, the cow treatment group did have decreased milk production which could cause the decrease in weaning weight. In a different study, heifers fed a low TDN diet during the last 90 days of gestation had calves with decreased birth weights, as well as a decrease in cow body condition likely leading to the decrease in reproductive performance (Bellows & Short, 1978). This and other research suggests that heifers may not be able to adapt to nutritional restriction as well as mature cows (Bellows et al., 1982). This is understandable as mature cows, in reasonable condition, have more body stores to partition towards fetal growth compared to a heifer or young

cow that is still growing creating a negative energy balance. This is a common production situation in animal agriculture.

Over-Nutrition

Compared to undernutrition, there is even less research in animal agriculture focused on over-nutrition as this would be costly to producers and inefficient. Nevertheless, in humans as we become more efficient at food production, availability of food increases and we live less physically demanding lifestyles obesity has become a paramount concern and the cause of many health problems. Obesity during gestation poses threats not only to the offspring, but also to the mother (Castro and Avina, 2002). Obese mothers are at higher risk during pregnancy for chronic hypertension and pre-eclampsia, diabetes, respiratory problems like asthma and sleep apnea, and infections (Castro and Avina, 2002). In addition to maternal consequences, offspring born to obese mothers are associated with increased birth weights and have a greater risk of developing childhood obesity and the subsequent morbidity associated with obesity (Castro and Avina, 2002). Similarly, Ramsay et al. (2002) found obese mothers had hyperinsulinemia, dyslipidemia, impaired endothelial function, high blood pressure, and inflammatory up-regulation that altered the environment in which the fetus was developing. Unfortunately in this study fetal measurements were not collected so maternal effects on the fetus are unknown. In sheep, Wallace et al. (2003) investigated the influence of over-nourished adolescent ewes during the last third of gestation on placental glucose transport. Over-nourished offspring had lighter fetal weights in response to reduced uterine and umbilical blood flow leading to hypoxia and

hypoglycemia in those fetuses. Ultimately the reduction in blood flow was contributed to the small size of the placenta in the over-nourished ewes (Wallace et al., 2003). In cattle, over-conditioning leads to decreased conception rates and poor milk production (Thomas, 1992). Additionally, Arnett et al. (1971) compared twin beef females raised separately: one fed at maintenance and the other fed at maintenance plus grain. The obese twin required more services per conception, had greater dystocia issues, increased calf mortality, decreased milk production, and gave birth to lighter calves (Arnett et al., 1971). As stated previously, little research has been conducted in cattle as it is not economically relevant to overfeed cattle. In general human data suggests obesity could potentially cause health problems similar to under-nutrition. In livestock, it is more difficult to draw conclusions from research because not many scientists have evaluated dam obesity in relation to offspring meat production. However, of the research conducted thus far implications on the maternal side suggest overfeeding livestock is costly not only in wasting feedstuffs, but also from a reproductive efficiency standpoint. On the fetal side over-nutrition during gestation suggests a decrease in fetal growth, but more research is required to validate whether or not fetal body composition is altered from over-nutrition of the dam, or if growth is simply restricted and offspring will be able to display compensatory growth after birth.

Protein Supplementation

Supplementing protein during late gestation is a common practice for producers. Generally producers want justification for this increased cost therefore a variety of research has evaluated protein supplementation and utilization of different winter grazing

systems to elucidate reasons for supplementation and the most applicable production system. Martin et al. (2007) investigated the influence of late gestation protein supplementation (42% CP supplement) to cows and determined protein supplementation did not affect birth weight, but increased adjusted 205 day weaning weight. Heifers born to protein supplemented cows had greater prebreeding weights and overall pregnancy rates. However, there were no differences in age at puberty, or the percentage of heifers cycling before breeding between the 2 treatment groups (Martin et al., 2007). In a similar research trial Stalker et al. (2006) supplemented protein (42% CP supplement) to cows to investigate the effects of prepartum nutrition during late gestation and its interactions on cow reproductive performance and calf growth through the feedlot. Birth weight was not different between treatments, but weaning weight was greater in progeny from dams supplemented protein during late gestation. No differences in feedlot performance or carcass characteristics were reported between progeny from either treatment group. Contrary to the previous trial, Stalker et al. (2006) reported protein supplementation improved pre-calving and pre-breeding body condition score (BCS) in the supplemented cows. Although many variables could contribute to the differences in offspring responses to maternal treatments in these studies they do demonstrate that maternal protein supplementation during the last trimester does have the potential to alter some aspects of offspring performance. Specifically, birth weight and weaning weight can be altered by third trimester protein supplementation. Therefore, it is necessary to determine how birth weight is altered in these fetuses. Research is still necessary to elucidate if carcass

composition is changed, if the change in weight is a product of milk production, or if growth potential was altered *in utero*.

Intrauterine Growth Retardation

One specific type of fetal programming, IUGR, can occur when a dam is young, genetic factors limit size, in litter bearing species or limited maternal nutrition occurs during gestation (Christenson and Prior, 1978; Ferrell, 1991a;b; Morriss et al., 1980; Wootton et al., 1983; Wu et al., 2006). Fetal growth is regulated by the size of the placenta and room in the uterus (Ferrell, 1991b). Intrauterine growth retardation occurs when normal growth and development of a fetus is impaired or stunted during pregnancy caused by a decrease in available space in the uterus (Wu et al., 2006). Impaired growth occurs through different mechanisms including impaired placental growth or reduced blood flow to the fetus which results in reduced nutrients to the fetus (Ferrell, 1991a;b; Ford, 1995; Reynolds and Redmer, 1995; Wu et al., 2006). One common cause of IUGR is age of the dam, which may have a large impact on fetal growth, especially when coupled with nutrient restriction. Robinson et al. (2012) accounted for over 17 percent of the variation in their nutritional treatment models due to age of the dam. Age also had the greatest effect on calf birth weight (Robinson et al., 2012). Similarly, cows in a nutrient restriction (68% NE_m) from days 30 to 125 of gestation and at a younger age, 3.5 years versus 5 years, demonstrated intrauterine growth restriction when fetal measurements were collected. Smaller fetal brain, heart, and liver weights were observed at 125 days of gestation. However, as a percentage of fetal body weight these organs were greater in size when compared to non-restricted fetuses (Long et al., 2009). This

increase in fetal organ weight could potentially be the fetus overcompensating for the lack of nutrients available during restriction to ensure growth and development of vital organs. Nevertheless, the effects on other tissue growth when this occurs are unknown. This data indicates younger cows could potentially be more susceptible to nutrient restriction and IUGR compared to older cows because younger animals are still trying to grow resulting in competition for nutrients, thus potentially altering growth rates in fetuses (Long et al., 2009). Conversely, after supplying adequate nutrients to gain 1 BCS following the early gestation nutrient restriction fetuses displayed compensatory growth resulting in no differences in fetal and organ weights at 245 days of gestation (Long et al., 2009; Meyer et al., 2010). However, there was no nutrient restricted IUGR group at this time point so it is difficult to determine if IUGR fetuses would also undergo compensatory growth. Similarly, in sheep fed 50% of NRC requirements during early to mid-gestation, which led to IUGR, resulted in normal birth weights, but carcass composition was altered towards an increase in adiposity and a decrease in skeletal muscle (Ford et al., 2007). This research suggests IUGR can result in reduced fetal growth, but the mechanism for reduced growth still needs to be elucidated.

One potential mechanism by which size is altered in response to IUGR is through nitric oxide (NO) and polyamines, which play roles in nutrient transport and cell growth respectively (Wu et al., 2006). Specifically nitric oxide is important in regulating placental-fetal blood flow as it is an endothelial vasorelaxing factor, which controls nutrient and oxygen transfer from mother to fetus (Bird et al., 2003; Flynn et al., 2002). Polyamines are known to regulate DNA and protein synthesis in tissues (Flynn et al.,

2002). In relation to altered maternal nutrition both nitric oxide and polyamine production are impaired in pigs and sheep exposed to both maternal over- and under-nutrition. (Kwon et al., 2004; Wu et al., 2004). Severe nutrient restriction in sheep reduced concentrations of polyamines in maternal and fetal plasma, as well as amniotic fluid (Kwon et al., 2004). Specifically, polyamines are used in the proliferation and differentiation of cells. It is also hypothesized that polyamines are needed for mediating growth of fetal muscle fibers and adipocytes (Flynn et al., 2002; Wu et al., 2006). Increased levels of nitric oxide inhibit growth of adipocytes in rats (Fu et al., 2005; Jobgen et al., 2006). Similarly in nutrient restricted fetal lamb adipose tissue, decreased levels of endothelial nitric oxide synthase would reduce the NO available leading to increased preadipocyte growth (Wu et al., 2006). Developmental differences occurring during gestation could be contributed to altered production of both polyamines and NO dependent on the dams' nutrient intake. These alterations in nitric oxide and polyamines can affect normal muscle growth as well as organ development (Wu et al., 2006). Specifically, nitric oxide and polyamines are important regulators in angiogenesis, embryogenesis, and placental and fetal growth (Reynolds and Redmer, 2001; Zheng et al., 2006). Nitric oxide is responsible for increased angiogenesis and blood flow and subsequently stimulates glucose uptake in insulin sensitive tissues (Jobgen et al., 2006). As a signaling molecule NO is also responsible for glucose and fatty acid oxidation in skeletal muscle and adipose tissue resulting in lipolysis in adipocytes, which is why increased levels of NO would inhibit adipocyte growth (Jobgen et al., 2006). The increased blood flow and nutrients to the fetus ultimately allows for expression of full

genetic potential of muscle in animals (Reynolds et al., 2010a; Reynolds and Redmer, 2001). Because of the ability of NO and polyamines to increase growth of the fetus it is important not to overlook the importance of these molecules.

In addition to genetic potential there are many factors that can affect fetal growth including maternal nutrition and subsequent absorption, diseases and toxins, environmental stresses, and placental function (Redmer et al., 2004). The effect of these environmental conditions on the fetus depend on the severity of the insult, the stage of gestation in which the insult occurred, and the duration of the insult (Wu et al., 2006). Evidence exists that carcass composition is shifted towards greater fat deposition when fetuses experience IUGR even when ewes were overfed (Matsuzaki et al., 2006). One example of this would be overfeeding a small framed animal, which would likely have smaller offspring because of genetic potential and a smaller uterine space could also contribute to a smaller offspring. Similarly, high birth weight lambs when compared to low birth weight lambs had less fat in the whole body regardless of rate of postnatal growth (Greenwood et al., 1998). These two examples illustrate that smaller and potentially IUGR fetuses have altered carcass composition towards less muscle and increased fat. Intrauterine growth restriction also limits the growth and development of vital organs, such as the liver (Widdowson, 1971), thus decreasing the functionality of metabolism of nutrients in those animals (Wu et al., 2006). Previous studies evaluating potential IUGR conditions have shown mixed results in postnatal feed efficiency (Greenwood and Cafe, 2007; Martin et al., 2007). Calves born at a significantly lower birth weight (~9 kg) likely experiencing IUGR did not display as much compensatory

growth and remained smaller prior to weaning. This difference also persisted in the feedlot, but the difference could be caused from the smaller entry weight into the feedlot and subsequently lower intake of nutrients because of the decrease in size (Greenwood and Cafe, 2007). Conversely, calves with lower birth weights were able to overcome weight differences when cows were fed adequate postpartum nutrition (Freetly et al., 2000). The differences in results is likely from the large difference in birth weight, the likelihood that the very small calves experienced IUGR, and the inability to consume enough nutrients in the feedlot to make up the weight differences prior to slaughter. As proven by a few of the previously mentioned experiments, IUGR is difficult to document in maintained pregnancies, but it is more likely to occur in smaller framed and younger animals. Therefore when evaluating data and research it is important to analyze cow size and age in relation to nutritional treatments and subsequent results. Intrauterine growth restriction is associated with subsequent progeny having altered body composition and altered composition of gain, also affecting DMI, and subsequently affecting feedlot performance. These consequences could have large impacts on cattle production systems when younger and smaller framed cattle potentially produce smaller calves, therefore producing smaller carcasses. This specific consequence is important with our current beef situation where we have decreasing cattle numbers and will need to increase the size of beef carcasses in order to feed the increasing population. Therefore, it is important to understand the mechanisms regulating fetal growth.

Immunity

Another area with limited research focus is the association of maternal nutrition on the development of the immune system of the resultant calf. Development of the immune system is necessary as it functions to protect the body from invasion of foreign substances. Immunity refers to how the body protects itself from invasion of foreign substances, such as bacteria, and can be divided into two different branches: innate (less specific) or acquired/ adaptive (specific). The innate immune system is the body's first line of defense and is non-specific in nature. This branch of immunity reacts very soon after appearance of an antigen within the body because most components are present in the body prior to infection. The innate system uses phagocytic cells, like macrophage cells, to clear threats from the body, but also uses these cells to connect with the adaptive immune system through the release of signaling proteins like cytokines. The adaptive immune system is specific and designed to recognize and remember specific pathogens. It is the second line of defense used to control pathogens that escape the innate immune response (Kindt et al., 2007; Lippolis, 2008). The adaptive immune response can be separated further into two different branches: humoral and cell-mediated immunity (Kindt et al., 2007). Humoral immunity utilizes B lymphocytes, which originate from bone marrow, to respond to antigens. These lymphocytes will become antibody-producing cells, or memory cells providing defense against infection (Galyean et al., 1999). Cell-mediated immunity utilizes T-lymphocytes, originating from the thymus, and corresponding cytokines to defend against intracellular pathogens (Galyean et al., 1999). Collectively, the body uses its adaptive immunity for memory of specific foreign

pathogens; therefore it will develop over time through the use of vaccines (Salak-Johnson and McGlone, 2007). Another part of the bodies' ability to fight infection includes acute phase response proteins. Proteins primarily made in the liver respond to signals from various cytokines during a local inflammatory response to induce a systemic response (Kindt et al., 2007). Acute phase proteins, like serum-amyloid-A and haptoglobin, are associated with induction of a fever, and increased production of white blood cells (Kindt et al., 2007). Acute phase proteins work in conjunction with cytokines to elicit the best inflammatory response possible.

Adaptive Immunity

Immunity can further be defined as active or passive. Active immunity develops when a body encounters a foreign pathogen, or receives a vaccination. Passive immunity develops from a mother passing her developed antibodies to her offspring usually through milk. Specifically, calves must acquire passive immunity soon after birth, since cows are unable to transfer antibodies from the dam to the fetus through the placenta. Passive transfer of immunoglobulins occurs through ingestion of colostrum, the secretions from the mammary gland right after birth. Not only does colostrum contain antibodies used to fight infection, but it also contains other immune cells like neutrophils and macrophages, which can be absorbed and used by the calf (Cortese, 2009). The protection a calf will receive from colostrum is a function of quality, quantity, and timing (Besser and Gay, 1994). Passive transfer is the only type of immunity a neonatal calf has, therefore it is necessary for the thriftiness of the calf. The immune status of a neonatal calf can have profound health implications for later in life. Neonatal calves with low 24 hr IgG levels

and total plasma protein levels had higher incidences of mortality and morbidity pre- and post-weaning (Wittum and Perino, 1995). In this study cow colostrum quality or quantity was not measured therefore it is difficult to determine if the calf was unable to properly absorb colostrum or if there was just inadequate colostrum produced by the dam. In relation to absorption of colostrum, Trahair et al. (1997) reported immature small intestines from sheep that experienced maternal nutrient restriction during gestation. This may indicate fetal programming can retard the animals' ability to absorb colostrum, leaving the animal vulnerable to disease. If these intestinal differences persisted in cattle, absorption of immunoglobulins could be negatively affected. However, Meyer et al. (2010) reported an increase in total intestinal vascularity in nutrient restricted fetuses suggesting the intestine was being programmed to scavenge nutrients more efficiently. Absorption of immunoglobulins may be a different situation than what Meyer saw, as absorption of immunoglobins occurs through pinocytosis in order to absorb whole proteins across the intestinal epithelium (Bush and Staley, 1980). The ability of a nutrient restricted calf to absorb whole proteins through pinocytosis may be a potential problem leading to increased morbidity and mortality rates postnatally.

Furthermore, differentiation of organs occur during the early stages of gestation (Funston et al., 2010). During this time, development of the thymus, responsible for T lymphocytes/ T cells will occur around day 25 (Hubbert et al., 1972) and will reach its maximum size as a percentage of body weight near mid-gestation (Cortese, 2009). As previously mentioned the thymus is responsible for cell-mediated immunity within the body to defend itself against foreign pathogens. This type of immunity evolves when the

animal encounters more pathogens. Most research has evaluated the effects of maternal nutrition and its effects on health in relation to the last trimester of gestation, as this is when the dam begins to start producing milk. Reducing the energy supplied to a gestating dam during the last 90 days of pregnancy results in increased morbidity and mortality rates in calves from those restricted dams (Corah et al., 1975). Heifers generally have more problems in relation to calf health likely due to stress related to dystocia and decreased concentrations of immunoglobulins; however this issue is likely caused by a decrease in the volume of colostrum produced by the heifer (Odde, 1988). This suboptimal immune function is troublesome as morbidity during the neonatal period not only increases the risk of mortality, but it also reduces performance later in life (Funston et al., 2010). Decreased performance alone during the postnatal period can reduce weaning weights up to 15 kg (Wittum et al, 1994). To date exact mechanisms have not been elucidated to make a connection between maternal nutrition and calf health. Where the disparity lies, between colostrum or the ability to absorb antibodies from the colostrum, still needs to be discovered through research (Funston et al., 2010). Health is an important issue to producers. Not only is poor health costly because of the treatment costs of a sick animal, but it is also time consuming to treat sick animals and it impacts the overall welfare of the individual. Currently little research exists to connect potential health problems with maternal nutrient alterations. Of that research, most evaluated the effects of late gestation protein supplementation on calf health. To date little, if any research has evaluated the influence of maternal nutrient restriction during mid-gestation on animal health.

Myogenesis

Animal agriculture produces three main consumable products: eggs, meat, and milk. Of these, meat animal production goals are often focused on maximizing the amount of skeletal muscle per animal. With the current world population it is necessary to maximize production by enhancing muscle growth, and potentially reducing fat accumulation, which would result in increased efficiency per animal (Du et al., 2011). Conversely, marbling or intramuscular fat is essential for palatability making efficiency and quality antagonistic goals. Furthermore, carcasses are composed of muscle, adipose tissue, and connective tissue all differentiating from mesenchymal multipotent cells (Du et al., 2011). Of the three tissue depots skeletal muscle has the most economic significance. Muscle development begins in early gestation during the embryonic stage (Du et al., 2010a). In the early stages of development there are many demands for nutrients ranging from brain development to muscle development, with important organs taking precedence over muscle development. This leaves muscle development vulnerable when there is a nutrient deficiency (Close and Pettigrew, 1990). This is of special note because there is no net increase in muscle fiber numbers after birth (Zhu et al., 2004). This consequence is of high value to producers because a pre-natal decrease in muscle fiber number will result in a permanent reduction in muscle mass, as well as negative effects on animal performance (Du et al., 2010a). Myogenesis, or the formation of muscle, spans the life of an animal; however muscle fiber formation generally occurs during the embryonic and fetal stages of development (Aberle, 2001). Muscle undergoes a significant level of hyperplasia during the embryonic and fetal phases of

development while undergoing hypertrophy during the last trimester of gestation (Aberle, 2001). Specifically during early gestation primary myogenesis is occurring, followed by secondary myogenesis during mid-gestation (Du et al., 2010a). As this tissue is not necessary for survival, muscle development could be compromised in the event of altered nutrient uptake by the fetus (Du et al., 2010a; Du et al., 2011; Zhu et al., 2004). Specifically, primary myofibers form within the first two months after conception, but these are limited in number and work primarily to form scaffolding for secondary muscle fibers to develop. Therefore events that impact development are not as detrimental to muscle mass at this point (Du et al., 2010a; Russell and Oteruelo, 1981). The majority of muscle mass develops from secondary myogenesis, which occurs from month 2 through 7 or 8 months of gestation (Beermann et al., 1978; Russell and Oteruelo, 1981). Secondary myogenesis and these secondary fibers constitute the bulk of muscle mass of the offspring. Nutrient alterations during this point in development can reduce fiber number and cause lasting effects on the subsequent progeny (Russell and Oteruelo, 1981). Sheep fetuses encountering a 50% nutrient restriction of TDN according to NRC requirements during early to mid-gestation resulted in a reduction of muscle fiber numbers in the resulting progeny (Zhu et al., 2004). This severe nutrient restriction resulted in permanently decreased muscle mass in nutrient restricted lambs. This disparity between treatment groups is likely caused by difference signaling pathways *in utero* in order to maintain pregnancy. This signaling pathway would commit mesenchymal multipotent cells towards adipogenesis and away from myogenesis in the developing fetus if the dam is trying to maintain pregnancy (Kollias and McDermott,

2008). Because a majority of muscle fibers form during this time a reduction in muscle fibers results in permanent negative consequences for the animal (Stannard and Johnson, 2004; Zhu et al., 2006). During mid-gestation adipogenesis begins in the ruminant animal and therefore could be competing with myogenesis for cell commitment (Feve, 2005; Gnanalingham et al., 2005). During the third trimester of gestation muscle fiber hypertrophy occurs (Du et al., 2010a). This stage of gestation contains most prenatal growth for the animal and therefore is most vulnerable to reduction in size. A common way researchers can quantify growth over time postnatally is through measuring an animals' weight. Weaning weights increased by 1.53 kg when birth weight was increased by 1 kg when dam factors were not taken into account. Robinson et al. (2012) saw an increase in final weight when birth weight and weaning weight were increased, suggesting frame size and growth potential can be altered by nutritional status of the cow during gestation if birth weight and weaning weight are affected by nutritional status of the cow. Feed intake was also affected by birth weight and weaning weight in a positive trend (Robinson et al., 2012). These results suggest a change in growth possibly relating to myogenesis, where decreased myogenesis result in lighter birth weights and ultimately produce lighter cattle. The factors controlling growth and myogenesis continue to be of interest to researchers, as manipulation of these regulators could lead to an improvement in beef production.

