IMPACTS OF BEEF COW NUTRITION ON CONCEPTUS DEVELOPMENT

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Two experiments were conducted to determine the effect of maternal nutrient restriction followed by realimentation during early to mid-gestation on uterine blood flow (BF), maternal performance, and conceptus development in pregnant beef cows. In Experiment 1, effects of maternal nutrient restriction followed by realimentation during mid-gestation on uterine BF of lactating, multiparous cows were evaluated. Nutrient restriction from d 30 until 140 of gestation did not alter total uterine BF. However, upon realimentation (from d 140 to 198 of gestation), there was enhanced ipsilateral uterine BF. In Experiment 2, effects of maternal nutrient restriction followed by realimentation during early to mid-gestation on late gestation uterine BF, maternal performance, and conceptus development was evaluated using non-lactating, multiparous cows. Slaughters were performed at d 85, 140, and 254 of gestation. During late gestation when all cows were receiving similar nutrition (100% of the NRC requirements), ipsilateral uterine BF and total BF were increased in cows that were previously nutrient restricted from d 30 until d 85 of gestation and realimented until d 254 of gestation. Therefore, results from both experiments suggest that the bovine placenta may be programmed to function differently after a period of nutrient restriction. Duration of restriction or realimentation impacted maternal performance and organ weights. The dam might become more efficient in the utilization of nutrients after being realimented as gestation advances. Nutrient restriction during early pregnancy tended to increase fetal and placental size by d 85. However, when cows were restricted longer or when realimented, there were no observable differences in placental or fetal growth. The maternal system may adapt to allow for fetal catch up growth during later gestation by enhancing uteroplacental nutrient transport capacity or placental function.

From the results obtained in these 2 experiments we can conclude that maternal nutrient restriction during early gestation enhances conceptus growth and uterine BF later in pregnancy. Perhaps, timely management strategies might result in enhanced conceptus development. Even though more research is necessary, opportunities to intervene appear to be available during times of poor nutrition in beef cow/calf systems.

Keywords: beef cows, nutrient restriction, pregnancy, uterine blood flow

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DEDICATION

To Doc,

Thank you for believing in me
and giving me the strength to continue my education,
without your encouragement and support
I would not be "doc".

***

"Para el logro del triunfo
siempre ha sido indispensable
pasar por la senda de los sacrificios."
Simon Bolivar

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ADF	acid detergent fiber
AI	artificial insemination
BCS	body condition score
BF	blood flow
BUN	blood urea nitrogen
BW	body weight
°C	degrees Celsius
Ca	calcium
CCR	curved crown rump length
CIDR	controlled internal drug release
cm	centimeters
CP	crude protein
CSA	cross sectional area
d	day
DM	dry matter
DMI	dry matter intake
EBW	empty body weight
EvBW	eviscerated body weight
g	gram
GnRH	gonadotropin-releasing hormone
h	hour
HR	heart rate

i.m	intramuscular
IU	international unit
IUGR	intrauterine growth restriction
K	potassium
kg	kilogram
L	liter
LM	longissimus muscle
LV	left ventricle
Mg	magnesium
mg	milligram
min	minutes
MHz	mega hertz
mL	milliliters
Mn	manganese
ND	non-detectable
NDF	neutral detergent fiber
NE	net energy
NEm	net energy maintenance
NRC	National Research Council
PGF	prostaglandins
PI	pulsatility index
REA	ribeye area
RI	resistance index

RV	right ventricle
s	seconds
SAS	Statistical Analytical Software
SD	standard deviation
SEM	standard error of the mean
TDN	total digestible nutrients
TAI	time artificial insemination
vs	versus
Zn	zinc

CHAPTER 1. LITERATURE REVIEW

Introduction

Maternal nutrition during pregnancy plays an important role for placental and fetal growth and development. Placental nutrient transport capacity and size determine the growth trajectory of the fetus (Redmer et al., 2004) as it provides nutrients and oxygen from the dam to the fetus greatly impacting size at birth (Reynolds and Redmer, 1995). In order to transfer nutrients and oxygen between the maternal and fetal system uterine and umbilical blood flows (**BF**) is necessary and these are dependent on placental vascularization. Quality of life in humans is in large part determined by intrauterine development and size at birth affecting not only the viability of the neonate but also rates of morbidity and mortality during adult life (Fowden et al., 2008).

Epidemiological studies have demonstrated that low birth weight is associated with a wide range of adult health complications including hypertension, cardiovascular disease, and diabetes. In 1989 the “fetal origins” hypothesis was proposed by Barker (Godfrey and Barker, 2001). This hypothesis states that an insult (e.g. improper maternal nutrition) during critical periods of fetal development can cause structural, metabolic, and physiologic alterations in utero that have negative long lasting effects on the offspring (Godfrey and Barker, 2001). This concept does not just apply to humans but also to other species including livestock. In fact, during the last couple of decades, great interest in fetal programming has grown in domestic animals and its long term consequences on production. Intrauterine growth restriction (**IUGR**) has negative consequences in animal production such as decreased neonatal survival, decreased offspring birth weight, and decreased postnatal growth and subsequent performance of the offspring (Wu et al., 2006).

Beef cows are commonly managed in grazing systems where the quality of forage varies according to the regional conditions. Forage often is of poor quality which affects nutritional and physiological status of the animal (Funston et al., 2010). Caton and Hess (2010) defined maternal nutrient restriction as a series of events where nutrient supply to the fetus during critical windows of development was reduced. In livestock, particularly ruminants, nutrient supply and environmental conditions are the most common causes of nutrient restriction (Wu et al., 2006; Caton and Hess, 2010). Therefore, pregnancy success and offspring health are dependent on nutrient intake and conceptus growth and development. Optimization of growth and development after birth is important for the profitability of the livestock industry.

Maternal Nutrition Influences During Pregnancy

Nutrition plays an important role during conceptus development. It is well-known that in livestock both over-nutrition and/or under-nutrition can negatively impact the establishment of pregnancy and the survival of the embryo. There are several reports on how nutrition and/or a particular nutrient impact conceptus development (Redmer et al., 2004; Wu et al., 2006; Caton and Hess, 2010; Funston et al., 2010). In addition, because most of the fetal growth in cattle occurs during late gestation an optimal condition is necessary for re-breeding. To increase pregnancy rates, much of the literature available in beef cows focuses on late gestation and the postpartum period (reviewed by Randel, 1990) and not during the earlier stages of pregnancy. It is important to mention that protein restriction during the first trimester of pregnancy followed by increased protein concentration during the second trimester enhances placental development and fetal growth (Perry et al., 1999). However, for the purpose of this review we will focus on global nutrient restriction and how this global restriction impacts placental and fetal growth and development.

