NUTRITIONAL PROGRAMMING OF PRENATAL BEEF HEIFER DEVELOPMENT AND POSTNATAL PERFORMANCE

A Thesis

by

JOHN MARK LONG JR.

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Chair of Committee,	M. Carey Satterfield
Committee Members,	Tryon A. Wickersham
	Jason E. Sawyer
	Charles R. Long
Head of Department,	G. Cliff Lamb

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ABSTRACT

Maternal nutrient restriction during pregnancy has been shown to impact postnatal life in beef cattle; examples include impaired immune, metabolic, and reproductive function, and reduced growth rate and carcass characteristics. Despite potential economic ramifications, epigenetic modifications to aforementioned physiological systems have yet to be elucidated in prenatal beef heifer development. To investigate cellular and molecular alterations associated with postnatal phenotype, we capitalized on embryo splitting technologies to develop a monozygotic twin model of maternal nutritional restriction. Each demi-embryo was individually transferred into phenotypically uniform virgin dams of similar breed type. Beginning on gestational day (GD) 158, dams carrying one identical twin were reduced to 70% NRC while dams carrying the other identical twin were assigned100% NRC. Dams carrying a twin whose identical counterpart was lost were labeled half-siblings, and randomly assigned to either control or 70% NRC. Identical twin pregnancies were necropsied on GD 265 while half-siblings were maintained through parturition.

Initially, there was no difference in dam BW, REA, or LRBF. Dams on a restricted diet gained less BW and had smaller REA at the end of treatment, while LRBF did not differ. Restricted twin fetuses exhibited lighter pancreases with lower insulin concentrations in fetal umbilical vein. At birth, half-sibling calves from restricted pregnancies were lighter than control. Restricted calves were lighter than control at postnatal day (PND) 35 and 70. There was no difference in calf BW from PND 105 through PND 485. At weaning (PND210), there was no difference in REA or LRBF. There were also no differences in REA or LRBF at PNDs 315, 420,

or 485, or time of puberty. At slaughter, heifers from restricted dams had greater internal fat and lighter pituitary glands, with impaired glucose clearance.

Collectively, results indicate that modest maternal nutrient restriction during late pregnancy alters pancreatic development, and these alterations contribute to compensatory growth in early postnatal life. Furthermore, nutrient restriction suggests increased visceral fat deposition and impaired blood glucose clearance. The observed alteration in development of the pituitary is of interest and future studies are needed to determine cellular and hormonal alterations and associated ramifications to performance.

DEDICATION

To Mr. and Mrs. John Mark Long:

For showing me what true perseverance is and always having my back.

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CHAPTER I

INTRODUCTION

Texas remains the largest beef producing state in the United States despite the estimated loss of 1.5 million head from 2006 to 2014 (a loss of 25% of the total cow herd; USDA-NASS, 2015). The primary factor contributing to this reduction was drought. More specifically, in the wake of declining nutrient resources producers were left with little option but to liquidate their herds. Climate change comes at greater and greater economic cost as land availability continues to decrease and cost of production continues to increase. Herd liquidation may serve as a viable short-term strategy, but over time this results in market saturation and an overall decline in total beef production. Not only does this create low selling prices during time of drought, but also leads to high female replacement costs when producers look to restock animals, as feedstuffs become more available. This ultimately compromises the sustainability of the beef industry and solidifies the need for alternative production strategies to mitigate the consequences of climatic shifts and reduced land availability. One management technique that has recently gained attention is semi-confinement feeding.

Semi-confinement feeding allows producers to confine-feed animals during annual periods of low forage-based nutrient availability, and graze animals during periods of high forage-based nutrient availability. This strategy reduces operating expenses by minimizing land requirements and more tightly controlling nutritional management of pregnant females, thus increasing beef production per unit area of land. This high degree of control over nutritional management also affords producers the potential opportunity to significantly improve the efficiency of nutrient utilization in beef cows. Improving maternal efficiency of nutrient

utilization is important, as feeding the cow remains the greatest direct cost to the producer. Previous work has found that when high quality feedstuffs are limit-fed, cow nutrient requirements are lower and diet digestion is greater than what is predicted by the NRC (Trubenbach et al., 2014). These findings support the concept of increased efficiency of nutrient utilization and represent a beneficial mechanism that could be of potential use to economically improve beef cow management. However, if this reduction in dietary intake does not truly meet the combined maintenance requirements of maternal tissue and pregnancy, the developing fetus may be subject to inappropriate metabolic programming relative to its extrauterine environment. If this were to occur, any economic gains made during management of pregnant females would subsequently be lost due to poor growth and performance of their offspring. Calves may be lighter at birth and weaning, and replacement females may not perform reproductively. These are merely two examples of a plethora of issues that may arise in the wake of inappropriate fetal programming, any of which would negatively impact a producer's bottom line.

Fetal programming is the concept that critical physiologic parameters, such as metabolism or stress tolerance, are patterned during the early stages of embryonic and fetal development and are established for the life of that individual and may in fact be heritable across generations. From a biological perspective, the concept of fetal programming has several advantages. It allows the developing fetus to interpret cues transmitted from its extra-uterine environment, predict the demands of that environment, and program his/her physiologic parameters in a manner that provides greatest opportunity for postnatal survival. This process gives each individual/generation a certain degree of adaptability beyond the comparatively rigid genetic code. However, when a calf is born from a poor uterine environment into the calorically abundant feedlot, the calf's metabolic parameters will have been inappropriately programmed

and several negative consequences will manifest; examples include impaired immune and metabolic function, as well as reduced growth rate and undesirable carcass characteristics (Satterfield and Dunlap, 2014a,b).

Despite the negative impacts on production efficiency, little has been done to investigate these phenotypes. It is this lack of knowledge that has hindered the development of prescriptive production practices that would ameliorate the consequences of a suboptimal intrauterine environment on the offspring's postnatal performance, when attempting to improve efficiency of nutrient utilization in the gestating cow. Therefore, our primary objective was to elucidate the impact of utilizing controlled maternal nutrient restriction to improve the energy utilization of a cow in late gestation on prenatal beef heifer development. Our secondary objective was to observe postnatal effects of the abovementioned prenatal insult on; heifer calves' growth from birth to weaning, feed efficiency from weaning to slaughter, age at attainment of puberty, ability to clear blood glucose post weaning and pre-slaughter, and carcass characteristics.

CHAPTER II

LITERATURE REVIEW

Bovine placental development

Early Development of the Embryo and Trophectoderm

Following fertilization, the embryo goes through a series of cellular divisions leading up to compaction of blastomeres at the morula stage. The outermost blastomeres form cell-to-cell adhesions known as tight junctions, creating the trophoblast cell layer. This occurs as the embryo transitions from the morula to the blastocyst stage. The blastocyst is characterized by a fluid filled cavity called the blastocoel, which will enlarge as the embryo proper migrates to one end of the cavity and forms the inner cell mass (ICM).

After the blastocyst hatches from its zona pellucida the primitive endoderm and mesoderm begin to take shape. The primitive endoderm will form beneath the ICM and grow downwards to line the inner surface of the trophectoderm, giving rise to the yolk sac. Concurrently, the mesoderm will grow between the trophectoderm and primitive endoderm and ultimately form a cavity around the yolk sac. This occurs as the embryo elongates, with the trophectoderm invaginating and fusing with cells of the mesoderm to create the amnion. As the embryo begins to form into a fetus, an out-pocketing of the hindgut extends from the fetus into the loose tissue of the mesoderm forming the allantois.

The extra-embryonic membranes of the pre-attachment embryo consist of the: yolk sac, amnion, allantois, and the chorion (Schlafer et al., 2000). Once the allantois is formed, it will continue to expand and come into apposition with the chorion, at which point the two cell layers fuse and produce the chorioallantois. The chorioallantois is the fetal contribution to the placenta

and will provide the surface for attachment to the endometrium. Attachment, referred to as implantation, occurs around week three of pregnancy in the cow (Hue et al., 2015).

Gross Anatomy

In the cow, the chorioallantois is described as cotyledonary in shape and is histologically classified as synepitheliochorial (Haeger et al., 2016). At about four weeks of gestation, the smooth surface of the chorioallantois will begin to become irregular over specialized maternal structures in the uterine epithelium, known as caruncles (Schlafer et al., 2000). Villous fingers extend from cotyledons into caruncular crypts between day 30 and 35 of pregnancy to from placentomes (King et al., 1979).

Placentomes can range from 100 to 140 in number and are largest in the horn that contains the developing fetus (Haeger et al., 2016). Placentomes are highly vascularized areas of interface between the chorioallantois and endometrium and represent the majority of nutrient and waste exchange between fetus and mother. The interplacentomal region of the chorioallantois is characterized by gentle folds and less aggressive endometrial invasion (Schlafer et al., 2000). Endometrial glands are located in these regions and secrete products that are essential for maintenance of pregnancy.

Histology

The chorionic epithelium consists mostly of two cell types: 80% of the cellular population is classified as mononuclear and the remaining 20% are binucleated (Schlafer et al., 2000). The mononucleate trophoblast cells (MTC) can be further divided into two subpopulations, both of which are phagocytic by nature. These cells can be found at the base of cotyledonary villi (arcade region of the placentome) where they phagocytize maternal erythrocytes, and in the interplacentomal regions of the chorioallantois situated directly above

the openings of endometrial glands where they phagocytize glandular secretions (Schlafer et al., 2000). Binucleate cells, also called trophoblast giant cells (TGC), migrate to the forefront of maternal-fetal interface and merge with tight junctions connecting MTCs (Wooding et al., 1994). Ultimately, TGCs will pass through these tight junctions to fuse with caruncular epithelial cells, forming a trinucleated cell. This TGC migration and fusion with maternal epithelial cells results in the formation of the fetal-maternal syncytium, setting up the major cellular pathways through which nutrients and waste are exchanged.

Bovine Nutrient Requirements During Gestation

Feeding the cow is the single greatest expense that producers face. This expense is often precariously balanced between what is cost effective and what is best for the developing fetus. Intake requirements increase as the cow reaches mid to late pregnancy, far surpassing what her intake requirements were during early pregnancy. If these increasing requirements are not met, important developmental events may be impacted causing the fetus to undergo gene expression changes that will result in suboptimal postnatal performance. It is therefore crucial that bovine nutrient requirements during gestation are met.

Net energy maintenance (NEm) is defined as the amount of feed energy intake required so that no net change (loss or gain) in energy will occur to the animal's tissues (NRC, 2000). Data from previous studies (Lofgreen and Garrett, 1968) have produced results leading to development of the net energy system which uses 0.077Mcal/EBW0.75 to calculate NEm, where EBW is the average empty body weight in kilograms (Caton and Dhuyvetter, 1997). This equation has proven to be quite useful for predicting maintenance requirements for penned cattle.

When nutrient requirements are taken into consideration, two components are focused on, protein and energy. Accurately calculating the requirements for these two components during gestation is primarily based off predicted calf birth weight (NRC, 2000). It is thereby assumed that factors influencing calf birth weight, such as breed of sire and dam, parity of dam, heterosis, and the environment the dam is gestating in, have a proportional effect on her nutrient requirements during pregnancy (Ferrell, 1991). Nutrient requirements follow an exponential curve, with requirements peaking in late gestation. Studies have shown a decrease in calf birth weight when energy or protein are severely restricted in mid to late gestation (Hight, 1966, 1968a,b; Café et al., 2005). Calf birth weight is not the only thing at jeopardy, as low feed intake in late pregnancy has also been associated with increased incidents of dystocia, longer post-partum intervals, and reduced milk production (Bellows and Short, 1978; Kroker and Cummins, 1979). Overfeeding can also produce the same negative effects on reproductive performance. Ideal feed intake can be associated with cow body condition score (BCS), as calf birth weight decreases when BCS falls below 3.5 or exceeds 7 (1 to 10 scale; NRC 2000).

Fetal Programming

Fetal programming is the concept that intrauterine representation of the extrauterine environment patterns prenatal development of lifelong phenotype and may in fact be heritable across generations (Hales and Barker, 2013). Fetal programming has the potential to turn genes on or off and provides the offspring with a certain amount of adaptability to its extrauterine environment. From an evolutionary standpoint, this is quite useful and can provide decided advantages in postnatal life. For instance, individuals who are gestated in drought or famine and spend their entire postnatal life in the same conditions will tend to have a metabolic advantage.

However, if the offspring's prenatal intrauterine environment is calorically scarce (i.e. gestational nutrient restriction), and it's postnatal extrauterine environment will be calorically abundant, then the offspring's metabolic parameters may be inappropriately programmed. This can lead to a myriad of problems in postnatal life such as adult onset of coronary heart disease, hypertension, and non-insulin dependent diabetes (Barker et al., 1993; Barker, 1998; Godfrey and Barker, 2000).

Several epidemiological studies in humans have revealed significant correlation between low weight and small size at birth to an increased risk of disease in later life (Barker and Osmond, 1986; Roseboom et al., 2001; Yajnik et al., 1995). A group of 468 men born in Hertfordshire, England between 1920 and 1930 were subjected to an oral glucose tolerance test. Of the 468 men, 93 exhibited impaired glucose tolerance. These men had a lower birth weight and lower weight at 1 year of age when compared to men who responded normally in testing (Hales et al., 1991). In another study, men and women were selected from a similar time era and geographic location and subjected to a glucose tolerance test. Twenty-seven percent of subjects who weighed less than 5.5 pounds at birth exhibited impaired glucose tolerance while only 6 percent of subjects weighing more than 7.5 pounds had impaired glucose tolerance (Phipps et al., 1992). These findings were independent of gestation length, indicating that increased occurrence of glucose intolerance could not be attributed to premature birth. These findings suggest that insults during critical periods of endocrine pancreas development appears to cause beta cell dysfunction in later life, and in severe cases may be the root cause of non-insulin dependent diabetes mellitus.

Another serious disease that appears to trace its origin back to insults incurred during fetal development is cardiovascular dysfunction. This relationship has been described in several

epidemiological studies conducted in humans. It was demonstrated that women who were below average birth weight but above average weight at one year of age had the highest rates of cardiovascular disease in late life (Osmund et al., 1993). In contrast, men had the highest rate of coronary heart disease when birth weight and weight at 1 year of age were both below average (Osmund et al., 1993). In an earlier study, 7,991 men were selected based on era (1911-1930) and location (Hertfordshire, England) of birth to investigate mortality rates as a result of ischemic heart disease in later life. Men weighing 18 pounds or less at one year of age were three times more likely to die of heart disease than men who weighed 27 pounds or more at one year of age (Barker et al., 1989).

