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THE EFFECTS OF LATE GESTATION MATERNAL NUTRIENT RESTRICTION WITH OR WITHOUT PROTEIN SUPPLEMENTATION IN ENDOCRINE REGULATION OF NEWBORN AND POSTNATAL BEEF CALVES

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THE EFFECTS OF LATE GESTATION MATERNAL NUTRIENT RESTRICTION
WITH OR WITHOUT PROTEIN SUPPLEMENTATION IN ENDOCRINE
REGULATION OF NEWBORN AND POSTNATL BEEF CALVES

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Presented to
the Graduate School of
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In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Animal and Veterinary Science

by
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August 2015

Accepted by:
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DEDICATION

This thesis is dedicated to my family. Their unconditional love and support brought me where I am. However their biggest contribution is instilling in me a foundation built on faith and a love of learning about my Creator and His creation.

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CHAPTER I

INTRODUCTION

The United States beef cattle herd is the smallest it has been in 50 years. The USDA (2015) estimated there were 29,693,100 beef cattle in early 2015. Some of the loss in cattle numbers are due to the natural rise and fall of the “cattle cycle.” However, recent drought years in the cattle dense area of the plains and intermountain region of the United States combined with the rapid loss of agricultural land to development have added to the liquidation of the US herd. With a decreased supply of cattle, relative steady consumer demand, and expanding export markets beef cattle prices have been the highest on record. Costs of feeding cattle have increased as less land is available to graze or grow forage. These factors have made it difficult to meet the nutrition requirements of cows at all stages of production in a cost effective manner. When there is a lack of quality or quantity of forage, producers often elect to limit first the non-lactating cows that have lower nutrient requirements. On an efficient operation this cow is most likely pregnant. Poor maternal nutrition during gestation has many adverse effects including effects on the dam and her offspring including altered postnatal growth and carcass characteristics, offspring appetite and metabolism, and offspring endocrine regulation (Bellows et al., 1982; Spitzer et al., 1995; Long et al., 2009; and Wu et al., 2006). Despite these consequences it is not always possible to provide adequate nutrition during gestation so methods like protein supplementation have been used to maintain cow BW and nutritional status (Sanson et al., 1990). This practice has been beneficial to dam and

fetus in multiple species (Bohnert et al., 2013; Langley-Evans, 1997). With less resources and a larger investment in producing a calf, research is warranted to develop practices that maintain profitability and performance characteristics even during times of nutrient restriction.

An objective of this work presented herein was to determine the effect of a small amount of maternal protein supplementation during late gestation nutrient restriction on maternal and calf performance. This study also demonstrated the effects of maternal protein supplementation and nutrient restriction on newborn calf plasma leptin and cortisol concentrations.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Fetal programming is defined as a *stimulus or insult during a critical period of development that has permanent effects* on the fetus (Lucas, 1991). The concept of fetal programming led to the developmental programming hypothesis. This hypothesis states that changes in fetal nutrition and/or endocrine status can create developmental adaptations that permanently effect fetal structure, physiology, and metabolism (Barker and Clark, 1997; Langley-Evans and Jackson, 1994; Osmond et al., 1993). This predisposes the postnatal offspring to metabolic and endocrine dysfunction and multiple diseases including obesity, cardiovascular disease, and certain cancers. Interests in fetal programming came from studies involving survivors of the Dutch Hunger Winter during World War II. During the winter of 1944 to 1945, the Nazis occupied western Netherland. They imposed nutrient restricted rations of roughly 400-800 calories per day per person (Roseboom et al., 2006). Studies involving the pregnant women exposed to the famine only during late gestation showed that the offspring were born small and remained relatively small the remainder of their lives. Comparatively, the offspring of the women that were exposed during early gestation and later returned back to adequate nutrition had higher occurrences of obesity, altered lipid profile, and cardiovascular disease (Roseboom et al., 2006).

These adverse effects have been duplicated in multiple animal models. Periods of nutrient restriction are very common to the beef industry. This can be caused by the lack of availability of feed, poor pasture conditions, overstocking, breeding young heifers, twins, and many other factors. Most cattle operations utilize forage via grazing pasture during gestation. Drought and poor management can create a lack of nutrients for cattle on pasture during gestation. Immature pregnant heifers and young cows have higher nutrient requirements than older cows as heifers and these young cows are growing to mature size while maintaining and growing a fetus (NRC, 2000). Lactation increases nutrient requirements. This scenario as well as carrying twins can create a negative nutrient environment for the dam and can affect the fetus during the critical periods of development. Fetal programming has important implications for livestock production. It can be used to explain postnatal growth retardation, reduced feed efficiency, decreased muscle quality, as well as poor reproductive performance (Wu et al., 2006). All of these factors affect profitability in animal agriculture.

Maternal Effects

In a cow-calf herd, producing a calf every year is the primary function of the dam. Anything less than optimal reproductive performance can result in economic loss for the producer. This is often caused by a prolonged postpartum anestrous interval. This is especially true for the primiparous heifer but also an issue in young multiparous cows (Short et al., 1990). Reduced maternal nutrient intake pre and post-calving increases adverse effects on postpartum reproduction in primiparous more than in mature cows

(Bellows et al., 1982; Grimard et al., 1995). Body condition score at parturition is one of the most important indicators affecting postpartum interval to estrus and pregnancy rate in cows (Richards et al., 1986; Selk et al., 1988). Spitzer et al. (1995) reported that an increase in BCS of cows calving at 4, 5, and 6 BCS resulted in an increase in birth weights with no association with increased dystocia. A study by Vizcarra et al. (1998) looked at the effects of BCS on calving and the post-partum period. During the last 90 days of gestation, cows were fed to calve at a BCS of 4, 5, or 6. From parturition to re-breeding, cows were assigned to a diet that allowed for a moderate gain or high gain. They found that cows with a BCS of 6 had greater glucose concentrations at re-breeding than those with BCS 4 and 5 (Vizcarra et al., 1998). Glucose is essential for lactation and during this period of early-lactation glucose demand is high. Hypoglycemia can occur during this period (Bickerstaffe et al., 1974). Lower glucose concentrations are associated with a loss of BW and BCS during periods of nutrient restriction (Vizcarra et al., 1998). A study by Cushman et al. (2014) examined the effects of nutrient restriction during the second and third trimester on daughter growth and reproductive performance of heifers born to mature cows. They found no negative effects on growth rates, age at puberty, or antral follicle counts on the heifer progeny. They also reported that an increase in maternal intake during the third trimester increased the percentage of heifers that calved during the first twenty-one days of the calving season. A mature cow responds to nutrient restriction more favorably than an immature heifer. Heifers that are nutritionally restricted during late gestation are more likely to have decreased calf birth weights than mature cows (Bellows and Short, 1978). The effects of maternal maturity

and nutrition are also observed in sheep (Wallace et al., 2002, 2005). Nutrient restriction in pregnant adolescent ewes exhibited associations of IUGR and preservation of fetal skeletal growth at the expense of fetal fat stores (Luther et al., 2005). Pregnant overnourished adolescent ewes demonstrated signs of IUGR and a reduced placentome size (Wallace et al., 2002) while pregnant overnourished nulliparous adult ewes had no reduction in cotyledonary weight or lamb birth weight (Wallace et al., 2005). They suggested that older animals are able to adequately partition nutrients to the fetus while younger animals appear not to. However, Peel et al (2012) demonstrated no effect of maternal overnourishment on birth weight or postnatal performance in lambs born to adolescent ewes. They suggested that the inconsistency may come from the different methods of conception or differences in the age or maturity of the adolescent ewes. Adolescent ewes in the study by Peel et al. (2012) were naturally serviced while the studies by Wallace et al. (2002, 2005) used embryo transfer and AI, respectively. George et al. (2010) suggested that the level of obesity could exceed the ewe's ability to deposit fat and subsequently affecting fetal growth and development.

Early to Mid Gestation

The effects of maternal nutrition during early to mid gestation are often overlooked. Despite lower nutrient requirements due to minimal fetal growth, this is still a critical period of development as many survival organs are developing. The fetus maintains the growth of survival organs (i.e., the brain and heart) and can reduce the growth of less critical organs and tissues in response to a negative stimulus (Hales and

Barker, 1992). Besides the brain, heart, and kidney nephrons the placental function is established during this period (Ford, 1995; Gopalakrishnan et al., 2005).

Placenta

The fetal growth trajectory is influenced by the size and nutrient transfer capacity of the placenta (Reynolds and Redmer, 1995). The placenta plays a crucial role in fetal programming and fetal development. It exchanges nutrients, respiratory gases, and metabolic waste from the fetus and dam (Meschia, 1983). It produces a variety of hormones and is important for endocrine functions.

The ruminant placenta has numerous button-like structures called placentomes. Bovine placentomes are convex and grow throughout gestation to provide increased surface area for transplacental exchange (King et al., 1980). A cotyledon is a placental unit originating from the trophoblast and consisting of abundant vasculature and connective tissue. The fetus cotyledon comes from the chorion. The fetal cotyledons attach to the maternal caruncles. These two structures together are called a placentome. The chorionic villi are the functional units of fetal placenta. Placentomal attachment happens around d 20 to 29 of gestation, when the chorion begins to attach to the caruncles (King et al., 1980). The chorionic villi invade crypts in caruncular tissue. However, the placenta grows the most during the first two trimesters of gestation (Reynold and Redmer, 1995). Epithelial cells in the placenta separate the fetus from dam. The maternal epithelial cells can slough and regrow. This causes a discontinuous exposure of the maternal capillaries to fetal epithelium. This is known as a

syndesmochorial placenta (Senger, 2005). Throughout gestation binucleate giant cells (BNGC) move from the fetal epithelium to the maternal epithelium. These cells are unique to the ruminant placenta. The placental BNGC secrete bovine placental lactogen (bPL) and bovine pregnancy associated glycoprotein (bPAG) (Senger, 2005).