Factors Controlling Myogenesis

As previously mentioned, skeletal muscle development begins during the embryonic stage with more differentiation of mesenchymal multipotent cells occurring

during the second trimester. The mesenchymal multipotent cells will commit or differentiate towards myogenesis following signaling factors that either promote or inhibit myogenesis. Most of these signals come from different transcription factors that are known to be necessary for myogenesis: myogenic regulatory factors (MRFs) including MyoD, myogenic factor 5 (Myf5), myogenic regulatory factor 4 (MRF4), and myogenin (Berkes and Tapscott, 2005). These factors are necessary for stem cell determination and terminal differentiation working through signaling cascades while repressing other factors, which result in gene expression that is very closely regulated (Berkes and Tapscott, 2005). Of the four discussed, MyoD and Myf5 are necessary as they are myogenic commitment factors for stem cells of myogenic lineage (Berkes and Tapscott, 2005). Myogenin can also initiate myogenic commitment, but is thought to be most necessary as a terminal differentiation factor (Berkes and Tapscott, 2005; Sabourin and Rudnicki, 2000). Myogenic regulatory factor 4 has been shown to work in both ways, as a commitment factor and a terminal differentiation factor (Berkes and Tapscott, 2005). Signals from Wingless and Int (Wnt) and Sonic hedgehog regulate the expression of other transcription factors (Kassar-Duchossoy et al., 2005). The Wnt pathway is a β-catenin-dependent signaling pathway that controls the expression of transcription factor Pax3 (Capdevila et al., 1998; Huelsken and Birchmeier, 2001). Pax3 and Pax7 also directly play a role in myogenesis by initiating the expression of the previously mentioned MRF's (Munsterberg et al., 1995). A double knockout mouse for the Pax3/Pax7 genes caused MyoD and Myf5 to not be activated in myogenic progenitor cells resulting in either cell death or incorporation of cells into other tissues like

adipocytes and fibroblasts, illustrating the regulation Wnt and Pax transcription factors play on myogenesis and the expression of MRFs (Buckingham et al., 2006; Munsterberg et al., 1995). In addition, the Wnt integration site family is associated with other developmental processes like postnatal muscle regeneration and differentiation, proliferation, and cell migration (Shang et al., 2007). In vitro experiments determined that Wnt3a activated Pax7, MyoD, Myf5, Myf4, and myogenin, while down regulating adipogenic differentiation factors like CAAT enhancer binding proteins (C/EBP) α, and peroxisome proliferator activated receptor (PPAR) γ (Shang et al., 2007). In short Wnt3a can induce myogenic signaling and inhibit adipogenic differentiation *in vitro*. Myocyte enhancer factor-2 (Mef2) plays a role in regulating cell proliferation by stopping a variety of different intra-cellular signaling pathways that prevent muscle differentiation (Black and Olson, 1998). This factor, Mef2, controls the transcription of genes that are also involved with cell proliferation in relation to muscle development (Black and Olson, 1998). Following the signaling of these specific factors myoblasts will undergo differentiation, followed by regulation of myogenesis by these same signaling factors (Du et al., 2010a; Kollias and McDermott, 2008). If the β-catenin pathway is blocked the total number of myocytes will be reduced and other tissues, like adipose tissue, will develop from the mesenchymal stem cells (Du et al., 2010a; Pan et al., 2005). On the contrary to most of the previously mentioned growth factors, myostatin is a growth and differentiation factor which negatively affects myogenesis by controlling proliferation of myoblasts. In vitro, myostatin inhibits MyoD function which will stop differentiation of myoblasts into myotubes (Langley et al., 2002). Stem cell differentiation is controlled

and regulated by a variety of signaling factors. Since 3 tissue types are derived from the same pool of mesenchymal stem cells these signaling factors likely alter gene transcription in response to stimuli from the fetus dependent on environmental conditions. One such condition could be nutrient availability, where muscle differentiation and growth could negatively be impacted if there is a lack of nutrients.

Adipogenesis

Along with muscle development, the amount of adipose tissue accretion within the animal can affect the value of the beef animal. Adipose tissue can be divided into four main depots: subcutaneous, visceral, intermuscular, and intramuscular fat. Each tissue is located in a different area; subcutaneous fat between the hide and the muscle of the animal, visceral fat surrounds the organs of the animal, intermuscular or seam fat is located between muscles, and intramuscular fat is located within the muscle. Subcutaneous fat is used to calculate USDA yield grades. Visceral fat is used to protect and insulate organs. Intramuscular fat or marbling deposition is critical to the flavor and juiciness of beef products. In beef production the two most relevant fat depots discussed are subcutaneous fat or backfat as it relates to cutability, and intramuscular fat or marbling as it relates to quality. Increased amounts of subcutaneous fat will negatively impact yield grade in cattle, which could cost beef producers' money. Yield grade is calculated based on carcass weight, muscling, and carcass fatness. Excessively fat cattle, which would have a higher yield grade, receive discounts at the packing plant. Conversely, marbling is one of the factors evaluated when assigning quality grades to carcasses and producers can receive premiums when they produce high grading cattle. In

the beef industry researchers are trying to elucidate mechanisms to increase marbling without also increasing backfat and decreasing muscle. We currently know that along with genetics, many postnatal strategies can affect marbling such as time on feed, environmental factors, management strategies, and plane of nutrition, which affect the number and size of intramuscular adipocytes (Du et al., 2010a). New directions of research are focused on elucidating other methods to positively influence marbling development in beef cattle. Before we can start manipulating development within a beef animal to maximize quality and cutability an understanding of adipose tissue development is needed.

Adipocytes and myocytes are both derived from mesenchymal stem cells, which are also precursors to many other cell lineages (Aberle, 2001; Du et al., 2010b). These stem cells are rich in skeletal muscle during development, but decrease as the animal ages (Du et al., 2010b). Most of the mesenchymal stem cells will differentiate to muscle fibers, but a few will become adipocytes, which are the cells collectively called marbling later in development (Du et al., 2010b; Tong et al., 2009). Mesenchymal stem cells or fibroblasts differentiate into adipoblasts as the precursor to preadipocytes (Gerrard and Grant, 2003). An adipoblast can continue to proliferate into new cells that will also become preadipocytes (Hausman et al., 2001). Preadipocytes are the precursors to adipocytes or fat cells which accumulate lipid composed mainly of triglycerides (Aberle et al., 2001). As the preadipocyte begins to fill with lipid it is committing towards a mature terminally differentiated adipocyte and to the adipogenic lineage (Aberle, 2001; Gregoire et al., 1998). Growth of the developing adipocyte will continue until the cell

has reached a maximum cell size (Faust et al., 1978). At this time mature adipocytes will signal for recruitment of preadipocytes to begin lipid accumulation, which is in contrast to muscle cells which cannot recruit more cells after birth (Hausman et al., 2001). This recruitment and lipid filling will continue as long as the animal is in a positive energy balance in order to store energy. The difference in postnatal cell recruitment between muscle and adipose tissue development has drastic implications for animal agriculture. The potential for postnatal alterations to adipose tissue development has far reaching implications for the enhancement of animal agriculture. Unfortunately this alteration to adipose tissue does not only apply to marbling, but also subcutaneous fat. We know marbling can be affected through postnatal alterations using different management practices. Current research is looking for new ways to alter adipose deposition, possibly by managing maternal nutrition during gestation.

As previously mentioned adipocytes are derived from the same pool of cells as muscle fibers during the fetal stages of development (Du et al., 2010a). Therefore marbling in cattle could also be affected by fetal programming. However, prior to marbling development, intramuscular adipocytes need to be in place prior for lipid accumulation to occur during the finishing phase. Additionally, the greater number of intramuscular adipocytes available for lipid accumulation during the finishing phase, the greater the chance for improving Quality grade. In sheep, adequate maternal nutrition allows for a greater number of mesenchymal cells available to the fetus, increasing the chances for those cells to be committed towards adipogenesis (Du et al., 2010a). If this cause-effect type relationship is occurring in sheep it could also occur in cattle. There

is minimal adipose tissue development prior to birth, but adipose depots grow during postnatal growth through the use of nutrition and energy content of the diet, as well as time on feed (Aberle et al., 2001). Before we can utilize postnatal strategies to improve carcass quality, we need to be able to recruit more precursor cells towards adipogenesis. Prior to manipulating mesenchymal multipotent stem cells an understanding of the mechanisms and signaling pathways that affect adipogenesis is needed.

Factors Controlling Adipogenesis

Since adipose tissue is derived from the same mesenchymal multipotent stem cells as muscle tissue, the differences in signaling pathways between the 2 tissue types should be discussed. Mesenchymal stem cells respond to a multitude of factors which will determine how those cells differentiate. There are 3 main transcription factor families that regulate adipogenesis (Saladin et al., 1999). Two of the better known regulators in adipogenesis are 1) C/EBP α, β, and δ and 2) PPAR α, β, δ, and γ (Saladin et al., 1999; Wu et al., 1999). Another transcription factor, helix-loop-helix adipocyte differentiation and determination factor-1, is not as researched in livestock compared to the other 2 transcription families, but still plays a part in regulation (Saladin et al., 1999). The main two transcription factor families, C/EBP and PPAR, influence the proliferation and differentiation of preadipocytes to mature adipocytes in a positive feedback loop stimulating each other to signal cells to differentiate (Wu et al., 1999), which is necessary for continued adipogenesis. It has also been shown that adipogenesis is also controlled by the Wnt signaling pathway (Du et al., 2010a). Specifically, PPARγ is regulated by β-catenin, which is part of the Wnt signaling pathway (Moldes et al., 2003). Upregulation

or downregulation of the Wnt pathway will affect both myogenesis and adipogenesis (Du et al., 2010a). If β-catenin is not degraded, then it will inhibit the expression of PPARγ, which will decrease the signaling towards adipogenesis (Okamura et al., 2009). Overfed ewes displayed downregulated Wnt/β-catenin signaling resulting in down-regulation of myogenesis and an up-regulation in adipogenesis illustrating maternal nutrition can affect signaling pathways of mesenchymal stem cells. Manipulation of these pathways due to maternal nutrition will help us to better understand development within the bovine animal (Tong et al., 2008; Zhu et al., 2008). Adipogenesis is an important developmental process within the animal, not only for storage of excess energy, but also from a meat quality standpoint. Marbling is a predictor of palatability used by USDA, and therefore is an essential marketing tool within the industry. An increase in quality grade results in an increase in revenue for the producer. Understanding the mechanisms that mediate marbling will continue to be a critical component in improving beef quality.

CONCLUSION

Unhindered fetal development is necessary in order to ensure proper growth and health of the resultant animal. Because there are numerous signaling factors at work directing satellite cells to different tissue lineages it is pertinent that the dam has adequate nutrients available to support the growing demands of the fetus. In cattle the average gestation length is nine months, which covers three seasons in the upper Midwest. Because of the changes in weather, cows can experience anything from heat and cold stress to inadequate nutrition due to drought or snow cover. Any of these stresses can alter the gestational environment of the fetus and potentially "program" it to deal with

similar situations when it is born. This is often at the expense of the fetus and the cow whether it is the cow losing body condition, low calf birth weight, poor calf health, or altered body composition in order to deal with being born into a nutrient sparse environment. In cattle this could be both positive and negative in that there will be increased fat deposition with decreased muscle, but improved flavor and juiciness of the product.

Because of all of the developmental processes occurring during gestation there is evidence to support supplementing gestating cows during the winter months. However, since feed costs are one of the largest expenses to a cow calf producer, many producers want justification for supplementing their cows during the winter months. Instead of a supplementation program, some producers will allow cattle to "rough it" during the winter months because of their low nutrient requirements in order to implement a low cost feeding program. But in doing this the fetus can encounter periods of nutrient restriction during critical periods of myogenesis and adipogenesis. To date most fetal programming research has investigated the results of first and third trimester nutrient restrictions and the effects on growth and carcass characteristics. Alterations in post-weaning growth in response to fetal programming have not been well characterized. Additionally, research has evaluated the effects of late gestation nutrient restriction on passive transfer in the resultant progeny. However, very little research has evaluated morbidity after weaning. In its entirety, very little research has evaluated the effects of mid-gestation nutrient restriction on offspring postnatal growth, feedlot performance, gene expression, and cattle health.

Therefore the objectives of this dissertation were:

1) To determine the effects of dietary energy restriction on measurements associated with cow body condition and metabolic indicators of energy status.

2) To determine the effects of maternal energy restriction during mid-gestation on birth weight, weaning weight, and growth performance of offspring.

3) To determine the effects of maternal energy status during mid-gestation on the humoral immune response in beef cattle during the receiving period by evaluating antibody titers to a novel antigen.

4) To determine the effects of maternal energy status during mid-gestation on gene expression in bovine subcutaneous adipose tissue at weaning and finished weight in the resultant offspring.

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CHAPTER II

The influence of energy restriction during mid-gestation on measurements associated with cow body condition and metabolic status

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ABSTRACT

Fetal programming research evaluates environmental changes a dam encounters during gestation that can have lasting effects on the resultant progeny. One of these changes can be alterations in nutrient intake of the dam due to forage quality and availability. In the upper Midwest cows on pasture encounter periods of inadequate forage quality during mid-gestation as this period commonly coincides with the winter months. Periods of inadequate forage quality can result in a negative energy balance for the cow during gestation, potentially leading to alterations in fetal development. An understanding of how changes in dietary energy influence measurements associated with cow body condition and metabolic blood metabolites will allow for a better understanding of fetal programming events. Therefore, the objective of this study was to determine the effects of dietary energy restriction on measurements associated with cow body condition and metabolic indicators of energy status. Naturally serviced crossbred beef cows (n=151) were assessed for pregnancy, day of gestation, cow body weight (BW) and body condition score (BCS). Cows were allotted to one of two treatment groups: 1) (76 cows)-fed to achieve and/or maintain BCS 5.0-5.5 (control diet; Positive Energy Status (PES)); or 2) (75 cows)-fed to lose 1 BCS over the ensuing 91 d treatment period of mid-gestation (restricted diet; Negative Energy Status (NES)). Cows were weighed

and blood was collected for analysis of insulin, blood urea nitrogen (BUN), and non-esterified fatty acids (NEFA) every 28 d throughout the treatment period. Ultrasound measurements of 12th rib subcutaneous fat thickness (FT) and ribeye area (REA), and visually assessed for BCS at the beginning and the end of the treatment period. After completion of the treatment period, all cows were managed as a common group on native range through subsequent weaning. Cow BW, BCS, REA, and FT were different *(P<0.05)* between the PES and the NES groups at the end of the treatment period. There were no differences between treatments when evaluating insulin at d 0, 28, 56, and 84 *(P>0.05*), as well as no differences in d 56 NEFA concentrations *(P>0.05)*. However, there were differences (*P<0.05*) in NEFA concentrations at days 0, d 28, and d 84 between treatments with greater concentrations in the control cows. There were no differences in PUN concentrations on d 56 and d 84 *(P>0.05)*, but PUN concentrations between management groups were different (*P<0.05*) on d 0 and d 28 of the treatment period with greater concentrations in the control cows. The measurements associated with cow body condition suggest the cows achieved the intended outcome of the dietary treatments. Positive energy status cows maintained their weight or gained weight and body condition and the NES cows lost weight or body condition during the mid-gestation treatment period. Differences identified in blood metabolite concentrations between the management groups were not expected. Upon further analysis of the data and literature it was determined blood collection procedures likely contributed to the counterintuitive results of blood metabolites.

INTRODUCTION

Fetal programming or developmental programming is an area of research that evaluates the effects of a stimulus or an insult encountered by the dam during gestation and the consequential effects on the developing fetus. Stimuli or insults that occur during critical periods of development have been shown to have lasting effects on offspring metabolism, physiology, and structure (Godfrey and Barker, 2000). In the agricultural industry it is common for livestock to encounter insults throughout the year, specifically caused by weather and its impacts on pasture conditions. In the United States many cows graze native range pasture as the primary source for nutrients. Depending on the region of the country cows can encounter low nutrient consumption due to drought, or dormant forages in relation to winter in the upper Midwest (Vavra and Raleigh, 1976). This can result in a period of inadequate nutrition to gestating cows at some point during pregnancy. In addition, producers ideally would like to implement low cost feeding programs in order to be more profitable. Often inadequate nutrition during the first half of gestation is overlooked because of the low nutrient requirements at that time (Funston et al., 2010; Reynolds and Redmer, 1995). While the majority of bovine fetal growth occurs during the last 2 m of gestation, many important events occur earlier in gestation such as cell differentiation, placental growth, vascularization, and fetal organogenesis which are necessary for normal fetal development (Bell et al., 1987; Funston et al., 2010). Specifically, cell differentiation can affect body composition of the fetus later in life as mesenchymal stem cells will differentiate into muscle, fat, or fibroblasts (Du et al., 2010). Because fetal cell differentiation and tissue growth could be affected by nutrient intake of the dam, the effects of poor cow condition during mid-gestation on progeny

characteristics should be evaluated. However, cow condition can often be difficult to determine. Practical ways of determining energy status of an animal include evaluating body condition and measuring weight. However, measuring blood metabolites and hormones provides a way to quantify cow energy status and understand the gestational environment. Measurement of different bi-products of metabolism aid in determining the nutritional and physiological state of the animal (Sletmoen-Olson et al., 2000) as these metabolites and hormones can be indicators of supply, use, and excretion of nutrients within an animal (Ellenberger et al., 1989). Therefore the objective of this study was to determine the effects of dietary energy restriction on measurements associated with cow body condition and metabolic indicators of energy status.

MATERIALS & METHODS

Animals

The South Dakota State University Animal Care and Use committee approved the following animal experiment. Naturally serviced crossbred beef cows (n=151) were bred to begin calving at the end of March. Approximately 38 d following removal of bulls from cow pastures, calves from the previous year were weaned and cows were transrectally ultrasounded for pregnancy, day of gestation, and calf gender. At this time cows were also evaluated for BW and BCS (1 to 9, 1 = extremely emaciated, 9 = extremely fleshy). Following pregnancy diagnosis cows from 2 different SDSU research stations were comingled and managed similarly on native range pastures at the SDSU Cottonwood Range and Livestock Research Station in Cottonwood, SD until allotted into treatment groups at mid-gestation. Allotment into mid-gestation treatment groups was based on day of gestation, source, body weight, age, and BCS. Cows were allotted to one of two treatment groups: Control-fed to achieve and/or maintain BCS 5.0-5.5 (n=76); or Restricted-fed to lose 1 BCS over the ensuing 91 d period of mid-gestation (n=75). At the time of allotment mean day of gestation was 84 ± 11 days, mean cow weight was 495 ± 58 kg, mean cow age was 4 ± 1 year, and mean BCS was 4.9 ± 0.5. After completion of the treatment period, all cows were managed as a common group on native range (NRC software predicts NE=10.9 Mcal/d with intake=20.75 lb/d) and provided a 20% CP supplement through calving.

Dietary Treatments

Diets were determined using the Nutrient Requirements of Beef Cattle (NRC, 2000) software for cows in the control group to gain 1 BCS over 175 d with a NE_m

balance of 1.4 Mcal/d, while restricted cow diets were formulated so cows would lose 1 BCS over a 91 d period with a NE_m balance of -1.8 Mcal/d. During treatment cows in the control group remained on dormant native pasture composed of mostly western wheatgrass (*Pascopyrum smithii*), as well as green needle grass (*Stripa viridula*), little bluestem (*Schizachyrium scoparium*), buffalo grass (*Buchloe dactyloides*), and blue grama (*Bouteloua gracilis*) and were supplemented to achieve energy balance relationships described by the Nutrient Requirements of Beef Cattle (NRC, 2000). (The amount of feed provided was calculated and reported relative to metabolic body size (MBS)). Grass consumption by these cows was not measured, but the NRC (2000) software predicted winter range intake to be approximately 87.5% of the diet (at 4.7% CP). Supplement for control cows (45.7% Crude Protein (CP), 1.65 Mcal/kg NE_m) was formulated to meet the remaining cow requirements which resulted in the supplement being fed at 12.5% of the diet fed every other day. Using these percentages the diet was estimated to provided approximately 88.9 g dry matter (DM)/kg MBS/ hd/ d of winter range (calculated estimate) and 12 .71 g DM/kg MBS/ hd/ d (actual amount of supplement fed). These 2 ingredients composed a diet that was estimated to be 9.8% CP (Table 2.1). Cows in the restricted treatment were blocked by weight into 10 pens containing 7 or 8 cows per pen and fed 65.83 g DM/ kg MBS/ hd/ d mature brome hay and 11.80 g DM/kg MBS/ hd/ d of a protein supplement (31.4% CP, 1.58 Mcal/kg NE_m) daily. Cow drylot diets consisted of 84.8% hay and were top-dressed with 15.2% supplement which provided 9.7% CP (NRC software predicts metabolizable protein=691 g/d) (Table 2.1). Feed samples of hay and both supplements were collected during the

treatment period in order to describe DM, CP, acid detergent fiber (ADF), and neutral detergent fiber (NDF) content (Table 2.2).

Cow measurements

Cows were weighed every 28 days throughout the treatment period, and ultrasound measurements (Aloka 500V real-time ultrasound machine, Aloka, Wallingford, CT) were collected to determine FT and REA at the beginning and the end of the treatment period. Body condition scores were also evaluated at the beginning and end of the treatment period using the average BCS of 4 trained evaluators. For calculation of BW change cows were weighed one week before initiation of the mid-gestation treatment period and one week after completion of the treatment period when cows were managed as a common group in order to normalize fill.

Blood Sample Collection

Blood samples from all cows were collected on day 0, 28, 56, and 84 of the treatment period. Blood samples were collected after cows had been withheld from water and feed overnight. Blood was collected via jugular venipuncture using an 18 gauge needle and commercial vacuum glass tube (Vacutainer, 10 mL, Benton Dickinson, Franklin Lakes, NJ). Blood was allowed to clot at 4°C for 1 h and then centrifuged at 1650 x g for 30 min at 4°C. After centrifugation serum was aliquotted into polypropylene microcentrifuge tubes and stored at -20°C for subsequent analyses of blood metabolites. Three animals were subsampled from each weight block for a total of 30 cows per treatment for analysis of blood metabolites. All blood sample analyses were analyzed allowing for a 5% coefficient of variation within each individual animal replicate in each assay.

Analysis of Blood Urea Nitrogen

Blood urea nitrogen concentration was determined using a colorimetric assay according to the procedures of Fawcett and Scott (1960) and Chaney and Marbach (1962). Each tube was read on a spectrophotometer (Lambda 25, PerkinElmer, Waltham, MA) at a wavelength of 634 nm.

Analysis of Insulin

Serum insulin concentrations were determined using duplicate 100 µL aliquots of serum with a Linco Porcine Insulin Radioimmunoassay (RIA) (PI-12K, Linco Research, St. Charles, MO), and bovine insulin (I5500, Sigma-Aldrich, St. Louis, MO) as the standard. The RIA was performed according to manufacturers' protocol. The bovine insulin standard was validated compared with the porcine insulin standard before initiation of the analyses. The precipitate was collected by centrifugation at 2,000 x g for 30 min at 4°C, and the supernatant was discarded. Assay tubes containing the precipitate pellet were counted for 1 min on a gamma counter (Wizard 1470 Automatic Gamma Counter, PerkinElmer, Waltham, MA).

Analysis of Non-Esterified Fatty Acids

Serum non-esterified fatty acid concentrations were determined in triplicate serum aliquots using a colorimetric assay according to manufacturers' protocol (HR Series NEFA-HR(2) Wako Diagnostics, Rickmond, VA). Each plate was read on a microplate reader (SpectraMAX 190, Molecular Devices, Sunnyvale, CA) at a wavelength of 550 nm.

Cow Management Analysis

Upon analysis of cow data it was determined that some cows within each dietary treatment group did not achieve the goals physiologically of the treatments. The intended treatment for the current experiment was to alter the uterine environment during mid-gestation. In order to achieve two physiological states different diets were used to maintain or lose body condition during mid-gestation. Because dietary treatment (control versus restricted) was not the intended treatment, cows and their calves were divided into energy status categories (PES versus NES). This re-classification of animals created 2 new treatment groups as there was a bimodal distribution within the population: PES and NES were calculated from metabolic indicators including BCS, REA, and BW collected during gestation. The formula used is as follows:

$$\left[\frac{(Obs\,BCS\;\Delta - BCS\;\Delta\,\bar{x})}{BCS\;\Delta\,S_d}\right] + \left[\frac{(Obs\,REA\;\Delta - REA\;\Delta\,\bar{x})}{REA\;\Delta\,S_d}\right] + \left[\frac{(Obs\,BW\;\Delta - BW\;\Delta\,\bar{x})}{BW\;\Delta\,S_d}\right]$$

The whole population of cows was used to calculate the mean and standard deviation for each variable. The bimodal distribution occurred around 0 and cows with a positive number were deemed PES, whereas cows with a negative number were deemed NES during mid-gestation. Two cows fell in the middle of this distribution and were removed from further analysis (both originally in the restricted dietary treatment). This resulted in the PES group containing 79 head and the NES group containing 70 head. This re-classification resulted in 6 cows moving from the restricted dietary treatment into the PES group and 3 cows from the control dietary treatment moving into the NES group (Mohrhauser, 2013). This re-classification of treatments allowed analysis to be more specific towards our intended treatment goals. Cow energy status was used to determine

differences in BW, BCS, REA, and FT. However, cow dietary treatment was used to evaluate differences in blood metabolites and insulin concentrations. We chose this method because the management of cows prior to blood collection based on dietary treatment likely had an influence on these blood parameters and therefore should be left in the original treatment for statistical analysis.