Rats provide an excellent experimental model when studying fetal programming. Their short generation intervals and molecular flexibility when is applied make them an ideal species to study molecular alterations following maternal nutrient restrictions. In studies where gestating rats are fed low protein diets, it is well documented that offspring exhibit hypertension (Woodall et al., 1996; Langley-Evans et al., 1996b). Furthermore, low protein diets increased heart size but decreased size of the liver (Langley-Evans et al., 1996b). In a study where dams were subjected to a total reduction in nutrient intake, litter size was not affected but the average pup weight at birth was significantly lighter (Woodall et al., 1996). Interestingly, the smaller pups exhibited compensatory growth and by 30 weeks of age had similar body weights to the control born pups. This increased rate of growth was coupled with higher systolic blood pressure at 30, 48, and 56 weeks of age (Woodall et al., 1996). In addition to low fetal weights following global nutrient restriction, protein restriction at GD 14 and GD 18 also caused low fetal weights (Gao et al., 2012).

Studies in the sheep and other large animal species are beginning to characterize the consequences of maternal nutrient restriction on livestock production and performance. Fetal lambs subjected to reduced nutrient availability in late gestation have exhibited metabolic perturbations postnatally, namely glucose intolerance and increased adiposity in early adulthood (Bell et al., 2006). In one ovine model of intrauterine growth restriction (IUGR) induced by thermal stress, fetal lambs were shown to have impaired pancreatic development. IUGR lambs not only had 58% lighter pancreases compared to their control counterparts, but the reduction in pancreatic mass occurred selectively in beta-cell mass (Limesand et al., 2005). This study also went on to show a decrease in mitotic activity of beta-cells in IUGR fetal pancreases. While this may not be a maternal nutrient restriction model, it is still an example of the negative impact maternal stress can have on fetal development. Offspring subjected to late maternal nutrient restriction were not different in BW at 1 year of age (Gardner et al., 2005). However, offspring from restricted pregnancies exhibited greater amounts of perirenal and omental adipose tissue as well as glucose intolerance in response to an intravenous glucose tolerance test (IVGTT) (Gardner et al., 2005). Results from rodent studies show there is a significant reduction of fetal pancreas mass in offspring from nutrient restricted dams (Dumortier et al., 2007; Garofano et al., 1997). Interestingly, supplementation of L-arginine to nutrient restricted ewes prevented a reduction fetal pancreas mass (Satterfield et al. 2013). Additionally, maternal supplementation of sildenafil citrate during pregnancy increases pancreas mass in fetuses carried by adequately fed and nutrient restricted ewes (Satterfield et al., 2010). These finding add to the growing body of evidence supporting altered pancreatic development following maternal nutrient restriction

Since the initial work by Barker and colleagues to unearth the connection between poor intrauterine environment and disease in adult life, a multitude of experiments have observed the

phenomenon of fetal programming in several other mammalian livestock species (Wu et al., 2006). Despite this, there is still much we do not understand about the role fetal programming plays in livestock production efficiency or the epigenetic processes through which it is mediated. Future work should continue to investigate the molecular alterations occurring in the epigenome and how these alterations translate to phenotype.

Effects of Malnutrition on Fetal Development

Epidemiological studies in humans began with the initial discovery of a correlation between low birth weight and disease onset in late adult life. As a result of this, fetal programming studies conducted in livestock use low birth weight as a proxy measurement for the quality of the intrauterine environment. Many studies in cattle have investigated the effects of suboptimal maternal nutrition on weight at birth, weaning and slaughter, yielding variable results. Timing and duration of nutrient restriction play a large part in the observed effects. Restriction during mid gestation has been shown to decrease fetal weights at GD125 and at birth (Long et al., 2009; Micke et al., 2010). This is in contrast with other studies that saw no difference in birth weight following maternal nutrient restriction (Martin et. al., 1997; Long et. al., 2009; Underwood et. al., 2010; Long et. al., 2010; Summers et. al., 2015; Paradis et. al., 2017). The variation in these findings may be due in large part to age and parity of the dams and the period and duration of gestation in which the mother was restricted. It must also be taken into consideration that fetal growth rate fluctuates a great deal during pregnancy. From d 70 to 100 that rate is 10 g/d, but from d 200 to 250 the fetus can grow up to 300 g/d (Lemeley et al., 2015). It would therefore be logical to conclude that cows restricted during early gestation but realimented during late gestation would not produce lighter calves at birth. This was

demonstrated by a study in which dams were restricted from d 30 to 125 of gestation, then subsequently realimented through d 245. At d 125, fetuses from restricted pregnancies were lighter, however at d 245 there was no difference in fetal weight (Long et al., 2009). The type of nutrients restricted also likely contributes to observed variation in fetal growth rates. Some studies choose total nutrient restriction (Long et al., 2010), while others choose to restrict only protein intake (Martin et al., 1997; Underwood et al., 2010; Summers et al., 2015) or energy intake (Long et al., 2009; Micke et al., 2010; Paradis et al., 2017). Two studies demonstrate the impact this added variable can have. One of the studies demonstrated that providing a high energy diet 100 d prepartum increased calf birth weight (Corah et al., 1975), while the other study demonstrated that protein supplementation during late gestation had no effect on calf weight at birth (Martin et al., 1997).

Organogenesis

Fetal organogenesis takes place after the conceptus has differentiated to form placental and fetal tissue. This occurs very early in pregnancy, and the bovine fetus's heartbeat can be seen on ultrasound as early as d 21 (Lemeley et al., 2015). Fetal limb development begins between d 25 and 30, and the gonadal ridge is apparent by d 28. Differentiation of the stomach into the rumen, reticulum, and omasum occurs between d 40 and 50 followed by sequential development of other organs, such as the pancreas, liver, lungs, adrenals, thyroid, spleen, brain, heart, thymus, and kidneys (Hubbert et al., 1972; Lemeley et al., 2015). Formation of sex organs occurs during this time as well. In male calves, testicular formation occurs by d 45 and in female calves the ovaries are apparent between d 50 and 60 of pregnancy. The genesis of the bovine fetus's organs may occur early during gestation, but the rate of hypertrophy for each tissue is different, leaving certain organs susceptible to suboptimal conditions all the way through late gestation.

The pancreas is no exception to this rule. Although its genesis takes place very early in gestation, critical developmental events occur very near parturition, specifically in the endocrine pancreas. During early pancreatic development and differentiation into endocrine and exocrine tissue, two distinguishable islet types form within the endocrine pancreas: perilobular giant islets & intralobular small islets (Merkwitz et al., 2013). Perilobular giant islets evolve synchronously in a single wave of hyperplasia during early gestation. These are near ganglia and nerve fibers and are comprised almost entirely of insulin producing beta-cells. Interestingly, perilobular giant islets undergo involution during the peri & antenatal periods whereas intralobular small islets are not subject to involution and persist into adulthood. Intralobular small islets can be further contrasted to giant islets, as they develop in multiple, asynchronous waves throughout mid to late gestation. They are large in number and embedded within the exocrine portion of the pancreas, with a considerable portion of the cells being glucagon and/or GLP-1-positive alpha-cells. However, there is a marked decrease in alpha-cells and concomitant increase in beta-cells during the peri & antenatal period. Importantly, it has been indicated that only intralobular small islets persist in the pancreases of calves and adult cattle, whereas perilobular giant islets dissipate almost entirely prior to parturition (Merkwitz et al. 2013). This makes insults during late pregnancy especially dangerous to proper final formation of intralobular small islets and the offspring's postnatal ability to regulate blood glucose. Maternal nutrient restriction during the latter third of gestation may inhibit the prenatal tipping of alpha to beta cell ratio, preventing adequate formation of healthy insulin producing beta cells.

A reduction in endogenous production of insulin in the adult ruminant could very well have significant effects on the animal's ability to produce efficiently. Offspring of rat mothers who were fed a low protein diet during pregnancy exhibited altered glucose production and

utilization and associated insulin secretion (Desai et al., 1995). Furthermore, it has been demonstrated in rodents that a suboptimal intrauterine environment causes prenatal alterations development of the structure and function of pancreatic islets (Fowden and Hill, 2001). Evidence of this in livestock species is less established at present. It has been reported that small changes in nutrient metabolism as a result of low insulin production reduces milk fat yield in dairy cattle (Murphy et al., 2000). In sheep, female offspring from dams restricted in early gestation have altered glucose metabolism (Effertz et al., 2007) and male offspring display hyperglycemia and altered patterns of insulin secretion (Ford et al., 2007). Lambs born from ewes restricted during late gestation appeared to be insulin resistant and exhibited greater adipose tissue mass but reduced GLUT4 expression in adipose but not muscle tissue (Gardner et al., 2005). These effects are difficult to characterize however, given that the adult ruminant is relatively insulin resistant to begin with (Funston et al., 2010).

Muscle and Fat Development

During fetal development, skeletal muscle and fat have a lower priority in nutrient partitioning than organs such as the heart or brain, rendering them particularly susceptible to maternal nutrient restriction (Zhu et al., 2006). Development of primary myofibers begins in the embryonic stage, while secondary myofibers form during fetal stages and make up the majority of adult muscle myofibers (Ward et al., 1991; Du et al., 2010). Concomitant with secondary myofiber development, adipocyte and fibroblast development occurs. These three cell types all arise from the same stem cell pool to create the structure of skeletal muscle (Du et al., 2010). The fetal period is crucial for muscle development, as postnatally no myofiber hyperplasia occurs, limiting the offspring to muscle growth via hypertrophy only (Glore and Layman 1983; Greenwood et al., 2000; Paradis et al., 2017). It has been postulated that proliferative rates of

fetal myonuclei may be reduced by maternal nutrient restriction in late pregnancy (Greenwood et al., 2000). This would be a serious limiting factor for muscle growth in postnatal life as well.

Several studies in other mammalian species have investigated the effects of maternal diet on fetal skeletal muscle development and have demonstrated that nutrient restriction can reduce the number of myofibers and myonuclei (Bedi et al., 1982; Wilson et al., 1988; Ward and Strickland, 1991). Offspring from sows fed a low-energy diet for the first 50 days of pregnancy exhibited fewer fast glycolytic fibers in semitendinosus muscle compared to offspring from sows fed a high-energy diet (Bee, 2004). Mice offspring from restricted dams were not different in muscle fiber number, however, did have 16% fewer myonuclei than control offspring (Bayol et al., 2004). In contrast, a decrease in muscle fiber number following early- to mid-gestational restriction has been observed in sheep progeny (Quigley et al., 2005; Zhu et al., 2006), pig progeny (Dwyer et al. 1994), and guinea pig progeny (Ward and Strickland, 1991). Another important component of skeletal muscle development is secondary muscle fiber type. Type I fibers exhibit low growth rates and high protein turnover while type II fibers have greater growth efficiency and reduced catabolic rates (Du et al., 2010). Nutrient restriction in gestating sheep has been shown to increase the number of type II muscle fibers in offspring (Zhu et al., 2006). Given the dramatic difference among myofiber type, altered development of type I and II myofiber ratios could have a major impact on skeletal muscle metabolism. Type II myofibers are primarily glycotic and have low insulin sensitivity while type I myofibers are primarily oxidative and are highly sensitive to insulin (Brown, 2014). In healthy individuals, about 80% of insulin-mediated glucose uptake occurs in skeletal muscle (Ferranini et al., 1982). It is therefore logical to conclude that alterations to fetal skeletal muscle development may pose significant threat to

postnatal ability to mediate glucose utilization and may ultimately impact production performance.

Effect of Malnutrition In Utero on Postnatal Performance

Alterations during fetal development have the potential to impact postnatal growth and performance. Carcass quality, feed efficiency, and reproductive performance are the main generators of revenue when postnatal production is taken into consideration in beef cattle. These traits (or phenotypes) are easily influenceable by prenatal supply of nutrients during critical development windows.

Feed Efficiency

The two biggest costs the feedlot sector faces are buying the calf and buying feed for the calf. Increasing body weight gain without increasing feed input would substantially improve a calf's profitability. For these reasons, feed efficiency has been an intense area of focus and research in the beef industry for decades. Several studies have shown maternal nutrient restriction impacts weaning and slaughter weight in a negative manner, decreasing carcass value (Funston et al., 2012; Larson et al., 2009; Martin et al., 2007; Underwood et al., 2010). Calves in these studies were fed similar to their control counterparts, indicating calves from restricted pregnancies have a reduced ability to convert feed into lean muscle.

Conversion of ingested feedstuffs into lean tissue mass is heavily mediated by hormones such as leptin, insulin, insulin like growth factor 1 (IGF-1), growth hormone (GH), and others known to regulate growth and development. Several studies have shown an imbalance of these hormones in calves born from restricted dams, and that these calves exhibit reduced growth rates (Gonzalez et al., 2013; Micke et al., 2011; Sullivan et al., 2010). The expression of metabolic

genes, such as glucose transporter 4 (GLUT 4) and fatty acid binding protein (AP2), have also been shown to be altered following maternal nutrient restriction (Long et al., 2010). These genes play a critical role in feed conversion and may lead to less desirable carcass characteristics.

Average daily gain (ADG) is another important measurement when it comes to evaluating feed efficiency. Sectors of growth in which ADG is typically measured include birth to weaning (pre-weaning phase), weaning to feedlot entry (stocker phase), and feedlot entry to slaughter (feedlot phase). In a study by Summers et al (2015), steer progeny from protein restricted cows in late gestation had a lower ADG during the stocker phase but were not different during the feedlot phase.

Carcass Composition and Meat Tenderness

Carcass performance of beef progeny is one of the most important economic factors to take into consideration when evaluating the effects of prenatal nutrient deficiency. To do so, it is important to understand what carcass value is based on. The primary criteria by which a carcass is valued are yield grade and quality grade. Yield grade describes how much lean muscle the carcass contains as compared to intermuscular and subcutaneous fat, with higher value given to carcasses with less fat. Carcass quality refers to the amount of intramuscular fat, also known as marbling, and quality increases with high amounts of marbling.

As discussed before, myocytes and adipocytes develop from the same stem cell pool during embryonic and fetal development (Du et al., 2010). Adipocytes migrate throughout fetal development to undergo further differentiation into subcutaneous, intermuscular, or intramuscular fat cells, aiding in the formation of skeletal muscle structure. Insults during this differentiation process may impact the fetus's postnatal ability to grow lean muscle tissue and deposit intramuscular fat, thereby decreasing quality grade and carcass value. This phenomenon

has been observed before in sheep. Dams restricted in mid gestation gave birth to lambs who matured to be fatter with a lower lean-to-fat ratio (Zhu et al., 2006). In cattle, studies have shown that steer progeny from dams restricted during late pregnancy exhibit reduced marbling and a lower percentage of those offspring grading choice at slaughter (Radunz et al., 2012; Larson et al., 2009). Another study demonstrated that calves born from nutrient restricted cows were lighter at final BW and had lighter HCW (Greenwood et al., 2004). These results contrasted with a study in which protein supplemented dams produced offspring that had higher final BWs and HCWs (Larson et al., 2009), thus significantly increasing carcass value.