The glycoproteins bPAG and bPL effect fetal growth and development by causing repartitioning of maternal nutrients to the fetus (Wooding, 1992; Bertolini et al., 2006). In a study by Sullivan and others bPL and bPAG were measured to indicate placenta function (2009). They observed that restricted protein intake (0.4 kg/ d during early and mid-gestation) transiently enhanced placental function. Restricted dietary protein dams had increased bPL during the second trimester as well as increased bPAG during the first trimester. In this study, estrogen sulphate (ES) was also used as an indicator of placental function since the placenta is a major source of estrogen and cotyledons convert estrogen to ES (Hirako et al., 2002). Circulating concentrations of ES also increased with protein restriction. They found that an increase in ES was associated to an increase in calf birth weight. This is one example how increased placenta function due to poor maternal nutrition can lead to post-natal effects. Sullivan et al. (2009) suggest that there may be a compensatory increase in cotyledonary size when fewer cotyledons are present.

Transplacental exchange relies on uterine and umbilical blood flow. This blood flow is influenced by placenta vascularization. Tissues that have lower metabolic rates have less priority during partitioning of maternal nutrients (Redmer et al., 2004). Angiogenesis is the prompt proliferation of blood vessels and is controlled by endometrial and placental factors. Angiogenesis occurs primarily in the placentome of

the pregnant ruminant uterus (Ford, 1995). In ewes, blood flow to the caruncles increases from 27% of total uterine blood of early gestation to 82% by late gestation (Rosenfeld et al., 1974). There is an increase in uterine arterial diameter and blood flow due to a decrease in arterial smooth muscle tone (Rosenfeld, 1984). Despite the increase in uterine diameter, the uterine vasculature is able to create short-term phasic vasoconstrictions due to circulating catecholamines (Shnider et al., 1979).

Catecholamines are released during acute maternal stress. This short term uterine vasoconstriction is important in allowing blood to shunt from the uterus to tissues involved in the “flight or fight” response (Ford, 1995). The sensitivity of this response decreases throughout gestation (Guenther et al., 1988). This helps prevent a long-term decrease in blood to the gravid uterus during chronic maternal stress. The decreased blood flow could inhibit fetal growth and vigor (Ford, 1995). Common production practices such as transport and handling can create chronic maternal stress. Lay and coauthors demonstrated that transport and handling of pregnant cows resulted in lower fetal BW, pituitary and heart weights (1997). During late gestation, long-term cortisol levels in the dam can lead to a reduction in placental size (Jenson et al., 2002).

Uteuroplacental blood flow may be influenced by maternal protein supplementation in ewes (Camacho et al., 2010). Pregnant ewes on an isocaloric diet received varying levels of dietary protein. At d 130 of gestation, ewes that received a high level of protein had heavier fetuses than ewes that received a low level of protein. There were no differences in placental weight but ewes receiving low protein had increased uterine blood flow.

Intrauterine Growth Restriction

There are many factors that lead to fetal intrauterine growth restriction (IUGR). It is the inhibited growth and development of the mammalian fetus or its organs during gestation. Uterine capacity, the physiological and biochemical limitations imposed on fetal growth in the uterus, greatly influences IUGR (Wu et al., 2006). Cows or heifers carrying twins demonstrate the effect of insufficient uterine capacity on IUGR. An increased number of fetuses leads to placental insufficiency, low birth weights and increased IUGR (Gootwine et al., 2006). Breeding at immature body weight is another common industry method that triggers IUGR (Wu et al., 2006). The dam must provide nutrients to a growing fetus as well continue to grow to mature size. Wu et al. (2006) explains that IUGR can be considered a mechanism for maternal survival during malnutrition. Fetal IUGR causes the reduced growth of less important organs and tissues, while maintaining the growth of critical organs (Hales and Barker, 1992). Fetal IUGR can lead to impaired renal development and reduced nephrons (Schreuder et al., 2006). A study involving ewes by Meyer-Gesch et al. (2013) surgically limited uterine space available for placental attachment. They found that metabolism, fetal growth, and kidney endocrine and excretory function were inhibited by this experimentally induced IUGR. Besides IUGR, early to mid-gestation maternal nutrient restriction can also result in altered organ development and reduced nephron numbers in sheep and cows (Vonnahme et al., 2003; Gilbert et al., 2007; Long et al., 2009).

The effects of IUGR from nutrient restriction during early gestation can be undetectable if cows are realimented during later gestation (Long et al., 2009).

Multiparous beef cows were fed to meet NRC recommendations (control; n = 15) or fed below NRC recommendation (nutrient restricted; n = 15) from d 30 to 125 of gestation (Long et al., 2009). Ten control and 10 nutrient restricted dams were necropsied at d 125 of gestation. The remaining 5 nutrient restricted cows were realimented to gain a similar BW and BCS of the 5 remaining control cows. Differences in fetal weights of nutrient restricted cows at d 125 of gestation suggested IUGR. Nutrient restricted non-IUGR cows had fetal weights similar to control cows however the nutrient restricted IUGR fetuses were lighter. Long and coauthors reported asymmetrical growth of organs in nutrient restricted IUGR fetuses (2009). In nutrient restricted IUGR fetuses, brain weight as a percentage of fetal weight was increased and fetal heart weight as a percentage of fetal weight also tended to be increased. Reduced cotyledonary weights and total placentome surface area in nutrient restricted IUGR cows when compared to nutrient restricted cows and control cows were associated with the decreased fetal growth. Fetal weights and caruncle weight were similar for nutrient restricted and control cows by d 245 of gestation suggesting that IUGR may go undetected if nutrient restricted cows are realimented during late gestation to assure normal birth weight (Long et al., 2009). Additionally Long et al. reported the non IUGR cows had greater cotyledonary tissue growth and higher plasma glucose concentrations than the nutrient restricted IUGR cows. Nutrient restricted non-IUGR cows were 18 mo older than the nutrient restricted IUGR cows. Long and coauthors suggest the greater cotyledonary tissue growth aided the older non IUGR cows in partitioning more glucose to the fetus than the younger cows that had IUGR fetuses.

Realimentation of nutrient restricted (50% of NRC recommendations) ewes during early to mid-gestation (d 28 to 78) resulted in similar birth weights of offspring of control ewes (100% NRC). However the offspring from restricted dams were heavier at 2 mo of age and remained heavier to 12 mo of age. They also had impaired glucose and insulin responses (Burt et al., 2007; Ford et al., 2007). It has been demonstrated in multiple species that maternal overnutrition or restriction during gestation can cause similar effects of IUGR (Long et al., 2009, 2010).

Varying Responses

The effect of nutrient restriction during different trimesters can vary. Low BW at breeding coupled with nutrient restriction during mid-pregnancy inhibits placental growth and decreases birth weights (Redmer et al., 2004). Fowden and others (1994) found that an intense nutrient restriction in adult sheep during the periconceptual period hastens maturation of the fetal hypothalamic-pituitary-adrenal axis and causes premature parturition. Kroker and Cummins (1979) showed that nutrient restriction of heifers during the last trimester of gestation leads to lower birth weights. Dietary protein restriction in heifers during the last trimester produced weak labor, more dystocia, increased perinatal deaths, and longer postpartum intervals due to anestrus (Kroker and Cummins, 1979). Da Silva et al. (2002; 2003) found a decrease in ovarian follicle number in fetuses from overfed adolescent sheep. This demonstrates that IUGR adversely affects reproductive performance of offspring.

Ewes fed at 50% of NRC recommendations from d 28 to 78 postmating followed by 100% of NRC had offspring with similar birth weights of ewes fed 100% NRC throughout gestation (Burt et al., 2007; Ford et al., 2007). These lambs from nutrient restricted ewes were heavier at 2 mo of age and remained heavier up to 12 mo of age than the lambs from ewes fed 100% NRC. These lambs from nutrient restricted ewes had increased baseline glucose concentrations. When administered an IV glucose bolus, these lambs had elevated baseline glucose and insulin concentrations.

Post-Natal Effect

Hales and Barker (1992) introduced the thrifty phenotype hypothesis stating that a fetus can make adaptations that will increase survival in a negative nutrient environment. This leads to adaptations of the postnatal fetus's metabolism that are advantageous for a poor nutrition environment but these adaptations can be adverse if there is an over abundance of nutrients (Hales and Barker, 2001). *Metabolic imprinting* is the ability of the fetus to adapt to variations in nutrient availability. These adaptations from early life can affect a variety of organs and tissues and can have permanent effects (Walterland and Garza, 1999).

There have been many bovine studies aimed at determining the effects of maternal BCS on fetal growth as well as post-natal growth. A cow's energy requirement greatly increases during late gestation as 75% of fetal growth occurs during the final two months of gestation (Robinson et al., 1977). A study by Bohnert et al. looked at possible consequences of inadequate BCS at calving as well as growth performance and carcass

traits of the offspring (2013). Cows were managed to start the last trimester of gestation with an approximate BCS of 4 or 6. Treatments were arranged in a 2 x 2 factorial in a randomized complete block design with 2 BCS with or without protein supplementation. All cows were fed low quality hay and supplemented cows received the equivalent of a daily 0.9 kg/ cow of dried distiller's grain (Bohnert et al. 2013). Bohnert et al. found that calves from dams with the high BCS had higher birth weights than the low BCS cows (2013). They also reported that calves from supplemented dams had higher weaning weights than non-supplemented. At rebreeding the high BCS cows had higher pregnancy rates than the low BCS cows. However this study reported that none of the treatments affected feedlot performance or carcass traits (Bohnert et al. 2013). This study again shows the importance of adequate BCS as well as protein supplementation during late gestation.

Reproductive Performance of Progeny

Reproductive organs begin developing early in gestation. The testicles begin development by gestation day 40 to 45 in bull calves and ovarian development begins by day 50-60 in the heifer (Gier and Marion, 1970). Folliculogenesis begins about day 80 of gestation (Nilson and Skinner, 2009). In sheep, folliculogenesis can be affected by maternal nutrient restriction during the first 110 days of gestation (Rae et al., 2001). Ultrasonography can be used to determine antral follicle count. This is helpful in predicting ovarian reserve. Heifers with low antral follicle count tend to have decreased pregnancy rates and less healthy oocytes and follicles (Cushman et al., 2009; Ireland et

al., 2008;). Maternal nutrient restriction has been shown to decrease offspring fertility (Long et al., 2010). Long and co-authors utilized primiparous ewes were fed either 50 or 100 % of NRC recommendations during early gestation to demonstrate the effects of gestational maternal nutrient restriction on reproductive characteristics of mature offspring. Mature offspring born to nutrient restricted ewes gave birth to fewer lambs compared to offspring of the control ewes (Long et al., 2010). Ewes born to nutrient restricted dams also had decreased levels of progesterone during their luteal phase of the estrous cycle however there was no effect on reproductive capability.