In order to ensure an appropriate nutrient supply to the growing and developing fetus, the nutrient requirements of the pregnant dam must be met. In addition to supply nutrients to the growing fetus, the dam needs to have an adequate body condition score (BCS) for calving, lactation, and be able to rebreed (NRC, 2000). An important difference for heifers and mature cows is that the heifers need to be in a good BCS and have enough reserves so that after calving and lactating they can also continue growing, while for mature cows nutrient requirements are lower (NRC, 2000).

Placental Impacts

Maternal nutrition and BCS in mature beef cows has been shown to impact placental development during late gestation. Rasby et al. (1990) have shown that cotyledonary weight was increased in thin (BCS = 4) cows compared to moderate BCS (BCS = 6). Amniotic fluid tended to decrease in thin vs. moderate BCS cows and fructose concentrations were also decreased in thin cows. Placental lactogen, estrone, and estradiol in maternal plasma were greater in thin cows compared to moderate BCS cows from d 240 to 259 of gestation. Fetal weight at d 259 of gestation was similar between treatments which indicate that the placenta can compensate in thin cows due to nutrient restriction and decreased body energy reserves (Rasby et al., 1990).

There is limited data on how realimentation after maternal nutrient restriction during early to mid-gestation impacts placental development in beef cattle. An experiment was conducted in which beef cows were nutrient restricted to 50% of the NRC recommendations from d 30 to d 125 of gestation. Results from this experiment indicate reduced caruncular and cotyledonary weights in nutrient restricted cows compared to cows receiving a control diet (100% of the NRC recommendations) towards the end of the restriction (Zhu et al., 2006). However, when cows were realimented, caruncular weight was similar between restricted and control cows at d 250 of gestation (Zhu et al., 2007). Vonnahme et al. (2007) demonstrated in the same experiment, that nutrient restriction from d 30 to 125 of gestation did not affect placental vascularity. Conversely, upon realimentation, placental vascularity was altered near term, indicating that the placenta compensated after restriction. Vonnahme et al. (2007) observed dramatic differences in capillary vascularity after the realimentation period (from d 125 to 250), suggesting an alteration of placental development and function by early nutrient restriction.

It appears that maternal nutrition can program the development of the placenta. Development of the placental vascular bed is imperative to support the growth and development of the fetus. While nutrient restriction from d 30 to 125 in the cow did not alter the vascular architecture of the bovine placenta, placental function must have been altered as fetal weight was reduced. It appears that realimentation after approximately 90 days of nutrient restriction is the stimulus not only for altering placental vascularity and development but also placental function in the cow.

Fetal and Calf Impacts

It has been previously shown that prepartum nutrient restriction impacts birth weight (Corah et al., 1975). Heifers and cows that were fed restricted during the last 100 d of gestation decreased calf birth weight and weaning weight (Corah et al., 1975). A series of studies were published looking at the effects of nutrition during late gestation and pre-partum period on calf birth weight and growth parameters (Tudor, 1981; Tudor and O’Rourke, 1980; Tudor et al., 1980). Hereford cows that received a low plane nutrition during the pre- (at 180 d of gestation) and post-partum period had decreased calf weight compared to the high plane nutrition (Tudor, 1981). After calves were born they were controlled fed (high plane and low plane nutrition) from d 4 to 200 and then finish either in pasture or intensively fed (Tudor and O’Rourke, 1980). Low plane nutrition after calves were born did not have a negative effect on growth rate and feed efficiency since they were able to obtained marketable weights similar to calves fed high plane diets (Tudor and O'Rourke, 1980). After females and males reached 370 and 400 kg, respectively, they were slaughtered and body composition was measured. Visceral organs, muscle and bone weight was similar between low- and high-plane diets during early postnatal life (Tudor et al., 1980). Beef cows that where nutrient restricted from d 30 to 125 of gestation, had fetuses with similar size and weight to those fetuses from dams receiving the control diet; however, when fetuses were separated into IUGR-nutrient restricted (reduce fetal weight and asymmetrical growth) those calves were lighter and empty carcass weight was also reduced compared to control and non-IUGR fetuses (Long et al., 2009). However, after maternal nutrient realimentation, fetuses from nutrient restricted cows had similar fetal weight compared to control fetuses at d 245 of gestation. Long et al. (2012) reported that when cows were nutrient restricted to 70% of NRC requirements from d 45 to 185 of gestation and thereafter fed the control diet (100% of NRC) until parturition, birth weights of the calves were similar between treatments. Maternal nutrition during early to mid-gestation has been shown to impact fetal growth as early as d 39 of gestation in beef heifers (Micke et al., 2009). In this particular experiment, heifers received either high or low nutritional planes during the 1st trimester (diets started at AI) and were switched to the opposite treatment during the 2nd trimester and by the 3rd trimester all heifers received similar diets. Calf BW was decreased and this was associated with the maternal low plane diet. Interestingly, those fetuses from dams receiving the high plane diet during early gestation had enhanced growth; however, after the 1st trimester the growth trajectory slowed (Micke et al., 2009).

Animal scientists are always looking for alternatives to improve calf production at the lowest cost, and feeds costs are the easiest to manipulate. During a 2 year study, cows were nutrient restricted to 57% of the NRC requirements or fed a control diet (100% NRC requirements) during the last 90 d of gestation. All cows received the same diet postpartum. Calf birth weight was not affected by maternal nutrient restriction but perhaps metabolism was. Calves born from nutrient restricted dams had an increase in cortisol and a decrease in T3 circulating concentrations from 0 to 36 h postpartum compared to calves born from control-fed dams; however, colostrum immunoglobin G (**IgG**) concentration and IgG in calves' serum was similar between treatments (Hough et al., 1990). Freetly et al. (2000) conducted an experiment where mature beef cows were allowed to lose and gain BW in different patterns thought the 2nd trimester of pregnancy and the subsequent breeding season. They found that cows that were maintained at a constant BCS (BCS = 5.5) and the group of cows that lost BW during the 2nd trimester and then gained BW during the last trimester and during lactation performed similarly and calf weights were also similar; therefore, BW fluctuation might be a good tool to decrease feed cost and improve calf production. Interestingly, in a recent study (Mossa et al., 2013) where beef heifers were nutrient restricted 11 d prior to insemination until d 110 of gestation decreased ovarian reserve was observed in the female calves. The author suggests that this could be due to the observed increase in maternal circulating testosterone. However, female calves' growth parameters were similar to the control counterparts (Mossa et al., 2013).