Carcass value can also be affected by meat tenderness. Tenderness is measured by Warner-Bratzler shear force and is inversely correlated with muscle fiber diameter. Several studies in cattle have shown that calves born to undernourished dams have an increased muscle fiber diameter (Long et al., 2010; Long et al., 2012; Micke et al., 2010). Increased muscle fiber diameter reduces meat tenderness (Underwood et al., 2010) and devalues the carcass.

Puberty

Heifer calves of most typical beef breeds (Hereford, Angus, Charolais, Limousine, etc.) attain puberty when they reach roughly 60% of their mature BW (Laster et al., 1972; Ferrell, 1982; Martin et al., 1992). Bos indicus breeds (Brahman, Nellore, etc.) tend to reach puberty at a later age and a heavier weight, usually 65% of mature BW. Pre- and early post-natal diet is therefore a huge factor in determining age at onset of puberty and subsequent economic success of production systems.

It is widely accepted that lifetime reproductive success is heavily dependent on a female's ability to calve at 2 years of age, meaning puberty must be reached no later than 14 months of age (Lesmeister et al., 1973). This is no small feat and can prove quite a managerial

challenge, especially when producers are dealing with a large amount of bos indicus influence. Several studies have investigated the use of diet regimes as a tool to hasten the onset of reproductive function in peripubertal females (Cardoso et al., 2018). More specifically, it has been demonstrated that increasing the plane of nutrition in young and developing females can induce puberty at a younger age (Gasser et al., 2006; Cardoso et al., 2014; Allen et al., 2017). This serves as a great managerial tool and highlights the importance of nutrient intake during the early postnatal period. However, if nutrient restriction occurs prenatally, advantageous effects observed in scenarios such as just described may be lost or unrealized due to developmental programming.

As discussed earlier, gonads are susceptible to developmental alterations following maternal malnutrition during pregnancy. The ramifications of this could reach as far as delaying onset of puberty and reducing lifetime reproductive performance. Antral follicle count (AFC) in progeny born from nutrient restricted dams has been shown to be greatly reduced (Mossa et al., 2013; Rae et al., 2001). Furthermore, lambs born from nutrient restricted ewes exhibited decreased proliferative rates in primordial follicles (Grazul-Bilska et al., 2009), shortening the reproductive lifespan of those lambs. Nutrient restriction has not been the only prenatal insult to produce undesirable results in relation to progeny reproductive performance. Maternal overnutrition also affected ovarian development in female progeny, reducing the density of primordial and primary follicles, as well as delaying the onset of puberty (Sullivan et al., 2009). This study also went on to show a reduction in circulating FSH concentrations of heifers born from restricted dams (Sullivan et al., 2009).

Maternal protein restriction has also been shown to alter development of reproductive performance in beef heifers. Dams supplemented during the last third of gestation gave birth to

heifers that had increased pregnancy rates when compared to heifers born from non-supplemented dams (Martin et al., 2007). Also, fewer heifers born from non-protein supplemented cows reached puberty by the end of their first breeding season (Funston et al., 2008). It is clear from these results that offspring born from malnourished dams experience a negative impact on their ability to attain puberty and reproductively perform at a high level.

CHAPTER III

NUTRITIONAL PROGRAMMING OF PRENATAL BEEF HEIFER DEVELOPMENT

Introduction

Fetal programming is the concept that intrauterine representation of the extrauterine environment patterns prenatal development of lifelong phenotype (Hales and Barker, 2013). If the offspring's prenatal intrauterine environment is calorically scarce (i.e. gestational nutrient restriction), and it's postnatal extrauterine environment will be calorically abundant, then the offspring's metabolic parameters may be inappropriately programmed. This can have a major impact on postnatal life, negatively effecting growth and performance and thereby valuable carcass characteristics (Satterfield and Dunlap, 2014).

Nutritionally programmable genes linked to regulation of metabolism have been an area lacking investigation in cattle. In other mammalian species, it has been demonstrated that maternal nutrient restriction negatively impacts the offspring's postnatal ability to regulate blood glucose. In rats, not only has it been demonstrated that pups from nutrient restricted dams have altered structure and function of pancreatic islets (Fowden and Hill, 2001), but it has also been shown that they have altered glucose production and utilization as well (Desai et al., 1995). Ewe lambs born from sheep restricted in late gestation exhibit reduced ability to metabolize glucose and have increased adiposity (Effertz et al., 2007). In another study, lambs born from nutrient restricted ewes were insulin resistant and had reduced expression of GLUT4 in adipose tissue, but not muscle tissue, leading to increased adiposity and decreased carcass value (Gardner et al., 2005).

Despite these wide-reaching economic ramifications, prenatal alterations to metabolically linked production traits have yet to be adequately identified in ruminant livestock. It was our

objective to investigate the effects of maternal nutrient restriction during late gestation on prenatal beef heifer development. We hypothesize that a modest maternal nutritional restriction during late gestation will negatively impact the development of the fetal pancreas and offspring will exhibit reduced gain-to-feed, impaired glucose utilization, increased age at attainment of puberty, and increased adiposity at slaughter.

Methods and Materials

All experimental procedures were approved by the Institutional Agricultural Animal Care and Use Committee of Texas A&M University.

Embryos were produced *In vitro* (IVP) utilizing oocytes collected from Angus-based slaughterhouse ovaries and semen from one Angus sire, sexed for X-bearing sperm. Oocytes were sourced from DeSoto Bioscience (Seymour, TN), shipped overnight at 38.5^{o}C in sealed sterile vials containing 5% CO2 in air-equilibrated Medium 199 with Earle's salts (Invitrogen, Life Technologies Inc., Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (HYCLONE, Logan, UT, USA), 1% penicillin-streptomycin (Invitrogen), 0.2-mM sodium pyruvate, 2-mM L-glutamine (Sigma Chemical Co., St. Louis, MO, USA), and 5.0 µg/µl of Folltropin (Vetoquinol, Pullman, WA, USA). The oocytes were matured in this medium for 22 to 24 hours prior to being washed twice in warm BoviPRO™ Wash medium (MOFA Global Verona, WI) in preparation for In vitro fertilization (IVF). Matured oocytes were placed in pre-equilibrated 100 µl micro-drops of BO-IVF medium (IVF Bioscience, Cornwall, UK) under oil (Irvine Scientific, Santa Ana, CA, USA) at 38.5^{o}C, 5% CO_2 in air humidified incubator until fertilized. Frozen sexed-semen was thawed at 35^{o}C for 30 seconds, then separated by centrifugation at 200 x g for 15 minutes in a density gradient medium (ISolate®; Irvine Scientific,

Santa Ana, CA, USA) 50% upper and 90% lower. Supernatant was removed; sperm pellet was re-suspended in 1 ml of BO-IVF and centrifuged as previously described for 5 minutes. Supernatant was removed and pellet was left in approximately 30-50 µl of medium. A total of 5 µl of semen was added to the IVF drops containing matured oocytes and left to culture for 16-18 hours (IVF = Day 0). Presumptive zygotes (up to 50 per 0.65-ml microtube) were cleaned of cumulus cells by a 2-minute vortex in 75 µl of BoviPRO™ Wash, washed twice post-vortex and placed in equilibrated 500 µl of BO-IVC (IVF Bioscience) medium covered in oil (Irvine Scientific) at 38.5°C, 5% CO_2/ 5% O_2/ 90% N_2 humidified incubator for six days. On day 7 post-IVF, only grade I or II bovine embryo blastocyst or compacted morula were manually split in 75 µl splitting medium (Vetoquinol) under microscopic conditions using a micro-blade (AB Technology, Pullman, WA, USA) to generate monozygotic twins. Embryo halves (demi-embryos) were washed, loaded in Vigro holding medium in ¼ cc straws, covered with a metal-tipped sheath and chemise (PETS, Canon, TX, USA) and singularly transferred non-surgically using a Cassou gun to synchronized recipient virgin dams of Angus-based composite breed-type uniform in age, body condition and frame score (n=72). Pregnancy was confirmed by ultrasound on gestational day (GD) 60. Care was taken to ensure that halves generated from a single embryo were transferred into dams of near identical frame size and body condition. Our first approach was to identify pregnancies generated when both halves of a split embryo establish pregnancy. For these individuals (Cohort 1), one recipient was assigned to the 100% NRC (control; n=4) and the other was assigned to the 70% NRC (restricted; n=4). This approach resulted in genetically identical fetuses developing simultaneously, but separately, in recipients exposed to different dietary treatments. For all remaining pregnancies where only one of the demi-halves established pregnancy (Cohort 2), recipient dams were randomly divided into groups to receive either 100%

NRC (control; n=9) or 70% NRC (restricted; n=9). Data collected from Cohort 2 will be presented in Chapter IV. Recipient dams were maintained on high-quality forage from GD -30 to 130, at which point they were moved into individual Calan gate feeding facilities (American Calan, Northwood, NH) to receive 100% dietary requirements for an acclimation period of 28 days. Initiation of dietary treatment began on GD 158 and continued through GD 265 for Cohort 1 dams, and through calving for Cohort 2 dams. Maternal body weight (BW) was assessed every 14 days, while rib eye area (REA) and last rib back fat (LRBF) were recorded every 28 days by ultrasonography, along with collection of blood serum and plasma samples from the jugular vein. Plasma samples were centrifuged at room temperature for 7.5 minutes x 2500 g and serum samples were centrifuged at room temperature for 15 minutes x 2500 g

Tissue collection and handling following necropsy

Immediately prior to necropsy on GD 265, dam BWs were recorded, and blood samples collected from the jugular vein. Dams were then intravenously administered phenytoin/pentobarbital at 0.20 mg/kg BW (Beuthanasia-D, Merck Animal Health, Madison, NJ). Immediately following euthanasia, maternal organ weights were recorded along with gravid uterus, empty uterus, placenta, fetus, and fetal organ weights. Amniotic and allantoic fluids, umbilical artery blood, and umbilical vein blood were collected along with samples from fetal organs which were preserved in either 4% paraformaldehyde or snap frozen in liquid nitrogen for histological and molecular biology analyses. Fetal blood samples were processed in the same manner as described above.

Hormone analysis

Concentrations of insulin in fetal umbilical vein (FUV) plasma on Day 265 were assayed using a bovine insulin ELISA kit (catalog no. 10-1201-01; Mercodia AB). Concentrations of

glucose in FUV on Day 265 were determined using a colorimetric glucose assay kit (catalog no. STA-680; Cell Biolabs, Inc.). Concentrations of glucagon in FUV plasma on day 265 were assayed using a bovine glucagon ELISA kit (catalog no. MBS2882609; MyBioSource, Inc.). Concentrations of non-esterified fatty acids (NEFAs) in FUV plasma on Day 265 were determined using the NEFA-HR enzymatic colorimetric method assay (protocol no. 1057; Mouse Metabolic Phenotyping Centers) and reagents, solvents, and standard were supplied by FUJIFILM Wako Chemicals Corporation (stock nos. 999-34691; 995-34791; 991-34891; 993-35191; 276-76491). All assays were conducted according to manufacturers' instruction and results calculated using the AssayZap Version 3.1 program (Biosoft, Ferguson, CA).

Immunohistochemistry

Immunohistochemistry (IHC) was performed to identify insulin producing beta-cells and glucagon producing alpha-cells in fetal pancreas tissue, as previously reported (Dunlap et al., 2011; Satterfield et al., 2010). In brief, 4% paraformaldehyde fixed tissue was embedded in paraffin, sectioned at a thickness of 5μm, and mounted to glasses slides. These paraffin sections were dewaxed in xylene and rehydrated to phosphate-buffered saline (PBS, pH 7.2) through a graded ethanol series. Boiling citrate buffer (pH 6.0) served as antigen retrieval method and endogenous peroxidase activity was destroyed with 1% H2O2 in methanol. Sections were then incubated with diluted protein blocker supplied by Vectastain Elite ABC kit (Universal IgG (Horse serum)) (Vectastain Laboratories). Following this, sections were incubated overnight at 4°C with primary antibodies diluted in 1%BSA/PBS (pH 7.2). The following morning sections were washed and incubated with diluted Biotinylated secondary antibody for 1 hour at 37°C, followed by a 30-minute incubation with immunoreactive protein from Vectastain Elite ABC kit (Vector Laboratories). Visualization was achieved using the chromogen 3,3′-diaminobenzidine

as a peroxidase substrate. Sections were rinsed and counter-stained with hematoxylin. Mouse anti-Insulin (catalog no. SAB4200691; Sigma-Aldrich) diluted 1:3600 was used to identify beta cells, and rabbit anti-Glucagon (catalog no. ab92517; Abcam) diluted 1:5000 was used to identify alpha cells.

Immunofluorescence

Proteins were localized in frozen fetal pancreas sections (8 μm) by immunofluorescence (IF) staining as previously reported (Johnson et al., 2001; Wang et al., 2014). Frozen sections were placed into methanol at -20°C for 10 minutes, washed with 3% Tween in 0.02 M PBS, blocked in 5% normal goat serum, and incubated overnight at 4°C with diluted primary antibody. The following morning immunoreactive protein was detected by incubating sections with fluorescein-conjugated secondary antibody at room temperature for 1 hour. Prolong anti-fade containing DAPI was applied, and slides were cover slipped (Molecular Probes, Eugene, OR). To identify proliferative cells, rabbit anti-Ki67 (catalog no. PA5-19462; Thermo Fisher) was applied at a 1:1000 dilution, and endocrine beta–cells were dual–labeled via mouse anti-Insulin (catalog no. SAB4200691; Sigma-Aldrich) diluted 1:3600. This allowed us to further classify proliferative cells as either exocrine or endocrine. Von Willebrand Factor (catalog no. ab6994; Abcam) diluted 1:1000, was applied for visualization of capillary development.

Images of representative fields of immunohistochemistry and immunofluorescence were recorded using a Nikon Eclipse E1000 photomicroscope fitted with a Nikon DXM 1200 digital camera. Image J software was used to quantify the intensity of immunohistochemically stained endocrine tissue by measuring reciprocal intensity (Nguyen, 2013). For immunofluorescence, proliferative cells were counted to determine the ratio of proliferative to non-proliferative cells within endocrine and exocrine tissue.