Protein Supplementation during Gestation

Maternal protein supplementation during a nutrient restricted gestation can increase cow BCS and BW at calving (Sanson et al., 1990; Bohnert et al., 2002). The goal of protein supplementation is to satisfy amino acid requirements. Amino acids contain both an amino and carboxyl group attached to a carbon skeleton. Proteins are long chains of amino acids. An animal's DNA specifies an order of amino acids when they are linked together to form a protein. There are 20 amino acids commonly found in plants and animals and a deficiency of amino acids can hinder protein synthesis. Protein is often the most expensive nutrient and consequently it is commonly deficient. A protein supplement may contain true protein and/or a nonprotein nitrogen (NPN) (Kellems and Church, 2010). Nonprotein nitrogen compounds contain N, but are not made up of amino acids. Rumen microbes synthesize their required amino acids by converting NPN to ammonia. Ammonia is also produced by the deamination of amino

acids in the rumen. Excess ammonia is converted to urea in the liver and excreted in the urine. Overfeeding NPN can cause decreased ruminant efficiency and increased N excretion (Kalscheur et al., 2006). Additionally it can be toxic to the animal if exceeds the liver's detoxification ability. Unlike monogastrics, ruminants receive a large portion of their protein requirement through the microbes present in the rumen (Kellems and Church, 2010). Rumen microbes break down rumen degradable protein into amino acids and ammonia that stimulates microbial growth. Rumen microbes are carried out of the rumen into the omasum and abomasum where they are killed and digested to amino acids. The amino acids from the microbial protein are absorbed in the small intestine. Rumen undegradable protein (RUP) passes through the rumen intact and is digested in the small intestine.

Sometimes a protein supplement is selected because of its crude protein (CP) value. The Proximate Analysis System estimates CP through multiplying the nitrogen content of a feedstuff by a conversion factor. This conversion factor reflects the average nitrogen component of protein in typical feedstuffs. More specifically it is the protein portion divided by nitrogen content ($100 / 16 = 6.25$). Some compounds have higher nitrogen levels and can give a higher CP analysis. Every protein has nitrogen but not all nitrogen is protein. Not all protein supplements will have the same effect to the cow. Oil seed meals such as cottonseed, soybean, and sunflower make good protein supplements since they are easily digested in the rumen (Kellems and Church, 2010). These oil seed meals have a high proportion of rumen degradable protein (RDP) (NRC, 2000). A deficiency in RDP can decrease the efficiency of the rumen by disturbing microbial

populations. This can cause depressed fiber digestion leading to reduced DMI and energy supply to the animal (Firkins et al., 1986; Allen, 2000).

Besides maternal benefits, further research has demonstrated benefits to the calf such as improved reproductive capability of heifer offspring (Martin et al., 2007), enhanced carcass characteristics (Larson et al., 2009), increased postnatal growth (Bohnert et al., 2013), and ultimately improved value of calves at weaning and slaughter (Funston et al., 2010). Martin and others evaluated the effects of late gestation or early lactation dam nutrition on subsequent heifer growth and reproduction (2007). In this study, late gestation or early lactation dam nutrition had no effect on age at puberty or the percent of heifers cycling before breeding. Heifers from protein supplemented dams had greater pregnancy rates and a greater percentage of heifers calved in the first 21 d of the heifers' first calving season. Protein supplementation during late gestation followed by poor maternal nutrition during the early lactation period resulted in heifers having increased DMI (Martin et al., 2007). Heifers from protein supplemented dams weighed more at weaning, prebreeding, first pregnancy diagnosis, and before their second breeding season. Heifer adjusted 205-d BW increased with slight maternal protein supplementation during maternal nutrient restriction in late gestation followed by meadow grazing during early lactation (Martin et al., 2007).

Late gestation cows on winter range receiving one pound of protein supplement per day (28 % CP range cubes) had higher pre-calving BW compared to cows that received no supplement (Larson et al., 2009). However cow BW and BCS at weaning was not affected by protein supplementation. Steer progeny were subjected to a 222 d

feeding trial following weaning. Steers from protein supplemented dams had increased final BW and HCW over non-supplemented progeny. There was no treatment effect on steer ADG, 12th rib area, LM area, and yield grade. However maternal protein supplementation during late gestation increased marbling in these steers and the proportion that graded USDA choice. Calf BW at weaning was increased in the protein supplementation progeny. This increased weaning weight along with the increases in carcass quality show value added by the protein supplement (Larson et al., 2009). Although this study demonstrates the effects of maternal nutrition during late gestation, the supplementation may be somewhat excessive. This study was not designed to mimic a nutrient restricted gestation, but cows grazing winter range have difficulty meeting energy requirements. Nutrient requirements are often higher than the available nutrient levels on winter range (NRC, 2000). Mild nutrient restriction of late gestation cows receiving protein supplement does not increase cow reproductive performance (Stalker et al., 2006). However the effects of protein supplementation are observed in the reproductive capability of the offspring (Stalker et al., 2006; Martin et al., 2007). The exact fetal programming mechanism responsible for the offspring improvements caused by maternal protein supplementation during gestation is unclear.

Postnatal Appetite

Multiple models of prenatal programming lead to increased postnatal appetite. It is believed that any alteration in postnatal plasma leptin can lead to an increased postnatal appetite. Long and others (2010) ran a 12 week feed trial involving 19.5 month old

offspring of ewes fed either 100% (con) or 150% (obese) of NRC recommendations from 60 days preconception to parturition. At the beginning of the trial, the groups had similar BW and body fat. At conclusion of the feeding trial, the obese ewe offspring had higher body fat percentage and consumed 10% more feed than the offspring from the control. During feeding, the offspring from the obese ewes had higher leptin concentrations when compared to control offspring. Intravenous glucose tolerance test indicated a decreased acute insulin response to glucose in the offspring from obese ewes (Long et al., 2010). A later study by Long and others used the same maternal obesity model and feeding challenge except the offspring were approximately 6 mo older at the start of the feeding challenge (2015). As expected the offspring born to obese dams consumed more feed and gained more weight compared to the control offspring. In comparison of the two experiments Long and others suggested older age appeared to elevate the appetite increase, glucose dysregulation and insulin secretion insufficiency, and leptin resistance.

Development of Appetite control centers

The development of many organs is affected by changes in maternal nutrition, including areas of the brain that regulate appetite and metabolism. The hypothalamus controls intake based on nutrient levels. Hunger and satiety signals stimulate the arcuate nucleus (ARC) of the hypothalamus. The ARC integrates signals from the brain stem and the periphery (Minor, 2009). The ARC has neurons that terminate past the blood-brain barrier in contact with the bloodstream near the carotid sinus. This contact with circulation enables the ARC to respond to hunger and satiety signals such as insulin,

leptin, ghrelin, and glucose (Martin-Gronert and Ozanne, 2005). The ARC regulates energy expenditure through a group of orexigenic neurons that co-express neuropeptide Y (NPY) and agouti-related peptide (Agrp). When nutrient levels are low, ghrelin is released from the gastric fundus and epsilon cells of the pancreas to promote hunger. Ghrelin activates neurons in the hypothalamic ARC that secrete NPY and Agrp. Aponte et al. (2011) used photostimulation of Agrp neurons in mice to demonstrate that Agrp neurons directly engage feeding circuits. They found that stimulation of these neurons resulted in voracious feeding. Feeding can be repressed by insulin and leptin. Insulin is released from the pancreas in response to elevated blood glucose concentrations. Insulin and leptin activate ARC neurons to express proopiomelanocortin (POMC). Proopiomelanocortin and Agrp work reciprocally (Aponte et al. 2011; Yang et al. 2011). Agouti-related peptide neurons in the paraventricular hypothalamic nucleus (PvN) inhibit oxytocin neurons. This inhibition is necessary for feed intake. Oxytocin neurons descend into the dorsal vagal complex of the medulla (Lee and Blackshaw, 2014). The ARC neurons connect to other neurons in the ventromedial hypothalamus (VMH), lateral hypothalamic area (LHA), paraventricular nucleus (PVN) and dorsomedial nucleus (DMH). These neurons project into the nucleus of the solitary tract (NTS) in the brainstem and the dorsomotor nucleus of the vagus. The central nervous system includes the NTS and the hypothalamus. The NTS integrates gastrointestinal signals while the hypothalamus mainly receives peripheral signals related to metabolic energy reserves (Minor et al., 2009). Cholecystokinin (CCK) a satiety signal released from the liver and gastrointestinal tract, that binds to its receptor in the stomach and its effects are

transmitted by the vagus nerve to the NTS (Moran and Kinzig, 2004). The dorsal vagal complex controls the periphery nervous system and includes the tractus solitarius nucleus. The NTS houses visceral sensory ganglia, that deliver input from the digestive, respiratory, and cardiovascular systems. In the altricial mouse and rat neonate, the connections between the ARC and DMH are still forming during the first few days after birth. However in more precocial species these neural connections are formed during late gestation (Koutcherov et al. 2003; Muhlhausler et al. 2004).