Statistical Analysis

Least squares means for phenotypic traits, blood metabolites, and hormones were computed using PROC GLM procedures of SAS (SAS Inc., Cary, N.C.). Differences due to the main effects of cow energy status and block were tested using the interaction of cow energy status and block as the error term. Means were tested to a predetermined significance level of $P<0.05$.

RESULTS

In order to determine the effects of dietary energy restriction on measurements associated with cow body condition and metabolic indicators of energy status BW, BCS, REA, FT, and blood were collected from cows in each treatment group. The change from the beginning of the treatment to the end of the treatment was significant for all of these measurements associated with cow body condition (Table 2.2). Specifically, the weight change between the two treatment groups was positive for the PES group and negative for the NES group *(P<0.05)* with the total difference in weight change equaling 74 kg between the groups. The change in BCS was significant between the two treatment groups with the PES group having a positive BCS and the NES group having a negative BCS *(P<0.05)*. The change in REA mirrored the BW and BCS responses as the PES group gained REA during the treatment period but the NES group decreased REA *(P<0.05)*. Fat thickness was also different between the two treatment groups with the PES group having an increase in FT during mid-gestation and the NES group had a decrease in FT *(P<0.05)*. These data suggest we were achieving the intended outcome of the treatment as the PES cows maintained their weight or gained weight and the NES cows lost weight during the mid-gestation treatment period.

In addition to measurements associated with cow body condition, cow blood metabolites were analyzed and results are shown in Table 2.3. No differences *(P>0.05)* in insulin were observed between the two treatment groups at any sampling time during mid-gestation. However, differences in NEFA concentrations were detected at d 0 *(P<0.05)*, 28 *(P<0.05)*, and 84 *(P<0.05)* with greater concentrations detected in the control cows. There were no differences in d 56 NEFA concentrations between treatment groups (P>0.05).

Additionally, control cows had greater BUN concentrations on d 0 *(P<0.01)* and 28 *(P<0.05)* of the treatment period, but there were no differences between treatment groups for BUN concentrations on d 56 and d 84 *(P>0.05).*

DISCUSSION

Cow-calf operations make use of non-tillable land, or rangeland, for production purposes. At times these pastures can be deficient in nutrients whether it is caused by drought, snow cover, or simply maturing forages (Vavra and Raleigh, 1976). Because cows are generally on pasture year round, periods of inadequate nutrition, specifically in energy, can occur. Many times these energy deficiencies come during the winter months where spring calving herds are in the middle of gestation. This period of time is developmentally sensitive as this is a time when most of muscle development is occurring and adipose tissue development is starting to occur (Du et al., 2010). Therefore, understanding how cows metabolically react to energy restriction during gestation will help determine alterations in the uterine environment and how that will affect a developing fetus.

Beef cow-calf producers are constantly striving to improve their production systems in order to develop more efficient practices for the production of beef. Two primary factors contribute to the profitability of cow-calf operations: reproductive performance and nutritional status of the animal (Hess et al., 2005). Lost income from reproductive unsoundness result in loss of income due to the lack of a calf and extra feed costs associated with feeding open cows (Bellows et al., 2002). Feed costs comprise over half the input costs annually for maintaining a cow and can have the greatest effect on commercial cow-calf operation profitability (Miller et al., 2001; Taylor, 1984). Of the factors that can influence profitability, nutrition demands the most attention as it is correlated to reproductive soundness (Hess et al., 2005) and livestock producers can control cow nutrition (Dunn and Moss, 1992). In addition to numerous reported effects

on reproduction, recent research suggests that maternal nutrition can impact the fetus later in life; this area of research is collectively termed fetal programming (Barker and Clark, 1997). These alterations to fetal environment can result in altered development of fat and muscle tissue in the resultant offspring (Symonds et al., 2004; Zhu et al., 2004).

Measurements associated with cow body condition are important for the practical application of the treatments applied in this experiment. Evaluating BCS and weighing cows are methods readily available to producers to evaluate cow body condition without the equipment necessary for running blood assays. Specifically, BCS is a valuable management tool allowing producers to estimate energy status and fat reserves based on visual appraisal (Edmonson et al., 1989; Tiezzi et al., 2013). The cows in the current experiment assigned to the PES treatment maintained a BCS and gained BW over the mid-gestation treatment period, similar to other research (Ciccioli et al., 2003; Selk et al., 1988). Cows in the NES treatment lost body condition and BW over the 91d mid-gestation treatment period which has been shown to result in longer postpartum intervals to first estrus and decreased pregnancy rates following parturition (Ciccioli et al., 2003; Hess et al., 2005). Cows in similar nutrient restriction studies have produced offspring with altered growth traits, carcass characteristics, and tenderness compared to non-restricted progeny (Long et al., 2012; Underwood et al., 2010).

Another technique used to report cow energy status is ultrasound measurements of REA and FT over the 12th rib. Research has shown these measurements can be correlated to nutrient intake in relation to determining cow body condition. Increased size of REA and increased FT are correlated with increased nutrient consumption and cow body condition (Hall et al., 1995). The reciprocal would likely be true where cows

with decreased nutrient intake would have a lower FT and a smaller REA as the current study reveals in the NES treatment group. Cattle in a negative energy balance will catabolize fat stores and lean body tissue to maintain pregnancy and continue with normal body functions when the diet is not providing the maintenance requirements for the animal (Freetly et al., 2008). These periods of time where nutrients can be inadequate are common in cattle grazing native range due to arid environments, dormancy of plants, and weather, all of which affect the quality and quantity of forage consumed by cattle (DelCurto et al., 2000). Additionally, cows will likely experience inadequate nutrition at some point during gestation if not properly supplemented (Martin et al., 2007; Underwood et al., 2010).

Hormone and metabolite analysis were used to assess the nutritional status of the cows during the treatment period, validating that the cow treatment altered the metabolic status of the cow during gestation. Results of blood metabolites and insulin analysis did not meet the expected outcomes of the treatments applied. We expected increased NEFA concentrations in NES cows because it would indicate mobilization of fat stores for maintenance energy and this should correlate to the decrease in BW and BCS that we saw in the NES group similar to Wertz-Lutz et al. (2006). However, cows in the control group had increased concentrations of NEFA and BUN compared to the NES group. But, there were no differences in insulin concentrations for either treatment group. The unexpected outcomes in the control group may be attributed to a few factors upon review of the procedures. Cows in the control group may have had elevated concentrations of NEFA and BUN in the blood as a result of the cows being held off pasture overnight prior to blood collection. Feed restriction in cattle has been shown to cause a negative

energy balance resulting in the mobilization of adipose tissue for energy (Grummer, 1995). Blood NEFA concentrations are negatively correlated with cow energy status as NEFA are one way to measure mobilization of fat stores (Lucy et al., 1991). The control cows were not accustomed to being off pasture and therefore were potentially undergoing a short term feed restriction. This likely caused the elevation in NEFA concentrations similar to what Marques et al. (2012) noted. Additionally, cows in the control group were supplemented every other day, which could have caused those cows to mobilize fat stores on days when no supplement was provided. Moriel et al. (2012) reported that developing heifers supplemented 3 times per week had elevated NEFA concentrations during days when no supplement was provided. Concentrations of NEFA in the restricted group in the current study were different from findings of Wertz-Lutz et al. (2008), where cattle fed at 0.8 times maintenance had greater NEFA concentrations then cattle fed 2.4 times maintenance. In the current study, 3 of the 4 sampling points resulted in greater NEFA concentrations in the control group compared to the restricted group, which is opposite of the Wertz-Lutz et al. (2008) study. When Wertz-Lutz et al. (2006) evaluated eating behaviors, cattle that were off feed for up to 48 h had greater NEFA concentrations than cattle on feed. This suggests similarities between the current study and Wertz-Lutz et al. (2006) where the control cows would have been withheld from feed overnight resulting in elevated NEFA concentrations.

Ruminant animals have the unique ability to recycle nitrogen when a deficiency of protein is encountered. The amount of nitrogen recycled is negatively related to the concentration of rumen ammonia and positively correlated to blood urea nitrogen levels (Owens and Bergen, 1983). Excess dietary protein is deaminated yielding ammonia in

the rumen. Without adequate available energy in the rumen to support bacterial crude protein synthesis, this ammonia leads to increased ruminal ammonia concentrations. Excess ammonia in the rumen enters the portal blood and is converted into urea in the liver (Owens and Bergen, 1983). Additionally, protein breakdown and turnover also contribute to circulating concentrations of amino acids and then contribute to urea concentrations after amino scids are deaminated in the liver (Church, 1993). Highly degradable protein like urea results in increased concentrations of ruminal ammonia leading to increased concentrations of BUN (Church, 1993). The rate of ammonia release ideally should be similar to the rate of fermentation in order to accomplish nutrient synchrony. If ammonia production is different from the rate of fermentation, ruminal concentrations of ammonia will fluctuate leading to changes in BUN concentrations (Church, 1993). In previous research, cattle were fed different sources of supplemental protein, either soybean meal which is a slow degrading protein versus urea which is a highly degradable protein, had different levels of plasma urea nitrogen (PUN). At each sampling time point soybean meal produced lower PUN concentrations than urea (Burris et al., 1975). Similarly heifers supplemented with urea while grazing pasture had higher BUN concentrations and lower average daily gains compared to supplementation of a slower degrading protein, casein (Hennessy and Williamson, 1990). In the current experiment control cows had greater concentrations of BUN at d 0 and 28 compared to the restricted group. The cause for this difference in BUN concentration is unknown, however it is hypothesized that because the cows were held off feed overnight the cows were potentially catabolizing non-essential amino acids in order to provide gluconeogenic substrates (Meijer et al., 1995). Free amino acids in the blood of the cows on this trial

were not analyzed. Additionally the supplement contained urea, a highly degradable source of nitrogen, which could have elevated BUN levels. However, urea was included in both supplements at the same percentage so this is not likely the cause of elevated BUN levels in the control group. Another important consideration is sampling time when determining concentrations of BUN. Peak BUN concentrations occur several hours following feeding (Elrod and Butler, 1993). If the control cows in the current experiment were supplemented the day before blood collection it is possible feeding supplement every other day was why unexpected differences in BUN concentrations occurred.

Insulin is a protein hormone directly involved with glucose regulation. Insulin is used to regulate blood glucose levels by use of insulin receptors found on specific tissues that use glucose as a fuel source. When glucose is high in the blood, insulin will bind to receptors on specific tissues to enhance the ability of the cell to absorb glucose (Hadley and Levine, 2007). Therefore, when animals are in a feed restricted state, insulin concentrations are likely low, allowing for increased mobilization of fat stores and potentially protein degradation if the restriction is severe. No differences were observed in insulin concentrations between the treatment groups in this study. These results do not agree with the results of Radunz et al. (2010), which evaluated insulin concentrations in response to different feedstuffs. The differences between these studies were likely caused by the substrates produced from the different feedstuffs. When evaluating only the hay treatment group in this study compared to the current study there were no differences in insulin concentrations which supports our findings (Radunz et al., 2010). Additionally, Richards et al. (1989) reported that cattle losing weight and decreasing BCS had decreasing concentrations of insulin. The current experiment did not have similar

results to this study, but the cows in the current experiment had very low initial levels of insulin. These differences in results may be caused by differences in sampling method where the cows in the current experiment were held off feed overnight before blood samples were collected and Richards et al. (1989) did not disclose feeding management and blood collection procedures.

It has been demonstrated in dairy cattle in a negative energy balance that the body will mobilize fat, glycogen, and protein for release into the blood for use by the animal (Ingvartsen and Andersen, 2000). It has also been reported that cows in a negative energy balance will lose muscle and fat stores in order to continue to produce milk and support a fetus (Kuhla et al., 2011). Even though there is a body of literature stating negative energy balance cows have increased concentrations of NEFA, PUN and decreased insulin, the blood metabolite profiles in the current study did not reflect the dietary treatments as expected. However, the performance measurements collected from the current experiment did reflect the intended changes in energy status. Cattle in the NES group were adapted to low feed intake. Since they were adapted to this management style holding that group of cattle off feed overnight potentially did not change anything as they had already consumed their feed for the day. In contrast, cattle in the PES group were used to grazing all day so when they were held off feed and water for 12-18 h they were likely experiencing a feed restriction, which is reflected in their elevated NEFA and BUN concentrations. Additionally the supplements included urea, which is quickly degradable. If the cattle in the PES group were supplemented the morning before blood collection it is possible the intake of the supplement elevated the BUN concentrations in that group since they were fed twice as much as they needed and

didn't likely limit their intake. It is undetermined why insulin concentrations did not change, but the initial concentrations were relatively low to begin the experiment. Even though blood metabolite profiles and insulin concentrations were not affected as expected we achieved our goal of creating differences in energy balance between the two treatment groups as evident by the decrease in weight, BCS, FT, and REA of the NES cows. Therefore these dietary treatments can be used to evaluate the effects of a NES during mid-gestation on the resultant fetus.

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Table 2.1. Formulations and compositions of mid-gestation treatment diets.[1]

Diet Composition	Control[2]	Restricted[3]
Estimated Dormant, Native Range, %[4]	87.50	-
Mature Brome Hay, %	-	84.80
Pelleted Supplement, %[5]	12.50	15.20
Soybean Meal[6]	(52.20)	(2.75)
Sunflower Meal[6]	(20.00)	(20.00)
Wheat Middlings[6]	(19.30)	(69.33)
Urea[6]	(3.06)	(3.04)
Vitamins & Minerals	(5.44)	(4.88)
Dry Matter Intake, kg/head/d[7]	10.79	7.69

Nutrient Composition	Control[2]		Restricted[3]	
	Dormant Range[4]	Supplement[8]	Mature Brome Hay[8]	Supplement[8]
Dry Matter, %	80.00	95.83	97.25	95.37
Crude Protein, %	4.70	45.65	5.76	31.39
Neutral Detergent Fiber (NDF), %	66.10	22.06	71.80	37.54
Ash, %	10.00	11.55	7.94	9.85

[1]All values except DM on DM basis
[2]Cows managed to maintain BCS during mid-gestation
[3]Cows managed to lose 1 BCS during mid-gestation
[4]Intake and composition estimated using Nutrient Requirements of Beef Cattle (NRC, 2000) estimates for winter range
[5]Fortified with vitamins and minerals to meet or exceed NRC requirements
[6]Values in parentheses are percent of pelleted supplement
[7]Average dry matter intake (DMI) per head per day throughout mid-gestation treatment; Control DMI based on Nutrient Requirements of Beef Cattle (NRC, 2000) estimates for intake of winter range
[8]Analyzed values determined through lab assays

Table 2.2. Least squares means for days of gestation at mid-gestation and cow body condition score (BCS), body weight (BW), ribeye area (REA), and fat thickness at the beginning and end of the mid-gestation treatment period.[1]

	Cow Energy Status			*P*-value	
Trait	Positive	Negative	SEM	Status	Block
Days of Gestation[2]	84	84	1.3	0.9730	**0.0215**
Initial BCS	4.78	4.94	0.051	0.1028	**0.0076**
Final BCS	4.92	4.29	0.046	**0.0001**	**0.0128**
Change in BCS	0.14	-0.65	0.050	**<0.0001**	0.4076
Initial BW, kg	462	462	2.4	0.9907	**<0.0001**
Final BW, kg	512	440	3.0	**<0.0001**	**<0.0001**
Change in BW, kg	50	-23	2.5	**<0.0001**	0.3197
Initial REA, cm^2	57.11	59.63	0.943	0.1035	**0.0007**
Final REA, cm^2	60.54	53.23	0.999	**0.0003**	**0.0004**
Change in REA,cm^2	3.43	-6.40	0.714	**<0.0001**	0.4460
Initial 12th Rib Fat Thickness, cm	0.39	0.40	0.013	0.7228	**0.0081**
Final 12th Rib Fat Thickness, cm	0.41	0.35	0.011	**0.0251**	**0.0418**
Change in 12th Rib Fat Thickness, cm	0.02	-0.05	0.009	**0.0083**	0.2907
Energy Status[3]	2.09	-2.32	0.146	**<0.0001**	0.9888

[1]Measurements taken at beginning and end of mid-gestation period normalized by fill

[2]Days of gestation at beginning of mid-gestation treatment as estimated by pregnancy ultrasound

[3]Energy status = $\left[\frac{(Obs\ BCS\ \Delta - BCS\ \Delta\ \bar{x})}{BCS\ \Delta\ S_d}\right] + \left[\frac{(Obs\ REA\ \Delta - REA\ \Delta\ \bar{x})}{REA\ \Delta\ S_d}\right] + \left[\frac{(Obs\ BW\ \Delta - BW\ \Delta\ \bar{x})}{BW\ \Delta\ S_d}\right]$

Table 2.3. Serum hormone and metabolite concentrations for cows fed a control diet or a restricted diet during mid-gestation[1]

	Treatment			
Metabolite	Control[2]	Restricted[3]	SEM	P-value
Insulin d0[a], ng/mL	0.19	0.26	0.07	0.2853
Insulin d 28, ng/mL	0.27	0.23	0.02	0.8267
Insulin d56[a], ng/mL	0.35	0.23	0.03	0.0850
Insulin d84, ng/mL	0.27	0.17	0.02	0.2155
NEFA d0[b], μEq/L	467.0	421.5	30.8	0.0268
NEFA d28[b], μEq/L	549.6	435.8	35.7	0.0222
NEFA d56[b], μEq/L	698.7	629.3	34.2	0.2453
NEFA d84[b], μEq/L	850.4	665.7	49.1	0.0076
PUN d0[c], mg/dL	8.35	4.71	0.36	0.0043
PUN d28[c], mg/dL	15.36	9.81	0.67	0.0276
PUN d56[c], mg/dL	16.91	13.41	0.49	0.1229
PUN d84[c], mg/dL	13.93	14.98	0.83	0.8199

[1]Diets were formulated using software from the Nutrient Requirements of Beef Cattle (NRC, 2000) for cows to maintain body condition score (BCS; control), or to lose 1 BCS over the 91 d treatment period.

[2]n=30

[3]n=30

[a]1 animal removed from the restricted treatment because non-estimable; control n=30, restricted n=29

[b]NEFA=non-esterified fatty acids

[c]PUN=plasma urea nitrogen

CHAPTER III

The influence of maternal energy restriction during mid-gestation on beef offspring growth and feedlot performance

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ABSTRACT

Fetal or developmental programming evaluates the effects of alterations in the gestational environment on the developing fetus. Specifically in beef, most fetal programming research has focused on under-nutrition of the dam, as cattle may experience a decrease in forage availability and quality during gestation. Therefore, the objective of this study was to determine the effects of maternal nutrient restriction during mid-gestation on birth weight, weaning weight, and growth performance of offspring. One hundred fifty one beef cows were allotted to one of two treatments: 1) Positive Energy Status (PES, also control dietary treatment; n = 76) fed to achieve and/or maintain body condition score (BCS) 5.0-5.5; or 2) Negative Energy Status (NES, also restricted dietary treatment; n = 75) fed to lose 1 BCS over the ensuing 91 d treatment period of mid-gestation. Measurements associated with cow body condition were collected throughout the treatment period. Following the end of the mid-gestation treatment cows were managed as a common group through weaning. At calving, calf birth weight, calving date, and calf gender were recorded. Following weaning, calves that met study protocol criteria (n=133) were allotted into feedlot pens according to cow treatment, gender, and weight. While in the feedlot dry matter intake, average daily gain, and feed

efficiency were assessed for the resultant offspring. Calves were fed to achieve 1 cm of backfat thickness. When calf birth weight was analyzed by cow dietary treatment there was a treatment by gender interaction ($P<0.05$). However, when birth weight was analyzed by energy status there was a tendency to be decreased in NES calves compared to PES calves ($P<0.10$). At weaning calves analyzed by cow dietary treatment were not different ($P>0.05$) for weaning weight, but calves analyzed by cow energy status had a tendency ($P<0.10$) to be decreased in NES calves. There were gender differences with steers having heavier birth weight and weaning weight when analyzed by cow dietary treatment or cow energy status ($P<0.05$). There were no differences ($P>0.05$) between treatments during the feeding period on growth performance measurements including average daily gain (ADG), dry matter intake (DMI) and feed efficiency (F:G). These results suggest NES during mid-gestation may have an effect on birth weight, and weaning weight. However, these differences in weight are overcome in the feeding phase and do not affect growth performance in progeny from NES cows during mid-gestation.

INTRODUCTION

In the agricultural industry it is common for livestock to undergo insults throughout the year, specifically caused by weather and its impacts on pasture conditions. In the upper Midwest forage availability and quality may be altered, especially during the winter months when snow cover is present and pasture is in a dormant state (Vavra and Raleigh, 1976). Often this results in a period of inadequate nutrition to gestating cows at some point during pregnancy. Specifically, spring calving cows would likely encounter a negative energy balance during the mid-gestation period (DelCurto et al., 2000).

Adaptations made by the fetus in response to an insult, stressor, or stimuli encountered by the dam during gestation is called fetal or developmental programming (Barker, 1998;1995). Fetal programming occurs *in utero*, but often manifests as a variety of problems in adult life for the offspring (Barker and Clark, 1997; Godfrey and Barker, 2000;2001). Previous research has shown adequate maternal nutrition is necessary for normal fetal development in livestock (Ford, 1995; Reynolds and Redmer, 1995). Specifically in livestock maternal under-nutrition at different times during gestation has been shown to alter muscle growth and adipose deposition (Larson et al., 2009; Long et al., 2012; Underwood et al., 2010). Severe maternal under-nutrition has been shown to create a "thrifty phenotype" in offspring where decreased muscle mass and increased adiposity occur after maturity (Hales and Barker, 1992). This phenotype possibly develops from a redirection of mesenchymal stem cell differentiation, likely programming the offspring to be born into a nutrient sparse environment (Du et al., 2010a; Du et al., 2010b; Hales and Barker, 1992). Since muscle is an expensive tissue to maintain, adipose tissue is the preferred tissue type in a sparse nutrient environment. However, this thrifty phenotype is calorically expensive to create. The amount of energy

needed to grow these tissues depends on the tissue type, with adipose tissue requiring more energy for growth according to the NRC (Brethour, 2004; NRC, 2000). With this in mind cattle performance can be affected by composition of gain. Because fat takes more energy for growth, feed efficiency decreases as fat depots increase in size, usually at the end of the feeding period (Garrett, 1980). With an understanding of this growth principle, a thrifty phenotype may be less efficient and have decreased performance in the feedlot. Therefore, the objective of this study was to determine the effects of mid-gestation nutrient restriction on offspring growth and performance.