Statistical Analysis

Data were analyzed using MIXED procedures in SAS 9.3 (SAS Inst. Inc., Cary, NC). Class variables included treatment, day, and cow. Changes in BW, maternal BW, backfat thickness, and REA were analyzed using the repeated measures technique. Model terms include treatment, day, and the treatment × day interaction. Day served as the repeated variable, with unstructured covariance and cow ID serving as the subject. For responses collected at necropsy, model terms include treatment and initial cow BW, which served as covariate. Means are reported as LSMeans ± SEM. *P*-Values less than or equal to 0.05 were considered statistically significant and less than or equal to 0.10 considered a trend towards significance.

Results

There were no differences in control (n=4) versus restricted (n=4) dam BW (432 vs 437±25 kg; P=0.83), REA (67.26 vs 66.05±3.44 cm^2; P=0.94) or LRBF (0.85 vs 0.87±0.16 cm, P=0.96) at the onset of nutrient restriction. From GD 158 to 265, control dams' BW increased, while restricted dams' BW decreased (15.44 vs -24.53±7.61 kg; P<0.01). Change in REA (-0.50 vs -4.59±3.17 cm^2; P=0.49) and LRBF (-0.06 vs -0.14±0.09 cm; P=0.46) were not different between control and restricted dams (fig. 1 – 3).

There was no difference (P>0.10) in weight of the gravid uterus, empty uterus, total placentome number, or total placentome mass between control and restricted dams. Fetal weight and sternal circumference were not different (P>0.10) between control and restricted dams. Weight of the fetal pancreas was reduced (24.41 vs 16.33±1.06 g; P<0.01) (table 1) in calves from restricted dams compared to controls, however there was no difference (P>0.10) in weights of the fetal brain, heart, lungs, liver, kidney, spleen, thymus, adrenals, stomach, intestine,

ovaries, omental fat, brown adipose tissue, or weights of the soleus, gastrocnemius, or longissimus dorsi muscles.

There was no difference ($P>0.10$) in the concentration of glucose (92.55 vs 2.61±0.77 ug/ml; $P=0.97$) in FUV plasma between control and restricted fetuses. In contrast, the concentration of insulin in fetal umbilical vein (FUV) plasma was higher in control fetuses than restricted fetuses (0.25 vs 0.16±0.02 ng/ml; $P<0.05$). Despite the reduction in insulin, there was no difference in concentrations of glucagon (0.120 vs 0.056±0.031 µg/ml; $P=0.19$) in FUV plasma between diets. Finally, there was no difference in concentrations of NEFAs (0.033 vs 0.027±0.004 mMol/L; $P=0.36$) in FUV plasma between diets (fig. 4).

No gross histoarchitectural differences of the endocrine or exocrine pancreas were observed. Immunohistochemical staining to identify alpha- and beta-cells revealed no differences between control and restricted fetuses (fig. 5). There was no difference in the number of proliferating cells in either the endocrine or exocrine fetal pancreas between maternal dietary treatments (fig. 6). Finally, there was no difference between maternal dietary treatments in vascularity of the fetal pancreas, assessed by immunostaining for von Willebrand's factor, in either perilobular giant islets or intralobular small islets of the endocrine pancreas (fig. 7).

Figure 1

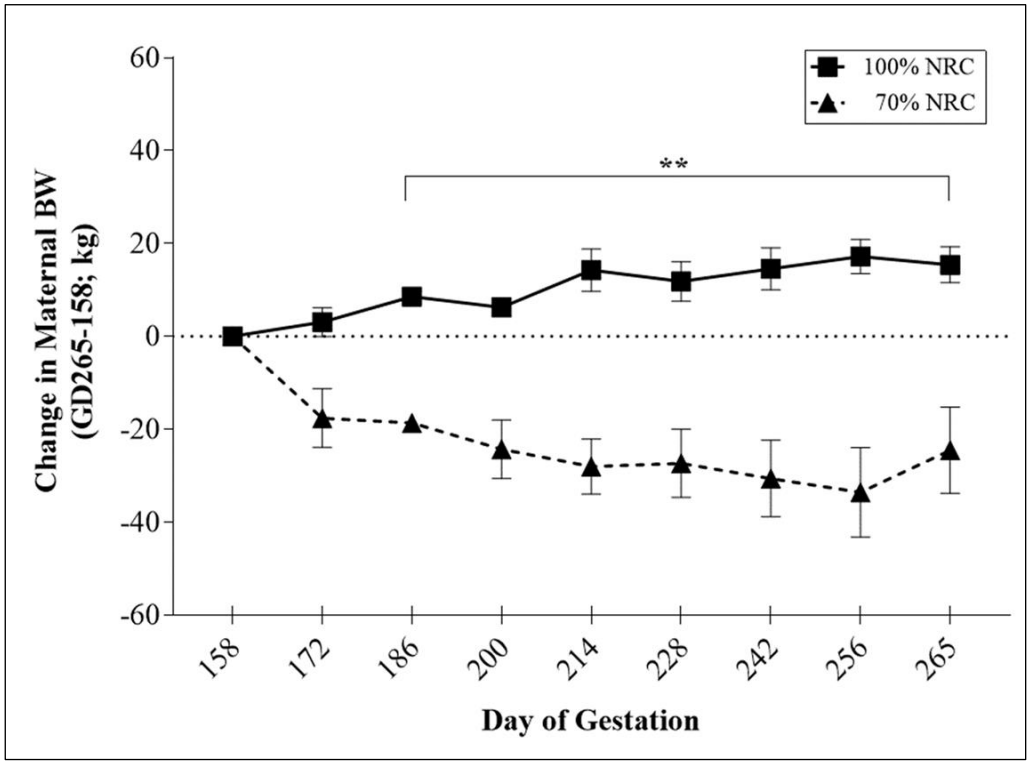

Figure 1. Effects of feeding 100%NRC or 70% NRC to pregnant beef heifers from GD 158 to 265 on change in maternal BW, kg. ** *P value < 0 .01*

Figure 2

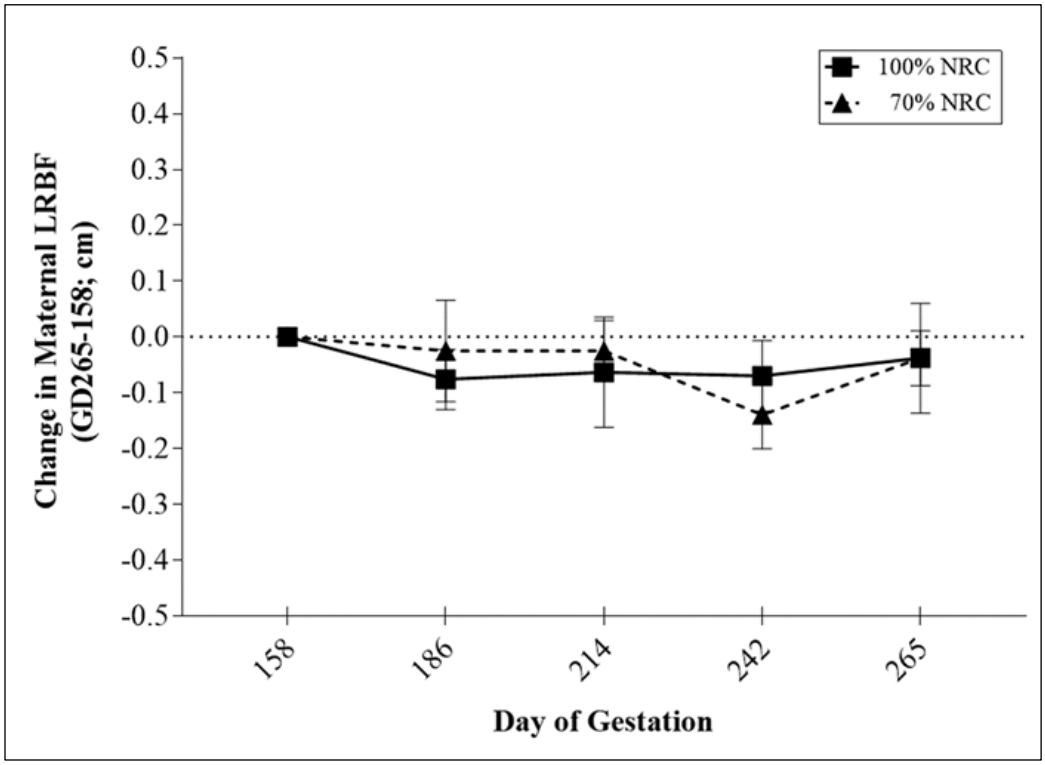

Figure 2. Effects of feeding 100%NRC or 70% NRC to pregnant beef heifers from GD 158 to 265 on change in maternal LRBF, cm.

Figure 3

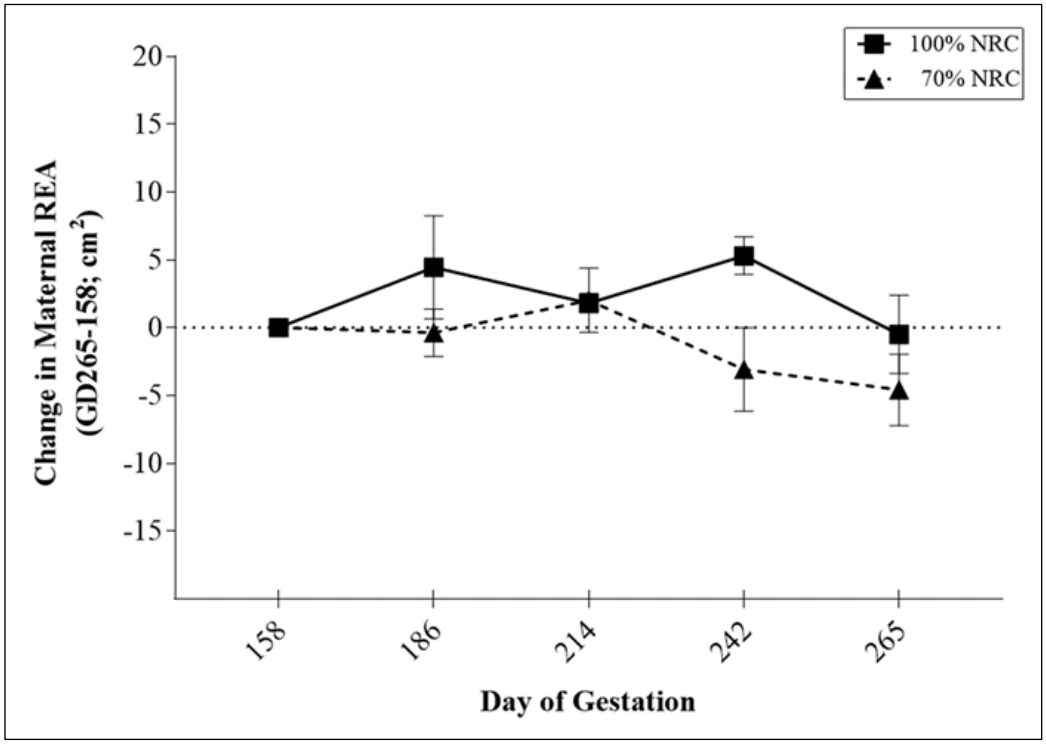

Figure 3. Effects of feeding 100%NRC or 70% NRC to pregnant beef heifers from GD 158 to 265 on change in maternal REA, cm².

Figure 4

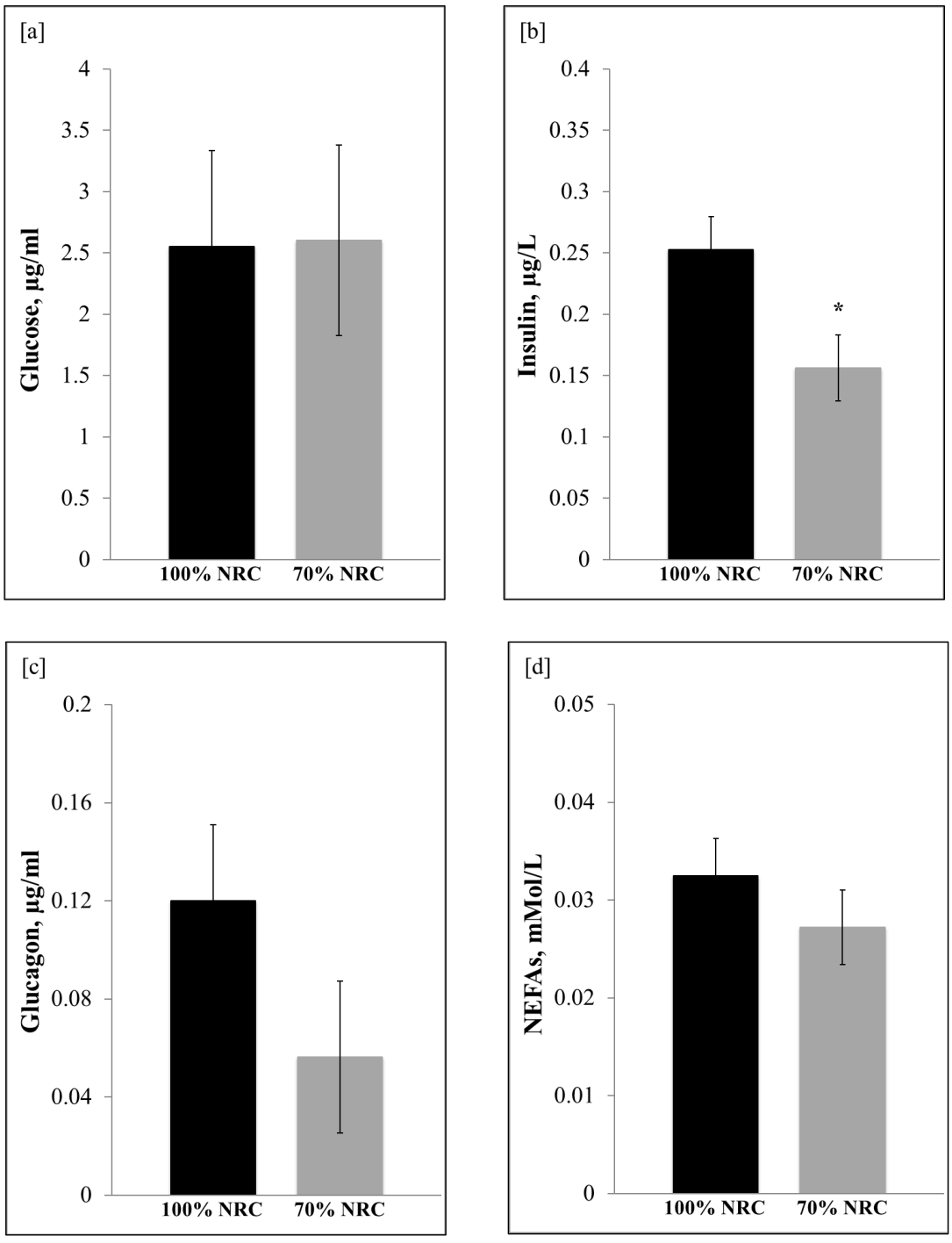

Figure 4. Effects of feeding 100%NRC or 70% NRC to pregnant beef heifers from GD 158 to 265 on concentrations of insulin **[a],** glucose **[b]** glucagon **[c]**, and non-esterified fatty acids (NEFAs) **[d]** in fetal umbilical vein (FUV) plasma collected at GD 265.