Leptin

There have been several hormones suggested to modulate the hypothalamic appetitive center formation. Leptin is used as an indicator of fat reserves as well as hormonal controller of food intake. Leptin is a cytokine produced in white adipose tissue. Leptin is produced by adipocytes and decreases food intake and increases energy expenditure (Friedman and Halaas, 1998). The use of an obese (ob/ob) mouse by Ingalls and others led to the description of a satiety factor, leptin (1950). This ob/ob mouse experienced severe hyperphagia and increased fat and weight gain. It was later shown that this obese mouse had a genetic defect that prevented leptin production (Zhang et al., 1994). Administration of exogenous leptin decreased the hyperphagia. Without leptin, the brain perceives starvation despite obesity through hypothalamic leptin receptors. This deficiency drives hunger, decreases energy expenditure, and lowers reproductive competence (Flier, 2004). Leptin deficient mice have reduced brain weights associated with a reduction and delay in ARC projection to PVN. A study that altered litter size of

rat pups to induce overfeeding or underfeeding indicated that overfeeding during this early postnatal period caused increased gains in overfed pups versus control pups (Oscai and McGarr, 1978). This caused the pups to remain overweight as adults. As early as seven days after parturition, these overfed pups had hyperinsulinemia and insulin resistance. There was an increase in body fat and leptin concentrations compared to control pups. Leptin and insulin have an inhibitive effect on the ARC such that the release of NPY and AGRP is diminished. This decreases appetite stimulation. However in these overfed neonates, this negative feedback is replaced by a partially positive feedback process. Conversely in control pups insulin and leptin have a stimulatory effect on the VMH neurons but the overfed pups had a reverse effect. The inhibitions may have caused the decreased energy expenditures in the overfed pups. They found that litters with increased pups had slower gains, reduced feed intake, and reduced body weights even after being fed control diets post-weaning. Oscai and McGarr (1978) report that controlling the food intake of neonate rats programs the animal's voluntary food intake later in life. This programming appears to happen postnatal in the rat, but in more developed neonate the programming may take place in utero. A more recent study involving mice fed either 50 % restricted diet or ad libitum during gestation demonstrated decreased birth weights in pups (Desai et al., 2011). These low birth weight pups had a reduction and dysregulation in hypothalamic appetitive pathways resulting in hyperphagia and obesity in adulthood. The pups had dysregulated hypothalamic leptin and insulin signaling that impaired anorexigenic responses, increased food intake leading to obesity. Desai and others (2011) suggest that these effects are caused by impairments

of neural stem progenitor cells that give rise to the neurons and glial cells of the appetite control pathway.

In mice, timing of maternal protein restriction during gestation and gender may program feeding behavior (Langley-Evans et al., 2005). Rats fed a low protein diet during gestation and lactation had progeny with altered hypothalamic appetite center such as an increase in VMH and PVN neuron density suggesting there is a minimal amount of maternal protein necessary to prevent feeding circuitry disorders (Plagemann et al., 2000). A subsequent study fed either a high protein or adequate protein diet during gestation that caused an increase in adipose tissue and a decrease in energy expenditure (Daenzer et al., 2002).

Leptin is a hormone that can cross and is produced by the placenta to transmit dam nutrient status. Insulin cannot cross the placenta (Buse et al., 1962). However the fetus produces insulin in pancreatic beta cells (Boskovic et al., 2003). It is proposed that insulin can regulate early formation of the appetite control centers since the fetus produces insulin before much of the hypothalamic neurogenesis begins. Insulin has been shown to stimulate in vitro leptin mRNA expression in explants of steer adipose (Houseknecht et al., 2000). Leptin and insulin are indicators of nutrition levels. Increased CP and energy in diets of primiparous heifers increased insulin and leptin levels (Ciccioli et al., 2003). Long and Schafer suggest that this leptin increase could be caused by elevated insulin due to the increased nutrient intake (2013). In a review of insulin's effects on developmental programming of body weight regulation, insulin is suggested to play a role in the programming of the appetite control centers (Plagemann,

2008). Maternal hyperglycemia can elevate fetal and early postnatal insulin levels that may lead to permanent malprogramming of hypothalamic regulation centers for metabolism, food intake, and body weight. Levels of glucose and insulin can affect the expression of hypothalamic neuropeptides that regulate appetite (Mühlhäusler et al., 2005). In that study, glucose or saline was infused into fetal sheep between gestational d 130 and 140 to demonstrate increases in fetal adiposity, leptin secretion and gene regulation of the hypothalamic appetite control neuropeptides, neuropeptide Y (NPY), agouti-related peptide (AGRP), pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) and receptors (leptin receptor (OB-Rb) and melanocortin 3 receptor (MC3R) (Mühlhäusler et al., 2005).

Leptin Surge

Plasma leptin levels in multiple neonatal species rise in a characteristic postnatal pattern (Long et al., 2011; Long and Schafert, 2013; Yura et al., 2005). This surge or elevation in circulating leptin is thought to influence the formation of the hypothalamic appetitive centers in rats (Yura et al., 2005). In rats, the characteristic peak occurs around postnatal d 16. In mice this surge is concomitant with the formations of the ARC projections (Martin-Gronert and Ozanne, 2005). Projections containing NPY develop between the ARC, the DMH and PVN of the hypothalamus during the first two weeks of life in the rat (Grove & Smith, 2003). Maternal nutrient restriction in rats likely inhibits the leptin surge consequently inhibiting the formation of ARC projections (Delahaye et al., 2008). In mice, the postnatal leptin surge can be altered by maternal undernutrition.

In offspring born to mice fed from post-conceptual day 10.5 to 18.5 either an *ad libitum* or nutrient restricted (70% of the food consumed by the *ad libitum* group) diet, maternal undernourishment caused a premature leptin surge that was greater than that of the control offspring (Yura, et al., 2005). In neonatal offspring of obese rats this leptin surge is amplified and prolonged (Kirk et al., 2009). A premature surge can increase the density of ARC to PVN projections (Lee and Blackshaw, 2014). In mice maternal energy restriction during lactation caused reduced pup BW until weaning. After weaning these offspring were heavier and consumed the same amount of food when compared to offspring from mice fed normal control diets (Teixeira et al., 2002). Maternal undernourishment during gestation causes a premature postnatal leptin surge. This surge is causally related to increased obesity in pups from (Yura, et al., 2005). This surge normally occurs around 16 days postpartum but maternal undernutrition advances the surge to 8 -10 d. Offspring from undernourished mice were born small and had decreased subcutaneous fat compared to the control group offspring. However the undernourished offspring did catch up in BW. When fed a high fat diet, the undernourished offspring had a pronounced weight gain and increased adipose tissue compared to the control offspring. During the high fat feeding trial the undernourished offspring were prone to obesity and had significantly higher leptin levels. Neonates from mice fed normal diets were treated with exogenous leptin to induce a similar premature postnatal leptin surge; when fed a high fat diet these offspring experienced the same increased weight gain and obesity as the undernourished offspring.

Leptin Resistance

Elevated leptin concentrations in the blood can lead to the resistance of the anorexigenic effects of leptin (Yura, et al., 2005). In the leptin deficient ob/ob mouse, leptin regulated pathways are permanently disrupted (Bouret et al., 2004). High calorie diets can cause obesity and leptin resistance through altering leptin transfer at the blood-brain barrier and impairing hypothalamic signal transfer. Pups (8 to 10 weeks) from undernourished and normal diet mice received an injection of leptin while on a regular chow diet. Unlike the high fat diet neither undernourished or normal diet developed obesity from leptin treatment on regular chow. However, 12 hr after leptin administration normal diet body weight increased was significantly reduced. Furthermore, the leptin treatment increased phosphorylation of signal transducer and activator of transcription 3 (STAT 3), a hypothalamic intracellular signaling mediator of leptin action. C-Fos protein is a metabolic marker used to trace neuroanatomical connections (Dragunow and Faull, 1989). After the leptin injection there was less c-Fos positive cells in the ARC and PVN of the undernourished offspring than the normal diet offspring. Yura and others suggested this impaired brain leptin transport from the exogenous leptin (2005). They also found a reduction mRNA expression of Ob-Ra in undernourished offspring hypothalami after leptin administration. The short-form leptin receptor, Ob-Ra, is involved in leptin transport across the blood-brain barrier. An injection of leptin induced a premature leptin surge that caused obesity and leptin resistance in the undernourished and normal diet offspring if they received a high fat diet instead of a regular diet. Yura and others suggest there is an unidentified compensatory

mechanism involved. These changes in structure, physiology, and metabolism to adapt to undernourishment are conclusive with the thrifty phenotype hypothesis (Neel, 1999; Godfrey and Barker, 2000).

The expression of hypothalamic leptin receptors, Ob-Rb, is decreased when mice neonates receive exogenous leptin (Toste et al., 2006). This underexpression leads to leptin resistance and programs the neonate for hyperleptinemia and hyperisulinemia in adulthood. Hyperleptinemia was also demonstrated in a sheep model of maternal obesity (Long et al., 2010). Offspring from obese ewes (fed 150 % of NRC requirements from 60 d prior to conception through parturition) and control ewes (100 %) were subjected to a 12-wk ad libitum feeding challenge at 2 to 3 y of age. This resulted in an elevation of visceral fat in the male offspring of the obese ewes compared to the control ewes' offspring. The mRNA expression of pituitary leptin was similar between the two groups, but the mRNA expression of OB-Rb were decreased in the obese offspring. This suggests leptin resistance. Offspring from the obese ewes also had reduced mRNA expression of GH, liver GH receptor, and IGF-1. Nurmamat and others suggest that stimulation of the GH/IGF-1 axis may be reduced by leptin resistance caused by gestational maternal obesity (2014).

These appetite control networks are developed during late gestation in more precocial species such as sheep and humans (Koutcherov et al. 2003; Muhlhausler et al. 2004). In a maternal obesity sheep model developed to demonstrate developmental programming, Long and others described the neonatal leptin profile of lambs (2011). Ewes were fed either 150 % of NRC recommendations or 100 % of NRC

recommendations from 60 d prior to conception until parturition. Blood was collected from the lambs daily from parturition to postnatal d 7 and then on d 9 and d 11. They reported a leptin peak between days 6 and 9 postpartum in lambs born to the control ewes (fed to 100 % of NRC recommendations) (Long et al., 2011). This surge is earlier than in rodents. There was no leptin surge in lambs born to obese ewes (fed 150% of NRC requirements). The leptin profile of beef calves has not been studied as much as the sheep model. Long and Schafer demonstrated the endocrine profile of postnatal beef calves from normal fed nulliparous dams (2013). Nulliparous cows received neither an unrestrictive or excessive diet during gestation and lactation. A daily blood collection was taken from the calves from parturition to postnatal d 8 and then every other day until postnatal d 18. They reported a leptin increase from birth until postnatal d 2 and then a decrease to d 16 of age. A similar plasma leptin surge has also been reported in dairy calves that were suckled on their dams (Blum et al., 2005). Long and Schafer report a sex effect on the leptin levels of the neonatal beef calves (2013) whereas in lambs, there is no difference in leptin levels due to sex (Bispham et al., 2002; McFadin et al., 2002; Long et al., 2011).