MATERIALS & METHODS

Animals

The South Dakota State University (SDSU) Animal Care and Use committee approved the following animal experiment.

Cow Management

Cows were managed as described in Chapter 2. Briefly, 151 naturally serviced crossbred beef cows were evaluated for pregnancy, day of gestation, fetal sex, cow body weight (BW) and BCS. Cows were allotted to one of two treatment groups: 1) (76 cows)-fed to achieve and/or maintain BCS 5.0-5.5 (control dietary treatment); or 2) (75 cows)-fed to lose 1 BCS over the ensuing 91 d period of mid-gestation (restricted dietary treatment). Cows were weighed every 28 d throughout the management phase. Ultrasound measurements were collected for 12^{th} rib subcutaneous fat thickness (FT) and ribeye area (REA), and BCS was evaluated at the beginning and the end of the management phase. After completion of the treatment period, all cows were managed as a common group on native range and allowed free choice of a 20% crude protein (CP) supplement through weaning. Cows were not weighed or evaluated for BCS prior to calving. At calving, calf birth weight, calving date, and calf gender were recorded. Bull calves were also castrated at this time. Following branding in late May 2011, cows and calves were moved to a summer grazing pasture at Fort Meade, SD and managed as a common group on native range until weaning.

Cow Management Analysis

Alteration of treatments was previously discussed in Chapter 2. Briefly, upon analysis of cow data it was determined that a few cows within each dietary treatment

group did not achieve the physiological goals of our treatments. The intended treatment for the current experiment was to alter the uterine environment during mid-gestation. In order to achieve two treatments different diets were used to maintain or lose body condition during mid-gestation. Because we could not establish biological or hormonal differences based on dietary treatment (control versus restricted), cows and their calves were divided into new energy status categories (PES versus NES). Birth weight and weaning weight were analyzed by both dietary treatment and energy status of the pregnant cow. This re-classification of animals created 2 treatment groups as there was a bimodal distribution within the population: PES and NES were calculated from metabolic indicators including BCS, REA, and BW collected during gestation. The formula used is as follows:

$$\left[\frac{(Obs\,BCS\;\Delta - BCS\;\Delta\,\vec{x})}{BCS\;\Delta\,S_d}\right] + \left[\frac{(Obs\,REA\;\Delta - REA\;\Delta\,\vec{x})}{REA\;\Delta\,S_d}\right] + \left[\frac{(Obs\,BW\;\Delta - BW\;\Delta\,\vec{x})}{BW\;\Delta\,S_d}\right]$$

This re-classification of treatments allowed analysis to be more specific towards our treatment goals. However, this re-classification occurred after calves had been allotted to pens in the feedlot. Therefore, feedlot performance data were analyzed by the cow dietary treatments (control versus restricted).

Postweaning Offspring Management

At weaning (October 12), calves meeting study protocol (n=133 head) were weaned and shipped 534 km to the SDSU Ruminant Nutrition Center (RNC) in Brookings, SD. Upon arrival to the feedlot calves had access to water and long-stem grass hay until the total mixed ration was delivered (approximately 24 h). Calves were individually weighed, ear-tagged, and vaccinated against viral antigens related to

respiratory disease using Bovishield Gold-5™ (Zoetis, Florham Park, NJ), clostridial organisms using Ultrabac 7™ (Zoetis, Florham Park, NJ) and treated for internal and external parasites using Cydectin™ (Zoetis, Florham Park, NJ) the day following arrival to the feedlot (October 13). Calves were then stratified by BW and randomly assigned to pens by gender and cow treatment (control versus restricted) where each gender/treatment combination consisted of 3 pens containing 11 or 12 head per pen. This was the pen assignment during the first 24 d of the receiving period. Following removal of a subsample (n=12 steers) remaining calves (n=121) were stratified by BW and randomly assigned to new pens within gender and gestation treatment where each gender/treatment combination consisted of 4 pens containing 7 or 8 head per pen. Dietary ingredients and nutrient composition are presented in Table 3.1 and Table 3.2 respectively. In order to ensure the only treatment applied to the calves was maternal dietary treatment all calves were fed similar diets throughout the feeding period. Feed bunks were managed according to a clean bunk management system described by Pritchard and Bruns (2003). Calves were fed once daily (1300) and feed refusals were quantified if feed went out of condition. Calves were implanted 35 d after entering the feedlot. Steer calves received a Synovex S (Zoetis, Florham Park, NJ) implant and heifer calves received a Component EH (Elanco, Greenfield, IN) implant. All calves were re-implanted 77 d later on d 112 in the feedlot with Revalor 200 (Merck, Summit, NJ). Calves were on feed from mid-October through early May.

Feed ingredients were individually sampled weekly throughout the trial and analyzed for dry matter (DM), CP, ash (Horwitz, 2000), neutral detergent fiber (NDF), and acid detergent fiber (ADF) (Goering and Van Soest, 1970). Nutrient and DMI were

calculated using weekly feed analyses and daily feed batching and delivery information for the feeding period (Table 3.2). Period BW gain was calculated using d 28, 57, 85, 112, 140, 168, and 208 non-shrunk BW and weekly DMI data. Cumulative BW gain was determined using d 208 adjusted for a 4% shrink. Cattle health was monitored daily with treatment practices following approved health protocols.

Calves were marketed when all of the progeny were estimated to average 1.0 cm of 12^{th} rib backfat thickness (208 d on feed). Both at 21 d and at 208 d in the feedlot, a subsample (n=12 at each date) of steers was harvested at the SDSU Meat Laboratory reducing the number of animals in this report to 109. Cumulative ADG and F:G were calculated two different ways: 1) Shrunk - final live weight with a 4% shrink and 2) Carcass Adjusted - hot carcass weight (HCW)/0.625.

Statistical Analysis

Statistical analysis of calf birth weight and weaning weight were conducted using each calf as the experimental unit. Least squares means were calculated for birth weight and weaning weight using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Differences in main effects were determined due to calf gender and gestation treatment, as well as the interaction of calf gender and gestation treatment. Differences in means were considered significant at $P<0.05$ with trends discussed at $P<0.10$.

Statistical analyses on offspring performance data were conducted using pen as the experimental unit. Least squares means for all performance data were computed using PROC GLM procedures of SAS, determining differences due to the main effects of gestation treatment, calf gender, and replication as well as the interaction of gestation treatment x calf gender, gestation treatment x replication, and calf gender x replication.

Means were tested to a predetermined significance level of $P<0.05$ with trends discussed at $P<0.10$.

RESULTS & DISCUSSION

Growth performance in cattle is dependent on many different variables including maternal influences. Not only are these postnatal influences like milking ability important for growth, but also prenatal influences can affect feedlot performance later in life. The most commonly reported prenatal influence is the influence of late gestation nutrient restriction on birth weight, as most fetal growth occurs during the last trimester in cattle (Robinson, 1977). During this time much of the growth occurring is due to hypertrophy of tissues (Du et al., 2010a). However, a lack of muscle fibers or pre-adipocytes would also decrease the fetus' ability to grow later in gestation and post-natally. Since secondary myogenesis, the time of greatest muscle fiber formation, and adipogenesis begin during mid-gestation it would be reasonable to assume a decrease in fetal nutrients during mid-gestation could alter muscle and fat development pre- and post-natally. If hyperplasia is decreased during development, hypertrophy could be limited pre- or post-natally (Du et al., 2010a). With this concept of growth and development in mind we chose to target an energy restriction during mid-gestation to elucidate the impact of altered maternal energy on offspring growth and feedlot performance traits.

In the current experiment, birth weight and weaning weight were analyzed both by cow dietary treatment and by cow energy status. Initially calves were evaluated on cow dietary treatment; however after further analysis of cow data some cows did not meet the treatment objectives. This was the reason for creating the new cow energy status classification. For the purposes of this dissertation it is important to show both evaluation methods (Table 3.3a and Table 3.3b). Birth weight for calves from different cow dietary treatments were decreased in restricted heifer calves compared to all other

treatment and gender combinations (*P<0.05;* Figure 3.1). Additionally, control heifers had a lower birth weight than restricted bull calves (*P<0.05*; Figure 3.1). There was also a tendency (*P<0.10*) for birth weights to be decreased in NES calves compared to PES calves (Table 3.3b). Birth weight was lower for heifer calves compared to bull calves when data were analyzed as energy status or cow dietary treatment (*P<0.0001*), which was expected. Most studies evaluating the effects of cow nutrition on calf birth weight have focused on late gestation and have produced varied results. Corah et al. (1975) found decreased birth weight when heifers and cows were fed below their requirements (65% and 50% of NRC requirements respectively) during the last 100 d of gestation even after re-alimenting the cows back to above their requirements 30 d prior to calving. Conversely, Prior and Laster (1979) did not find differences in fetal weight as a result of differences in dietary energy fed from mid-gestation through late gestation. However, in that study all diets were formulated for some degree of growth, not for maintenance or loss of body condition like in the previous study by Corah et al. (1975). In another study, heifers fed a low total digestible nutrients (TDN) diet during the last 90 d of gestation had calves with decreased birth weights (Bellows & Short, 1978). This and other research suggests heifers may not be able to adapt to nutritional restriction as well as mature cows (Bellows et al., 1982). This is understandable as mature cows in good condition have more body stores to partition towards fetal growth compared to a heifer or young cow that is still growing, resulting in a competition for nutrients for growth of the heifer or growth of the fetus.

Additionally, cows in a weight cycling management program did not have negative effects on birth weight when cows were either maintained at a constant BCS or

lost BCS during mid-gestation, but gained condition during the last trimester. However, cows that lost condition during the second and third trimester gave birth to lighter weight calves compared to cows maintaining or gaining body condition (Freetly et al., 2000). These findings suggest calf birth weight is more dependent on nutrition supplied to the cow during the last trimester of gestation than any other time period. Furthermore, different winter grazing systems during the last trimester affect bull birth weight. Bull birth weight was increased when cows grazed corn residue compared to native range pasture during the last trimester (Larson et al., 2009). This difference may be attributable to more available energy in the feedstuff and subsequently more energy for fetal growth. Other experiments during the last trimester evaluating protein supplementation did not affect calf birth weight (Martin et al., 2007; Stalker et al., 2006).

In experiments with gestational treatment periods similar to the current experiment, nutrient restriction of heifers during the last two thirds of gestation caused decreased birth weights compared to their non-restricted heifer contemporaries (Warrington et al., 1988). Likewise, Micke et al. (2010) also demonstrated nutrient restricted heifers during mid-gestation had calves with decreased birth weights. Radunz et al. (2012) evaluated different energy sources from 160 d of gestation through parturition. Cows fed grass hay had lower calf birth weights compared to cows fed corn or dried distillers grains (DDGS). During mid-gestation cows grazing improved pasture versus native range that was likely deficient in CP, did not have differences in birth weight (Underwood et al., 2010). Additionally, multiparous cows receiving a control diet, a restricted diet, or a restricted plus protein diet during early to mid-gestation produced calves with similar birth weights among the treatment groups (Long et al.,

2012). Similarly, early gestational global nutrient restriction in heifers did not affect calf birth weight when nutrient restricted heifers were fed to meet their requirements later in gestation (Long et al., 2010).

Results of fetal programming effects are varied depending on the timing of the nutritional insult and the type of dietary change the dam encounters during gestation. The current results are similar to other researchers findings even though the timing and type of cow nutrient alteration is not the same among all these experiments (Corah et al., 1975; Larson et al., 2009; Micke et al., 2010; Radunz et al., 2012). In contrast, our results do not agree with Long et al. (2010, 2012) and Underwood et al. (2010) who had more similar dam dietary treatments and timing of the treatments to our experiment. One reason there might be differences between experiments is we included both genders, however Underwood et al. (2010) only used steer progeny. When evaluating only steer progeny we also did not detect any differences between our treatments. Additionally, cow weight, age, and whether or not cows were fed to gain back condition or maintain their current condition appear to have an impact on how the animal can handle a nutritional insult and the effects it will have on the calf (Robinson et al., 2012). In the current experiment, many of the cows were young (cow age was 3 and 4 years), potentially still growing, and they were not fed above their requirements during the last trimester. If the cows were still growing they would likely not have extra body stores to partition towards fetal growth. For analysis of birth weight, calves were grouped according to whether or not their dams were in a positive or negative energy status, as well as evaluated cow dietary treatments. The energy status classification allowed analysis of more than one variable to validate whether or not the intended treatment was

met, which was a change in the uterine environment. It also ensured cows were indeed losing condition in order to support maintenance requirements and fetal growth. None of the previously mentioned authors evaluated their treatments similar to this method which may be why there are differences in results.

Weaning weight was evaluated both by cow dietary treatment and cow energy status. Similar to birth weight, there were no differences in weaning weight when calves were analyzed using cow dietary treatment as the main effect (*P>0.05;* Table 3.3a). However, when calves were evaluated using cow energy status there was a tendency for calves in the NES treatment to be lighter than calves in the PES treatment *(P<0.10*; Table 3.3b). As for birth weight, steer calves were heavier than heifer calves independent of the way data was analyzed (*P<0.05;* Table 3.3a & Table 3.3b).

Similar to birth weight, weaning weight results from other maternal nutrition studies are varied. As previously mentioned Long et al. (2010a,b) evaluated the effects of feeding heifers half of their energy and protein requirements during the first trimester followed by feeding excess of their daily requirements. The progeny weaning weight was not affected by early gestational nutrient restriction. Again, in a similar study, multiparous cows were nutrient restricted or nutrient restricted and supplemented protein with no adverse effects on weaning weight (Long et al., 2012). Radunz et al. (2012) evaluated the effects of different energy sources fed to cows from 160 d of gestation through parturition and reported a tendency for weaning body weight to be lower in the progeny from hay fed cows versus progeny from corn fed cows. Additionally,Underwood et al. (2010) had steer progeny from cows grazing native range with a lighter weaning weight compared to steer progeny from cows grazing improved

pasture. However, adjusted 205 d weaning weights were similar showing these differences are potentially caused by differences in age of the calf. Conversely, when heifers and cows are severely energy restricted (65% and 50% of NRC requirements respectively in late gestation) the resultant calves were also lighter at weaning even after the cows were fed at or above their maintenance requirements (Corah et al., 1975). In contrast, heifer calves from cows supplemented protein during late gestation had a greater adjusted 205 d weaning weight, but there was no difference in actual weaning weight when compared to heifer calves from non-supplemented cows (Martin et al., 2007). The discrepancy between actual weaning weight and adjusted 205 d weaning weight was potentially from a difference in age of the calf. Another variable to consider is the difference in weaning weight may be caused by an increase in cow body condition leading to an increase in milk production since there was no difference between supplementation groups for birth weight. Furthermore, late gestation cow protein supplementation increased weaning weights in calves compared to calves from non-supplemented cows (Stalker et al., 2006). Also winter grazing system and protein supplementation affect weaning weight. Calves from cows grazing native range without a protein supplement during the last trimester had lighter weaning weights than calves from cows grazing native range that were given a protein supplement (Larson et al., 2009).

Therefore, supplementation to the cow pre- and post-partum appears to have an effect on calf weaning weight. This increase in weaning weight with the addition of supplementation is potentially the result of increased cow body condition and the ability to produce ample milk for growth. However, the current study did not evaluate

supplementation programs during the postpartum period, as all cows were managed as a common group. The tendency for the difference in weaning weight between different energy status groups is likely a result of cows not gaining enough condition prior to calving and after calving not being able to produce enough milk to compensate for lower birth weight. Lower weaning weight in the NES calves was likely related to having a lower birth weight and/ or dam milk production. However, it is possible that growth potential was altered *in utero* in these heifer calves. Corah et al. (1975) displayed decreased birth and weaning weights when dams were severely nutrient restricted. This may lead to decreased myogenesis and result in a permanent decrease in growth potential of these offspring. If fetal programming did occur then calves would have differences in weight throughout the feeding period and differences in carcasses characteristics following harvest. If differences in weaning weight do not persist through the feedlot, then the differences are likely caused by maternal influences.

Receiving period and grow-finish performance data can be found in Tables 3.4 and 3.5, respectively and were analyzed using the original cow treatments (control versus restricted) as calves were allotted to pens consistent with cow dietary treatments. Any subsequent analysis was also analyzed using the pen mean. During the receiving period (the first 28 d in the feedlot) there was a tendency for calves from restricted cows to have a lower ADG ($P<0.10$), but no differences for BW at 28 d in the feedlot ($P>0.05$). Likewise, there was a tendency ($P<0.10$) for BW at d 57 to be lighter in calves from restricted dams and a tendency ($P<0.10$) for decreased feed efficiency in calves from restricted dams from d 29 to d 57 in the feedlot. No differences between treatments were observed after d 57 for BW, or growth performance characteristics ($P>0.05$). Steers were

heavier ($P<0.05$) then heifers and throughout the feeding period (Tables 3.4 and 3.5). There were differences or tendencies for heifers and steers to be different for ADG, DMI, and feed efficiency throughout the feeding period, as would be expected. No differences were observed in cumulative post-weaning performance ($P>0.05$) between treatments when evaluated on a live weight basis or a carcass adjusted basis (Table 3.6).

Similar to the results in the current study, Radunz et al. (2012) observed no differences in progeny performance when dams were fed different energy sources from mid-gestation through calving. Larson et al. (2009) and Stalker et al. (2006) found no differences in ADG, DMI, or feed efficiency when protein supplementation or no protein supplementation occurred during the last trimester of gestation, or with different grazing systems. However, steers from cows grazing winter range with no protein supplement during the last trimester had lighter final weights than steer progeny from cows grazing winter range that did receive a protein supplement during the last trimester (Larson et al., 2009). Additionally, steers from cows that had grazed native range pasture during gestation had lower ADG, total BW gain, and live weight than steers from cows grazing improved pasture (Underwood et al., 2010). These progeny were penned together during the growth study making it impossible to determine whether the growth response was caused by differences in DMI or biological efficiency. In contrast to the previous study, Long et al. (2010a,b) did not find any differences in feedlot performance in offspring from heifers severely nutrient restricted during the first trimester of gestation.

Initial weight into the feedlot, which for calf-feds can be similar to weaning weight, can be correlated with increased DMI, and potentially final BW. Additionally, DMI can affect other feedlot performance response variables (Galyean et al., 2011;

McMeniman et al., 2010; NRC, 2000). Cattle that have a smaller mature size will eat less and usually convert poorly at the same weight as their larger framed counterparts. On that note, one potential reason cattle would convert poorly when they are smaller is the creation of a "thrifty phenotype" in which offspring prepare to be born into a nutrient sparse environment in response to encountering a challenge *in utero*. This "thrifty phenotype" is markedly fatter with less muscling than other similar progeny. Additionally, it is thought the "thrifty phenotype" was severely growth restricted either during fetal development and/or early post-natal growth and would have different requirements then a non-restricted counterpart. These individuals have an increased chance of getting fat likely because of decreased maintenance energy requirements and they are likely further along in their growth curve relative to non-restricted individuals. These reasons allow for the creation of a "thrifty phenotype" indirectly during growth (Robinson et al., 2012).

In the current study, one potential reason no differences were observed in performance may be from the timing and severity of the gestational insult. Cows were restricted to 80% of their requirements during mid-gestation to determine the effects on offspring growth and performance. It is likely that the restriction implied in the current study, while production relevant, was not severe enough or long enough to create a drastic disparity in energy within the cow to adversely affect cumulative feedlot performance of the offspring.

Another reason differences in offspring performance was limited is that cows were commingled following the treatment period and re-alimented to a common diet so the nutrient restricted cows likely underwent some compensatory growth which would

add body condition just prior to calving. Since birth weight was not drastically reduced it is not likely the calves from restricted cows were severely restricted *in utero*. Fetuses also potentially underwent compensatory growth during the last trimester with increased nutrient availability allowing growth of tissues.

Likewise, factors affecting pre-weaning calf growth are dependent on maternal influences like lactational ability of the cow and nutritional quality of the pasture (Bartle et al., 1984; Robinson et al., 2012). Another variable in weaning weight is calf birth weight. Calves that are born with a larger birth weight have the potential to have a heavier weaning weight, and ultimately finishing weight (Robinson et al., 2012). However, if lighter birth weight calves have the genetic makeup to grow, but did not receive enough nutrients during the last few months of gestation to allow for hypertrophy of tissues they could catch up prior to weaning if the cow can adequately support calf growth (Martin et al., 2007; Stalker et al., 2006). On this note cow body weight can directly affect calf weaning weight as it is indicative of cow condition if the mature size of the cow is known (Greenwood and Cafe, 2007). Unfortunately, in the current study we did not weigh cows or evaluate body condition after they were commingled during the last trimester and therefore do not have a good idea of cow condition at calving. Even without this information we can conclude that calf growth potential was not ultimately affected as there were no differences in growth and performance after the receiving period in the feedlot. Therefore, supplying the calf with adequate nutrients during the pre-weaning period is crucial for offspring growth performance in order to ameliorate any adverse effects from mid-gestation nutrient restriction.

IMPLICATIONS

Cows in a negative energy status during mid-gestation may produce heifers with decreased birth weights. Additionally progeny weaning weights are affected by negative energy status of cows during gestation. However, calves overcome birth weight and weaning weight differences in the feedlot. In addition, calves from nutrient restricted cows during mid-gestation perform similarly to calves from non-restricted cows. Therefore, there are limited post-weaning effects on growth performance on offspring from cows experiencing a mid-gestation nutrient restriction.

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Table 3.1. Diet composition[1]

	Days on Feed											
Item	1-28		29-69		70-95		96-128		129-162		163-175		176-208	
n	5		6		5		5		5		2		4	
Sorghum Silage, %	34.29	(1.51)[6]	27.01	(1.96)	30.70	(2.89)	17.52	(0.94)	3.02	(0.48)	-		-	
Alfalfa, %	9.74	(0.21)	10.71	(0.23)	10.20	(0.44)	-		-		-		-	
Dry Rolled Corn, %	38.31	(1.02)	42.20	(1.09)	40.56	(1.72)	30.31	(0.50)	-		42.92	(0.39)	42.49	(0.46)
Dried Distiller's Grain W/ Solubles, %	13.32	(0.31)	-		13.99	(0.55)	11.66	(0.09)	12.59	(1.18)	9.55	(0.20)	10.29	(0.09)
Wet Distiller's Grain W/ Solubles, %	-		15.33	(0.51)	-		-		-		-		-	
Chopped Ear Corn, %	-		-		-		36.61	(0.55)	-		-		-	
High Moisture Ear Corn	-		-		-		-		80.17	(1.92)	43.49	(0.66)	40.20	(0.62)
Grower Supplement[2], %	4.34	(0.08)	4.76	(0.15)	4.54	(0.20)	-		-		-		-	
Liquid Supplement[3], %	-						3.89	(0.03)	4.22	(0.31)	4.04	(0.07)	-	
Finisher Supplement[4], %													7.02	(0.10)

[1] DM basis

[2]Pelleted supplement formulated to provide vitamins and minerals to meet or exceed nutrient requirements (NRC, 2000) using soybean meal, ground corn, limestone, trace mineral salts, and a vitamins and minerals premix.
Supplement was formulated to provided 22 g/ton (DMB) Rumensin 90 (Elanco, Greednfield, IN) of the diet.