Table 1. Effects of feeding 100%NRC or 70%NRC to pregnant beef heifers from day 158 to 265 of gestation (GD) on identical twin female offspring* BW (kg), organ weight (g), sternum circumference (cm), placentome weight (kg), placentome number, as well as maternal uterine weight (kg)

	Treatment			
	Control	Restricted	SE	P value
Weight (kg)				
Gravid Uterus	59.1	61.8	5.2	0.73
Fetus	36.8	38.1	2.6	0.74
Empty Uterus	12.2	12.7	1.0	0.74
Placentome Weight	4.3	4.7	0.3	0.42
Placentome Number	100.0	91.0	9.9	0.54
Sternum Circumference (cm)	67.6	68.0	1.5	0.86
Fetal Organ Weights (g)				
Heart	237.5	253.6	23.3	0.64
Lungs	671.5	807.5	74.6	0.24
Thymus	63.9	74.3	9.0	0.45
Brain	202.5	197.5	3.7	0.37
Liver	700.5	711.0	67.6	0.92
Spleen	117.9	107.2	14.0	0.61
Pancreas	24.4	16.3	1.1	< 0.01
Kidneys	138.8	135.2	17.1	0.89
Adrenals	2.37	2.31	0.22	0.89
Ovaries	1.81	1.17	0.66	0.53
Intestine	717.5	787.0	47.7	0.34
Stomach	393.5	374.0	19.7	0.51
Brown Adipose	108.0	109.7	6.7	0.86
Omental Adipose	30.4	33.2	2.3	0.42
Gastrocnemius	128.0	131.4	10.8	0.83
Soleus	42.1	47.7	7.0	0.58
Longissimus Dorsi	274.5	292.0	28.6	0.68

Figure 5

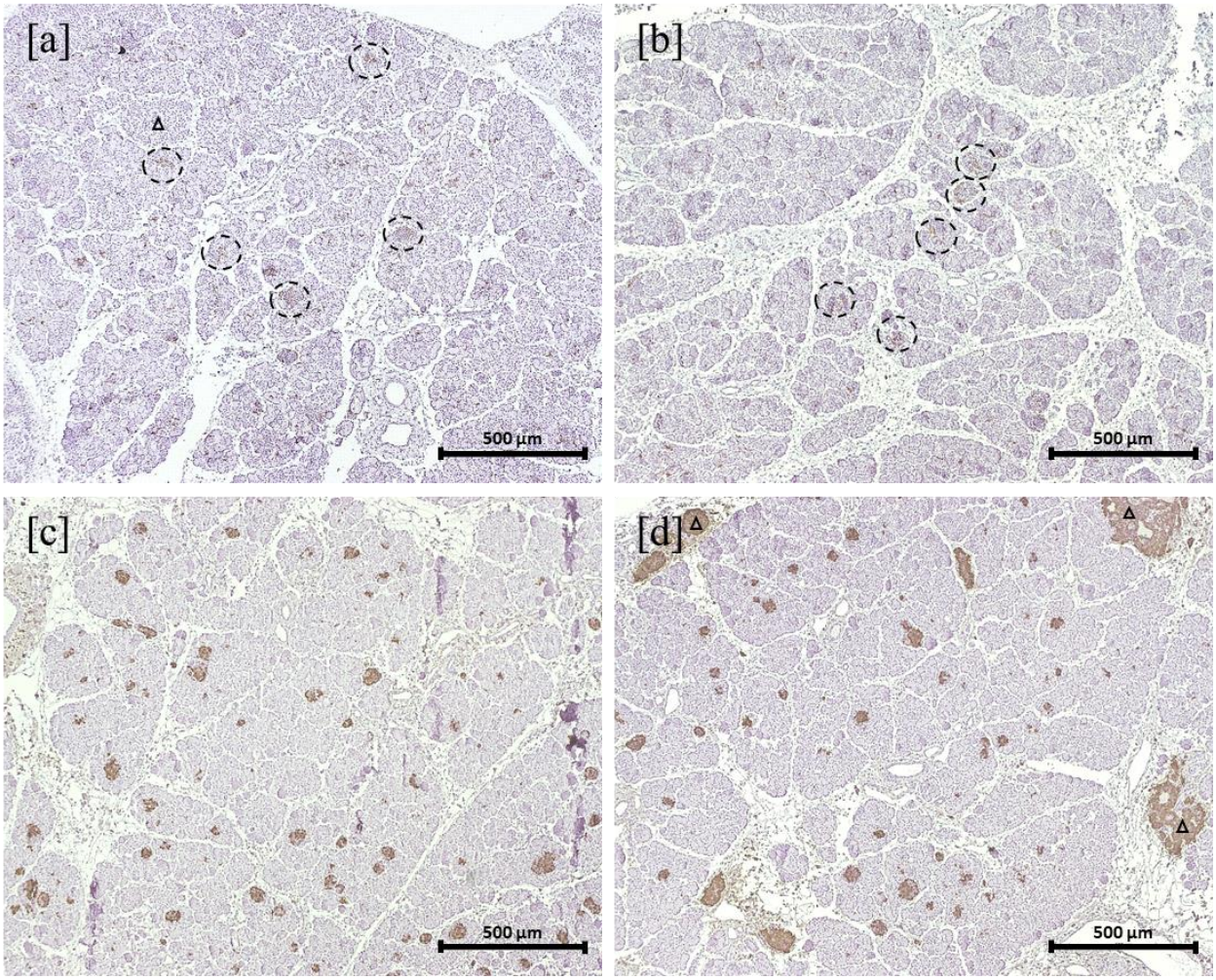

Figure 5. Immunohistochemical staining of paraffin embedded fetal pancreases harvested at GD 265 visualized here at 4x magnification. Discrete areas of brown stain can be seen in the dash-circles and represent glucagon producing alpha cells [a - b]. Insulin producing beta cells are also represented by the brown stain and are more easily visualized [c - d]. Hematoxylin was used as counterstain following exposure to DAB. Immunohistochemical staining revealed no differences in quantity or distribution of alpha cells between control [a] and restricted [b] fetuses or beta cells between control [d] or restricted [e] fetuses. Perilobular giant islets are represented by triangles.

Figure 6

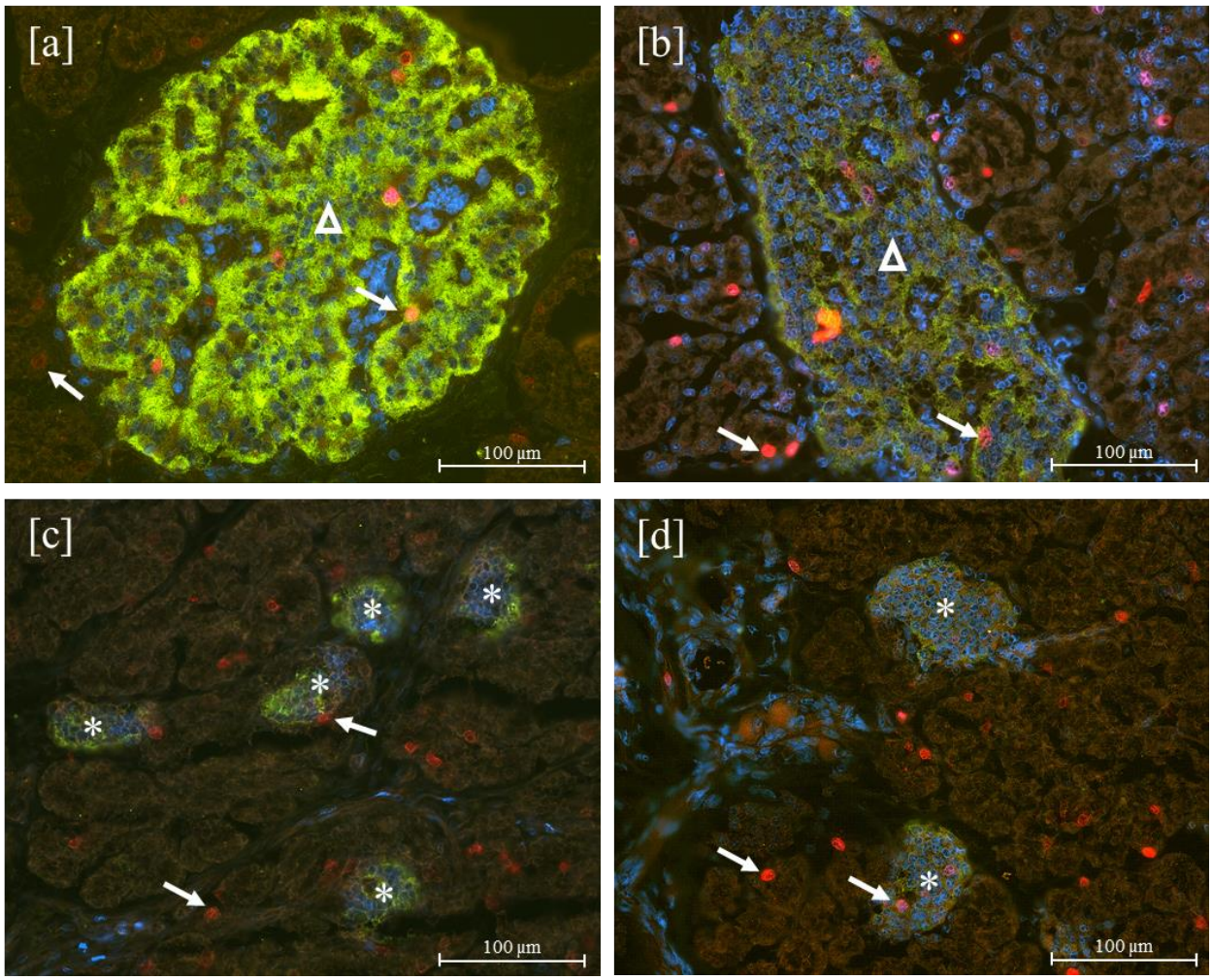

Figure 6. OCT embedded fetal pancreas tissue collected at GD 265 were subject to immunofluorescence dual labeling of insulin producing beta cells (green fluorescence) and cells undergoing proliferation (red fluorescence; arrows) with DAPi (blue fluorescence) providing background staining. The endocrine pancreas was further subdivided in this figure by islet size. Perilobular giant islets (triangle) [a – b] and intralobular small islets (asterisk) [c – d]. No differences were observed cell proliferation in the exocrine pancreas. No differences were observed in large or small islets between control [a & c] or restricted [b & d] fetuses. Images are 20x magnification.

Figure 7

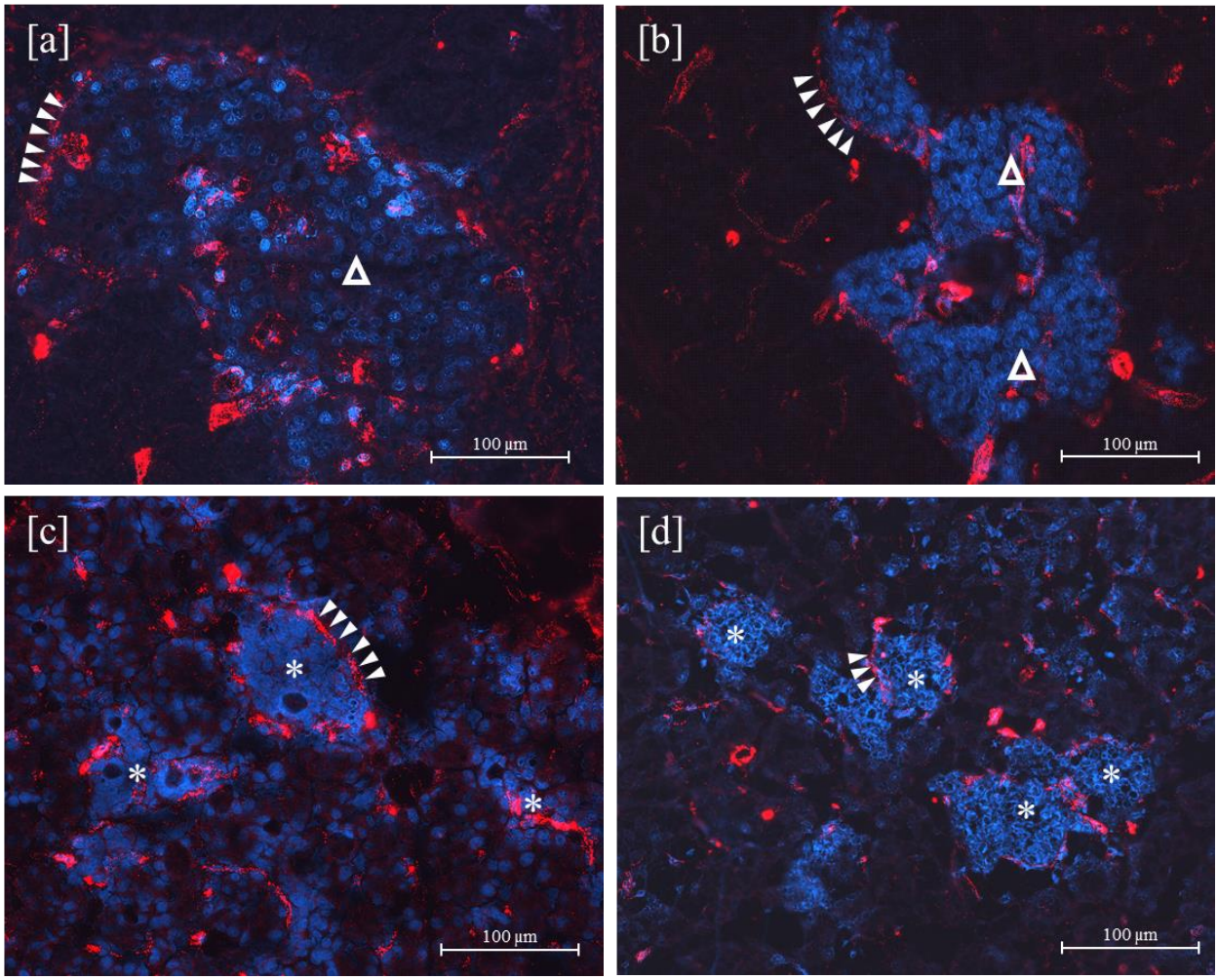

Figure 7. OCT embedded fetal pancreas tissue collected at GD 265 were subject to immunofluorescence labeling of capillary endothelial cells (red fluorescence) with DAPi (blue fluorescence) providing background staining. The endocrine pancreas was further subdivided in this figure by islet size. Perilobular giant islets (triangle) [a – b] and intralobular small islets (asterisk) [c – d]. No differences were observed in vascularity of the exocrine pancreas. No differences were observed in vascularity of large or small islets (arrowheads) between control [a & c] or restricted [b & d] fetuses. Images are 20x magnification.