The elimination of the leptin surge due to maternal obesity during gestation can be observed across multiple generations. A developmental programming study utilized the sheep model previously described to demonstrate the transgenerational effect of maternal obesity on lamb leptin levels (Shasa et al., 2014). Their dams (F0) received adequate nutrition during lactation (ad libitum access to high quality alfalfa hay and supplementation as per NRC recommendations for a lactating ewe). The F1 ewes

received 100 % of NRC of recommendations from weaning to breeding. These F1 continued to receive 100 % of NRC of recommendation until the weaning of their offspring. Lambs whose grandmothers were obese during gestation lacked a characteristic postnatal leptin surge. Similarly the effects of maternal synthetic glucocorticoid administration on leptin can be observed across multiple generations (Long et al., 2013a,b).

Synthetic glucocorticoids administration to ewes during gestation days 103 to 104 eliminates the postnatal leptin surge two generations later (Long et al., 2013a,b). It is suggested that the leptin surge may be blocked by elevated cortisol levels. This generation experienced increased appetite, gains, adipose tissue, and a decrease in insulin response. Neonates of obese rats have increased leptin mRNA in their adipose tissue (Kirk et al., 2009). Adipocytes are probably the source of leptin for the postnatal surge (Long et al., 2011).

Cortisol

Glucocorticoids (GC) have been shown to control fetal and adult leptin production (Soret et al., 1999). Cortisol is a naturally occurring glucocorticoid that can regulate metabolic, cardiovascular, immune, and behavioral responses (Smith and Vale, 2006). In sheep, an increase in fetal cortisol levels begins around 20 d prepartum (Magyar et al., 1980). This increase has a maturation effect on the fetus and is necessary to prepare the fetus for parturition and postpartum life (Fowden et al., 1998). Glucocorticoids have a maturational effect on fetal organs. Prior to parturition cortisol

initiates lung maturation, increases metabolism, and prepares the liver for gluconeogenesis (Liggins, 1994). Its role is critical to the survival of fetus and adults alike. Synthetic GCs are often administered to women in danger of early parturition to progress premature organ development. Synthetic GC can reduce birth weight and postnatal growth (Long et al., 2013a,b). Elevated levels of GC can create an IUGR environment (Long et al., 2013a,b). In animals, synthetic GC can cause endocrine, renal, and metabolic defects (Sloboda et al., 2000; Drake et al., 2005; Shasa et al., 2014). Poor nutrition can also lead to an increased maternal and fetal level of GC showing that GC can be thought of as a common factor across multiple models of fetal programming (Sloboda et al., 2000; Langley-Evans and McMullen, 2010).

Precocial species such as the lamb and calf can have elevated cortisol levels due to maternal nutrition. Calves born to dams fed 57% of NRC protein and energy requirements had higher cortisol levels compared to calves born to dams fed at 100% (Hough et al., 1990). Likewise maternal obesity can cause an increase in neonatal cortisol levels (Long et al., 2011). This increase is likely involved in disrupting the normal leptin surge. Both nutritional extremes are known to shorten gestation length.

The adverse effects of undernutrition can be mitigated by inhibiting the GC response. During gestation rats were fed either a low protein diet or a protein moderate diet (Langley-Evans, 1997). Offspring of the low protein dams had elevated blood pressure at 7 wk of age compared to the protein moderate offspring. Low protein dams that were administered a corticosterone inhibitor during gestation gave birth to offspring

with normal blood pressure. Inhibition of maternal GC synthesis reduced hypertension (Langley-Evans, 1997).

Many factors have been suggested to program the appetite control centers through the regulation of leptin. Soret et al. (1999) suggests that glucocorticoids, glucose, insulin, thyroid hormones and IGF-1 control fetal and adult leptin production in rodents. In a more precocial species, obese sheep model, Long and others found no clear relation to the leptin surge of control lambs from any changes in plasma cortisol, insulin, triiodothyronine, IGF-1 or glucose (2011). However the increase in cortisol levels reported in the lambs born to obese ewes was suggested to play a role in disrupting the leptin peak. Previous studies suggest that insulin has a large role in leptin regulation. More recent data suggests that cortisol may be more critical in leptin regulation and appetite control. Administration of a synthetic GC to ewes during gestation has been shown to increase lamb plasma cortisol levels during the postnatal period but does not have an effect on the insulin levels (Long et al., 2013b).

As previously mentioned, cortisol levels rise during late gestation. Similarly, plasma leptin and perirenal adipose tissue leptin mRNA expression increase during the final 15 d of ovine gestation (O'Connor et al., 2007). However, fetal adrenalectomy during this period prevents this leptin increase. Conversely, GC exposure to the intact fetus during this period produced a premature elevation of plasma leptin and perirenal adipose tissue leptin mRNA. Late gestation intracerebroventricular infusion of leptin to the sheep fetus inhibits the pre-parturient rise in ACTH and cortisol concentrations (Howe et al., 2002).

Hypothalamic-Pituitary Adrenal Axis

The hypothalamic-pituitary adrenal (HPA) axis is highly involved in the body's response to stress. Stress is the real or perceived threat to homeostasis (Smith and Vale, 2006). The stress response can affect the cardiovascular, respiratory, digestive, reproductive, and immune systems. Poor nutrition is a form of stress that can alter the HPA axis and cause permanent effects. The HPA axis growth and function can have long-lasting changes caused by poor nutrition. In sheep, the development and function of the HPA can be altered by maternal nutrient restriction (Kapoor et al., 2006). The HPA axis includes the parvocellular hypothalamic nuclei (PVN), the anterior lobe of the pituitary gland, and the adrenal gland. Fetal IUGR in humans can inhibit the HPA axis response to stress (Schaffer et al., 2009). The role of the HPA axis on control of food intake and reproductive axis is mediated in part through the secretion of corticotrophin-releasing hormone (CRH) (Mastorakos and Zapanti, 2004). Corticotropin-releasing hormone is a 41-amino acid peptide that is synthesized by neurons of the PVN. It is then secreted into the hypophyseal portal blood by axons projecting to the median eminence. Corticotropin-releasing hormone is also synthesized by corticotroph cells in the anterior pituitary. In the anterior pituitary, CRH binds to CRH receptor and causes adenylcyclase activation. This adenylcyclase activation results in secretion of ACTH and other POMC-derived peptides within a few seconds (Solomon, 1987). Proopiomelanocortin is a precursor hormone produced by the corticotrophs of the anterior pituitary. Proopiomelanocortin is post-translationally cleaved to ACTH, beta-endorphin, and

melanocyte stimulating hormone. The primary product of POMC cleavage, ACTH, stimulates the adrenals to secrete cortisol and other adrenal steroids, such as dehydroepiandrosterone (DHEA) and aldosterone (Conaglen et al., 1984). Adrenocorticotrophic hormone binds to the melanocortin type 2 receptor in zona fasciculata parenchymal cells of the adrenal cortex. This promotes GC synthesis (Simpson and Waterman, 1988). Adrenocorticotrophic hormone stimulates the conversion of cholesterol to pregnenolone.

The CRH and AVP neurons in the PVN and other areas of the brain, the locus ceruleus/norepinephrine, and the central autonomic sympathetic system in the brainstem are critical components of the stress response. Corticotropin- releasing hormone and AVP are the main central nervous system regulators of the HPA axis. Arginine vassopressin is a nonapeptide that is primarily expressed in the PVN, supraoptic, and suprachiasmatic nuclei of the hypothalamus. Projections of the PVN and supraoptic nuclei magnocellular neurons terminate in the posterior pituitary. The magnocellular neurons release AVP directly into systemic circulation. The primary role of AVP is regulation of osmotic homeostasis (Brownstein et al., 1980; Verbalis, 1993). Arginine vassopressin can also increase the effects of CRF on ACTH release from the anterior pituitary (Rivier and Vale, 1983). Parvocellular neurons of the PVN synthesize and release AVP into the portal circulation via the median eminence.

There are two subtypes of CRH receptors, CRH-R1 and CRH-R2. Both are G protein-coupled receptors with seven trans-membrane domains each. The CRH-R1 is predominately found in the pituitary and the cerebral cortex and the CRH-R2 is mainly

found in the limbic region, however both receptors are found in the cerebellum, brainstem, and hypothalamus (Hillebrand et al., 2002). The CRH-R1 has a higher affinity to CRH than CRH-R2.

Neuropeptide Y, AGRP, alpha MSH, and CART influence the HPA axis activity (Smith and Vale, 2006). In rats, central injection of NPY activates the HPA axis (Wahlestedt et al., 1987). Agouti-related peptide can increase CRH release in rats (Dhillon et al., 2002). MSH and CART can increase ACTH and corticosterone and stimulate CRH release in rodents (Dhillon et al., 2002). Energy balance can cause activation of the HPA axis (Smith and Vale, 2006). The forebrain may contribute to HPA axis regulation. The limbic system of the forebrain is primarily responsible for memory formation and emotional responses. Limbic structures have direct connections to the pituitary neurons via the PVN instead they regulate the HPA axis through neurons in the BNST, hypothalamus, and brain stem. Lambs born to nutrient restricted dams had a greater ACTH response after an intravenous CRH/AVP injection than ewes from mother fed 100 % of NRC (Long et al., 2010). Long and others suggest that maternal nutrient restriction during early gestation may affect progeny stress responses but may have little effect on HPA axis sensitivity. Interestingly a similar response was observed in offspring from an obese dam (Long et al., 2012).