[3]Provided vitamins and minerals to meet or exceed nutrient requirements (NRC, 2000) using urea.
Supplement was included in the diet to provide 27.14 g/ton monensin and 6.57 g/ton tylosin (Tylan, Elanco, Greenfield, IN) (DMB).

[4]Pelleted supplement formulated to provide vitamins and minerals to meet or exceed nutrient requirements (NRC, 2000) of the diet using soybean meal, limestone, trace mineral salts, urea, and a vitamins and minerals premix.
Supplement was formulated to supply 30 g/ton (DMB) Rumensin 90.

[5]Calculated from weekly feed analysis.

[6]Mean (S_d)

Table 3.2. Nutrient composition of diets[1]

	Days on Feed													
Item	1-28		29-69		70-95		96-128		129-162		163-175		176-208	
n	5		6		5		5		5		2		4	
DM, %	66.01	(1.54)[2]	52.27	(1.60)	61.53	(2.80)	72.56	(0.63)	66.64	(2.84)	77.45	(1.46)	77.13	(1.10)
CP, %	12.58	(0.31)	13.17	(0.32)	12.71	(0.15)	12.02	(0.07)	12.32	(0.54)	11.85	(0.28)	12.65	(0.13)
NDF, %	33.14	(0.53)	31.26	(0.85)	31.03	(0.83)	26.38	(1.23)	21.67	(1.13)	15.24	(0.06)	15.65	(0.30)
ADF, %	17.95	(0.30)	16.47	(0.52)	17.26	(0.72)	12.00	(0.78)	9.28	(0.26)	6.06	(0.10)	5.73	(0.23)
Ash, %	7.47	(0.18)	6.88	(0.21)	7.33	(0.28)	4.43	(0.11)	3.01	(0.20)	2.39	(0.07)	4.31	(0.06)
Ether Extract, %	3.41	(0.04)	3.66	(0.07)	3.61	(0.10)	3.77	(0.07)	3.82	(0.22)	3.52	(0.08)	3.39	(0.04)
NE_m, Mcal/ kg	1.80	(0.013)	1.86	(0.016)	1.83	(0.023)	1.90	(0.007)	1.92	(0.004)	2.04	(0.001)	2.06	(0.001)
NE_g, Mcal/ kg	1.09	(0.013)	1.15	(0.016)	1.12	(0.023)	1.22	(0.008)	1.27	(0.003)	1.37	(0.001)	1.38	(0.001)

[1] DM basis; Calculated from weekly feed analysis.

[2] Mean (S_d)

Table 3.3a. Body weights of calves from cows fed different diets during mid-gestation[1]

	Cow Dietary Treatment[2]			Gender			*P*-value		
	Control	Restricted	SEM	Steers	Heifers	SEM	Diet	Gender	D x G
Birth Weight[3a], kg	38	38	0.5	40	36	0.5	0.312	<0.000	0.026
Weaning Weight[4b], kg	217	213	2.9	220	210	3.0	0.293	0.010	0.261

[1]Birth weight and weaning weight data were analyzed by individual calf

[2]Control-calves from cows managed to maintain body condition during mid-gestation;
Restricted-calves from cows managed to lose one body condition score during mid-gestation

[3]Control n=73; Restricted n=77

[4]Control n=69; Restricted n=64

[a] Steers n=74; Heifers n=66

[b]Steers n=72; Heifers n=61

Table 3.3b. Body weights of calves from cows in different energy status during mid-gestation[1]

	Energy Status[2]			Gender			*P*-value		
	PES	NES	SEM	Steers	Heifers	SEM	Energy Status	Gender	ES x G
Birth Weight[3a], kg	39	37	0.5	40	36	0.5	0.090	<0.000	0.207
Weaning Weight[4b], kg	218	211	3.0	220	209	2.9	0.055	0.010	0.396

[1]Birth weight and weaning weight data were analyzed by individual calf

[2]PES-calves from cows in a positive energy status during mid-gestation;
NES-calves from cows in a negative energy status during mid-gestation.

[3]PES n=76; NES n=64

[4]PES n=73; NES n=60

[a]Steers n=74; Heifers n=66

[b]Steers n=72; Heifers n=61

Table 3.4. Receiving period performance of steers and heifers by treatment[1]

	Treatment[2]			Gender			P-value	
	Control	Restricted	SEM	Steers	Heifers	SEM	Treatment	Gender
Receiving Period (1-28 d)								
Weaning Weight, kg	218	212	3.2	221[a]	209[b]	3.2	0.257	0.015
d 28 BW, kg	262	254	3.4	266[a]	250[b]	3.3	0.119	0.001
ADG	1.58	1.50	0.033	1.62[a]	1.45[b]	0.032	0.079	0.000

[1]Receiving period performance was analyzed by pen with 6 replications.

[2]Control-calves from cows fed to maintain body condition during mid-gestation
Restricted-fed to lose 1 body condition score during mid-gestation

[a,b]Means within main effects differ $P<0.05$

Table 3.5. Grow-Finish performance of steers and heifers by treatment[1]

	Treatment[2]			Gender			P-value	
29-57 d[3]	Control	Restricted	SEM	Steers	Heifers	SEM	Treatment	Gender
d 57 BW[4]	314	305	2.0	320[a]	298[b]	2.0	0.054	0.005
ADG	1.80	1.75	0.032	1.89[a]	1.67[b]	0.032	0.272	0.016
DMI	7.77	7.80	0.086	7.96	7.60	0.086	0.847	0.062
F/G	4.33	4.48	0.039	4.24[a]	4.57[b]	0.039	0.070	0.009
58-85 d								
d 85 BW	362	353	3.4	373[a]	343[b]	3.4	0.161	0.009
ADG	1.74	1.74	0.060	1.87	1.61	0.060	0.986	0.055
DMI	8.69	8.59	0.155	8.87	8.41	0.155	0.661	0.128
F/G	5.06	5.01	0.163	4.81	5.26	0.163	0.866	0.143
86-112 d								
d 112 BW	404	395	3.5	416[a]	382[b]	3.5	0.163	0.006
ADG	1.53	1.53	0.039	1.62[a]	1.44[b]	0.039	0.915	0.044
DMI	8.63	8.50	0.127	8.58	8.56	0.127	0.528	0.910
F/G	5.65	5.68	0.090	5.38[a]	5.95[b]	0.090	0.817	0.021
113-140 d								
d 140 BW	448	440	3.8	464[a]	424[b]	3.8	0.259	0.006
ADG	1.58	1.63	0.025	1.69[a]	1.52[b]	0.025	0.237	0.020
DMI	8.91	9.17	0.129	9.28	8.81	0.129	0.247	0.082
F/G	5.68	5.66	0.071	5.52	5.81	0.071	0.822	0.061
141-168 d								
d 168 BW	486	478	4.2	505[a]	458[b]	4.2	0.288	0.004
ADG	1.35	1.35	0.035	1.49[a]	1.21[b]	0.035	1.000	0.011
DMI	9.41	9.35	0.102	9.85[a]	8.91[b]	0.102	0.685	0.007
F/G	6.99	6.98	0.183	6.63	7.35	0.183	0.971	0.067
169-208 d								
d 208 BW	545	541	2.9	574[a]	511[b]	2.9	0.404	0.001
ADG	1.48	1.56	0.054	1.72[a]	1.32[b]	0.054	0.334	0.013
DMI	9.73	9.89	0.079	10.45[a]	9.17[b]	0.079	0.264	0.001
F/G	6.69	6.41	0.138	6.12[a]	6.98[b]	0.138	0.254	0.021

[1]Performance data was analyzed by pen with 8 replications; no shrink applied to BW
[2]Control-calves from cows fed to achieve and/ or maintain body condition during mid-gestation
Restricted-calves from cows fed to lose 1 body condition score during mid-gestation
[3]Period
[4]BW=body weight, kg

[a,b]Means within main effects differ $P<0.05$.

Table 3.6. Cumulative post-weaning performance of steers and heifers from cows in altered nutrient status during mid-gestation[1]

	Treatment[2]			Gender			P-Value	
Live Weight Basis[3]	Control	Restricted	SEM	Steers	Heifers	SEM	Treatment	Gender
Final BW	523	519	2.8	551[a]	491[b]	2.8	0.404	0.001
ADG	1.45	1.47	0.016	1.59[a]	1.34[b]	0.016	0.471	0.002
DMI	8.91	8.95	0.062	9.25[a]	8.61[b]	0.062	0.734	0.006
F/G	6.15	6.1	0.059	5.83[a]	6.43[b]	0.059	0.602	0.006
Carcass Adjusted[4]								
Final BW	526	521	1.5	551[a]	497[b]	2.8	0.278	0.001
ADG	1.47	1.48	0.015	1.58[a]	1.37[b]	0.015	0.666	0.002
DMI	8.91	8.95	0.062	9.25[a]	8.61[b]	0.062	0.734	0.006
F/G	6.07	6.05	0.060	5.84[a]	6.28[b]	0.060	0.818	0.014

[1]Performance data was analyzed by pen with 8 repetitions.

[2]Control-calves from cows fed to maintain body condition during mid-gestation

Restricted-calves from cows fed to lose 1 body condition score during mid-gestation

[3]Calculated using final live body weight with a 4% shrink

[4]Calculated as HCW/0.625

[a,b]Means within main effects differ $P<0.05$.

Figure 3.1.

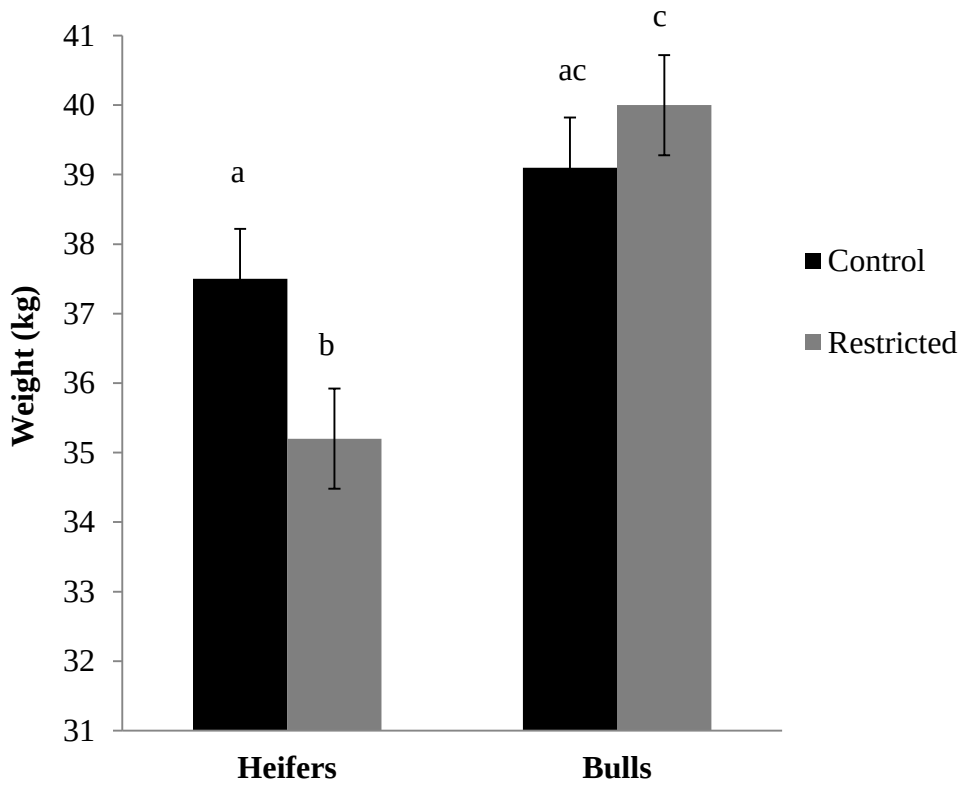

Figure 3.1. Calving birth weights of progeny from cows fed different diets during mid-gestation. Control-calves from cows fed to maintain body condition during mid-gestation; Restricted-calves from cows fed to lose one body condition score during mid-gestation.

[a,b,c]Means without common superscripts differ ($P<0.05$); Gender x cow treatment interaction ($P=0.026$).

CHAPTER IV

Maternal energy status during mid-gestation affects the immune response in the resultant beef progeny

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ABSTRACT

Fetal or developmental programming evaluates the effects of maternal alterations on the developing fetus and the potential consequences later in life. Specifically in beef, most fetal programming research has focused on under-nutrition of the dam, as cattle may experience a decrease in forage availability and quality. A lack of available nutrients during gestation could lead to altered development within the offspring. This altered development could affect how organs within the body function later in life, which could influence the immune system of the animal. Poor immune system development in cattle could result in health problems during the feeding period costing the industry millions of dollars every year. Therefore, the objective of this study was to determine the effects of maternal energy status during mid-gestation on the humoral immune response in beef cattle during the receiving period by evaluating antibody titers to a novel antigen. Beef cows were allotted to one of two mid-gestation treatment groups: 1) Positive Energy Status (PES; n = 76)-fed to achieve and/or maintain body condition score (BCS) 5.0-5.5; or 2) Negative Energy Status (NES; n = 75)-fed to lose 1 BCS over the ensuing 91 day period of mid-gestation. Following the end of the targeted treatment period cows were commingled (last trimester) and managed as a common group through weaning. Calves

were weaned and shipped to a research feedlot. A subsample (n=36) of calves were subcutaneously injected with 4 mg ovalbumin antigen at d 0 of antigen challenge and again on d 28 of antigen challenge. In order to measure antibody production in response to the antigen, blood samples were collected every 7 d via jugular venipuncture from d 0 through d 56. Serum was isolated and an enzyme-linked immunosorbant assay (ELISA) was used to determine antibody titers in response to the ovalbumin challenge. Data was analyzed as a repeated measures model using the PROC MIXED procedure of SAS (SAS Inc., Cary, N.C.). There were no differences ($P>0.05$) in any interactions between treatment, day, or gender, as well as no gender main effects. There was a difference ($P<0.05$) between gestational treatments over the sampling period with calves from PES cows having a greater antibody titer to ovalbumin than calves from NES cows. These results suggest cows in a NES during mid-gestation produce calves with a decreased ability to produce antibodies to a novel antigen and thus a decreased humoral immune response. However, the decrease in antibody titer may not ultimately affect the health of the animal as antibody production was still relatively high in the NES treatment.

INTRODUCTION

A healthy immune system in livestock is critical for the health, well-being, and productiveness of the animal. Cattle encounter many different immune challenges throughout life. The most vulnerable times for cattle immunologically are shortly after birth and following weaning as these are times when calves are immunocompromised (Duff and Galyean, 2007; Tizard, 2004). Weaning health issues can be the result of environmental stressors and pathogens (Duff and Galyean, 2007). In cattle, bovine respiratory disease (BRD) is one of the most economically devastating cattle diseases costing the beef industry millions of dollars every year. These losses come not only from increased mortality rates, but also additional costs associated with increased morbidity. Some hidden costs associated with morbidity include feed inefficiencies and poor growth performance due to health, as well as pharmaceutical cost, and labor involved with treating cattle (Loerch and Fluharty, 1999). In addition, BRD occurs commonly after weaning when cattle are moving into the feedlot, ultimately affecting performance of the animal (Duff and Galyean, 2007). Therefore the potential exists to improve animal welfare and producer profitability through improved immune function.

Achieving optimal health throughout life requires sufficient passive immunity. Ingestion of colostrum to ensure passive transfer of immunoglobulins from the dam to the calf is essential for proper immune function in cattle (Galyean et al., 1999). Passive transfer is necessary for the calf until the calf is able to develop its own antibodies to environmental pathogens. Previous research has demonstrated poor prepartum nutrition can have detrimental effects on postpartum calf health (Hough et al., 1990; Quigley Iii and Drewry, 1998). Many research projects have focused on passive transfer of colostral

immunoglobulins and subsequent cattle health, as well as the effects of vitamin and mineral supplementation on immune function. Other research has focused on maternal nutrition, both pre- and postnatally, and how it affects passive transfer of immunoglobulins (Wittum and Perino, 1995). However, little research to date has been conducted to evaluate fetal programming and health post-weaning. Since late gestation nutrient restriction negatively impacts health post-natally there is a chance that there are also adverse health effects post-weaning. Therefore the objective of the following study was to evaluate the effects of negative energy status during the second trimester on the development of the immune system in response to an antigen challenge during the receiving period in the resultant progeny.

MATERIALS & METHODS

Animals

The South Dakota State University (SDSU) Animal Care and Use committee approved the following animal experiment.

Cow Management

Cows were managed as described in Chapter 2. Briefly, 151 naturally serviced crossbred beef cows were evaluated for pregnancy, day of gestation, cow body weight (BW) and BCS. Cows were allotted to one of two treatment groups: 1) (76 cows)-fed to achieve and/or maintain BCS 5.0-5.5 (control dietary treatment); or 2) (75 cows)-fed to lose 1 BCS over the ensuing 91 day period of mid-gestation (restricted dietary treatment). Cows were weighed every 28 days throughout the treatment phase. Ultrasound measurements were collected for 12^{th} rib subcutaneous fat thickness (FT) and ribeye area (REA), and BCS were evaluated at the beginning and the end of the treatment phase. After completion of the treatment period, all cows were managed as a common group on native range and allowed free choice of a 20% crude protein (CP) supplement through weaning. Cows were not weighed or evaluated for BCS prior to calving. At calving, calf birth weight, calving date, and calf gender were recorded. Bull calves were also castrated at this time. Following completion of calving, cows and calves were moved to a summer grazing pasture and managed as a single common group on native range until weaning.

Cow Management Analysis

Treatment re-classification was previously discussed in Chapter 2. Briefly, upon analysis of cow data it was determined that a few cows within each dietary treatment group did not achieve the goals of our treatments physiologically. The intended

treatment for the current experiment was to alter the uterine environment during mid-gestation. In order to achieve two treatments different diets were used to maintain or lose body condition during mid-gestation. Because we could not establish biological differences based on dietary treatment (control versus restricted), cows and their calves were divided into new energy status categories (PES versus NES). This re-classification of animals created 2 treatment groups as there was a bimodal distribution within the population: PES and NES were calculated from metabolic indicators including BCS, REA, and BW collected during gestation. The formula used is as follows:

$$\left[\frac{(Obs\,BCS\;\Delta - BCS\;\Delta\,\bar{x})}{BCS\;\Delta\,S_d}\right] + \left[\frac{(Obs\,REA\;\Delta - REA\;\Delta\,\bar{x})}{REA\;\Delta\,S_d}\right] + \left[\frac{(Obs\,BW\;\Delta - BW\;\Delta\,\bar{x})}{BW\;\Delta\,S_d}\right]$$

This re-classification of treatments allowed analysis to be more specific towards our treatment goals. The reasons for the re-classification was previously discussed in detail in Chapter 2.

Calf Management

At weaning (October 12), calves meeting study protocol (n=133 head) were weaned and shipped 534 km to the SDSU Ruminant Nutrition Center (RNC) in Brookings, SD. Upon arrival to the feedlot calves had access to water and long-stem grass hay until the total mixed ration was delivered (approximately 24 h). Calves were individually weighed, ear-tagged, and vaccinated against viral antigens related to respiratory disease using Bovishield Gold-5™ (Zoetis, Florham Park, NJ), clostridial organisms using Ultrabac 7™ (Zoetis, Florham Park, NJ) and treated for internal and external parasites using Cydectin™ (Zoetis, Florham Park, NJ) the day following arrival to the feedlot (October 13). Calf health was monitored daily with treatment practices

following approved health protocols. Calves were allotted into pens by gender and cow treatment as described in Chapter 3.

Following adjustment of calves to the feedlot environment (19 d after arrival to the feedlot) a subsample (n=36) of steers and heifers were randomly selected for the ovalbumin challenge. The only selection criteria were calves may not have been previously treated for illness or have been selected for use in another experiment. All subsampled calves remained in their original pens throughout the application of the ovalbumin protocol. On d 0 of the ovalbumin challenge blood (10 mL) was collected via jugular venipuncture from each calf immediately prior to subcutaneous vaccination with ovalbumin. Ovalbumin (4 mL) were prepared by mixing 2 mg of crystallized ovalbumin (chicken egg albumin, Grade V, F-5503, Sigma Chemical, St. Louis, MO) per milliliter of phosphate buffered saline (PBS). The PBS/ ovalbumin solution was diluted 1:1 (vol:vol) with Freund's incomplete adjuvant (F-5506, Sigma Chemical, St. Louis, MO) emulsified and stored at 4°C until injection. Blood was also collected via jugular venipuncture on d 7, 14, 21, 28, 35, 42, 49, and 56 from initial ovalbumin vaccination. Calves were revaccinated with the ovalbumin mixture on d 28. Following a 4 h clotting time, blood was separated by centrifugation at 2,000 x g for 25 min at 4°C and serum was collected and stored at -20°C for later analysis.

ELISA Ovalbumin Assay

For the purpose of this project a subsample of days post vaccination was chosen to proceed. Days used for subsequent analysis were d 0, 14, 28, 42, and 56. Serum was analyzed to determine specific IgG titers to ovalbumin using an ELISA that was modified from Rivera et al. (2002). All reagents were made fresh daily prior to beginning the

ELISA. Specifically, individual wells on a 96-well plate (Immulon 1B, 14-245-78, Fisher Scientific, Pittsburgh, PA) were coated by placing 500 ng of ovalbumin (chicken egg albumin, Grade V, Product # F-5503, Sigma Chemical, St. Louis, MO) in each well by pipetting 100 µL per well of a solution containing 0.005 mg of ovalbumin/mL of PBS. Plates were incubated at 4°C for at least 12 h (overnight) to allow the ovalbumin antigen to adhere to each well. The top row for each animal was used as the control row, to account for nonspecific binding, where no ovalbumin was added. All subsequent steps were performed on the control row. Following the overnight incubation wells, were emptied and 200 µL of a PBS-0.05% polyoxyethylene sorbitan monolaurate (Tween 20; (PBST)) (P5927, Sigma Chemical, St. Louis, MO) 2% Casein (C7078, Sigma Chemical, St. Louis, MO) blocking solution was added to each well for 1 h at 22°C to decrease nonspecific binding. Following incubation of blocking buffer, wells were emptied, washed 3 times with PBST (wash buffer) and blotted dry. Serum samples were thawed and diluted with PBST-0.1% bovine serum albumin (BSA) (A3059, Sigma Chemical, St. Louis, MO) in a two-fold serial dilution. Serum samples were diluted from their initial concentration such that d 0 was diluted 1:100, d 14 was diluted 1:200, d 28 was diluted 1:400 and d 42 and d 56 were diluted 1:1800. One hundred µL of diluted serum samples were added to the wells in triplicate and incubated for 1 h at room temperature (~22°C). Following incubation, samples were removed and plates were washed 3 times with a PBST wash buffer and blotted dry. The second antibody, alkaline phosphatase anti-bovine IgG (A-0705, Sigma Chemical, St. Louis, MO) was diluted to a concentration of 1:5000 in PBST-0.1% BSA, added to each well at 100 µL/well, and incubated at 22°C for 1 h. Well contents were discarded and plates were washed 3 times with PBST wash

buffer and blotted dry. Substrate, SIGMAFAST™ p-nitrophenyl phosphate tablets (N1891, Sigma Chemical, St. Louis, MO), was added at 200 µL/well and incubated in the dark at 22°C for 30 min. The substrate solution was made fresh daily according to manufactures protocol in the absence of light. The reaction was stopped by adding 50 µL of a 2 M NaOH solution to each well. The optical density of each well was determined using a 96-well plate reader (SpectraMAX 190, Molecular Devices, Sunnyvale, CA) at an optical wavelength of 405 nm. The optical density of the control well (no ovalbumin antigen) was subtracted from the corresponding optical density reading for the value of the sample well.