Discussion

Maternal nutritional intake during pregnancy is a critical determinant of fetal growth and has more recently been shown to have lifelong impacts on health and performance efficiency of the offspring. In any production enterprise, input costs such as feed, must be balanced with output values, such as calf BW at market or carcass value. To maximize profitability and sustainability of the enterprise we must fully understand maternal and fetal dietary needs, critical windows of development, and physiological consequences of varying nutritional intake levels on postnatal performance. Results of the present study indicate that a modest maternal nutritional restriction during the last half of gestation results in a reduction of the weight of the fetal pancreas on GD 265. This reduction in pancreas mass coincided with a reduction in circulating insulin levels in the fetus, but no difference in gross histology of the pancreas including size or number of insulin producing islets.

Birth weight is a frequently used proxy measurement for assessing the quality of the uterine environment during pregnancy. Nonetheless, studies show a varied impact of maternal nutritional restriction on birth weight, presumably due to differences in level and duration of the restriction and the timing during gestation when the insult is applied. As example, Long et al. (2009 & 2010) found no effect of maternal nutritional restriction in early gestation on fetal weight at day 245 or birth, respectively. These studies are in contrast to a study by LeMaster et al., (2017) in which birth weights were reduced when pregnant dams were nutritionally restricted for the last 100 days of gestation. Results of the present study found no difference in fetal weights at day 265 of pregnancy between restricted and control fed dams. This is likely due to the modest level of nutritional restriction which can be evidenced by no loss of maternal LRBF, REA, or circulating leptin concentrations at the time of necropsy. Results did indicate a reduction

in total body weight of the dam which may be reflected as a reduction in gastrointestinal fill, abdominal adipose tissue mass, and/or visceral organ mass.

A lack of difference in fetal weight is also supported by a lack of difference in weight or number of placentomes. Unlike the sheep whose placental mass reaches its maximum by day 90 of gestation, placentome mass in cattle continues to increase throughout gestation (Laven and Peters, 2001). It is well established that placental weight, placental blood flow, and fetal weight are highly correlate. Indeed, factors known to alter fetal weight such as genotype, maternal nutritional intake, heat stress, altitude, and fecundity have been shown to have parallel effects on placental weight and uteroplacental blood flow (Reynold et al., 2006).

Several studies have found that maternal nutritional restriction from mid to late gestation reduce weaning and/or hot carcass weight of calves (Larson et al., 2009, Funston et al., 2012, Underwood et al., 2010). Despite these observations, to date, the effect of maternal nutritional restriction on metabolic parameters of the growing offspring, such as glucose clearance and insulin sensitivity have not been described in cattle. In the sheep, maternal nutrient restriction during late gestation resulted in the development of insulin resistance and increased adiposity at one year of age compared to lambs born from control fed ewes or ewes nutrient restricted during early gestation, although lamb body weights were similar (Gardner et al., 2005). In the present study, we observed a decrease in the weight of the fetal pancreas and a reduction in circulating insulin concentrations. Importantly, insulin does not cross the bovine placenta and thus a reduction in insulin within the fetal circulation is due to an alteration in fetal synthesis or clearance, not placental transport. Immunohistochemical analyses of pancreatic tissues indicate that the size and number of beta cell containing islets of Langerhans did not differ between dietary treatments, suggesting that the reduction in circulating insulin is simply due to the

development of a smaller organ rather than an altered differentiated state. We also did not identify differences in the number of proliferating cells either within the endocrine or exocrine pancreas. The ontogeny of pancreatic development has been insufficiently studied, to date. While functional endocrine cells are observed very early in the developing fetus (Merkwitz et al., 2013), specific periods of large-scale pancreatic cell proliferation have not been adequately described. That our tissue is smaller in size yet appears functionally normal suggests that a period of proliferation is occurring between day 158 and 265 of gestation, although the Ki-67 immunofluorescence would indicate that proliferation rates are similarly low in both well fed and restricted fetuses as they near term.

Despite the reduction in circulating insulin, we observed no difference in circulating glucagon, which is produced by alpha cells of the endocrine pancreas. Alpha cells make up only one-fifth of the cells in the endocrine pancreas. It should be noted that a moderately high degree of variation in circulating glucagon levels was observed which may have contributed to the lack of detected differences in glucagon levels despite a smaller pancreatic mass. To that end, we did not observe an increase in the number and/or intensity of staining of glucagon producing alpha cells within the pancreatic tissue. Interestingly, we previously found that late gestation maternal arginine supplementation to nutrient restricted pregnant ewes increased the weight of the fetal pancreas at necropsy at day 125 of gestation (Satterfield et al., 2013). Thus, prescriptive maternal arginine supplementation to the late gestation beef cow may support normal pancreatic development while allowing producers to reduce total dietary intake as a means to reduce production costs.

In conclusion, results of the present study indicate that a modest late gestation nutritional restriction to the pregnant beef cow impairs development of the fetal pancreas. The decrease in

pancreatic mass was associated with a reduction in circulating insulin levels. These developmental changes may lead to altered glucose and insulin hemostasis in the adult and contribute to adult onset of metabolic syndrome. Further, these results provide a potential mechanism for observed reductions in postnatal growth rates in other studies. Future studies are needed to further characterize the effect of our late gestation nutritional model on postnatal growth and reproductive and feedlot performance.

CHAPTER IV

POSTNATAL PERFORMANCE OF BEEF HEIFERS SUBJECTED TO PRENATAL NUTRIENT RESTRICTION

Introduction

Worldwide demand for protein continues to grow as land and other resources become increasingly scarce. These accelerating trends have turned producers' attention to implementing confinement, or semi-confinement feeding in an attempt to: increase production per unit area of land and improve animal nutrient utilization by reducing feed intake (Trubenbach et al., 2013). Improving a pregnant cow's feed efficiency has the potential to produce a myriad of benefits. However, in many instances this attempt results in gestational maternal nutrient restriction and may offset the present benefits of reduced cow maintenance costs with future reductions in offspring performance.

Offspring phenotype is the product of inherited genes and the environment in which those genes are developed. Fetal programming is the concept that expression or function of inherited genes is altered by external stimulus during pre- and perinatal development (Barker, 1997). This phenomenon has been observed in livestock species. Maternal nutrient restriction during gestation has been shown to decrease postnatal growth rates in sheep progeny (Quigley et al., 2005; Zhu et al., 2006), pig progeny (Dwyer et al. 1994), and cattle progeny (Funston et al., 2010; Underwood et al., 2010). Furthermore, economically important carcass traits in cattle have been shown to be negatively impacted. Reductions in hot carcass weight (HCW), yield grade, quality grade, and meat tenderness have been observed in numerous cattle studies (Underwood et al., 2010; Sullivan et al., 2010; Long et al., 2012; Larson et al., 2009). Many of these studies have observed steer progeny performance in a feedlot setting (Underwood et al., 2010; Long et

al., 2010) and studies have also observed heifer progeny reproductive performance (Martin et al., 1992; Martin et al., 2007; Summers et al., 2015).

The objective of this study was to investigate postnatal growth efficiency, attainment of puberty, and carcass characteristics in heifer progeny born to nutrient restricted dams. We hypothesize that heifer calves born from dams nutrient restricted during late gestation will exhibit compensatory growth rates, reduced gain to feed ratios, increased carcass adiposity, decreased tenderness, and delayed onset of puberty.

Methods and Materials

All experimental procedures were approved by the Institutional Agricultural Animal Care and Use Committee of Texas A&M University.

Cohort 2 dams were described in the previous chapter. To reiterate, these dams were assigned to receive either 70% NRC or 100% NRC from GD 158 through parturition. Maternal body weight (BW) was assessed every 14 days, while rib eye area (REA) and last rib back fat (LRBF) was recorded every 28 days by ultrasonography along with collection of blood serum and plasma samples from the jugular vein. Plasma samples were centrifuged at room temperature for 7.5 minutes x 2500 g and serum samples were centrifuged at room temperature for 15 minutes x 2500 g.

Fetuses from Cohort 2 dams were all female half-siblings to the identical twin fetuses carried by Cohort 1 dams. At birth, Cohort 2 calves (CON=9; RES=9) were weighed, and a venous blood sample collected prior to suckling. Lactating cows received 100% NRC requirements for the duration of lactation. Calf weights and blood were collected at 35-day intervals until slaughter at postnatal day (PND) 485. Two weeks following weaning (PND 210),

calves were placed into a grower facility to undergo an acclimation period prior to the initiation of a feed efficiency trial.

Glucose tolerance test

At PNDs 301 and 482 heifers were subjected to an intravenous glucose tolerance test (IVGTT) performed as previously reported (Vasconcelos et al., 2009). Heifers were fitted with an indwelling jugular cannula and samples were collected prior to dextrose infusion to provide initial insulin and glucose values. Dextrose was infused (50%; wt/vol) at 0.5 mL/kg BW (0.25 g/kg BW) immediately following baseline sample. After completion of dextrose infusion, blood samples were collected at 2.5, 5, 7.5, 10, 15, 30, 60, 90, and 120 minutes, in 10-mL tubes containing sodium-heparin (Monoject, Sherwood Medical, St. Louis, MO), placed on ice for 30 minutes, and centrifuged at room temperature for 7.5 minutes x 2500 g. Plasma was aliquoted and stored at -20 C for insulin and glucose analyses.

Postnatal feed trial

Beginning at PND 315, feed was provided ad libitum and daily intake was monitored to determine average daily gain and gain to feed ratio. Body weights were recorded weekly and blood was collected at 35-day intervals throughout the trial. Calf REA and LRBF were determined via ultrasound at weaning (PND 210), initiation of feed trial (PND 315), and prior to slaughter (PND 420). As heifers neared the peri-pubertal period, bleeding frequency was increased to twice weekly to allow for determination of approximate age at onset of puberty, as determined by a rise in circulating progesterone concentrations (3 consecutive samples equaling or exceeding 1 ng/ml) (Cardoso et al., 2014). Once heifers reached an appropriate slaughter weight (~535 kilograms), a second glucose tolerance test was conducted 3 days prior to slaughter. Two heifers were humanely euthanized prior to end of study due to injuries incurred

during study, one heifer from control pregnancy (reducing n to 8)and one heifer from restricted pregnancy (reducing n to 8).

Tissue collection and handling following necropsy

Necropsy took place on PND 485, procedures followed those previously described in Cohort 1 collections. Heifer body weights were recorded, and blood samples collected from the jugular vein. Heifers were then intravenously administered phenytoin/pentobarbital at 0.20 mg/kg BW (Beuthanasia-D, Merck Animal Health, Madison, NJ). Immediately following euthanasia, organ weights were recorded and samples from each organ were preserved in either 4% paraformaldehyde or snap frozen in liquid nitrogen for future histological and molecular biology analyses. Also, at the time of slaughter, we determined rib eye area, 12th rib fat thickness, KPH, marbling score, and lean color score. We evaluated a sample of the longissimus dorsi muscle for fiber diameter and performed a Warner-Bratzler shear force test as previously reported (Destefanis et al., 2008).

Hormone analysis

Concentrations of insulin and glucose in calf plasma on PND 0 were assayed in duplicates using a bovine insulin ELISA kit (catalog no. 10-1201-01; Mercodia AB). Glucose concentrations in calf plasma from all GTT timepoints were determined using a colorimetric glucose assay kit (catalog no. STA-680; Cell Biolabs, Inc.). Progesterone concentrations in heifer serum were assayed using a commercial RIA kit (catalog no. 07-270102; ImmuChem, MP Biomedicals).

Statistical Analysis

Data was analyzed using MIXED procedures in SAS 9.3 (SAS Inst. Inc., Cary, NC). Class variables included treatment, day, and heifer. Changes in BW, maternal BW, backfat thickness, and REA were analyzed using the repeated measures technique. Model terms include treatment, day, and the treatment × day interaction. Day served as the repeated variable, with unstructured covariance and heifer ID serving as the subject. For responses collected at necropsy, model terms include treatment. Means are reported as LSMeans ± SEM. *P*-Values less than or equal to 0.05 were considered statistically significant and less than or equal to 0.10 considered a trend towards significance.

Results

A reduction in maternal BW became evident (P<0.01) by GD 186 and continued to expand to GD 270 in nutrient restricted dams compared to control fed dams (fig. 8). In contrast to Cohort 1 dams, a reduction in REA (P<0.01) was observed in restricted dams by GD 270 compared to control fed dams (fig. 10). There was no difference (P>0.10) in maternal LRBF throughout the nutritional restriction period between diets (fig. 9). At birth, calves born to control fed dams were heavier (39.09 vs 32.39±2.97 kg; P<0.05), than calves born to nutrient restricted dams. This trend in calf weight continued to both PND 35 (64.91 vs 53.22±3.57 kg; P<0.01) and PND 70 (105.94 vs 90.62±7.31 kg; P=0.05). There was no difference (P<0.10) in calf BW at PNDs 105, 140, 175, 210, 245, 315, 350, 385, 420, or 455 (table 2).

Calf REA from control and restricted dams was not different at PND 210 (8.16 vs 7.73±0.50 cm^2; P=0.45), PND 315 (10.17 vs 9.90±0.50 cm^2; P=0.70), or PND 420 (13.78 vs 12.38±0.50 cm^2; P=0.70). Similarly, calf LRBF did not differ at PND 210 (2.89 vs 2.77±0.20

cm; P=0.39), PND 315 (2.90 vs 2.90±0.06 cm; P=0.96), or PND 420 (3.30 vs 3.36±0.06 cm; P=0.48) between calves from control and restricted dams (table 3).