Conclusion

In the past 60 years the understanding of the effect of maternal nutrient status on fetal development and postnatal growth has evolved. Many studies with multiple models

have demonstrated the effect of maternal nutrient status during gestation on offspring postnatal growth, carcass characteristics, appetite and metabolism, and endocrine regulation (Bellows et al., 1982; Spitzer et al., 1995; Long et al., 2009; and Wu et al., 2006). The adverse effects of IUGR have been demonstrated in multiple species in either a maternal obesity or nutrient restricted model (Yura et al., 2005; Long et al., 2010, 2011; Ford and Long, 2012). Obesity is not common in production livestock as overfeeding is a costly practice. However data from animal studies using an obese maternal model has important implications to human medicine where overnutrition is much more common. With the increased frequency of drought and higher feeding costs, it is likely that more animals will be nutrient restricted. Often it is the dry pregnant cow that a producer will allow to lose BCS. As more research is conducted we will be able to better identify animals affected by IUGR. As Long et al. (2009) demonstrated, IUGR may occur without notice if dams were realimented after a transient period of nutrient restriction during gestation. The multigenerational studies have demonstrated the long lasting effects of maternal nutrition and GC concentrations on fetal growth, postnatal growth, appetite, and offspring cortisol regulation (Long et al., 2012; Long et al., 2013a,b; and Shasa et al., 2014). A greater understanding of periparturient regulation of fetal and neonatal concentrations of cortisol and leptin may lead to a better knowledge of programming of the hypothalamic appetite control centers.

Although many of the mechanisms that maternal nutrition effects progeny performance are unknown, there are studies aimed at mitigating these adverse consequences. It is well established that protein supplementation during gestation is

beneficial to the dam and will maintain maternal BCS and BW (Sanson et al., 1990).

Further studies have demonstrated that maternal protein supplementation during nutrient restriction increased offspring performance. With the increasing value of cattle, these adverse effects of maternal nutrient restriction are going to cost more.

CHAPTER III

The Effects of Maternal Nutrient Restriction with or without Protein Supplementation on Endocrine Regulation of Postnatal Beef Calves

Abstract

A study was conducted to evaluate late gestation maternal nutrient restriction with or without protein supplementation on endocrine regulation in newborn beef calves. This study utilized multiparous cows (4 and 5 yr of age, $n = 57$) randomly assigned to one of three treatments for the last 100 d of gestation. The control ($n = 19$) cows were fed to increase BCS while the nutrient restricted (NR, $n = 19$) and nutrient restricted with protein supplement (NRS, $n = 19$) cows were fed to lose 1.2 ± 0.2 BCS during the last 100 d of gestation. Control cows were allowed ad libitum access to tall fescue/crabgrass paddock and when grazing became insufficient, ad libitum hay was provided along with 1.3 kg of corn gluten feed 5 d/wk. Tall fescue paddocks were strip grazed to limit forage availability for NR and NRS. The NRS treatment were individually penned and fed 0.45 kg of soybean meal 3 d/wk. As forage became dormant the nutrient restricted paddocks received limited fescue hay. After parturition cow/calf pairs were moved to one common pasture and received ad libitum silage and 1.8 kg of high concentrate feed. Maternal nutrient restriction regardless of supplementation reduced cow plasma glucose and insulin concentrations during late gestation ($P < 0.0001$ and $P = 0.0051$ respectively). Calves from NR dams weighed less at parturition and the NRS calves were intermediate to control calves (33.4 ± 1.2 , 35.0 ± 1.3 and 37.2 ± 1.3 kg respectively; $P = 0.04$).

Plasma glucose concentrations of unsuckled calves at parturition was reduced ($P = 0.037$) in NR and NRS calves compared to control (67.7 ± 6.5 , 60.1 ± 6.9 and 83.7 ± 6.1 mg/dl respectively). At parturition, control and NRS calves had increased ($P = 0.0037$) plasma leptin concentrations compared to NR calves while calf plasma cortisol concentrations were greater for the nutrient restricted groups than the control (trt x day $P = 0.0135$). This research demonstrates that late gestation nutrient restriction reduces postnatal calf birth weight, plasma glucose and leads to reduced plasma leptin. Maternal protein supplementation appears to alleviate effects of late gestation nutrient restriction. This research demonstrates that maternal protein supplementation during late gestation nutrient restriction may alleviate effects of late gestation nutrient restriction.

Keywords: Nutrient Restriction, Cortisol, Leptin, Late Gestation Protein Supplementation

Introduction

Periods of nutrient restriction occur frequently in cattle production. Many factors can contribute to a negative nutrient environment during gestation including drought, overstocking, poor forage stockpiling management, twinning, or breeding immature heifers. These stimuli during a critical period of development such as late gestation can have permanent effects on progeny postnatally (Barker and Clark, 1997). The developmental programming hypothesis states that these changes in fetal nutrition and endocrine status can create adaptations that permanently affect fetal structure, physiology, and metabolism and predispose the offspring to postnatal metabolic and endocrine diseases. Decreased maternal BCS and BW prior to calving can increase

postpartum interval, decrease calf birth weights and decrease postnatal growth (Bellows and Short, 1978; Dunn and Kaltenbach, 1980; Kroker and Cummins, 1979). Maternal protein supplementation during late gestation can mitigate some of the negative effects of maternal nutrient restriction by improving reproductive capability of heifer offspring (Martin et al., 2007), enhancing carcass characteristics (Larson et al., 2009), increasing postnatal growth (Bohnert et al., 2013), and ultimately improving value of calves at weaning and slaughter (Funston et al., 2010). Offspring appetite can be altered by changes in postnatal leptin concentrations. Multiple species have a characteristic postnatal leptin surge that can program the hypothalamic appetite control centers (Yura et al., 2005). Cortisol appears to regulate the leptin surge in multiple species (Long et al., 2011; O'Connor et al., 2007). Long and Schafer (2013) recently published the endocrine profile of neonatal beef calves; however, there is limited data on maternal nutrient restriction impacts on subsequent beef calf endocrine regulation. This study investigated the effects of nutrient restriction on neonatal calf endocrine regulation and postnatal growth. The authors hypothesize that a small amount of maternal protein supplementation will alleviate some of the adverse effects of nutrient restriction.

MATERIALS AND METHODS

All procedures were approved by the Clemson University Animal Care and Use Committee (AUP # 2013-044).

Animals

Multiparous cows, (4-5 yr of age, n = 57) bred to multiple Angus sires with similar EPD, were blocked by breed (Angus n = 15, Simmental x Angus n = 22, and Polled Hereford X Angus n = 20), BCS (Wagner et al., 1988; range 4 to 7 BCS), age, and breeding dates. The cows were randomly assigned to one of three treatments for the last 90 to 100 d of gestation. The control (Con, n = 19) treatment was fed to maintain or slightly increase BCS. The nutrient restricted (NR, n = 19) treatment and the nutrient restricted with protein supplement (NRS, n = 19) were fed to lose 1 to 1.5 BCS during the last 90 to 100 d of gestation resulting in a loss of 1.5 to 2.5 kg BW per week. Strip grazing was used to restrict the amount of forage for the NR and NRS treatments. The NRS treatment were individually penned and fed 0.45 kg of soybean meal (2.06 Mcal/kg NE_m, 47.50% CP; DM basis) 3 d/wk. Each treatment was divided into two paddocks for a total of six paddocks. Control cows were allowed ad libitum access to a tall fescue (*Festuca arundinacea* cv. Kentucky 31) or a tall fescue/crabgrass (*Digitaria sanguinalis*) paddock. The two control pastures were switched weekly to prevent any paddock effect due to forage. As forage became dormant the control cows received ad libitum fescue hay with 1.3 kg of high concentrate feed 5 d/wk. As the nutrient restricted paddocks became dormant, the NR and NRS paddocks received fescue hay. The allowed amount of forage/hay was adjusted weekly according to animal BCS and BW. An initial BW and BCS were taken as the animal entered the study followed by a weekly BW collection and BCS was taken every three weeks until parturition. Body condition score was assigned by the same trained individual throughout the study. Along with BCS, blood was collected (~ 9 ml) by jugular venipuncture into 9 ml blood collection tubes (Starstedt,

Newton, NC) containing sodium heparin. The samples were immediately placed on ice then centrifuged within thirty minutes (2,000 X g for 20 min at 4°C) and plasma was collected and stored at -20°C until analysis. After parturition cow-calf pairs were moved to one pasture where they received ad libitum silage and 1.8 kg of high concentrate feed or ad libitum hay and 2.9 kg of high concentrate feed.

Within 4 h of parturition, a blood sample (~ 4 ml) was collected via jugular venipuncture from the calf. Blood collection was continued daily from d 0 (parturition) to d 8, then on d 10, 12, 14, 16, 18, and 20. The calf blood samples were handled in a similar fashion as the cow blood samples. Commercial bull calves were castrated at 20 d of age and received an anabolic implant (RALGRO, Intervet Inc., Merck Animal Health, Summit, NJ). Angus bull calves remained intact. Calf BW and sex (bulls n = 22, heifers n = 35) were recorded within 24 hours postpartum. Calf BW was collected every 30 d ± 7 d until weaning and adjusted to an every 30 d BW.

Hormone and Metabolite Quantification

For laboratory assays, calves were blocked by dam treatment and calf sex. At least one bull from each treatment was in every block (n = 6). Sample order was randomly assigned within the assay blocks. Plasma cortisol levels were measured in duplicate in using Coat-A-Count cortisol RIA with a sensitivity of 0.5 µg/dL (Siemens Medical Solutions Diagnostics, Los Angeles, CA) that has been validated in our lab (Long and Schafer, 2013). Plasma leptin concentrations were determined by RIA in duplicate in multiple assays (n=6) (Multispecies leptin RIA, Linco Research, St. Charles, MO) with an intraassay CV of 4.1 % and an interassay CV of 8.8 % and sensitivity of 0.5

ng/mL. Leptin assay validation comes from previous work in our lab by Long and Schafer (2013b). Maternal insulin was measured in duplicate in 2 assays by Coat-A-Count insulin RIA with an intraassay CV of 6.2 % and an interassay CV of 8.1 % and a sensitivity of 0.20 ng/mL. This was previously validated in our lab (Long and Schafer, 2013b). Plasma glucose concentration was measured colorimetrically in triplicate (Liquid Glucose Hexokinase Reagent, Pointe Scientific Inc., Canton, MI) using previously published procedures (Long and Schafer, 2013b) with an intra- and interassay CV of 4.1% and 2.5 % respectively.