Bovine Placenta

In ruminants the placenta is morphologically classified as cotyledonary and histologically as epitheliochorial. However, the bovine placenta can also be classified as syndesmochorial because its unique formation of binucleate cells (Björkman, 1965). On the uterine wall there are specialized areas of endometrium called caruncles with a button-like appearance (Silver et al., 1972). In non-pregnant ruminants, the caruncles are organized into two dorsal and two ventral rows that run lengthwise along the uterine horns. There are approximately 100 caruncles and they can be seen even in uterus from fetal calves (Shlafer et al., 2000). The chorioallantois which has a flat surface, becomes irregular when it starts covering the caruncles; because of the growth and expansion of the extra embryonic fetal membranes in the lumen of the uterus. This process is followed by the recognition of the cotyledons (Shlafer et al., 2000). Therefore, the caruncular-cotyledonary unit called the placentome and is formed from the growth and interdigitation of fetal villi and caruncular crypt adopting a convex shape (Silver et al., 1972). Contact surface area is enhanced because the cotyledon finger-like projections enter the crypts formed in the caruncles. Placentomes vary in size; however they are bigger in the base of the horn and decrease in size close to the tip of the uterine horns (Shlafer et al., 2000). The placentome is the primary functional area of physiological exchanges between mother and fetus.

Due to massive amounts of data in sheep and the fact that sheep has cotyledonary type of placenta we tend to compared sheep and cattle. However, placental growth between sheep and cattle differ as in sheep placental growth plateaus around d 70 to 80 of pregnancy and has limited growth until term (Stegeman, 1974); whereas the placental growth in cattle is continuous throughout pregnancy (Vonnahme et al., 2007). Placentation is composed of extensive angiogenesis and vascularization and subsequently a rapidly increase in uterine and umbilical BF (Reynolds and Redmer, 1995). More specifically, increases in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on growth of the placenta during early pregnancy followed by dramatic development and reorganization of the uteroplacental vasculature during the last half of gestation (Meschia, 1983; Reynolds and Redmer, 1995).

The placenta plays a major role in the regulation of fetal growth. Placental nutrient transport efficiency is directly related to utero-placenta blood flow (Reynolds and Redmer, 1995). Gases, nutrients, and metabolic end products are exchanged between maternal and fetal circulation via the placenta (Bleul et al., 2007; Reynolds and Redmer, 1995; Reynolds and Redmer, 2001).

Uterine Blood Flow

Pregnancy is associated with an increase in both cardiac output and uterine BF and a reduction in systemic vascular resistance. This increase in uterine BF supports exponential fetal growth during the last trimester of gestation and provides adequate oxygen and nutrient delivery. Of the overall increase in uterine BF during late gestation, more than 85% is directed toward the caruncular vascular beds in sheep, which transfer oxygen and nutrients to the placenta and fetus through the associated cotyledonary vasculature (Rosenfeld and Fixler, 1977). Also, some non-reproductive organs, for example kidneys and gastrointestinal tract, have increased blood flow demands because their involvement with physiological adaptations during pregnancy (Itskovitz-Eldor and Thaler, 2005), but as a percentage of cardiac output, there is less blood volume going to those organs (Rosenfeld, 1984).

Several techniques (electromagnetic probes, heavy water, and Doppler) have been used to characterize uterine BF in pregnant cows. Because all these techniques have been conducted in different cow models, at different stages of gestation, and because of the variation among techniques, we will be discussing in detail the literature available in pregnant cows uterine BF. A summary of all the literature available to our knowledge in uterine BF will be summarized and presented in tables in the Appendix section (A.1, A.2, and A.3) at the end of the dissertation. During early pregnancy and around the time of maternal recognition of pregnancy, uterine BF was characterized using electromagnetic flow probes (Ford et al., 1979). Ipsilateral (pregnant horn) uterine BF was constant during the first 13 d and from d 14 to 18 there was a 2 to 3-fold increase in ipsilateral uterine BF. In contrast, contralateral uterine BF remained low during this period. However, from d 19 to 25 of gestation, there was a decrease in ipsilateral BF but from d 25 until d 30 of gestation ipsilateral BF was increased and contralateral BF decreased (Ford et al., 1979). Therefore, it was suggested that ipsilateral and contralateral uterine BF responds differently during maternal recognition and may be due to conceptus secretions during early establishment of pregnancy (Ford et al., 1979). Ferrell and Ford (1980) also measured BF utilizing electromagnetic probes on the ipsilateral uterine artery at d 30 and 80 of gestation. Uterine BF from d 31 to 45 was 0.12 L/min whereas they report an increase at d 77 to 92 where uterine BF was 0.20 L/min. Panarace et al. (2006) measured uterine BF at d 30 and 84 of gestation on the ipsilateral and contralateral uterine arteries using Doppler ultrasonography. At d 30 of gestation, ipsilateral BF was 0.08 ± 0.01 L/min and contralateral BF was 0.04 ± 0.01 L/min. Therefore total BF was approximately 0.12 L/min. By d 84, ipsilateral BF (0.59 ± 0.05 L/min) and contralateral BF (0.17 ± 0.03 L/min) provided the total uterus with approximately 0.79 L/min of BF. Another study (Bollwein et al., 2002) characterizing BF during early gestation was conducted at d 30, 60, and 90 of gestation utilizing Doppler ultrasonography. Average total BF at d 30 was 0.10 L/min, at d 60 was 0.14 L/min, and at d 90 was 0.30 L/min. Even though uterine BF during the first trimester has been characterized in different cow breeds and with different techniques, uterine blood flow in all experiments was low and constant from d 30 to d 90 of gestation.

Briefly, uterine BF at d 140 and 180 of gestation was measured utilizing electromagnetic probes on the ipsilateral uterine artery. At d 139-155 the ipsilateral uterine BF was 2.0 ± 0.6 L/min and by d 178-199 BF was 3.2 ± 0.4 L/min. In 1983, Ferrell and coworkers characterized uterine BF using steady state diffusion with antipyrin solution, since differences in uterine BF from d 163 to 176 of gestation were not found; the average uterine BF reported was 5.9 ± 0.5 L/min. Also, uterine BF was measured at d 137 to 180 of gestation utilizing heavy water infusion. Uterine BF reported with this technique was 2.9 L/min at d 137 and 4.8 L/min at d 180 of gestation (Reynolds et al., 1986). Three more recent studies have been conducted with Doppler ultrasonography. The first study reported ipsilateral and contralateral uterine BF at d 120, 150, and 180 of gestation (Bollwein et al., 2002). Ipsilateral uterine BF was always greater than contralateral uterine BF regardless of day of gestation. Moreover, ipsilateral uterine BF increases from 0.7 L/min on d 120 to 1.7 L/min and 4.0 L/min by days 150 and 180, respectively. The contralateral uterine BF also increased, but at overall lower volumes (0.4 L/min, 0.9 L/min and 1.3 L/min on d 120, 150 and 180, respectively; Bollwein et al., 2002). Panarace et al. (2006) reported ipsilateral BF (5.5 ± 0.4 L/min) and contralateral (0.6 ± 0.1 L/min) BF on d 168 of gestation. The third Doppler ultrasonography study reported uterine BF in Holstein cows at d 147 and 175 of gestation (Herzog et al., 2011). This particular study split cows into heavy and light weight categories and also into cows calving heavy and light calves. Heavy cows had greater BF compared to light cows at both time points (d 147: 2.6 L/min vs. 3.4 L/min; d 175: 4.2 L/min vs. 5.4 L/min; light vs. heavy cow weight respectively). In addition, cows had similar total uterine BF at d 147 of gestation when carrying heavy or light calves but at d 175 of gestation cows carrying heavy calves had increased uterine BF vs. light calves (Herzog et al., 2011).