There were no differences (P>0.10) in feed intake, total weight gain, or gain to feed ratio between calves from control and restricted dams in response to ad libitum feeding from PND 315 to PND 485 (table 4). Intravenous glucose tolerance tests (IVGTT) conducted at PNDs 301 and 482 found that calves born to restricted dams exhibited a greater area under the curve compared to heifer calves born to control fed dams. Maximum concentration of plasma glucose (Cmax; µg/ml) in response to infusion was not different amongst treatments, nor was time to maximum concentration of plasma glucose (Tmax; minutes) in response to infusion (table 5).

There was no difference (P>0.10) in the age and/or weight at which heifers attained puberty between maternal dietary treatments (fig. 11).

At slaughter (PND 485) heifer BW did not differ between maternal dietary treatments. Weight of the pituitary gland was larger in heifers born to control fed dams compared to heifers born to restricted dams (2.45 vs 2.09 ± 0.12 g; P=0.04). Further, heifers born to control fed dams possessed less internal fat deposition (19.02 vs 23.22±1.28 kg; P=0.04) when compared to their restricted counterparts. There was no difference (P>0.10) in weights of the brain, heart, lungs, liver, spleen, kidneys, pancreas, adrenal glands, ovary, uterus, rumen, small intestine, or gastrocnemius muscle at slaughter between maternal dietary treatments (table 6).

Parameters indicative of carcass quality and cutability such as quality grade, REA, 12th rib fat thickness, and Warner-Bratzler shear force of the longissimus dorsi muscle did not differ (P>0.10) between treatments (tables 7 – 8).

Figure 8

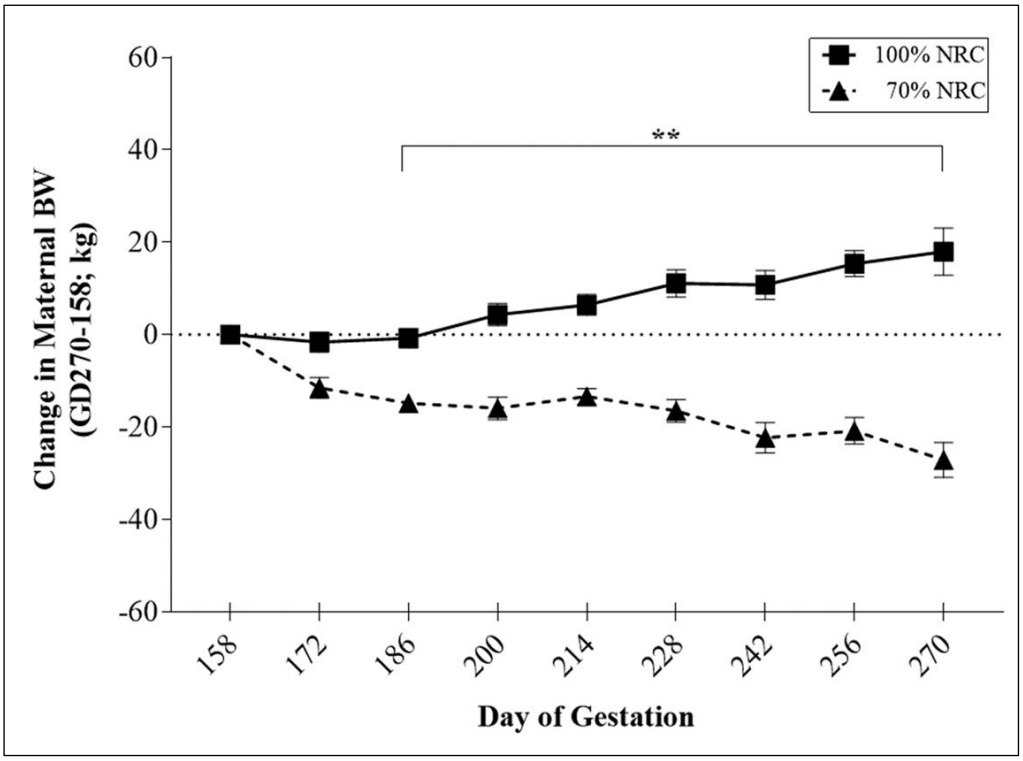

Figure 8. Effects of feeding 100%NRC or 70% NRC to pregnant beef heifers from GD 158 to 270 on change in maternal BW, kg. ** *P value < 0 .01*

Figure 9

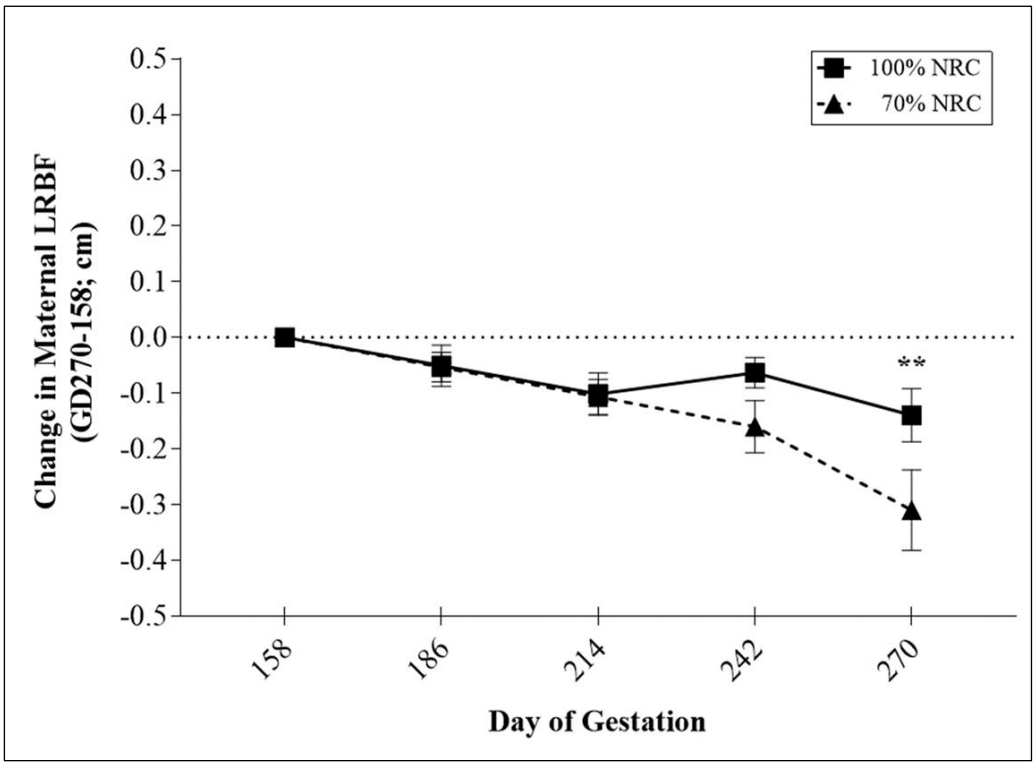

Figure 9. Effects of feeding 100%NRC or 70% NRC to pregnant beef heifers from GD 158 to 270 on change in maternal LRBF, cm. ** *P value < 0 .01*

Figure 10

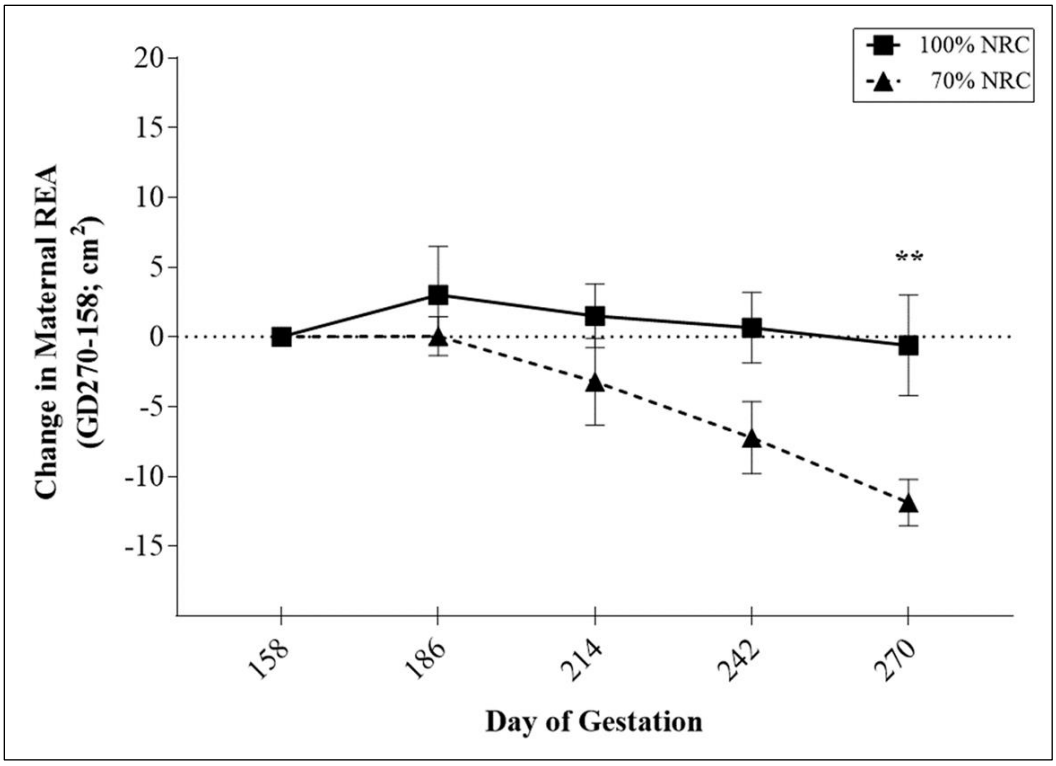

Figure 10. Effects of feeding 100%NRC or 70% NRC to pregnant beef heifers from GD 158 to 270 on change in maternal REA, cm². ** *P value < 0 .01*

Figure 11

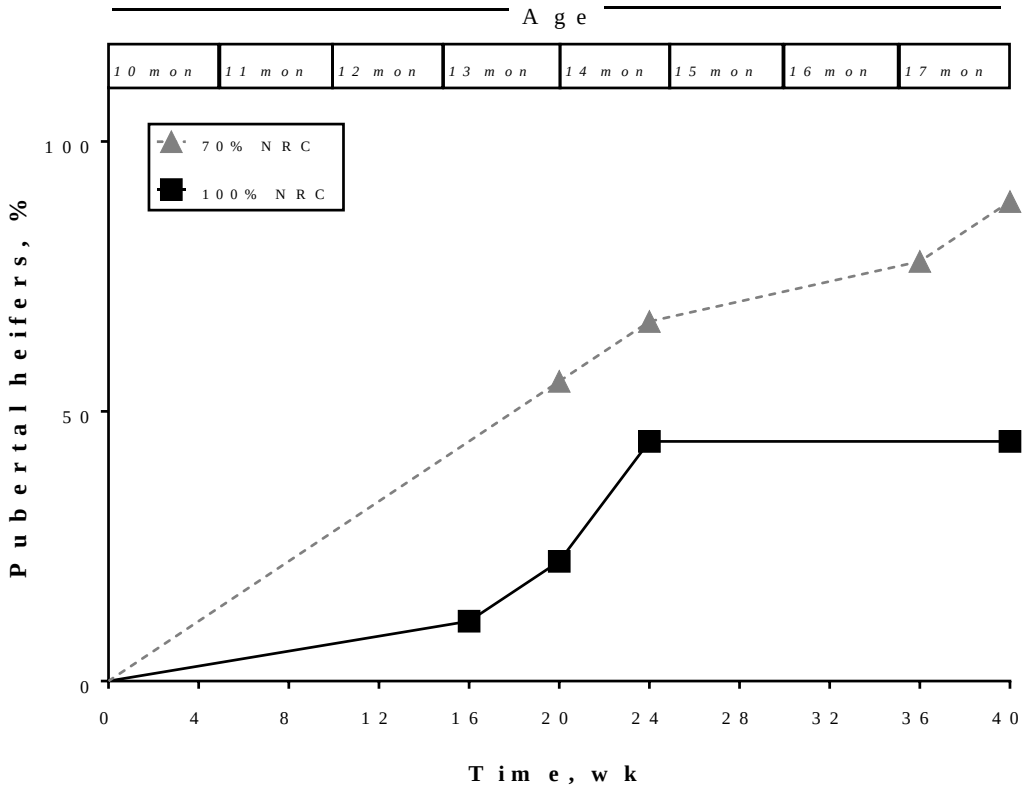

Figure 11. Effects of feeding 100%NRC or 70% NRC to pregnant beef heifers from GD 158 to parturition on offspring's age at attainment of puberty. No significant differences were observed. Time in weeks is reported as number of weeks post weaning.

Table 2. Effects of maternal nutritional restriction from GD158 to parturition on offspring BW (kg) from PND 0 to slaughter (PND 485)

	Treatment			
	Control	Restricted	SE	P value
PND 0	39.09	32.39	2.10	0.04
PND 35 ± 3	64.91	53.22	2.52	<0.01
PND 70 ± 3	105.94	90.62	5.17	0.05
PND 105 ± 3	151.70	136.78	6.38	0.12
PND 140 ± 3	195.75	184.16	7.68	0.30
PND 175 ± 3	239.50	222.36	8.36	0.17
PND 210 ± 3	269.43	252.80	10.04	0.26
PND 245 ± 3	283.34	268.43	11.22	0.36
PND 280 ± 3	324.89	311.44	11.79	0.43
PND 315 ± 3	347.37	337.65	13.03	0.61
PND 350 ± 3	403.89	389.00	16.33	0.53
PND 385 ± 3	454.00	431.00	17.52	0.37
PND 420 ± 3	475.56	477.33	15.35	0.94
PND 455 ± 3	522.00	504.56	16.96	0.48
PND 485 ± 3	532.38	516.00	18.25	0.54

Table 3. Effects of maternal nutritional restriction from GD158 to parturition on REA and LRBF of offspring fed ad libitum from PND 315 to slaughter (PND 485)

	Treatment			
	Control	Restricted	SE	P value
REA (cm^2)				
PND 210	8.16	7.73	0.40	0.45
PND 315	10.17	9.90	0.50	0.70
PND 420	13.78	12.38	0.54	0.08
LRBF (cm)				
PND 210	2.89	2.77	0.10	0.39
PND 315	2.90	2.90	0.06	0.96
PND 420	3.30	3.36	0.06	0.48

Table 4. Effects of maternal nutritional restriction from GD158 to parturition on feed intake and feed efficiency of offspring fed ad libitum from PND 315 to slaughter (PND 485)

	Treatment			
	Control	Restricted	SE	P value
Initial BW (kg)	347.37	337.65	13.03	0.61
Final BW (kg)	537.33	525.11	16.38	0.61
Total BW gain (kg)	190.00	187.44	11.61	0.88
Overall ADG (kg/d)	1.16	1.13	0.07	0.81
Total Intake (kg)	1588.17	1476.86	72.01	0.29
Intake (kg/d)	9.45	8.79	0.43	0.29
Intake (% mean BW)	2.133%	2.038%	0.0007	0.32
Gain to Feed, total	0.1199	0.1272	0.0060	0.40

Table 5. Effects of maternal nutritional restriction from GD158 to parturition on glucose tolerance of offspring at PND 315, initiation of ad libitum feed trial, and at PND 482, end of ad libitum feed trial.