Statistical Analysis

Cow BW and BCS at beginning, middle and precalving and change over the whole period along with number of d on treatment were analyzed using the MIXED model of SAS (SAS Institute Inc., Cary, NC) with treatment in the model and block and paddock as a random variable. Cow breed was initially included in the model but was found to be nonsignificant. Cow plasma glucose and insulin was analyzed as a repeated measure using the MIXED model with treatment, day, and their interaction in the model statement and lab assay block as a random variable. Calf BW at birth was analyzed using the MIXED procedure with treatment, sex, and their interaction in the model. Maternal breed was initially included in the model and found to be non-significant and was removed from the final model. Unsuckled calf plasma glucose concentrations were analyzed using the MIXED procedure with treatment in the model and paddock as a random variable. Maternal breed and calf sex was initially included in the model and found to be non-significant and was removed from the final model. Calf postnatal plasma

leptin and cortisol concentrations was analyzed as a repeated measure using the MIXED model with treatment, day, breed, and their 2 way interaction in the model statement and lab assay block as a random variable. Calf adjusted BW from d 30 to 205 of age was analyzed as a repeated measure using the MIXED model with treatment, day, sex, and their 2 way interaction in the model statement and cow block as a random variable. The three way interaction was found to be non-significant and was removed from the model. Data are presented as least squares means $\pm$ SEM and considered significantly different when $P \leq 0.05$ and a tendency was indicated when $P \leq 0.10$.

RESULTS

Maternal BW and BCS are shown in Table 1. Initial BW and BCS were similar ($P = 0.80$ and 0.98 , respectively) among treatment groups. After approximately 55 days on treatment the NR and NRS cows had a reduced BW ($P < 0.0001$) compared to control. By parturition the NR and NRS groups had lost 39.4 ± 8.0 kg and 23.1 ± 7.6 kg respectively, while the Con treatments gained 60.8 ± 7.4 kg ($P < 0.0001$, NR and NRS vs. Con). As intended the Con group gained 0.3 ± 0.1 BCS over the study while the NR and NRS groups lost 1.2 ± 0.1 , 1.2 ± 0.1 BCS respectively ($P < 0.0001$, NR and NRS vs. Con). There was no difference ($P = 0.123$, Table 2) in number of days from start of study until parturition between treatments.

Maternal plasma glucose and insulin concentrations are shown in Figure 1 and Figure 2, respectively. Nutrient restricted and NRS dam plasma glucose concentrations decreased from the beginning of the study until partition ($P < 0.0001$, Fig. 1). Control

plasma glucose concentration remained constant throughout gestation with a slight increase at parturition. Plasma insulin concentrations (Figure 2) were decreased by treatment (NR and NRS vs. Con, $P = 0.0051$) and day ($P < 0.0001$). There was no significant treatment x day effect on plasma insulin concentrations ($P = 0.1484$).

Calf birth weights are shown in Table 3. Calves from NR cows weighed less at birth compared to Con calves with the NRS calves intermediate ($P = 0.04$). Sex effect on birth weight is shown in Table 4. There was no difference in birth weight due to calf sex or treatment x sex interaction ($P = 0.38$, $P = 0.54$ respectively).

Plasma glucose concentrations of calves prior to suckling were reflective of maternal glucose levels at parturition (Figure 3). Plasma glucose levels were greater ($P = 0.0369$) in calves born to Con dams compared to calves born to NR and NRS dams.

There was a significant treatment and day effect ($P = 0.0037$, $P < 0.0001$ respectively, Fig. 4) on plasma leptin concentrations of postnatal calves. Sex or breed did not affect plasma leptin concentrations ($P = 0.1990$, $P = 0.2128$ respectively). At parturition, NRS calves had greater plasma leptin levels than Con or NR. Control calves had an increase in plasma leptin levels from parturition until d 3 and then a plateau to d 20. Plasma leptin levels increased from birth to d 2 in NR and NRS calves and then plateaued to d 20.

There was a significant treatment x day effect ($P < 0.0001$, Fig. 5) for plasma cortisol concentrations. At parturition, the NR calves had greater plasma cortisol concentrations than Con ($P = 0.05$) with an intermediate NRS. By one d after parturition, NRS and Con plasma cortisol concentration had decreased to 3.1 ± 0.4 . NRS and Con

offspring had reduced plasma cortisol concentrations at d 1 compared to NR offspring ($P < 0.05$, Con and NRS vs. NR).

Calf BW from d 30 to weaning is displayed in Figure 6. Calves born to Con dams were heavier from d 30 to weaning while the NRS offspring were intermediate to the NR offspring which weighed the least. Figure 7 shows calf BW by sex from d 30 to weaning. Bulls were heaviest while the steers and heifers were lighter.

Discussion

To the authors knowledge, this is the first experiment to demonstrate the relationship of late gestation maternal nutrient restriction, plasma leptin, and plasma cortisol levels of beef calves. This study also reports that maternal protein supplementation can mitigate adverse effects of nutrient restriction during late gestation on calf birth weight, postnatal calf plasma leptin and cortisol levels, weaning weights.

On objective of this study was to mimic a severe nutrient restriction period during late gestation. Many studies of maternal undernutrition during late gestation may not realize full fetal programming effects due to insufficient nutrient restriction (Martin et al., 2007; Bohnert et al., 2013). The decrease in BW and BCS in our nutrient restricted cows was severe enough to result in developmental adaptations. Body condition score is a better indicator of overall long-term nutrition than changes in BW (Wagner et al., 1988). The identical change in BCS of the NRS and NR demonstrate that the protein supplementation did not contribute enough to the energy status of the NRS cow to alter BW or BCS losses. Excess amino acids are ultimately excreted but some can be

deaminated and diverted to glucose synthesis. With this experiment using global nutrient restriction and a small amount of protein supplementation, it is unlikely that there were any excess amino acids to be diverted to glucogenic energy. Soybean meal is often used as a protein supplement because it is palatable, highly digestible, and has a high energy value (Kellems and Church, 2010). Dried distiller's grain with solubles has been used as a protein supplement in other maternal nutrient restriction studies (Larson et al., 2009; Bohnert et al., 2013). However, it has a lower proportion of RDP than soybean meal (NRC, 2000). This can decrease rumen microbial populations and limit the rumen's efficiency to convert fiber to energy (Firkins et al., 1986; Allen, 2000). Besides BCS and BW, the decreased plasma glucose levels in the NRS and NR treatments were also indicative of nutrient restriction. In the Con, the slight decrease in plasma glucose followed by a spike prior to parturition is probably caused by the increase in fetal size and normal gestational insulin resistance (Kronfield, 1982). Reduced glucose levels were expectedly mirrored in cow plasma insulin levels. Unsuckled calf plasma glucose levels were highest in calves born to Con dams. Additionally decreased insulin levels of NRS and NR further demonstrated the severity of the nutrient restriction imposed on the cows in this study.

Nutrition requirements rise during late gestation due to 75 % of the fetal weight increase occurring in this period (Robinson et al., 1977). Restriction during this period affects myogenesis, adipogenesis, and appetite control (Tong et al., 2008; Du et al., 2010; Underwood et al., 2010). It is well documented in multiple species that fetal and postnatal growth is hindered by maternal nutrient restriction. Maternal malnutrition

resulting in decreased BCS and BW prior to calving decreases BW of calves at parturition (Tudor, 1972; Kroker and Cummins, 1979; Spitzer et al., 1995). Dietary protein restriction in heifers during the last trimester produced weak labor, more dystocia, increased perinatal deaths, and longer postpartum intervals due to anestrus (Kroker and Cummins 1979). A mature cow responds to nutrient restriction more favorably than an immature heifer (Bellows and Short, 1978; Wallace et al., 2005). In heifers, a reduction in TDN intake during late gestation resulted in reduced calf birth weights, decreased maternal tissues, and an increased post-partum anestrus period (Bellows and Short, 1978). In our study, the reduced birth weight of calves born to NR dams and the intermediate birth weight of NRS calves is conclusive with data from a similar study by Bohnert and others (2013). They reported increased maternal BCS at calving resulted in higher birth weights and maternal protein supplementation during late gestation increased birth weights.

Multiple species have a characteristic postnatal surge of plasma leptin levels (Long et al., 2011; Long and Schafer, 2013; Yura et al., 2005). This surge influences the formation of the hypothalamic appetitive centers in rats (Yura et al., 2005). This programming appears to happen postnatally in the rat, but in the more developed neonate programming may take place in utero. In the altricial mouse and rat neonate, the connections in the appetite control centers of the brain are still forming during the first few days after birth. However, in more precocial species such as the Meschian pig and sheep these neural connections are formed during late gestation (Lee and Blackshaw, 2014). Lambs have a postnatal leptin peak between days 6 and 9 postpartum (Long et al.,

2011). Lambs born to obese ewes lacked the characteristic postnatal leptin surge between d 6 and 9 instead they had slightly elevated leptin levels between d 0 – 4 (Long et al., 2011).

Unlike postnatal lambs, beef calves have a different leptin surge profile (Long and Schafer, 2013). They reported an increase in leptin level from birth to postpartum d 2 followed by a plateau and a decrease at d 16. Our control calves increased to d 3 and then followed a similar pattern. The nutrient restricted treatments (NR and NRS) only increased to d 2. This is similar to the lesser and premature leptin increase reported in lambs born to obese ewes (Long et al., 2011). Long and others (2011) suggest that adipose tissue is the source of the leptin surge in sheep but the amount of leptin produced is not related to adipose tissue mass. This disruption of the normal neonatal surge is accompanied by an increase in lamb appetite (Long et al., 2010; Long et al., 2011).

Lambs born to ewes fed 150 % of NRC (1985) recommendations from 60 d preconception to parturition were subjected to an ad libitum feeding challenge at 19.5 mo of age. These offspring consumed 10 % more feed and had increased BW gain compared to offspring born to ewes fed at 100 % recommendations. Elimination of characteristic leptin peaks from maternal obesity can be transgenerational. The offspring of the obese ewes were fed 100% of NRC recommendations during gestation and gave birth to lambs that showed a lack of leptin surge (Long et al., 2011).