Statistical Analysis

Titers from the ELISA procedure were calculated as described by Rivera et al. (2002). Briefly, binding that occurred on d 0 was considered non-specific, and was regarded as the baseline. Titer values were equal to the inverse of the dilution at which the optical density was equal to or less than the baseline/ d 0 value. Four animals were removed from any future analysis because titer values on d 14, 28, 42, & 56 were lower than the baseline. Titer value was transformed to $\log_2$ before statistical analysis to reduce variation among samples. Data were analyzed as a treatment by gender factorial design with repeated measures model using the PROC MIXED procedure of SAS (SAS Inc., Cary, N.C.). Individual animal was the experimental unit with fixed effects including gender, cow treatment, day, and the interactions of these effects. Day was the repeated measure and was analyzed using the REPEATED statement using the covariance structure Autoregressive(1) in the MIXED procedure of SAS after it was determined as

the best fit for the model based on fit statistics and residual analysis. Means were tested to a predetermined significance level of $P<0.05$ with trends discussed at $P<0.10$.

RESULTS & DISCUSSION

The ability of an animal to respond and adapt to a challenge influences the potential for infection or sickness to occur, and ultimately the well-being of the animal. Cattle health is necessary for well-being and optimum performance no matter the stage of production or sector of the industry. Cattle well-being is unattainable without a strong, well developed immune system that will protect the animal from foreign pathogens. Immune system development occurs through 2 different mechanisms: Innate and adaptive/ acquired immunity. Adaptive immunity can further be divided into active or passive, where an animal either actively develops antibodies to an antigen, or an animal passively absorbs antibodies produced by its mother and delivered to the calf in the form of colostrum (Tizard, 2004). Calves encountering poor passive transfer or failure of passive transfer have been shown to have increased morbidity and mortality rates, ultimately affecting feedlot profitability (Galyean et al., 1999). Passive transfer is affected by colostrum production, ingestion of colostrum, and absorption of colostrum. A large amount of research has focused on these three areas as it relates to calf health and calf growth prior to weaning. Little research exists between prenatal nutrition and energy status of beef cows as it relates to immune system development and function in the resultant calves. Of the research that does exist, most is focused on the last trimester evaluating colostrum, passive transfer, and morbidity and mortality at birth. While knowledge of passive transfer is important for calf health we will discuss our results during the receiving period in the feedlot, a period of time when cattle can be immunocompromised and BRD is prevalent (Duff and Galyean, 2007; Loerch and Fluharty, 1999).

In the current study no difference was detected in the 3 way interaction of gender, cow treatment, or day for ovalbumin titer. There also was no difference in gender by cow treatment (Figure 4.1 & 4.2), gender by day, or cow treatment by day interactions ($P>0.05$). Additionally, there was no difference in the gender main effect. There was a difference ($P<0.05$) between treatments over the sampling period with calves from PES cows having a greater antibody titer to ovalbumin than calves from NES cows (Figure 4.3). There was also an anamnestic response ($P<0.0001$) in the day main effect, where there is an increase in antibody titer on d 14, a decrease on d 28, the highest peak on d 42 and declines again on d 56. This type of response is expected when evaluating a response to a vaccination over time.

To date there is little knowledge or understanding of fetal programming effects in relation to health status in beef offspring, especially when evaluated during the feeding phase. Of the research that has been conducted most of it relates to last trimester nutrient alteration. Stalker et al. (2006) investigated the effects of cow protein supplementation and grazing system during the last trimester of gestation on calf IgG titers and found no differences in titers of calves at up to 2 d of age. This suggests there was no difference in absorption of colostrum, as well as no difference in colostrum quality among the supplementation and grazing management systems (Stalker et al., 2006). However, passive transfer was not directly measured therefore these are inferences made from IgG titers. Also a comparison between Stalker et al. (2006) and the current experiment cannot be made since IgG titers at birth were not collected in the current study. Conversely, Larson et al. (2009) found late gestation protein supplementation of cows decreased feedlot morbidity in the resultant calves compared to calves from non-

supplemented cows. Similar to the previous experiment, passive transfer was not measured and calves did not have health differences prior to weaning; therefore these differences are unknown and difficult to explain. Additionally in this experiment, percent of steers treated was evaluated, not a specific challenge to measure the acquired immune response. Because of the way data were collected in the experiment, this data may be extrapolating immune function information without critically evaluating the ability of those cattle to mount an immune response. The current research does agree with Larson et al. (2009) such that cows fed according to their NRC requirements will have healthier calves in the feedlot. The percent of animals treated in the current study was not different between treatments or genders. Similarly, calf survival was decreased when cows were severely nutrient restricted during the last trimester (Corah et al., 1975). While these cows still produced ample milk, there is a good possibility that colostrum quality was affected by severe nutrient restriction. Unfortunately in that study colostrum quality was not discussed so it is hard to determine the ability of a calf to absorb colostrum was a problem, or the colostrum itself was a problem. Another factor that may affect calf morbidity and mortality in that study was calf birth weight, and likely subsequent lower body energy reserves, which is associated with decreased survival rate (Azzam et al., 1993; Berger et al., 1992). It has been shown previously that low birth weight tends to be associated with greater incidences of morbidity (Azzam et al., 1993). This may be connected to the energy needed to mount an immune response as a consequence of a lack of energy reserves. Morbidity during the neonatal period in the current study was not evaluated and calves that had lower birth weights had gained weight such that there were no differences in weight at the time of the ovalbumin challenge.

Protein restriction in heifers during the last 100 days of gestation has been shown to negatively affect the absorption of IgG antibodies from colostrum in progeny. However, heifer colostrum was collected and found no difference in antibody concentration among the treatments. Thus it is likely absorption of immunoglobulins within the calf was affected by altered dam plane of nutrition. Intestinal morphology within the calf was not evaluated and therefore is difficult to determine the mechanism behind altered absorption of colostrum (Blecha et al., 1981). Conversely, Hough et al. (1990) did not see any differences in calf serum IgG concentration when cows were fed a restricted energy and protein diet during the last 90 days of gestation. However, calves from control dams that were fed colostrum from nutrient restricted dams did have a lower serum IgG concentration suggesting a difference in colostrum composition or colostrum specificity from dam to calf (Hough et al., 1990). Neonatal calves with low 24 hr IgG levels, suggestive of failure of passive transfer, had higher incidences of mortality and morbidity pre- and post-weaning (Wittum and Perino, 1995). In that study cow colostrum quality or quantity was not measured therefore it is difficult to determine if the calf was unable to properly absorb colostrum or if there was just inadequate colostrum provided by the dam. The differences in these studies could be related to age of the dam, where heifers are likely unable to adapt to nutritional restrictions as well as the restrictions in the diet. Additionally, a protein restriction may have more of an effect on the development of the immune system as immunoglobulins are essentially proteins. The effect on colostrum quantity, quality, failure of passive transfer, and calf birth weight is likely dependent on previously mentioned factors such as age of the animal, previous body condition of the animal, and severity of the nutrient restriction during gestation.

In relation to absorption of colostrum, Trahair et al. (1997) reported immature small intestines from sheep that experienced maternal nutrient restriction during early gestation as well as Intra-Uterine Growth Retardation (IUGR). Gastrointestinal tract (GIT) growth is greatest during the last trimester in species with long gestation periods (Trahair et al., 1997; Weaver et al., 1991). But growth restricted sheep display well established abnormal development of the GIT by late gestation likely resulting in suboptimal absorption in those animals (Avila et al., 1989; Trahair et al., 1997). This decrease in absorption of sheep has health implications for other species likely indicating fetal programming during other times of gestation outside of the last trimester can retard the animals' ability to absorb colostrum, leaving the animal vulnerable to disease-causing pathogens. If these intestinal differences persisted in cattle, absorption of immunoglobulins could be negatively affected. However, Meyer et al. (2010) reported an increase in total intestinal vascularity in early to mid-gestation nutrient restricted fetuses suggesting the intestine was being programmed to scavenge nutrients more efficiently (Meyer et al., 2010). Absorption of immunoglobulins may be a different situation than observed by Meyer et al. (2010) as absorption of immunoglobulins occurs through pinocytosis in order to absorb whole proteins across the intestinal epithelium (Bush and Staley, 1980). The ability of a nutrient restricted calf to absorb whole proteins through pinocytosis may be a potential problem leading to increased morbidity and mortality rates postnatally. Research has also shown that the efficiency with which immunoglobulins are absorbed from colostrum is variable. Absorption of IgG from colostrum ranges from 6% - 88%, although a more likely estimate is 20%-35% (Quigley Iii et al., 2002; Stokka, 2010). Because efficiency of absorption of immunoglobulins is

low in calves, colostrum quality and quantity must be high in order to achieve optimal immunity.

Other factors involved with morbidity issues in cattle include post-natal factors in addition to pre-natal factors. Stressors encountered by an animal usually have adverse health effects causing immunosuppression (Blecha et al., 1984). Active immunity specifically evaluates challenges presented to the animal usually through exposure or vaccination (Redman, 1979). The magnitude of the immune response is dependent upon the immune state of the animal and the number of times an antigen has been presented to the animal (Tizard, 2004). In the current study calves from NES cows had a lower magnitude of response to the ovalbumin vaccination over time during the receiving period in the feedlot. It is unknown whether the decrease in antibody titer is related to a lack of stimulation by the immune system, or a lack of response from immune cells. This is important to understand, as the receiving period is a common time for feedlot cattle to be immunosuppressed and experience disease challenges due to numerous environmental changes such as shipping stress, commingling, and other receiving practices. Therefore the ability of cattle to mount an immune response during this time is imperative. However, at this time, to the authors knowledge there is no threshold antibody titer in response to ovalbumin as it relates to actual immune protection. So it is possible that both of these titers, even though different, may protect the animal from a foreign pathogen.

Some limitations of the study include not measuring passive transfer, not measuring mineral status of the animal, and the lack of specificity of the assay. Not having a good understanding of immune status at the beginning of life does present some

problems, however, we were evaluating the humoral immune response within each calf in response to maternal nutrition, not the cows' ability to provide adequate colostrum and the calf's ability to absorb colostrum. But understanding intestinal morphology in relation to mid-gestation nutrient restriction may help answer why there was a difference in antibody titer between the two treatment groups. Generally calves are born with all of the essential immune components and organs necessary for immune function, but are not functional until 2-4 weeks of age with full development occurring around puberty (Chase et al., 2008; Wilson et al., 1996). In the current experiment, immune function organs were not collected or evaluated. Evaluation of the spleen, lymph nodes, and bone marrow would potentially shed light on the differences between the two treatment groups as these are the organs essential in humoral immunity. Additionally, vaccination against a foreign antigen will stimulate antibody synthesis of IgM prior to IgG in the primary immune response. During an enhanced secondary immune response the antibodies primarily formed are IgG. And the magnitude of the secondary immune response is greater and lasts longer than the primary immune response (Redman, 1979). The production of these antibodies may be one cause of increased background within the assay. Immunoglobulin G is a single Y-shaped structure, whereas IgM is a pentamer of the Y structure (Tizard, 2004). This could cause some cross-reactivity within the assay as the antibody used was a whole molecule antibody. Also, since a whole molecule IgG antibody was used in the assay it is likely IgG_1 and IgG_2 would bind whether those antibodies were produced in relation to vaccination with ovalbumin or a different stimulation like a subclinical disease (Tizard, 2004). Cattle were also initially vaccinated

upon arrival to the feedlot, with some of the background coming from antibodies produced in response to those initial vaccinations.

The next step would be to evaluate calves encountering potential fetal programming in relation to immune function and stress. We did not evaluate our samples for any markers related to stress within each calf, but it has been shown stress can have adverse effects on morbidity (Azzam et al., 1993). Another unanswered question in relation to fetal programming and immune function is the role vitamins and minerals play within the animal. There are a few key minerals, zinc, copper, and selenium, known to impact immune function and those minerals could potentially interact with development in the fetus (Galyean et al., 1999). It would also be important to evaluate immune cell production to determine where the disparity between the PES and NES groups' antibody titers are occurring. Therefore research is still needed to evaluate fetal programming effects on morbidity and mortality of the resultant offspring from birth through the feeding period.

IMPLICATIONS

Cows encountering a negative energy status during mid-gestation produce offspring with decreased antibody titers to ovalbumin. This suggests that the humoral immune response is decreased in offspring from cows that were energy restricted during gestation. Cattle producers should be aware of the potential effects maternal plane of nutrition can have on calf health later in life. However, the exact mechanism for a decreased antibody titer is unknown and still needs to be elucidated.

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Figure 4.1.

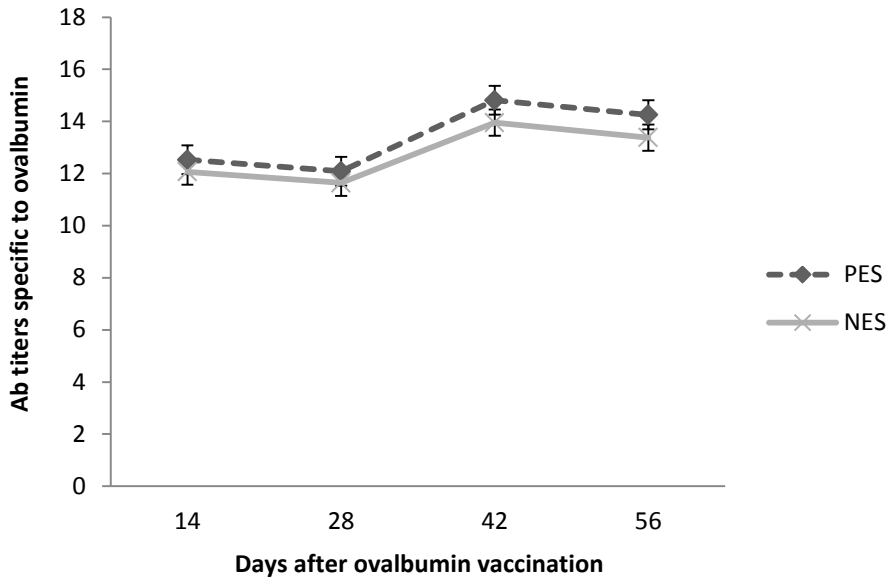

Figure 4.1. Influence of maternal energy status on the humoral immune response in heifers from cows in a positive energy status (PES; n=9) or a negative energy status (NES; n=7) during mid-gestation. No gender by treatment interaction was detected *(P>0.05)* for antibody titers specific to ovalbumin. Calves were injected subcutaneously with 4 mg of a PBS/ ovalbumin solution diluted 1:1 (vol:vol) with Freund's incomplete adjuvant on d 0 and d 28.

Figure 4.2.

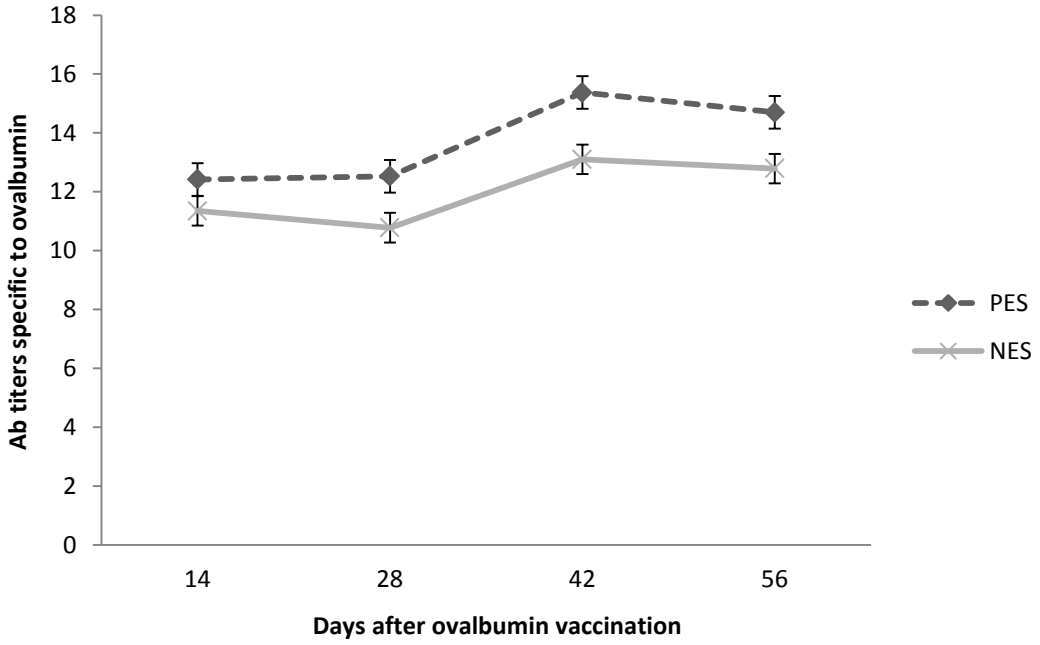

Figure 4.2. Influence of maternal energy status on the humoral immune response steers from cows in a positive energy status (PES; n=9) or a negative energy status (NES; n=7) during mid-gestation. No gender by treatment interaction was detected *(P>0.05)* for antibody titers specific to ovalbumin. Calves were injected subcutaneously with 4 mg of a PBS/ ovalbumin solution diluted 1:1 (vol:vol) with Freund's incomplete adjuvant on d 0 and d 28.

Figure 4.3.

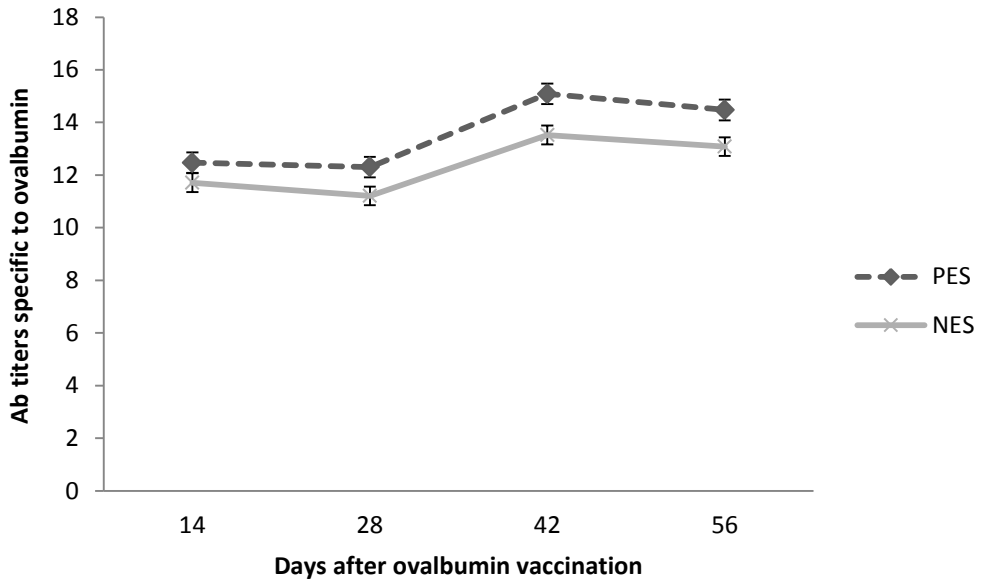

Figure 4.3. Influence of maternal energy status on the humoral immune response in calves from cows in a positive energy status (PES; n=18) or a negative energy status (NES; n=14) during mid-gestation. A treatment main effect was detected ($P<0.05$) over time for antibody titers specific to ovalbumin. Calves were injected subcutaneously with 4 mg of a PBS/ ovalbumin solution diluted 1:1 (vol:vol) with Freund's incomplete adjuvant on d 0 and d 28.

CHAPTER V

Maternal energy status during the second trimester of gestation does not alter gene transcription in subcutaneous adipose tissue of the resultant offspring

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ABSTRACT

Within the beef industry there is an unfavorable distribution of carcasses with a low quality grade and a high yield grade. Many factors are known to affect quality grade such as time on feed, plane of nutrition, health of the animal, and implant strategies. Factors such as increasing days on feed and plane of nutrition have been shown to increase quality grade by increasing marbling deposition during the growing period. While many postnatal influences on growth are understood, prenatal influences on growth are not well defined. Specifically, the effects of maternal nutrient restriction and cow energy status during mid-gestation on fat deposition in the resultant calf are not well understood. Therefore the objective of this study was to evaluate the effects of altered maternal energy status during mid-gestation on gene expression of subcutaneous fat depots in the resultant fetus at 2 different time points during the feedlot phase using Real-Time Polymerase Chain Reaction (RT-PCR) techniques. Briefly, 151 pregnant crossbred beef cows were allotted to one of two treatment groups: 1) (76 cows)-fed to achieve and/or maintain a body condition score (BCS) 5.0-5.5 (Positive Energy Status (PES)); or 2) (75 cows)-fed to lose 1 BCS over the ensuing 91 day treatment period of mid-gestation (Negative Energy Status (NES)). After completion of the treatment period, all cows were

managed as a common group on native range through weaning. Following weaning calves were acclimated to the feedlot and 12 steers at weaning and 12 steers at the end of the finishing phase of similar age and weight per day of age (WDA=2.2±.14) were selected from each of the treatment groups for harvest. Following exsanguination samples from subcutaneous fat (removed from over the LD (SUBQ)) were excised from each animal, diced, snap frozen, and stored at - 80°C for evaluation of gene expression. using RT-PCR. A comparison of the relative gene expression for peroxisome proliferator activated receptor gamma (PPARγ), CCAAT enhanced binder protein alpha (CEBPα), AMP-activated protein kinase alpha 2 catalytic subunit ($AMPK\alpha_2$), AMP-activated protein kinase gamma 2 non-catalytic subunit ($AMPK\gamma_2$), acetyl CoA carboxylase (ACC), fatty acid synthase (FASN), stearoyl CoA desaturase (SCD), lipoprotein lipase (LPL), and leptin (LEP) was performed to evaluate adipogenesis in SUBQ. No differences in genes of interest were detected in the weaning subsample due to maternal energy status ($P>0.05$). However, there was a trend for PPARγ ($P<0.10$) and CEBPα ($P<0.10$) to be up-regulated in the NES treatment group of the weaning subsample compared to the PES treatment group. Analysis of SUBQ fat samples revealed no differences ($P>0.05$) between treatment groups in the final subsample for any of the genes of interest. These results suggest negative energy status during mid-gestation may alter differentiation of SUBQ fat around weaning, but these changes are not maintained through the feeding period.

INTRODUCTION

Excess fat deposition in relation to lean tissue continues to challenge production efficiency in the beef industry. Because muscle, fat, and connective tissue originate from the same pool of mesenchymal stem cells these tissue depots are competing against each other for progenitor cells (Du et al., 2010a). This begins early during the fetal stages of growth in beef animals, with muscle fibers differentiating first followed by adipose tissue later during mid-gestation (Du et al., 2010a). Specifically, it is estimated that muscle fiber formation occurs from 2-8 months, and adipogenesis does not begin until mid-gestation (Feve, 2005; Russell and Oteruelo, 1981). Because competition for stem cells can occur during fetal development it is necessary to understand the consequences of altering the maternal environment and the resulting effects on the developing fetus.