The greatest increase in uterine BF has been shown in late gestation. This increase in uterine BF during the third trimester of pregnancy also corresponds to the greatest increase in fetal weight deposition. The variation in reported BF during late gestation may be a result of measurement technique as well as individual animal variation and/or environmental conditions in which the animals were exposed. Comline and Silver (1976) reported uterine BF between d 250 and 270 of gestation using a diffusion equilibrium technique. The mean BF for this study was 330 ± 18.2 ml/kg/min. A few years later, Ferrell and Ford (1980) measured uterine BF with electromagnetic probes approximately at d 210 and 240 of gestation, reporting BF at d 202-224 being 4.0 ± 0.5 L/min and at d 230-258 BF was 3.1 ± 0.2 L/min. Three studies conducted by the same laboratory reported late gestation BF utilizing the heavy water infusion technique. Utilizing this technique, ipsilateral uterine BF at d 226 (2.9 L/min) and 250 (13.1 L/min) of gestation were reported (Reynolds et al., 1986; Reynolds and Ferrell, 1987). In addition, there was a 4.5-fold increase in ipsilateral BF from d 137 to 250 and maternal heart rate was also increased (Reynolds et al., 1986; Reynolds and Ferrell, 1987). The next experiment used Charolais cows carrying either Charolais or Brahman fetuses and Brahman cows carrying either Charolais or Brahman fetuses. This experiment was design to test the hypothesis that uterine environment plays an import role on fetal and postnatal growth by suing a large and small breed of cattle. At d 220 of gestation ipsilateral uterine BF was measured and their findings indicated that BF of Brahman cows carrying Charolais (4.7 L/min) or Brahman fetuses (5.0 L/min) were similar but lower than Charolais cows carrying either Charolais (9.2 L/min) or Brahman (7.18 L/min). However, when looking at the Charolais cows, Charolais carrying Charolais fetuses had increased BF vs. Charolais carrying Brahman fetuses. This study suggested that late gestation BF and function of the uteroplacenta may be the limiting factors for fetal growth. In addition, it was also shown that fetal genotype is a very important component of fetal growth (Ferrell, 1991). The third study also used 2 different breeds (Charolais and Hereford) but also looked at single vs. twin fetuses. At d 190 of gestation, there was a decrease in ipsilateral uterine BF for Hereford (4.8 L/min) vs. Charolais (7.1 L/min). Another observation from this experiment was the cows carrying twins (5.2 L/min) had decreased uterine BF per fetus vs. cows carrying singletons (6.7 L/min) regardless of cow breed. The authors concluded that the reason calves from twin pregnancies often exhibit reduced birth weight compared to a singleton might be due to the reduce BF per fetus that was observed (Ferrell and Reynolds, 1992). More recently 3 other studies were conducted utilizing Doppler ultrasonography during late gestation. Bollwein et al. (2002) reported ipsilateral and contralateral uterine arteries BF at d 210 (5.0 vs. 1.3 L/min), 240 (6.0 vs. 2.8 L/min), 270 (10.5 vs. 3.9 L/min), and 284 (13.1 vs. 4.5 L/min) of gestation. They observed an increase in BF and arterial diameter during gestation and decreased resistance of the uterine arteries during the first 8 months of gestation; however, during the last month of gestation resistance of the uterine artery was similar. Another experiment using Doppler, BF was measured at d 266 of gestation in both ipsilateral (14.1 L/min) and contralateral (2.0 L/min) uterine artery. Therefore, total BF at d 266 of gestation is approximately 16.1 L/min (Panarace et al., 2006). They also reported a 20-fold increase in ipsilateral BF and 17-fold increase in ipsilateral uterine artery diameter by d 266 of gestation compared to d 30 of gestation measurements. However, contralateral BF remained steady (Panarace et al., 2006). The third experiment conducted by Herzog et al. (2011) was previously described and also measured BF at d 203 (light 6.1 L/min vs. heavy cow 8.7 L/min), d 231 (light 10.5 L/min vs. heavy cow 13.1 L/min), d 259 (light 13.6 L/min vs. heavy 16.9 L/min) and d 273 (light 14.1 L/min vs. heavy cow 19.2 L/min) of gestation. Throughout the experiment a linear increase in total uterine BF was reported. Also, heavy cows had a greater increase in uterine BF vs. light cows; however, within a weight category, cows carrying heavy fetuses had greater uterine BF compared to light fetuses.

Summarizing all the data presented above, different techniques and animal models have been used over the years to characterize uterine artery BF. All this work has been very important and lead to different paths for research during gestation in the beef cow. While several techniques have been used to measure uterine BF and resistance indices of the uterine artery, there is wide variation across techniques but also within a similar technique. However, it is very important that the same technique is implemented during a study to address a specific problem.

In all models of placental insufficiency in the sheep, uterine and umbilical BF were reduced (Reynolds et al., 2006). There is a lack of information on how diet impacts uterine or umbilical BF in beef cattle. However, uterine and umbilical BF in models of nutrient restriction (or nutrient excess), have not been measured upon realimentation in sheep or cattle. This is probably due to the fact that the several methods of determining BF are very invasive and require increased numbers of animals to determine BF at different time points during pregnancy. While these are effective, Doppler ultrasonography is a more refined method to obtained uterine and umbilical BF since surgical procedures are not necessary. In addition, we can use fewer animals since we can monitor the same animal throughout gestation. Recently publications by several laboratories have successfully monitored uterine BF with color-Doppler ultrasonography in cattle (Bollwein et al., 2000; Panarace et al., 2006). However, to our knowledge, the impacts of nutrient restriction, followed by realimentation, on uterine and umbilical BF in the cow has not been done throughout pregnancy.