Glucocorticoids have been shown to control fetal and adult leptin production (Soret et al., 1999). Cortisol is a glucocorticoid that can regulate metabolic, cardiovascular, immune, and behavioral responses (Smith and Vale, 2006). In sheep, an

increase in fetal cortisol levels begins around 20 d antepartum (Magyar et al., 1980). This increase has a maturation effect on the fetus and is necessary for parturition and postpartum life (Fowden et al., 1998). In the current study, the cortisol levels for all treatments were elevated at birth. This spike was followed by a plasma leptin level increase in all treatments. Similarly, plasma leptin and perirenal adipose tissue leptin mRNA increase during the final 15 d of ovine gestation (O'Connor et al., 2007). The cortisol increase must program for the leptin surge. However fetal adrenalectomy during this period prevents this leptin increase (O'Connor et al., 2007). Conversely glucocorticoid exposure to the intact fetus during this period produces a premature elevation of plasma leptin and perirenal adipose tissue leptin mRNA (O'Connor et al., 2007). Maternal nutrition can greatly affect maternal, fetal, and neonatal cortisol levels. Both nutrient restriction and overnutrition elevate cortisol levels in precocial species such as the cow and ewe (Hough et al., 1990; Long et al., 2013b). In our study the nutrient restricted treatments had higher cortisol levels compared to Con. Maternal protein supplementation during late gestation is known to benefit the dam and growth characteristics of the neonate; in our study it appears to have lessened the NRS elevated cortisol level.

Our finding of a lack of a sex effect on postnatal leptin agrees with multiple lamb studies (Bispham et al., 2002; McFadin et al., 2002; Long et al., 2011). However in a postnatal calf study by Long and Schafer plasma leptin levels were greater in bulls than heifers (2013). This disagreement can be attributed to the larger sample size of the present study and our use of multiparous cows rather than nulliparous heifers. Immature

pregnant heifers have higher nutrient requirements than older cows as heifers are growing to mature size while maintaining a fetus (NRC, 2000). A heifer or adolescent ewe may shunt nutrients from the fetus to maintain her growth or nutritional status (Bellows and Short, 1978; Wallace et al., 2005; Luther et al., 2005). Smaller uterine capacity of the heifers may have caused intrauterine growth restriction. The lack of sex effect on cortisol levels from our data is conclusive to Long and Schafer (2013).

As with leptin, cortisol dysfunction can be transgenerational. Late gestation administration of synthetic GC to F0 ewes elevated GC in F1 and F2 offspring (Long et al., 2013a,b). This periparturition increase in the cortisol levels of the offspring likely caused the elimination of the leptin surge and consequently the increased appetite. Long and others demonstrated that the HPA axis of the F1 ewe is dysregulated (2013a). Administration of synthetic GC can be likened to maternal nutrient restriction as both cause IUGR and GC play a significant role in programming regardless of model (Long et al., 2012).

In some studies maternal protein supplementation during late gestation can improve postnatal growth characteristics of offspring (Martin et al., 2007; Larson et al., 2009; Bohnert et al., 2013). Calf BW from 100 d of age to weaning were heavier for Con offspring compared to NR offspring, however NRS had intermediate BW at weaning. Improved weaning weights for our NRS calves is conclusive with other studies utilizing maternal protein supplementation during late gestation nutrient restriction to improve postnatal growth characteristics of offspring (Martin et al., 2007; Larson et al., 2009; Bohnert et al., 2013). In addition to an increase in calf BW at weaning, Larson and

others reported maternal that protein supplementation increased HCW, marbling, and proportion that grade USDA choice at slaughter (2009). These improvements suggest that maternal protein supplementation can add value to the offspring (Larson et al., 2009).

This study demonstrates that even 0.45 kg of protein supplementation is beneficial during severe late gestational nutrient restriction. Future work may indicate that protein supplementation may be decreased even further than the current study and still be effective. This has the potential to be used in drought years or other events that cause global nutrient restriction. More research is warranted to study the mechanism by which the protein supplement alleviates the negative effects of maternal nutrient restriction on calf endocrine regulation. Although our experiment did not measure intake, a similar maternal nutrient restriction study has reported an increase in DMI at 1 yr of age in calves born to protein supplemented dams (Martin, 2007). A greater understanding of maternal protein supplementation's effect on the offspring hypothalamic appetite control centers may prove to be a tool to program the offspring's appetite. In conclusion, a small amount (0.45 kg) of protein supplement provided just 3 d per week appears to mitigate many of the adverse effects of maternal nutrient restriction during late gestation.

Table 1. Body weight (kg) and BCS of multiparous cows fed either a control (Con), nutrient restricted (NR), or nutrient restricted with protein supplementation (NRS) diet during the last 100 d of gestation.

Item	Treatment			<i>P</i> -value
	Con	NR	NRS	
Cow, <i>n</i>	19	19	19	
BW				
Initial ¹	618.7 ± 19.0	621.0 ± 20.4	606.1 ± 19.3	0.80
Middle	667.0 ± 18.5	595.0 ± 19.9	589.8 ± 18.8	< 0.0001
Calving	679.4 ± 17.9	581.6 ± 19.3	583 ± 18.2	< 0.0001
Change	60.8 ± 7.4	-39.36 ± 8.0	-23.1 ± 7.6	< 0.0001
BCS ²				
Initial	5.4 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	0.98
Calving	5.7 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	< 0.0001
Change	0.3 ± 0.2	-1.2 ± 0.2	-1.2 ± 0.2	< 0.0001

Data presented LSM ± SEM.

¹Wagner et al., 1988.

²Initial BW was collected at initiation of treatment, approximately d 186 of gestation.

Table 2. Days from initiation of treatments until parturition in multiparous cows fed either a control (Con), nutrient restricted (NR), or nutrient restricted with protein supplementation (NRS) diet during the last 100 d of gestation.

Item	Treatment			<i>P</i> -value
	Con	NR	NRS	
Cow, <i>n</i>	19	19	19	
d	103.6 ± 2.9	97.1 ± 3.1	103.8 ± 2.9	0.123

Data presented LSM ± SEM.

Table 3. Calf birth weight (kg) born to multiparous cows fed either a control (Con), nutrient restricted (NR), or nutrient restricted with protein supplementation (NRS) diet during the last 100 d of gestation.

Item	Treatment			<i>P</i> -value
	Con	NR	NRS	
Calf, <i>n</i>	19	19	19	
Birth Weight	37.2 ± 1.2	33.4 ± 1.3	35.0 ± 1.3	0.0406

Data presented LSM ± SEM.

Table 4. Calf birth weight by sex born to cows fed either a control (Con), nutrient restricted (NR), or nutrient restricted with protein supplementation (NRS) diet during the last 100 d of gestation.

Item	Treatment			Sex	Treatment x Sex
	Con	NR	NRS		
<i>P</i> -value				0.38	0.54
Bulls					
<i>n</i>	8	8	6		
Birth weight	37.7 ± 1.8	32.2 ± 1.7	35.1 ± 2.0		
Heifers					
<i>n</i>	11	11	13		
Birth weight	35.2 ± 1.5	33.2 ± 1.5	33.3 ± 1.3		
Data presented LSM ± SEM.					

Figure 1. Plasma glucose concentrations of multiparous cows fed either a control (n = 19), nutrient restricted (NR, n = 19), or nutrient restricted with protein supplementation (NRS, n = 19) diet during the last 100 d of gestation. Values are means $\pm$ SEM. Treatment, $P < 0.0001$; Day, $P < 0.0001$; Treatment*Day, $P = 0.0135$.

Figure 2. Maternal plasma insulin concentrations during late gestation. Values are means $\pm$ SEM. Treatment $P = 0.0051$; Day $P < 0.0001$; Treatment*Day $P = 0.1484$

Figure 3. Calf plasma glucose concentrations prior to suckling. ^{a,b} means differ $P < 0.05$ between treatment. Control (n = 7), nutrient restricted (NR, n = 5), nutrient restricted with supplement (NRS, n = 7).

Figure 4. Plasma leptin concentrations from birth to postnatal d 8 and every other day until d 20 in calves born to multiparous control dams (n = 19), nutrient restricted (NR, n = 19), nutrient restricted with protein supplement (NRS, n = 19). Values are means $\pm$ SEM. Treatment $P = 0.0037$; Day $P < 0.0001$; Treatment*Day $P = 0.7499$

Figure 5. Plasma cortisol concentrations from birth to postnatal d 5 in calves born to multiparous control dams (n = 19), nutrient restricted (NR, n = 19), nutrient restricted with protein supplement (NRS, n = 19). Values are means $\pm$ SEM.

*a, b, c means differ $P < 0.05$ between treatment with in a d. Treatment $P = 0.0103$; Day $P < 0.0001$; Treatment*Day $P < 0.0001$

Figure 6. Calf BW (Kg) from d 30 to d 205 of age by maternal treatment. Values are means $\pm$ SEM. Treatment*Day $P = 0.038$

Figure 7. Calf BW (Kg) from d 30 to d 205 of age by sex. Values are means $\pm$ SEM. Day*Sex $P = 0.004$.

CHAPTER IV

Conclusion

This study presented data that can be applied to all aspects of the cattle industry. Fetal programming studies are not limited to cow-calf producers they can be applied to stocker and feeder systems as well. This data reinforces the importance of a beneficial nutrient environment for the fetus. The major economic factors of a cattle operation, reproduction, nutrition, and performance on feed, are influenced by maternal nutritional status during gestation. It may not always be possible to maintain adequate nutrition for the cow herd but this data indicates that a small amount of protein supplementation can alleviate some of the adverse effects of gestational maternal nutrient restriction. There is justification for future studies examining a way to employ appetite programming. There is a potential that the appetites of animals destined for the feedlot could be increased to take advantage of a dilution of maintenance requirements allowing more feed to be used for growth.

Outside of the cattle industry this data can be applied to human medicine. Fetal programming studies in humans are justly limited by ethics. This data from a precocial species can more accurately apply to humans than that from altricial species such as the mouse. In developing countries where nutrient restricted pregnancies are more prevalent, it may be beneficial for pregnant women to receive small amount of protein supplement during late gestation.

CHAPTER V

LITERATURE CITED

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