Fetal or developmental programming is the result of a stimulus or an insult to the mother during a critical period of development that has lasting effects on metabolism, physiology, and/or structure of the offspring (Godfrey and Barker, 2000). Throughout gestation many alterations in nutrition are imposed on the mother and these dietary variations have demonstrated the potential to influence fetal development (Barker, 1995; Ramsay et al., 2002). Many cow-calf producers graze native range, however, it is common for beef cows to experience periods of under-nutrition, due to limited forage availability and poor forage quality, which often coincides with some point during gestation (Vavra and Raleigh, 1976). In the upper Midwest spring calving cows experience adverse conditions during mid-gestation, as this period is generally during the winter months where there is the potential for snow cover and forage is in a dormant state (DelCurto et al., 2000).

Variation in offspring health, weight, and carcass characteristics have been evaluated in many livestock species as a result of altered maternal nutrition (Munoz et al., 2009; Pond et al., 1969; Underwood et al., 2010). In beef cattle nutrient restriction during gestation has varied results where Long et al. (2012) reported an increase in yield grade in nutrient restricted offspring, but Underwood et al. (2010) saw a decrease in SUBQ fat with no change in yield grade from native range offspring. However, few studies have evaluated the effects of altered nutrient intake during mid-gestation on gene transcription in adipose depots, which is critical for meat animals as this is the period of gestation that correlates to secondary myogenesis and the initiation of adipogenesis as previously mentioned (Du et al., 2010a). Therefore altered nutrient intake affects signaling pathways driving cell differentiation towards connective tissue development or muscle development (Du et al., 2010b). Most commonly noted is the creation of a thrifty phenotype where an animal prepares itself to be born into a nutrient sparse environment resulting in increased adiposity (Hales and Barker, 1992). Conversely, having excess nutrients available, or overfeeding, has also been shown to alter carcass characteristics (Castro and Avina, 2002; Wallace et al., 1996). Thus, research is needed to understand the signaling mechanisms responsible for alterations to adipose deposition in carcass composition due to altered maternal nutrition. We hypothesized that restricting the maternal plane of nutrition during mid-gestation will result in differential expression of genes in bovine adipose tissue at 2 different time points during the feeding phase. Therefore the objective of this study was to determine the effects of maternal energy status during mid-gestation on gene expression in bovine subcutaneous adipose tissue at weaning and finished weight in the resultant offspring.

MATERIALS & METHODS

Animals

The South Dakota State University (SDSU) Animal Care and Use committee approved the following animal experiment.

Dietary Treatments

Cows were managed as described in Chapter 2. Briefly, 151 naturally serviced crossbred beef cows were evaluated for pregnancy, day of gestation, fetal sex, cow body weight (BW) and BCS. Cows were allotted to one of two treatments: 1) (76 cows)-fed to achieve and/or maintain BCS 5.0-5.5 (control dietary treatment); or 2) (75 cows)-fed to lose 1 BCS over the ensuing 91 day treatment period of mid-gestation (restricted dietary treatment). Cows were weighed every 28 days throughout the treatment period. Ultrasound measurements were collected for 12^{th} rib subcutaneous fat thickness (FT) and ribeye area (REA), and BCS were evaluated at the beginning and the end of the treatment period. After completion of the treatment period, all cows were managed as a common group on native range and allowed free choice of a 20% crude protein supplement through weaning. Cows were not weighed or evaluated for BCS prior to calving.

Offspring Management

In October, calves that met study protocol criteria (133 head) were weaned and immediately shipped 534 km to the SDSU Research Feedlot in Brookings, SD. Calves were managed and fed similarly to reach 1 cm of 12^{th} rib subcutaneous fat by June 1, 2012, as well as maintain health throughout the trial. Calves were housed in outdoor pens and allocated such that each pen only represented one gender and one treatment per pen. Bodyweight was stratified across pens. Calves were fed the same diet once daily through the feeding period.

Selection of Subsample Animals

From the 133 head an initial subsample (n = 12; 6 steers per treatment group) was harvested at weaning after calves had been acclimated to the feedlot (approximately 22 days post-weaning). A final subsample (n = 12; 6 steers per treatment group) was harvested at the targeted finishing endpoint of 1 cm of 12^{th} rib fat.

Prior to the weaning harvest calves were preselected to be in either the weaning or final subsample as follows. Thirty head of steers (n = 15 per treatment) were initially selected based on having a similar birth date to the average birth date (BD; April 13) for the entire group of calves and a similar WDA ((Weaning weight - Birth weight)/age). From this initial group of 30 steers, 24 head were randomly assigned to either the weaning (n=12; WDA=2.2 ± 0.09, BD=4/16/11 ± 6) or final subsample (n=12; WDA=2.2 ± 0.14, BD=4/14/11 ± 6). Steers in the final subsample were evaluated for performance data and weight gain throughout the feeding phase prior to final harvest in order to compare performance relative to the whole group.

Sample Collection

Prior to harvest calves were sorted from their pens, and feed was withheld overnight. The morning of harvest cattle were weighed at the research feedlot prior to shipping. Following shipping (3.2 km) steers were harvested at the South Dakota State University abattoir following standard procedures. Immediately following exsanguination subcutaneous fat was removed from over the *longissimus dorsi* at approximately the 13^{th} rib. Subcutaneous fat from each individual was minced and snap frozen in liquid nitrogen for later analysis of gene expression. Tissue samples were stored at -80°C until expression of target genes was evaluated.

Cow Management Analysis

Changes in treatment classification are explained in Chapter 2. Briefly, upon analysis of cow data it was determined that a few cows within each dietary treatment group did not achieve the goals of our treatments physiologically. The intended treatment for the current experiment was to alter the uterine environment during mid-gestation. In order to achieve two treatments different diets were used to maintain or lose body condition during mid-gestation. Because we could not establish biological differences based on dietary treatment (control versus restricted), cows and their calves were divided into new energy status categories (PES versus NES). This re-classification of animals created 2 treatment groups as there was a bimodal distribution within the population: PES and NES were calculated from metabolic indicators including BCS, REA, and BW collected during gestation. The formula used is as follows:

$$\left[\frac{(Obs\,BCS\;\Delta - BCS\;\Delta\,\vec{x})}{BCS\;\Delta\,S_d}\right] + \left[\frac{(Obs\,REA\;\Delta - REA\;\Delta\,\vec{x})}{REA\;\Delta\,S_d}\right] + \left[\frac{(Obs\,BW\;\Delta - BW\;\Delta\,\vec{x})}{BW\;\Delta\,S_d}\right]$$

This re-classification resulted in 1 animal moving in both the weaning and final subsample from the NES group to the PES group (NES=5 steers, PES=7 steers).

RNA Extraction

Total RNA was isolated from bovine SUBQ using TRI Reagent® RT (#RT111, Molecular Research Center, Inc. Cincinnati, OH). Samples were powdered using a mortar, pestle, and liquid nitrogen. Approximately 200 mg of powdered sample was placed into 1 mL of TRI Reagent RT. To achieve phase separation 50 µl of bromoanisol (BN 191, Molecular Research Center, Inc. Cincinnati, OH) was added to each sample, vortexed for 10 sec, and centrifuged at 12,000 x g for 15 min at 4°C. Following phase

separation from centrifugation the aqueous RNA phase was collected and placed in a clean microcentrifuge tube. Isopropanol (0.5 ml) was added to precipitate the RNA. The sample was allowed to incubate at room temperature (approximately 21°C) for 5-10 min followed by centrifugation at 12,000 x *g* for 5 min at 4°C. After confirming formation of an RNA pellet attached to the tube, a mixture of 75% ethanol and 25% RNA free water was added, then the sample was vortexed in order to wash the pellet. The sample was then centrifuged at 5000 x *g* for 5 min at 4°C. After confirmation of the presence of a pellet, ethanol was decanted and tubes were inverted for 3 min to air dry. The RNA pellet was resuspended with nuclease-free water. Concentration of RNA was determined spectrophotometrically (Nanodrop 2000, Thermo Scientific, Wilmington, DE, USA). Absorbance ratios at 260 and 280 nM were used as a determination of purity. Extracted RNA with a 260/280 ratio between 1.6 and 2.0 with a concentration greater than 200 ng/µL was deemed acceptable. To improve RNA quality a DNase I Amplification Grade kit (# 18068015, Invitrogen, Roche Molecular Systems, Inc., Foster City, California) was used to remove genomic DNA contamination, and RNA concentration was diluted to 200 ng/µL.

cDNA Synthesis

A high-capacity cDNA reverse transcription kit (# 4368813, Applied Biosystems, Carlsbad, CA, USA) was used to convert RNA to cDNA using a Bio-Rad MyCycler Thermocycler (# 170-9703; Bio-Rad Laboratories, Hercules, CA, USA) with thermal cycling parameters recommended by the manufacturer which included one cycle of 37°C for 60 min followed by 85 ºC for 5 min.

Cytokine Primers

Previously published mRNA sequences for the genes of interest were used to design specific forward and reverse primers. Messenger RNA sequences were found utilizing the National Center for Biotechnology Information (NCBI; United States Library of Medicine, Bethesda, MD, USA) database. GeneBank Accession number was then used to design primers with the PrimerQuest software from Integrated DNA Technologies (IDT, Coralville, IA, USA). Primer sequences for genes of interest can be found in Table 5.1. Primers were brought up to a working dilution of 10 µM in 10X TRIS.

Real Time- PCR

Measurement of the relative quantity of the cDNA of interest was carried out using RT^2 Real-Time™ SYBR Green/ROX PCR Master Mix (# 330523, Applied Biosystems, Foster City, CA) with appropriate forward and reverse primers (400 nM), and 1 µL cDNA mixture. Assays were performed using the Mx3005P thermal cycler (Agilent Technologies, Stratagene Product Division, La Jolla, CA, USA) with thermal cycling parameters recommended by the manufacturer which included 95°C for 10 min, 40 cycles of (95ºC, 30 sec; 55ºC, 60 sec; 72°C, 60 sec) and 1 cycle of (95°C, 60 sec; 55°C, 30 sec; 95°C, 30 sec). Each amplicon was electrophoresed on a 2% agarose gel to verify the existence of a single amplicon of the correct length.

Statistical Analysis

Least squares means for cow measurements taken during the mid-gestation treatment period were calculated using PROC GLM procedures of SAS (SAS Inc., Cary,

NC, USA). Differences in the main effect of cow energy status were tested to a predetermined significance level of $P<0.05$ with trends discussed at $P<0.10$.

Fold differences in gene expression between PES and NES treatment groups were determined using Relative Expression Software Tool (REST; Corbett Research & M. Pfaffl, Technical University Munich) and according to the procedures of Pfaffl (Pfaffl, 2001). Relative expression is based on the expression ratio of a target gene versus a reference gene and is adequate for most purposes to investigate physiological changes in gene expression levels. The expression of a target gene is standardized by a non-regulated reference-gene. The expression ratio results of the investigated transcripts were tested for significance by a Pair Wise Fixed Reallocation Randomization Test (Rest.gene-quantification.info). In SUBQ, lipoprotein receptor-related protein 10 (LRP10), RNA polymerase 11 polypeptide A (POLR2A), eukaryotic translation initiation factor 3, subunit K (EIF3K), and emerin (EMD) were used as reference genes (Table 5.2) . These genes have been identified as suitable housekeeping genes in bovine fat depots (Saremi et al., 2012). Means of the main effect of dam dietary treatment were tested to a predetermined significance level of $P<0.05$ with trends discussed at $P<0.10$.

RESULTS & DISCUSSION

Results of dietary treatments on cows of the subsampled steers are presented in Table 5.3. After reclassifying the dietary treatments (control versus restricted) into energy status groups (PES versus NES) some of the measurements associated with cow body condition were no longer significant in the weaning and final subsample. However, the entire treatment groups were different ($P<0.05$) for change in BCS, BW, REA, and FT. In the weaning subsample while both groups of cows lost body condition, the NES group lost a greater amount ($P<0.05$) of condition during the treatment period. Cows in the PES group gained weight while cows in the NES group lost weight during the treatment period ($P<0.001$). Ribeye area for cows in the PES group increased while cows in the NES group had a tendency to decrease ($P<0.10$) during mid-gestation. This same comparison resulted in a tendency for a change in FT ($P<0.10$) between treatment groups. When these performance measures were combined to determine cow energy status, a difference was observed ($P<0.0001$) between the two groups in the weaning subsample. In the final subsample cow BCS had a tendency to be different ($P<0.10$) between the treatment groups with the PES cows slightly gaining body condition and the NES cows losing body condition. The change in cow BW between the treatment groups was different ($P<0.001$) with the PES cows gaining weight and the NES cows losing weight during the management period. The change in REA had a tendency to be different ($P<0.10$), but no differences in FT ($P>0.05$) were found between the treatment groups. However, the energy status was different ($P<0.001$) between the groups of cows, with cows in the PES group having a positive energy status and a negative energy status in the NES group. These data suggest we were achieving the intended outcome of the treatment in that the PES cows were in a

positive energy state and the NES cows were in a negative energy balance during the mid-gestation treatment period. Additionally, cow performance data for the entire population was similar to the cow subsample data. Therefore the subsamples of offspring selected from each treatment group at weaning and final harvest were representative of the population. The performance measurements collected and used to calculate energy status from the cows allowed for quantification of the energy status of each animal. Body condition scoring and change in weight are measures that producers and researchers can use to evaluate the metabolic status of the animal (Roche et al., 2009). Additionally, cows in a negative energy balance will catabolize fat stores along with lean tissue in order to meet the demands of the fetus and the individuals' maintenance requirements (Kuhla et al., 2011; Roche et al., 2009). When a negative energy balance occurs in the dam, depending on the severity of the nutrient restriction, pregnancy can be aborted or fetal alterations can occur (Funston et al., 2010). Some of these alterations to fetal development have been reported to manifest as permanent adaptations later in life for livestock (Larson et al., 2009; Underwood et al., 2010; Zhu et al., 2006). Changes in carcass characteristics related to changes in carcass composition in regards to fetal programming from altered nutrient intake have been reviewed previously (Du et al., 2013; Du et al., 2010a; Funston et al., 2010). But very few experiments have been performed evaluating gene transcription in adipose tissue relative to mid-gestation negative energy status at weaning and at a final finishing time point.

Real-Time semi-quantitative PCR was used to determine the expression of genes associated with adipogenesis relative to a battery of housekeeping genes. A comparison of the relative gene expression for PPARγ, CEBPα, AMPKα_2, AMPKγ_2, ACC, LPL, SCD, LEP, and FASN are presented in Figure 5.1. At the weaning sample period there

were no differences between treatment groups for any of the adipose transcription factors. However, there was a trend for PPARγ (*P<0.10*) and CEBPα (*P<0.10*) to be up-regulated in the NES treatment group in the weaning subsample. Analysis of SUBQ fat samples revealed no differences between treatments in the final subsample period for these genes of interest. Figure 5.2 displays the comparison of relative gene expression for PPARγ, CEBPα, AMPKα_2, AMPKγ_2, ACC, LPL, SCD, LEP, and FASN at the final subsample harvest.

Adipogenesis begins during mid-gestation when mesenchymal stem cells are differentiating into muscle fibers, or connective tissue composed of either adipocytes or fibroblasts (Du et al., 2013; Feve, 2005). The *de novo* synthesis of adipocytes is accomplished in two stages termed determination and differentiation (MacDougald and Mandrup, 2002). The current study focuses on differentiation factors associated with adipogenesis since gene expression regulates cell differentiation, as well as growth and development. Some factors evaluated in this study differentiate preadipocytes into mature adipocytes, like PPARγ and CEBPα (Avram et al., 2007).

Adipose tissue is derived from the same mesenchymal stem cells as muscle tissue. However, adipose tissue follows a different signaling pathway and those mesenchymal stem cells respond to different factors that regulate determination towards adipogenesis. There are three main transcription factor families that regulate differentiation during adipogenesis (Saladin et al., 1999). Two of the most researched regulators of adipogenesis are 1) C/EBP with isoforms α, β, and δ and 2) and PPAR γ1 and γ2 (Saladin et al., 1999; Wu et al., 1999). Another transcription factor, helix-loop-helix adipocyte differentiation and determination factor-1, is not as well understood in

livestock compared to the other two transcription families, but still performs a role in regulation (Saladin et al., 1999).

The main two transcription factor families, C/EBP and PPAR, influence the proliferation and differentiation of preadipocytes to mature adipocytes in a positive feedback loop stimulating each other to signal cells to differentiate (Wu et al., 1999), which is necessary for adipogenesis. It has also been shown that adipogenesis is also controlled by the Wnt signaling pathway (Du et al., 2010a). Specifically, PPARγ is regulated by β-catenin, which is part of the Wnt signaling pathway (Moldes et al., 2003). Up-regulation or down-regulation of the Wnt pathway will affect both myogenesis and adipogenesis (Du et al., 2010a). Therefore it is important to understand the interaction between β-catenin and PPARγ, and the outcomes associated with a change in expression in either transcription factor.

There are 3 common isotypes of PPAR, which are a nuclear hormone receptor family activated by ligands (Michalik et al., 2006). This paper focuses on PPARγ, the isoform known to be essential for adipose tissue differentiation (Rosen et al., 2000). The gamma isoform receptor appears in the early stages of adipose cell differentiation having higher expression levels in preadipocytes than other connective tissue cell types (Spiegelman and Flier, 1996). This transcription factor appears to be the regulator for initiation of adipogenesis entirely, directly affecting other fat specific genes (Saladin et al., 1999; Spiegelman and Flier, 1996; Tontonoz and Spiegelman, 2008).

The transcription factor CEBPα increases expression late during adipogenesis (Spiegelman and Flier, 1996). High expression levels created pharmacologically can cause differentiation in connective tissue cells, but normal levels of CEBPα expression

observed in mature fat cells result in low adipogenic action when PPARγ is not present (Freytag et al., 1994; Tontonoz et al., 1994). Together PPARγ and CEBPα, have a synergistic mechanism when expressed together creating abundant differentiation (Tontonoz et al., 1994). This co-expression even has the potential to transdifferentiate myoblast cells into adipocytes, thus illustrating the power of co-activation (Hu et al., 1995). These differentiation factors, CEBPα and PPARγ, also maintain the differentiated state of adipocytes regulating each other as necessary (Rosen, 2005). In order for terminal differentiation of an adipocyte to occur both PPARγ and CEBPα need to be expressed simultaneously to initiate lipid synthesis and other lipid programming events (Fernyhough et al., 2007; Hausman et al., 2009).

Calves from the NES dams had a tendency to have up-regulation of PPARγ expression and CEBPα expression at the weaning subsample and no differences in the final subsample. This is likely because at the final subsample adipocytes were at the lipid filling stage of growth and not the differentiation stage like at the weaning harvest. Considering previous research it makes sense that these two key regulators of differentiation had a tendency to be different when maternal nutrition was altered. At weaning, calves were likely differentiating their SUBQ stores from determined cells developing them into preadipocytes and mature adipocytes. Some research has suggested the number of adipocytes is set when reaching the end of adolescence in other species, but most cattle are slaughtered prior to full maturity and are still undergoing some rate of adipocyte growth (Du et al., 2013; Goessling et al., 2009). In addition, the majority of adipose tissue growth results from adipose hypertrophy or lipid filling as the animal ages (Robelin, 1981). Subcutaneous fat, unlike the other 3 fat depots, has been shown to

develop later in life, where adipocytes appear to go through hyperplasia later in the growing period and at a faster rate than other adipose depots (Robelin, 1981). However, this research from Robelin (1981) only evaluated adipose deposition through 65% of the growing period, not at full maturity.

Furthermore, subcutaneous adipocyte determination occurs in mid- to late gestation and around weaning from mesenchymal stem cells (Hood and Allen, 1973). This is followed by differentiation factors further stimulating proliferation of adipocytes which include transcription factors like PPARγ and CEBPα (Avram et al., 2007). Because subcutaneous adipocyte formation is occurring around weaning this potentially could explain the tendency observed to up-regulate differentiation factors in the NES group at weaning, but not at the final harvest. Up-regulation of PPARγ and CEBPα in the NES group may be from fetal programming effects and creation of a "thrifty phenotype" in those steers since the fetus was developing in a limited nutrient environment (Hales and Barker, 1992). Additionally it takes less energy to maintain fat tissue than it does muscle, lowering the maintenance energy expenditure (Thompson et al., 1983).

Conversely, one transcription factor that regulates adipogenesis is AMPK, known for regulating energy metabolism through fat and carbohydrate catabolism (Aschenbach et al., 2002). This gene is a heterotrimer consisting of α, β, and γ subunits, which also have multiple isoforms (Gao et al., 1996; Winder and Hardie, 1999). The α subunit contains the catalytic domain for activity, but the β and γ regulatory subunits are also necessary for proper enzyme activity (Gao et al., 1996). AMP-activated kinase oversees the metabolic status of the animal by indirectly increasing the rate of fat oxidation and

glucose metabolism in response to low energy charge of the cell (Winder and Hardie, 1999). Previous research has shown an increase in AMPK activity when cattle have more muscling than their counterparts (Underwood et al., 2007). As muscle takes more energy to maintain and AMPK regulates cell energy status, this increase in activity is understandable. In the current experiment cattle in the weaning subsample were not different in weight (Mohrhauser, 2013), so they likely did not have differences in muscularity either, resulting in no differences in expression of either AMPK subunit. Steers were also of similar weight between groups at the final subsample thus potentially not having any differences in muscularity resulting in no differences in expression or subsequent activity of either AMPK subunit. Because cattle were on a similar diet throughout the growing and finishing periods, both diets formulated for growth, both groups of steers were in a positive cellular energy state. This positive energy charge would not increase expression of AMPK as additional energy substrates were not needed by the animal. Additionally, activation of AMPK has been shown to inhibit expression of PPARγ and CEBP *in vitro* and in mice (Giri et al., 2006; Habinowski and Witters, 2001). Since there is a tendency in the weaning subsample for PPARγ and CEBPα to be up-regulated in the NES treatment, expression of AMPK would not be up-regulated as well. In relation to fetal programming, ewes in an overfed state produced fetuses that had inhibited AMPK activity in skeletal muscle, but increased expression of PPARγ suggesting these fetuses were not in a nutrient sparse environment and capable of adipogenesis (Zhu et al., 2008). In the current experiment the animals evaluated were nutrient restricted *in utero* during mid-gestation. If the response is opposite of the aforementioned experiment then there should be increased expression of AMPK and

decreased expression of PPARγ and CEBPα. The reason these results are different may be two-fold: 1) in the current experiment there was no fetal subsample collected and AMPK expression was evaluated in subcutaneous fat, not skeletal muscle unlike Zhu et al. (2008). and 2) the restriction was potentially not severe enough to alter gene transcription permanently. The lack of severity in the diet restriction likely did not produce epigenetic-type effects where DNA methylation and histone modification did not take place on the resultant progeny (Bird, 2007).

In relation to adipogenesis, there are 3 enzymes addressed in this paper associated with the terminal phase of differentiation in adipocytes: ACC, FASN, and SCD (Gregoire et al., 1998). These enzymes are also known to be active in fatty acid formation. Because of their anabolic role in fatty acid synthesis during times of fasting, enzyme activity, and likely expression, are depressed. Subsequently during re-feeding periods or times of compensatory gain these enzymes are up-regulated (Sul et al., 2000).