		Treatment			P value		
	Age	Control	Restricted	SEM	Trt	Age	Trt*Age
Glucose AUC	301	1606906	1620842	44391.00	< 0.05	0.058	0.14
	482	1458555	1602036				
Cmax (ug/ml)	301	22844	19899	2219.45	0.68	0.18	0.33
	482	17686	18996				
Tmax (minutes)	301	3.25	4.06	0.46	0.28	0.42	0.42
	482	3.25	3.35				

Table 6. Effects of maternal nutritional restriction from GD158 to parturition on organ weights of offspring at slaughter (PND 485)

	Treatment			
	Control	Restricted	SE	P value
BW (kg)	532.38	516.00	18.25	0.54
Organ Weights				
Brain (g)	343.92	357.17	11.62	0.43
Whole Pituitary (g)	2.45	2.09	0.12	0.04
Heart (kg)	1.98	1.85	0.08	0.25
Lungs (kg)	2.82	2.66	0.14	0.45
Liver (kg)	7.65	8.00	0.42	0.56
Pancreas (g)	261.02	281.68	23.95	0.55
Spleen (kg)	1.73	1.77	0.14	0.84
Kidneys (kg)	1.25	1.20	0.09	0.67
Adrenals (g)	24.74	23.31	1.88	0.59
Ovaries (g)	15.79	19.19	2.06	0.26
Uterus (g)	138.86	143.96	13.04	0.79
Small Intestine (kg)	8.02	8.49	0.56	0.56
Rumen (kg)	20.36	19.33	0.89	0.41
Ttl. Internal Fat (kg)	19.02	23.22	1.28	0.04
Gastrocnemius Muscle (kg)	1.64	1.54	0.07	0.29

Table 7. Effects of maternal nutritional restriction from GD158 to parturition on carcass quality of offspring fed ad libitum from PND 315 to slaughter (PND 485)

	Treatment			
	Control	Restricted	SE	P value
Fat Thickness (cm)	0.75	0.71	0.08	0.75
REA (cm^2)	13.80	12.66	0.77	0.32
Numerical Marbling	447.50	435.00	29.90	0.77
QG	408.33	400.00	14.22	0.68

Table 8. Effects of maternal nutritional restriction from GD158 to parturition on carcass shear and cook yield of offspring fed ad libitum from PND 315 to slaughter (PND 485)

	Treatment			
	Control	Restricted	SE	P value
Time Off	0.40	0.41	0.02	0.91
Cook Yield	82.45	82.89	1.62	0.85
Warner-Bratzler shear force (kg)	3.38	2.78	0.34	0.23

Discussion

A growing body of evidence highlights the link between maternal nutritional and environmental exposures during pregnancy and associated alterations in the postnatal phenotypes of the offspring. More specifically, it has been suggested in mammalian species, that in response to maternal malnutrition, the fetus will alter its metabolism to preferentially store consumed calories as fat for future utilization during periods of hardship rather than for the deposition of lean tissue mass (Symonds et al., 2004; Gardner et al., 2005; Greenwood et al., 1998). Despite these observations, limited data exists in beef cattle regarding the effects of a modest late gestation nutritional restriction on postnatal growth and performance. Results of the present study highlight a reduction in birth and postnatal weights to PND 105 at which time offspring from restricted dams exhibit compensatory growth and become equivalent in weight to calves from control fed dams. There were no observed differences in age at attainment of puberty, glucose utilization, or feed efficiency. Consistent with observations from other species, we observed an increase in abdominal fat at PND 485 in calves born to restricted dams. We also observed a decrease in the weight of the whole pituitary gland in calves born to restricted dams.

Phenotype of the offspring in response to an environmental insult, such as maternal nutritional restriction, is dependent upon a variety of factors including timing of the insult, duration of the insult, severity or dosage of the insult, sex of offspring, and more. Similar to observations from Cohort 1, nutritionally restricted pregnant dams in Cohort 2 exhibited a reduction in body weight. However, unlike dams from Cohort 1, dams from Cohort 2 also exhibited a decrease in LRBF and REA beginning at GD 270 suggesting that as the duration of nutritional restriction extends further into the last third of gestation the dam is forced to catabolize her adipose and skeletal muscle stores to support the increasing demands of the

exponentially growing fetus. Indeed, at this stage of gestation the fetus can grow up to 250 g/d (Lemley et al., 2014).

In the present study, birth weight of calves born to restricted dams was reduced 6.7 kg compared to controls, unlike calves in Cohort 1 necropsied at GD 265, which had similar fetal weights between control fed and restricted dams. It is likely that the additional ~18 days of nutritionally restricted gestation contributed to the observed reduction in fetal weight. LeMaster et al., (2017) observed a similar 3.8 kg reduction in calf birth weight in response to a similarly modest nutritional restriction over the course of the final 100 days of gestation.

Following parturition, nutrient restricted calves in the present study exhibited rapid compensatory growth such that by PND 105 and continuing until slaughter on PND 485 weights were similar between calves born from control fed and restricted dams. While the ability of calves to exhibit compensatory growth is well established (Greenwood and Cafe, 2007) several studies have observed differences in body weights of calves born to restricted dams to weaning (~7 months of age) (Larson et al., 2009; Funston et al., 2012) and even at the age at slaughter (Larson et al., 2009; Underwood et al., 2010).

We observed no difference in post-weaning feed intake or gain to feed ratio between groups. However, areas under the curve for glucose following IVGTT on PNDs 301 and 482 were greater in heifers born from nutrient restricted dams. This is a novel and important finding as, to our knowledge, cattle studies have yet to investigate adult glucose metabolism of progeny born from dams nutrient restricted in late gestation. It is important to note that our findings may be a key underlying mechanism that could in part explain observations made in previous studies of reduced carcass yield and altered postnatal growth rates following late-gestation maternal nutrient restriction (Larson et al., 2009; Underwood et al., 2010; Funston et al., 2012; LeMaster

et al., 2017). It remains unclear whether this reduced ability to clear peripheral glucose is the result of reduced insulin production or sensitivity. In either case, it is a major alteration in energetic efficiency that warrants further study. Similar results have been reported in retrospective human studies. Women who experienced the Dutch Famine late in pregnancy gave birth to children who exhibited an inability to properly regulate blood glucose as adults (Ravelli et al., 1998). Studies in sheep have also indicated that late-gestation nutrient restriction alters offspring's postnatal glucose metabolism (Gardner et al., 2005; Dellschaft 2015). Glucose intolerance is one of several risk factors related to the adult onset of type II diabetes. These suboptimal metabolic parameters threaten the postnatal growth and performance of beef progeny. Our findings provide further insight into the underlying metabolic mechanisms controlling economically important production traits.

Despite not observing differences in whole body weight at the time of slaughter, calves born to restricted dams exhibited an increased accumulation of internal fat compared to calves born from well fed controls. There was no difference in either subcutaneous or intramuscular fat depots between dietary groups. These three adipose depots represent potential for devaluation of the bovine carcass as their quantities are directly utilized to calculate yield and quality grade. From an economic perspective, a 4.2 kg increase in abdominal fat in calves born to restricted dams would be easily offset by the 30% reduction in feed inputs over the last ~125 days of gestation. Nonetheless, these results clearly highlight that energetic efficiency is altered in heifer calves born to modestly nutrient restricted dams. What the implications of an altered energetic efficiency would be had we chosen to retain this heifer in the cow herd remains to be seen. It should also be noted, that extrapolation of findings from the present study to male offspring would be inappropriate. A rapidly growing body of literature highlights the relationship between

environmental insults during pregnancy, sex of the fetus/offspring, and the observed postnatal phenotype (Sundrani et al., 2017). One relevant example in sheep, found that a peri-conceptional dietary deficiency in methionine and specific B vitamins resulted in the development of hypertension in male but not female lambs at 9 months of age (Sinclair et al, 2007). A more recent study in pregnant beef cows fed differing levels of protein during the first and second trimesters observed sexual dimorphism in regulation of the thyroid hormone axis associated with differences in milk intake and postnatal growth rates (Micke et al., 2015).

While muscle tenderness does not have a direct economic impact on the value of the carcass, tenderness is strongly associated with consumer satisfaction and has long-term economic consequences to the beef industry if poor palatability impact product demand. Underwood et al., (2010) detected an increase in muscle fiber diameter and a reduction in meat tenderness in steer calves, when cows were nutritionally restricted during mid to late gestation. A number of studies have identified a similar increase in muscle fiber diameter when the nutritional restriction is applied from early to mid-gestation (Long et al., 2012; Gonzalez et al., 2013; and Micke et al., 2011), likely due to differences in the stage at which the muscle fibers are differentiating at during the nutritional insult. In the present study, we found no difference in Warner-Bratzler shear force measures in longissimus dorsi muscles from calves born to nutrient restricted and control fed dams. We also found no difference in marbling score of the longissimus dorsi between groups. These data suggest that a modest late gestation nutritional restriction does not negatively impact carcass quality in heifer calves. It remains to be seen if the differences observed between the present study and the study conducted by Underwood et al., (2010) are due to differences in the nutritional paradigm or if this is another sexually dimorphic trait.

A novel and potentially important finding of the present study is that a modest late gestation nutritional restriction impairs the development of the pituitary gland in the heifer offspring. In support of our observation that the pituitary is a nutritionally sensitive organ, Sullivan et al., (2009) observed a reduction in circulating FSH concentrations in heifer calves born to cows that had been nutritionally restricted during early to mid-gestation. Sullivan also observed a reduction in the follicular pool of nutritionally restricted heifer calves. The pituitary is comprised of the anterior and posterior lobes. Within the anterior lobe are tropic cells that support numerous physiological processes including reproduction, growth, metabolism, lactation, and response to stress. Our observation of reduced pituitary mass necessitates a microscopic examination of this tissue to determine if these tropic cells are uniformly impacted by the prior maternal nutritional restriction or if individual cell types are more susceptible to late gestation nutritional insult. Importantly, while there was limited negative impact of our nutritional restriction to PND 485 it remains to be seen how these programmed effects will impact production efficiency if that female is kept in the cow herd. Martin et al., (2007) found that protein supplementation to pregnant cows resulted in heifer progeny that exhibited a 7% higher pregnancy rate in their first breeding compared to heifers born to un-supplemented dams. If these effects are due to functional alterations in the anterior pituitary including an altered pattern of FSH secretion is unknown.

When put in context of the beef industry, results of the present study suggest that a modest nutritional restriction during the last half of gestation does not negatively impact the production efficiency of heifer offspring to slaughter and may improve profitability of the cow/calf enterprise by reducing input costs. Two critically important caveats to this statement are that results are limited to heifer calves. How male offspring will respond to an identical

nutritional paradigm cannot be inferred from the present study. Results also highlight a critical need to extend the current study paradigm to the replacement heifer to determine if prenatal nutritional restriction programs poor reproductive performance of the calf.

CHAPTER V

CONCLUSION

It is evident from the findings in Chapter IV that moderate maternal nutrient restriction does not have a significant negative economic impact on heifer progeny destined for the feedlot, regarding growth rates and feed efficiency. It is important to note that results from this study do not include steer calves, and it would be inappropriate to assume similar results in male offspring. Also, had these females been retained as replacements, it is likely that lifetime reproductive performance may have been impacted by the altered energetic efficiency observed in heifers born from nutrient restricted dams. Reduced ability to clear blood glucose has been closely associated with adult onset of type II diabetes and metabolic syndrome in humans (Hales et al., 1991; Hales and Barker, 2013) and obesity in sheep (Gardner et al., 2005; Dellschaft et al., 2015). Factors contributing to metabolic syndrome in humans such as type II diabetes and obesity have also been linked with cardiovascular dysfunction and impaired fertility (Barker et al., 1989; Barker, 1995; Barker, 1997). It is important to note here that these changes are mediated in part by epigenetic modification to chromatin, and these effects may in fact be heritable across generations.

A replacement heifer's profitability is based in her ability to maintain body tissue while also conceiving, growing, and raising a healthy calf. The energetic demands of gestation and lactation are incredibly high and require efficiency of nutrient utilization. Metabolic perturbations such as those observed in Chapter IV do not lend themselves to a lifetime of high reproductive performance. This combined with reduced pituitary weights in heifers born from nutrient restricted dams could be an indicator of reduced tropic cell masses critical to endocrine

regulation of reproductive function. Sullivan et al. (2009) observed lower concentrations of plasma FSH in heifers born from protein restricted dams. These heifers also had a lower density of primordial and primary follicles, as well as reduced healthy antral follicles at the time of slaughter (~23 months).

Furthermore, the combination of reduced pancreatic weight and lower circulating insulin in restricted fetuses from Chapter III clearly indicate there are prenatal developmental alterations occurring to metabolic parameters. It is interesting to note that while restricted twins had smaller pancreases, there was no difference in proliferative rates in the endocrine or exocrine pancreas. This suggests there is a critical window of pancreatic cell proliferation sometime between GD 158 and 265. The exact timing of this window and the underlying mechanisms driving it remain unclear and warrant further study.

The prenatal metabolic alterations observed in Chapter III proved to be non-impactful on postnatal feed efficiency or carcass quality of the half-sibling heifers observed in Chapter IV. Results from this study indicate that economic gains made in reduced feed costs of a moderate nutrient restriction in late gestation are not subsequently lost when female progeny enter the feedlot and is therefore an economically justifiable production practice. Steer calves are not included in this statement, and further work is needed to determine the effects of gestational nutrient restriction on male progeny. Furthermore, reproductive performance of females in the present study is unknown. If lifetime calf production were to be reduced by even one calf, this would pose a significant threat to the sustainability of the cow-calf sector. Further work is needed to determine the effects of moderate maternal nutrient restriction in late gestation on female progeny lifetime reproduction efficiency, as well as male progeny performance in the feedlot sector.

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