Doppler Ultrasonography

The Doppler effect was named after Christian Andreas Doppler and it is defined as change in frequency of waves due to motion between source of wave and receiver (Abramowicz and Sheriner, 2008; Maulik, 2005). Doppler technology has been widely used in humans for medical diagnoses of either healthy or particularly compromised pregnancies. In addition, use of the Doppler ultrasonography can predict complications that might happen later during pregnancy or the outcome of a high-risk pregnancy (Yangel et al., 1998; Abramowicz and Sheriner, 2008). However, during the last decade Doppler ultrasonography is gaining more interest for livestock use in particular to measure uterine BF (Bollwein et al., 2002; Panarace et al., 2006), umbilical BF and fetal growth parameters (Lemley et al., 2012; Harris et al., 2013) during pregnancy. It is important to mention that Doppler ultrasonography is also used to assess other reproductive and non-reproductive parameters in livestock including uterine BF during the estrous cycle (Bollwein et al., 2000), luteal BF (Acosta et al., 2002; Ginther et al., 2007), mammary gland BF (Potapow et al., 2010), and portal BF (Starke et al., 2011). Doppler ultrasonography has considerable potential to increase the understanding of different systems in livestock.

The Doppler effect, as mentioned before, is the change in frequency of energy wave due to motion between source of wave and receiver. The change in frequency is known as Doppler frequency shift or Doppler shift [**fd**; fd = ft (transmitted frequency) – fr (received frequency); Maulik, 2005]. When talking about blood vessels one can say that the stationary object is the transducer and the red blood cells are the moving reflectors that produce the returning echoes (Ginther and Utt, 2004; Figure 1.1).

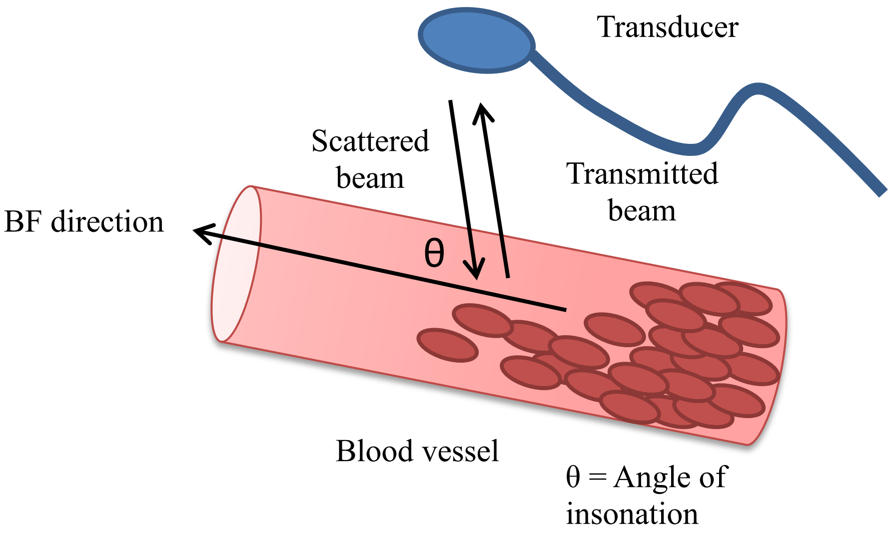

Figure 1.1. Doppler ultrasonography of a blood vessel (Adapted from Maulik)

Blood flow of a vessel can be assessed in Doppler spectral mode by placing a sample gate cursor on the image of vessel of interest. As shown in Figure 1.1, an angle of insonation is important because it represents the angle of intersection between the ultrasound beams and the direction of the blood flow. It is important to understand that the sound transmission changes depending on where the sound is going. For example, if the source and the receiver move closer the Doppler shift sound is of higher frequency but if the source moves away from the receiver the Doppler shift sound is of lower frequency (Maulik, 2005; Figure 1.2)

In order to assess BF is imperative to have 2 pieces of information being angle of insonation and CSA of the vessel. When performing Doppler ultrasonography there are 2 different modalities that are most commonly used in measuring BF, the spectral and color-flow mode. For spectral mode which could be used in B-mode or color-mode image, the sample gate cursor is placed in the lumen of the vessel image. Sample gate can be adjusted and should not touch the walls of the vessel of interest (Ginther and Utt, 2004).

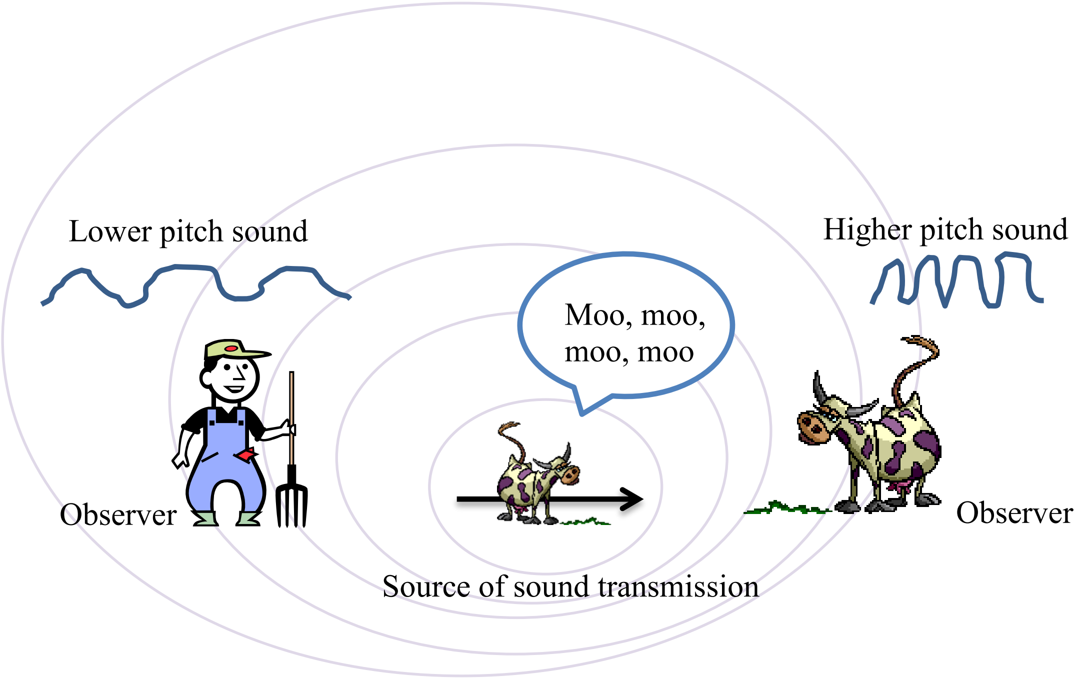

Figure 1.2. Doppler shift sound (Adapted from Maulik, 2005)

For the color-mode, as its name describes it, areas where BF is present are colored. This is important when measuring BF as you can make sure you are in an area of BF. Normally, color-flow mode represents movement away and towards the transducer in different shades of red and blue and depending on the intensity of velocity that can be identified (Ginther and Utt, 2004). Angle of insonation is another important characteristic (Figure 1.1) when measuring BF. The desirable angle of insonation is between 30 and 60 degrees but it is important to keep it below 90 degrees to decrease error in calculating velocity; anything close to or at 90 degrees will produce weak Doppler signaling (Ginther and Utt, 2004; Maulik, 2005). In other words, as the angle of insonation increases, the frequency shift decreases, and vice versa (Maulik, 2005). The angle of insonation can be controlled by the machine operator by placing the cursor on the vessel and determining the angle.