The first gene involved in the regulation of lipid metabolism is ACC, where AMPK regulates cell energy status through phosphorylation of ACC (Tong et al., 2008). Acetyl Co-A carboxylase is involved with the production of malonyl-CoA, which is the first committed and rate limiting step in the production of long-chain fatty acids (Wakil et al., 1983). AMP-activated kinase is an up-stream regulator of ACC for lipid metabolism (Park et al., 2002). Sheep fetuses from ewes in a positive energy balance/overfed state had a decrease in AMPK activity leading to an increase in ACC activity allowing for lipid accumulation in fetal muscle (Tong et al., 2008). Since AMPK is negatively associated with ACC inverse activity would be expected. In the current experiment there were no differences between treatment groups within subsampling times for ACC or

AMPK. However, in the final subsample ACC expression was numerically greater than AMPK showing a potential increase in available energy, which can be utilized for fat deposition. Since a greater amount of subcutaneous fat accumulates during the finishing period greater activity from ACC would be expected compared to AMPK (Du et al., 2013).

The second enzyme responsible for the formation of fatty acids *de novo* is FASN, which catalyzes the reaction of acetyl-CoA and malonyl-CoA to palmitate later producing long-chain fatty acids (Sul et al., 2000; Wakil, 1989). This is the last step in the biosynthetic pathway for fatty acid synthesis (Clarke, 1993). Tissue concentration of FASN is also a good indicator of the maximum capacity of that tissue to synthesize fatty acids (Clarke, 1993). Previous research has shown an increase in FASN expression is correlated with an increase in adipose tissue deposition in cattle (Jeong et al., 2012). The current study showed no differences in FASN expression between treatment groups in either the weaning or final subsample. At the weaning subsample steers were not likely storing much energy as there would be a significant amount of lean tissue growth occurring at that time and most energy would go toward that type of growth. At the final subsample cattle would be depositing more fat than lean tissue. It is likely there were no differences in the final subsample because both groups were on the same diet. Steers in this subsample were also not over-finished and thus potentially another reason there were no differences between treatment groups.

The last enzyme involved in fatty acid synthesis evaluated in this paper is SCD, an enzyme active in lipogenesis. In the lipogenesis pathway SCD converts saturated fatty acids into monounsaturated fatty acids, a preferred substrate for triglyceride synthesis

(Brown and Rudel, 2010). Finished cattle have an increase in SCD activity and mRNA expression in subcutaneous adipose tissue indicating this enzyme may be a marker for terminal differentiation of preadipocytes (Martin et al., 1999; St John et al., 1991). This period of time when preadipocytes leave the proliferative phase and begin to fill with lipid happens prior to differentiation, thereby being classified as an early differentiation factor for preadipocytes resulting in adipocyte hypertrophy (Cornelius et al., 1994; Martin et al., 1999). Knock-out mice for this gene display significant reductions in adiposity, possibly through feedback inhibition of ACC (Cohen and Friedman, 2004). The present study found no differences in SCD expression between the treatment groups. However, in the weaning subsample the NES group had a greater expression level approaching a tendency compared to the PES group. This suggests steers in the NES group may have the potential to store more SUBQ fat than steers in the PES group. However, this trend for increased expression did not persist through the final subsample.

Conversely to lipogenesis, LPL is used for its ability to metabolize lipids, like chylomicrons and very low density lipoproteins, efficiently and deliver energy to the appropriate tissues. Lipoprotein lipase is necessary for releasing fatty acids from circulating triglycerides (Braun and Severson, 1992; Salinelli et al., 1996). This hydrolysis of the triacylglycerol portion of lipoproteins produces free fatty acids and 2-monoacylglycerol for use in tissues as energy substrates (Braun and Severson, 1992). The free fatty acids are either re-esterified for fat storage, or used for metabolic energy (Obunike et al., 2001). Lipoprotein lipase is used to overcome changes that occur within the body, particularly in energy requirements. This enzyme can be regulated in adipocytes through activation or inhibition of protein kinase C (Cruz et al., 2001).

Regulation of LPL can occur through transcriptional and post-transcriptional control, depending on the mechanism controlling LPL gene expression, such as up or down regulation of message RNA (Mead et al., 2002). Post-transcriptional control is the more prominent form, where regulation comes from changes in nutritional status and subsequently the hormones that accompany changes in blood glucose levels like insulin (Mead et al., 2002). Glucose can also regulate this enzyme in adipocytes (Cruz et al., 2001). This regulation occurs in a tissue and cell specific manner (Mead et al., 2002). In rats there is an increase in LPL activity in cardiac muscles and a decrease in activity in adipose tissue during fasting. However, just after a meal, the increase and decrease in activities between the two tissues is reversed (Doolittle et al., 1990). Steers in this study had no differences in LPL expression at either subsampling point between treatment groups. This is likely from the lack of differences in dietary treatment in the feedlot and no differences in carcass characteristics within the subsample groups (Mohrhauser, 2013). Similarly, Long et al. (2012) reported cattle from nutrient restricted cows supplemented with and without protein during early to mid-gestation did not have differences in expression of LPL in SUBQ tissue.

In addition to LPL, LEP is also produced by adipose tissue and its function is to regulate caloric intake or appetite within an animal (Hausman et al., 2009; Hollenberg et al., 1997; Zhang et al., 1994). When animals were given a dose of LEP intakes were depressed, there was a decrease in body weight, fat depots were depleted, and there was an increase in energy metabolism (Levin et al., 1996; Pelleymounter et al., 1995). Because of the decrease in body weight during food deprivation LEP gene expression declines very quickly, likely in response to reduced adipose tissue that was producing

LEP (Cusin et al., 1995; Frederich et al., 1995). Leptin expression is also correlated with adipocyte size and whole body fatness (Houseknecht et al., 1998). When body weight is decreased by 10%, serum LEP concentrations decrease by about 50%, but when body weight increases by 10%, serum LEP concentrations increase by 300% (Considine et al., 1996; Kolaczynski et al., 1996). Studies on the LEP gene have been of interest in recent years as there is a polymorphism within the gene that has economic impacts for producers (Buchanan et al., 2003; DeVuyst et al., 2008). A variety of traits have been shown to be influenced by LEP such as milk production, calf weaning weight, growth, carcass quality, backfat measurements, and cow productive life (Buchanan et al., 2003; DeVuyst et al., 2008; Kononoff et al., 2005; Lusk, 2007; Mitchell et al., 2009). Although we did not evaluate these aforementioned traits, they do have implications for gene expression in the current study. However, there were no differences in LEP expression between treatments in either subsample. Again, the lack of weight differences and carcass characteristics between the groups at a subsampling time point is likely the reason why there were no differences between treatment groups at a given subsample period.

Overall the lack of differences between treatment groups was not expected as other researchers have reported differences in activity levels of genes in offspring when dams were either overfed or underfed during gestation in fetuses (Tong et al., 2008; Underwood et al., 2008). However, those experiments were conducted differently in that one used fetal tissue and both evaluated gene expression in muscle. These experiments also evaluated gene expression as it relates to intramuscular fat, not SUBQ tissue. Additionally, the four fat depots do not act similarly in the way and time that hypertrophy and hyperplasia of these depots take place and therefore cannot be directly compared to

each other. Intramuscular adipogenesis during the prenatal stage is desirable as it creates sites for fat deposition in muscle resulting in marbling and increasing meat quality (Du et al., 2013). But SUBQ fat is also developing during the prenatal stage, which can be costly to producers as it decreases cutability. Genes known to be active in adipogenesis and differentiation had greater expression levels in SUBQ fat than in intramuscular fat of beef carcasses illustrating a greater amount of fat accumulation in the SUBQ fat depot (Pickworth et al., 2011). This increase in expression likely results in differentiation and hypertrophy of adipocytes as adipose tissue is growing (Pickworth et al., 2011). Because of these differences in SUBQ fat and marbling it is hard to compare gene expression between these two fat depots. This is especially true because this comparison was not made directly in this experiment.

Another possible reason no differences were detected in gene expression between treatment groups may be that the subsample animals were not encountering any challenge, nutrition or otherwise, at the time of sampling. Cattle from both treatment groups were on the same diet being used for growth, not maintenance, and were healthy. This could explain why genes associated with energy metabolism were not different between groups since growth rates between groups of steers were not different. Additionally, the treatment was applied to the offspring *in utero*. The only way changes would persist into adulthood is through epigenetic effects on the fetus. Moreover, the nutritional challenge imposed on the cows during gestation was not extreme, which is likely the cause of a lack of epigenetic-type effects in our results. Further, changes that occur *in utero* with gene expression are difficult to evaluate without an initial subsample

during gestation. This would help to understand the physiological state of the fetus and whether those differences would persist into adulthood resulting in epigenetic effects.

Since steers in this experiment consumed similar diets, fetal programming during mid-gestation would have had to decrease the amount of mesenchymal stem cells available, or altered mesenchymal stem cell determination away from myogenesis towards adipogenesis; otherwise we would not expect differences in adipogenic differentiation factors. We did hypothesize fetal programming during mid-gestation would shift determination from myogenesis towards adipogenesis, but carcass characteristics like FT do not agree with the hypothesis (Mohrhauser, 2013). Therefore it is not likely the dietary treatments imposed during mid-gestation affected mesenchymal stem cell determination. This, coupled with the fact that the steers in the final subsample were not over-finished and that there were no differences in backfat (Mohrhauser, 2013) between the treatment groups makes it understandable that no differences in gene expression were found. If cattle between the treatment groups had differences in FT, then differences in expression of PPARγ, CEBPα, ACC, FASN, SCD, and LEP would have been expected in the group with increased FT. But this did not occur and gene expression results agree with a lack of differences in carcass characteristics.

It is hard to determine if steers would have been on different diets if gene expression would have been different. Potentially steers within the same treatment group fed different diets, a restricted diet, a maintenance diet, and an overfed diet, may have showed differences in gene expression. The difficulty in this example would be whether the changes in gene expression were from the original management *in utero* or in

response to energy intake at that time. This example is something that still needs to be determined.

One last reason we may have not found any differences in gene expression could be from our differences in numbers between treatment groups. Because of the intensive and costly nature of this project it would be difficult to have a large group of animals for subsampling. Also, upon further scrutiny of the original treatments (control versus restricted), we decided the treatment was whether or not cows lost condition or gained condition during mid-gestation. Once the treatments were evaluated in this nature a few steers changed treatment groups resulting in NES=5 and PES=7 at each subsampling time. This could have made the NES subsampling groups weaker than intended. Ideally treatment groups would be at least equal to one another, making the experiment stronger.

IMPLICATIONS

Overall steers from NES cows during mid-gestation do not have altered gene expression in SUBQ fat compared to steers from PES cows. Offspring are able to overcome any adverse effects that might have occurred *in utero*. These findings suggest offspring can encounter nutritional insults during gestation without experiencing long term effects on carcass characteristics as it relates to backfat.

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Table 5.1 Primer sequence for genes of interest.

Gene[a]		Primer Sequence	Accension Number
bPPARγ	forward	5' - CTGCGAAAGCCCTTTGGTGACTTT -3'	NM_181024
	reverse	5' - CCAAGGCTTGCAGCAGATTGTCTT - 3'	
bC/EBP-α	forward	5' - AGAAGTCCGTGGACAAGAACAGCA - 3'	NM_176784
	reverse	5' - ATTGTCACTGGTCAGCTCCAGCA - 3'	
bSCD	forward	5' - CCAGAGGAGGTACTACAAACCTG - 3'	NM_173959
	reverse	5'- AGCCAGGTGACGTTGAGC - 3'	
bFAS	forward	5' - GGTGTGGACATGGTGACAGA - 3'	NM_001012669
	reverse	5' - ACAATGGCCTCGTAGGTGAC - 3'	
bLPL	forward	5'- GACTCGTTCTCAGATGCCTTAC - 3'	NM_001075120
	reverse	5' - GGCCTGGTTGGTGTATGTATTA - 3'	
bLeptin	forward	5' - CCCAAAGTCCAGGGAAGAAA - 3'	NM_173928
	reverse	5' - TGAGAGGAGCGAGAGAGAAA - 3'	
bAMPKα_2	forward	5' - CTGAATACAACGAAGCCCAAATC - 3'	NM_001205605
	reverse	5' - GCTCGGTAAACTTCAGCCATA - 3'	
bAMPKγ_2	forward	5' - GAGACCATCGTGGACAGAATC - 3'	XM_002686979
	reverse	5' - GCAGAATATCGGACAGGGAAATA - 3'	
bACC	forward	5' - CTCCAACTTCCTTCACTCCTTAG - 3'	NM_174224

[a]bPPARγ = bovine Peroxisome Proliferator Activated Receptor gamma; bC/EBPα = bovine CCAAT Enhanced Binder Protein alpha; bSCD = bovine Stearoyl CoA Desaturase; bFAS = bovine Fatty Acid Synthase; bLPL = bovine Lipoprotein Lipase; bAMPKα_2 = bovine AMP-Activated Protein Kinase alpha 2 catalytic subunit; bAMPKγ_2 = AMP-Activated Protein Kinase gamma 2 non-catalytic subunit; bACC = Acetyl CoA Carboxylase

Table 5.2. Primer sequence for housekeeping genes.

Gene[a]		Primer Sequence	Accension Number
bEIF3K	forward	5' - CTGACAGACAGCCAGCTAAA -3'	NM_001034489
	reverse	5' - CACGATGTTCTTGGGCTTTATG - 3'	
bEMD	forward	5' - GCACACTACCGCCCTATTT - 3'	NM_203361
	reverse	5' - CCGAAGATGAAGATGAGGACAC - 3'	
bLRP10	forward	5' - CAGCTTCCCATCCACTACTTC - 3'	NM_001100371
	reverse	5'- GAGGGACACCTAACTTGATAGC - 3'	
bPOLR2A	forward	5' - GGACTCCATCGCTGATTCTAAG - 3'	NM_001206313
	reverse	5' - GCTCCAGCTCGTTGTTATGT - 3'	

[a]bEIF3K = bovine Eukaryotic translation Initiation Factor 3, Subunit K; bEMD = bovine Emerin; bLRP10 = bovine Low density Lipoprotein Receptor-related Protein 10; bPOLR2A = bovine Polymerase (RNA) II (DNA directed) polypeptide A;

Table 5.3. Least squares means for days of gestation at mid-gestation and cow BCS, body weight (BW), ribeye area (REA), and fat thickness at the beginning and end of the mid-gestation treatment period.[1]

	Weaning Subsample				Final Subsample			
Trait	Positive[4]	Negative[5]	SEM	*P*-value	Positive[4]	Negative[5]	SEM	*P*-value
Days of Gestation[2]	89	85	2.7	0.3699	84	87	3.8	0.6186
Initial BCS	4.96	5.18	0.263	0.5536	4.45	4.40	0.236	0.8836
Final BCS	4.80	4.43	0.294	0.3488	4.59	4.03	0.158	**0.0215**
Change in BCS	-0.16	-0.75	0.163	**0.0203**	0.14	-0.38	0.208	**0.0868**
Initial BW, kg	475	458	39.2	0.7380	450	443	29.6	0.8496
Final BW, kg	525	435	31.8	**0.0562**	505	414	23.3	**0.0133**
Change in BW, kg	49	-23	11.3	**0.0006**	55	-29	10.9	**0.0002**
Initial REA, cm^2	59.51	59.68	6.764	0.9854	54.04	52.15	4.269	0.7433
Final REA, cm^2	63.75	53.75	6.322	0.2550	55.40	46.57	4.601	0.1733
Change in REA, cm^2	4.24	-5.92	3.608	**0.0569**	1.36	-5.59	2.520	**0.0613**
Initial 12th Rib Fat Thickness, cm	0.40	0.50	0.116	0.5231	0.39	0.33	0.050	0.3280
Final 12th Rib Fat Thickness, cm	0.42	0.38	0.071	0.6787	0.37	0.32	0.044	0.4431
Change in 12th Rib Fat Thickness, cm	0.02	-0.12	0.056	**0.0858**	-0.03	-0.01	0.031	0.6327
Energy Status[3]	1.66	-2.45	0.482	**<0.0001**	1.95	-1.90	0.496	**0.0001**

[1]Measurements taken at beginning and end of mid-gestation period normalized by fill

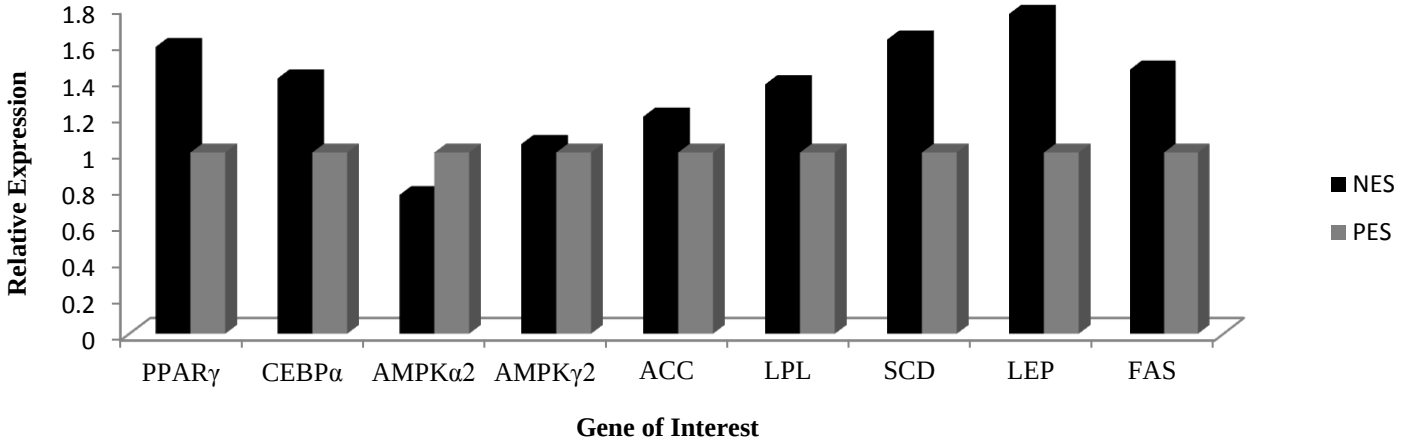
[2]Days of gestation at beginning of mid-gestation treatment as estimated by pregnancy ultrasound

[3]Energy status = $\left[\frac{(Obs\,BCS\,\Delta - BCS\,\Delta\,\vec{x})}{BCS\,\Delta\,S_d}\right] + \left[\frac{(Obs\,REA\,\Delta - REA\,\Delta\,\vec{x})}{REA\,\Delta\,S_d}\right] + \left[\frac{(Obs\,BW\,\Delta - BW\,\Delta\,\vec{x})}{BW\,\Delta\,S_d}\right]$

[4]Positive-n=7

[5]Negative-n=5

Figure 5.1. Influence of maternal energy status during mid-gestation on relative expression of transcription factors in bovine offspring subcutaneous adipose tissue at weaning.



	PPARγ[a]	CEBPα	AMPKα$_2$	AMPKγ$_2$	ACC	LPL	SCD	LEP	FAS
PES[b]	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
NES[b]	1.581	1.408	0.766	1.047	1.198	1.376	1.622	1.763	1.457
P-value	0.079	0.103	0.278	0.742	0.32	0.431	0.145	0.197	0.945

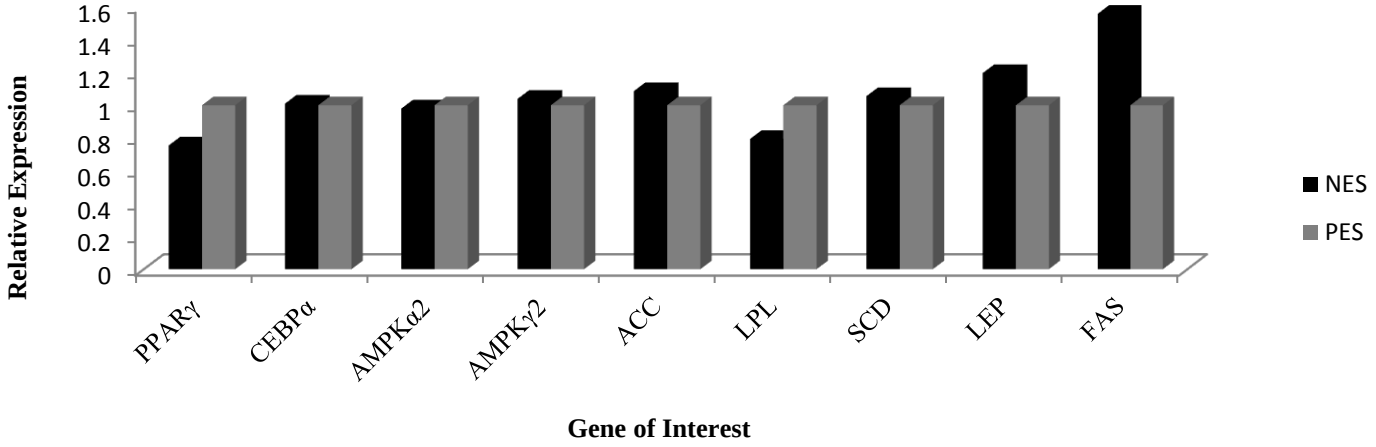
[a]bPPARγ = bovine Peroxisome Proliferator Activated Receptor gamma; bC/EBPα = bovine CCAAT Enhanced Binder Protein alpha; bSCD = bovine Stearoyl CoA Desaturase; bFASN = bovine Fatty Acid Synthase; bLPL = bovine Lipoprotein Lipase; bAMPKα$_2$ = bovine AMP-Activated Protein Kinase alpha 2 catalytic subunit; bAMPKγ$_2$ = AMP-Activated Protein Kinase gamma 2 non-catalytic subunit; bACC = Acetyl CoA Carboxylase.

[b]PES=calves from cows in a Positive Energy Status during mid-gestation, n=7; NES=calves from cows in a Negative Energy Status during mid-gestation, n=5.

[c]Statistical comparisons were made using REST 2008 (Corbett Research Pty, Ltd., Sydney, Australia) expressed as fold change difference from PES treatment group.

[d]Significance was determined at $P \leq 0.05$.

Figure 5.2. Influence of maternal energy status during mid-gestation on relative expression of transcription factors in bovine offspring subcutaneous adipose tissue from steers harvested after 208 d in the feedlot.



	PPARγ[a]	CEBPα	AMPKα2	AMPKγ2	ACC	LPL	SCD	LEP	FAS
PES[b]	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
NES[b]	0.756	1.01	0.98	1.039	1.086	0.796	1.055	1.195	1.555
P-value	0.294	0.975	0.902	0.799	0.723	0.559	0.853	0.501	0.827

[a]bPPARγ = bovine Peroxisome Proliferator Activated Receptor gamma; bC/EBPα = bovine CCAAT Enhanced Binder Protein alpha; bSCD = bovine Stearoyl CoA Desaturase; bFASN = bovine Fatty Acid Synthase; bLPL = bovine Lipoprotein Lipase; bAMPKα_2 = bovine AMP-Activated Protein Kinase alpha 2 catalytic subunit; bAMPKγ_2 = AMP-Activated Protein Kinase gamma 2 non-catalytic subunit; bACC = Acetyl CoA Carboxylase.

[b]PES=calves from cows in a positive energy status during mid-gestation, n=7; NES=calves from cows in a negative energy status during mid-gestation, n=5.

[c]Statistical comparisons were made using REST 2008 (Corbett Research Pty, Ltd., Sydney, Australia) expressed as fold change difference from PES treatment group.

[d]Significance was determined at $P \leq 0.05$.