We commonly use Doppler indices which are independent of the angle of insonation and are ratios of velocity measurements. Doppler indices are: pulsatility index (**PI**) and resistance index (**RI**) which are calculated by pre-programmed Doppler software. The equations are PI = (peak systolic velocity - end diastolic velocity)/mean velocity and RI = (peak systolic velocity - end diastolic velocity)/ peak systolic velocity (Figure 1.3). Because the PI includes the entire cardiac cycle (denominator in the above equation), it is believed to provide more hemodynamic information than RI. However, both PI and RI are widely used in obstetrics. The RI can represent the negative relationship between resistance and vascular perfusion of a tissue. In other words, the higher the resistance, the lower the perfusion. The Doppler software also calculates BF, which is described by the equation below:

BF (mL/min) = mean velocity (cm/s) x cross-sectional area of the vessel (cm^2) x 60 s.

Because this equation takes cross-sectional area of the vessel of interest into consideration (i.e., π x $radius^2$) an error of 15% in diameter measurement can cause a major error in BF determination (Ferrazi and Rigano, 2005). In summary, as with all methods and techniques use in science we need to be aware of the advantages and disadvantages that each technique provides. As mention previously, utilizing the same technique and allowing the control of certain variables (i.e. angle of insonation and CSA) is important in order to obtain appropriate information. Doppler ultrasonography continues to develop and more sophisticated equipment is available; however, there are areas where more research can be done and techniques can be improved.

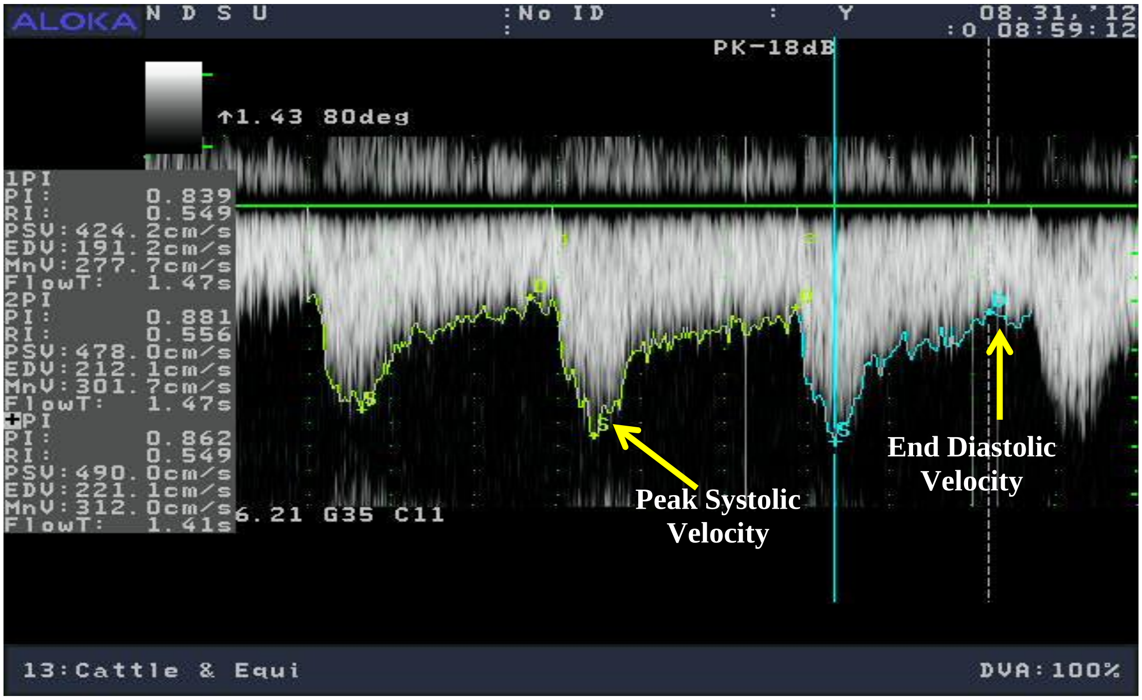

Figure 1.3. Doppler waveforms of bovine uterine artery

Statement of the Problem

Beef cows are commonly managed in grazing systems where the quality of forage varies according to the regional conditions and this can negatively impact the nutritional and physiological status of the dam as well as the development of their offspring (Wu et al., 2006; Funston et al., 2010). Intrauterine growth restriction is associated with altered fetal organ development and subsequent performance of offspring (Godfrey and Barker, 2000; Wu et al., 2006). As previously reviewed in this chapter, maternal and fetal responses to nutritional impacts have been studied in the beef cow; however, most of the relevant research in this area has been conducted in other models including sheep. Even though sheep are ruminants, they might not be the appropriate model to compare with beef cows. The most common, and easiest therapeutic to administer, is to realiment the undernourished dam; however, there is a scarcity of information on how realimentation after maternal nutrient restriction impacts placental and fetal development in any species. To our knowledge, the only study done in beef cows using a nutrient restriction/realimentation model is Vonnahme et al. (2007); where they demonstrated that placental vascularity was augmented when previously restricted beef cows were realimented to nutritional planes similar to controls.

In all models of placental insufficiency in the sheep, uterine and umbilical BF were reduced (Reynolds et al., 2006). There is a lack of information on how diet impacts uterine or umbilical BF in beef cattle. However, uterine and umbilical BF in models of nutrient restriction (or nutrient excess), have not been measured upon realimentation in sheep or cattle. Foundational uterine BF work in beef cattle has been conducted utilizing more invasive techniques such as electromagnetic blood flow transducers (Ford and Christensen, 1979) or infusion of deuterium-labeled water (Reynolds and Ferrell, 1987). By using color Doppler ultrasonography to assess uterine BF and vascular resistance throughout gestation in pregnant beef cows, we were able to examine the same animal continuously throughout gestation with no surgical preparation and with minimal interference to the dam. Recently, publications by several laboratories have successfully monitored uterine blood flow with color-Doppler ultrasonography in cattle (Bollwein et al., 2000; Panarace et al., 2006). However, to our knowledge, the impacts of nutrient restriction, followed by realimentation, on uterine and umbilical BF in the cow has not been done throughout pregnancy. Thus, the ability to develop a reliable method of monitoring uterine BF utilizing Doppler ultrasound technology which allows us to examine the same animal continuously through gestation, reducing the number of animals required to conduct an experiment.

Therefore, the overall hypothesis is that maternal nutrient restriction in the cow during key developmental stages of the placenta will alter uteroplacental vascular function, stunting fetal growth and development. However, paradoxically, nutrition restriction during the proper time of placental development may stimulate, upon increased nutrient availability, vasodilatory properties within the gravid uterus increasing its efficiency in supplying nutrients to the fetus having measurable effects on postnatal growth and potentially reproductive capacity/end product quality.

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CHAPTER 2. EFFECTS OF MATERNAL NUTRIENT RESTRICTION FOLLOWED BY RE-ALIMENTATION DURING MID-GESTATION ON UTERINE BLOOD FLOW IN BEEF COWS

Abstract

The objective of this study was to examine the effect of maternal nutrient restriction followed by realimentation during mid-gestation on uterine blood flow (**BF**). Lactating, multiparous Simmental beef cows (n = 10; 714.8 ± 23.4 kg of BW) were placed in a pen equipped with Insentec B. V. roughage individual intake control system feeders. On d 30 of pregnancy, cows were assigned randomly to treatments: control (**CON**; 100% NRC; n = 6) and nutrient restriction (**RES**; 60% of CON; n = 4) from d 30 to 140 (Period 1) and, thereafter, realimented to CON until d 198 of gestation (Period 2). Calves were weaned from their dams at d 90 of gestation. Uterine BF, pulsatility index (**PI**), and resistance index (**RI**) were obtained from both the ipsilateral and contralateral uterine arteries on d 30, 58, 86, 114, 140, 152, 159, 166, and 198 of gestation via Doppler ultrasonography. Ipsilateral uterine BF in both groups increased quadratically ($P < 0.01$) during period 1 and linearly ($P < 0.01$) during Period 2. There was a treatment ($P = 0.05$) effect during Period 2; where RES cows had greater ipsilateral BF vs. CON. Ipsilateral uterine PI and RI did not show an interaction or treatment effect ($P \geq 0.13$) during either period; however, PI and RI decreased linearly ($P \leq 0.01$) during Period 1 across treatments. Contralateral uterine BF in CON cows tended ($P < 0.09$) to be greater vs. RES in both periods. Uterine BF increased linearly ($P \leq 0.04$) during both periods. There was no interaction or treatment effect ($P \geq 0.15$) for contralateral uterine PI; however, contralateral PI in both groups increased linearly ($P \leq 0.01$) during Period 1. Contralateral uterine RI was increased

($P \leq 0.05$) in RES cows vs. CON in both periods. Regardless of treatment, there was a quadratic day ($P < 0.01$) response for contralateral RI during Period 1. There was no interaction or treatment effect ($P \geq 0.24$) for total BF during either period. Regardless of treatment, total BF responded quadratically ($P < 0.01$) during Period 1 and linearly ($P < 0.01$) during Period 2. Nutrient restriction does not alter total uterine BF, but it may increase vascular resistance. However, upon realimentation, local conceptus derived vasoactive factors appear to influence ipsilateral uterine BF.

Keywords: beef cows, nutrient restriction, pregnancy, uterine blood flow

Introduction

Beef cows are commonly managed in grazing systems where the quality of forage varies according to the regional conditions and this can negatively impact the nutritional and physiological status of the dam as well as the development of their offspring (Funston et al., 2010). Intrauterine growth restriction is associated with altered fetal organ development and subsequent performance of offspring (Godfrey and Barker, 2000; Wu et al., 2006). The most common, and easiest therapeutic to administer is to realiment the undernourished dam; however, there is a scarcity of information on how realimentation impacts placental and fetal development. Vonnahme et al. (2007) demonstrated that placental vascularity was augmented when previously restricted beef cows were realimented to nutritional planes similar to controls.

Placental nutrient transport efficiency is directly related to utero-placental blood flow (**BF**; Reynolds and Redmer, 1995). Increases in transplacental exchange, which supports the rapid increase in fetal growth during the last half of gestation, depends primarily on growth of the placenta during early pregnancy followed by dramatic development and reorganization of the uteroplacental vasculature during the last half of gestation (Meschia, 1983; Reynolds and Redmer, 1995).

Color Doppler ultrasonography is a non-invasive technique which has been used to measure uterine BF and arterial indices of resistance in cattle (Bollwein et al., 2000, 2002; Herzog and Bollwein, 2007; Panarace et al., 2006). However, to our knowledge, uterine BF in models of nutrient restriction has not been measured upon realimentation. We hypothesized that uterine BF in nutrient restricted cows would be reduced during the restriction period, but upon realimentation, uterine BF would surpass that of adequately fed control cows. The objective of this study was to examine the effect of maternal nutrient restriction followed by realimentation during mid-gestation on uterine BF and other hemodynamics.

Materials and Methods

All animal procedures were approved by the North Dakota State University (**NDSU**) Animal Care and Use Committee (#A12046).

Animals and Management

A total of 18 lactating, multiparous Simmental beef cows were transported from the NDSU Beef Research and Teaching Unit (Fargo, ND) to the NDSU Beef Cattle Research complex within 3 d of artificial insemination. All cows were inseminated the same day (April 13th, 2012) and randomly assigned to be bred to 2 different bulls. Upon arrival, radio frequency identification tags were placed in the right ear of cows and BW was measured. Cows were placed in a pen equipped with 8 individual Insentec roughage intake control system feeders (Insentec B. V., Marknesse, Netherlands). Cows were trained to use the Insentec system and fed a common diet until d 30 of gestation. Cows were limit-fed using the Insentec feeding system to provide the desired NE intake. Dietary net energy of grass hay was estimated using approaches described by Weiss et al. (1992) and NRC (2000). Limestone was added to the total mixed diet to maintain a Ca:P ratio of approximately 1.3:1. Cows were fed once daily at 0800 h and had free access to water and traced mineralized salt blocks (American Stockman, North American Salt Company, Overland Park, KS; 95.5-98.5% NaCl, 3500 mg/kg Zn, 2000 mg/kg Fe, 1800 mg/kg Mn, 280-420 mg/kg Cu, 100 mg/kg I, 60 mg/kg Co).

On d 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). Moreover, the corpus luteum was identified and the gravid uterine horn was determined so that the ipsilateral uterine artery could be identified. On d 30 of pregnancy, 12 lactating (714.8 ± 23.4 kg of BW), multiparous beef cows were assigned randomly to dietary treatments: control (**CON**; n = 6) and nutrient restriction (**RES**; n = 6) from d 30 to 140 and, thereafter, realimented to control until d 198 of gestation. Cows were fed the same diet (Table 2.1) at either 100% or 60% of NRC recommendations for NE for maintenance, lactation (until weaned at d 90), and fetal growth (NRC, 2000), and to meet or exceed the recommendations for MP. Feed intake was adjusted relative to predicted NE requirements for the following periods (d 30 – 85, d 86 – 140, and d 141 – 198 of gestation).

Per experimental design, DMI in Period 1 was reduced ($P = 0.05$) in RES cows compared to CON (6.01 vs. 12.02 ± 0.45 kg DM). This resulted in RES cows consuming less ($P < 0.01$) as a percentage of BW compared to CON (1.00 vs. 1.75 ± 0.02% of DM/kg BW). During Period 2, formerly RES cows continued to have less ($P = 0.05$) DMI than CON (8.22 vs. 10.13 ± 0.65 kg DM); however as a percentage of DM per BW, they were similar ($P = 0.22$; 1.54 vs. 1.63 ± 0.05%, RES vs. CON cows, respectively). One RES cow had to be removed from the project because she was carrying twins, and a second RES cow was also removed from the project because of early embryonic loss resulting in n = 6 for CON and n = 4 for RES. On d 90 of gestation, all cows were weaned and diets were adjusted to meet their nutrient requirements according to their stage of gestation.

Body condition was estimated monthly using a 1 to 9 scale (with 1 = emaciated and 9 = obese; Wagner, 1988) from d 30 to 198 of gestation. Cows were weighed every 2 wk at approximately 0700 h throughout the experiment and dietary intake adjusted relative to BW. Percentage of BW change was calculated by BW difference (final BW - initial BW) divided by initial BW times 100, where initial BW was BW at d 30 of gestation. At d 198, all cows were fed a common diet until calving.

Table 2.1. Diet composition and nutrient analyses.

Ingredient	% of dietary DM
Grass hay	92.5
Corn condensed distiller’s solubles	7.0
Limestone	0.5
Analyses	
Ash, %	11.5
CP, %	9.3
NDF, %	67.3
ADF, %	40.1

Feed Analysis

Diet samples were collected weekly and dried in a 55^{o}C oven, ground to pass a 1-mm screen, and analyzed for DM, ash, and CP (Kjeldahl) by standard procedures (AOAC, 1990). Neutral detergent fiber and ADF concentration was determined by the method of Robertson and Van Soest (1981) using an Ankom fiber analyzer (Ankom Technology Crop., Fairport, NY).

Ultrasonography Evaluation

Hemodynamic measurements of the uterine artery ipsilateral and contralateral to the conceptus were obtained via a color Doppler ultrasonography (model SSD-3500; Aloka America, Wallingford, CT) fitted with a 7.5 MHz finger transducer (Aloka UST-995) on d 30, 58, 86, 114, 140, 152, 159, 166, and 198 of gestation. Ultrasonic evaluations were taken at the same time of day between 0800 and 1200 h and lasted approximately 30 min per cow. Cows were examined via ultrasonography within 2 d of every reported sampling time. Briefly, the probe was inserted through the rectum and the aorta was located. In B mode using the finger probe, the origin of the external iliac, ipsilateral to the gravid uterine horn, was located and the transducer was moved caudally to locate the internal iliac artery. The umbilical artery begins as a major branch of the internal iliac, and gives rise to the uterine artery (Bollwein et al., 2000). After the uterine artery was identified as a movable and pulsating artery, a longitudinal section was visualized by manually turning the transducer of the probe. The probe was aligned to the uterine artery at an average angle of insonation of 79.0 ± 0.2 degrees and uterine artery hemodynamic measurements were collected.

Three similar cardiac cycle waveforms from 3 separate ultrasonography evaluations from each side (ipsilateral and contralateral uterine artery; Figure 2.1) were obtained with spectral Doppler and averaged per cow within a gestational day (9 measurements per side per sampling day). Maternal heart rate (**HR**), pulsatility index (**PI**), resistance index (**RI**), and uterine artery blood flow (**BF**) were calculated by pre-programmed Doppler software where PI = (peak systolic velocity - end diastolic velocity)/mean velocity; RI = (peak systolic velocity - end diastolic velocity)/ peak systolic velocity; and BF (mL/min) = mean velocity (cm/s) x cross-sectional area

(**CSA**; cm^2) x 60 s. Total BF was calculated as the sum of ipsilateral and contralateral uterine artery BF.

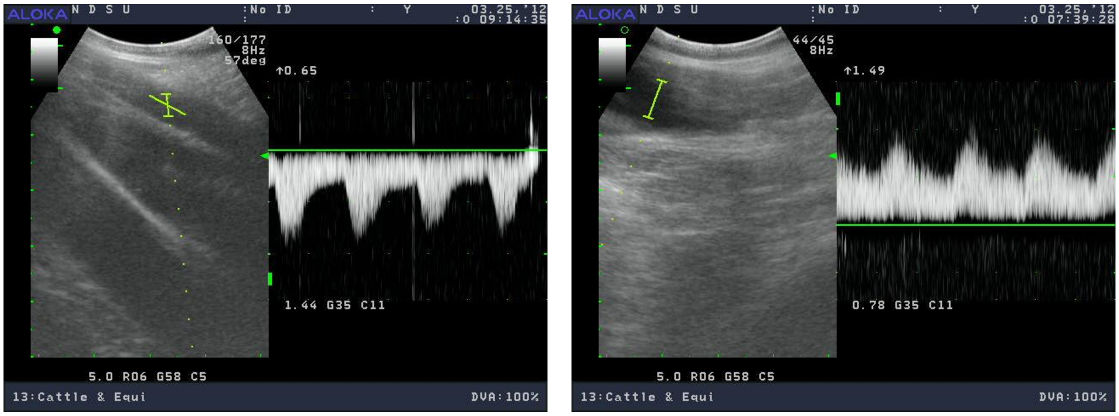

Figure 2.1. Images of uterine arteries obtained by Doppler ultrasonography. Left-hand side image represents the contralateral uterine artery and the right-hand side image represents the ipsilateral uterine artery. Both images were taken from the same cow at the same stage of gestation.

Statistical Analysis

All data were analyzed as a completely randomized design using generalized least squares (mixed procedure, SAS Inst. Inc., Cary, NC). Effects of maternal nutrition on dependent variables were examined by repeated measures analysis within periods (Period 1 = nutrient restriction and Period 2 = realimentation). Factors included in the model were treatment (i.e., CON and RES), day, fetus sire, and treatment × day where day was the repeated variable and cow nested within treatment was the subject. Fetus sire had no influence ($P > 0.25$) on the variables tested and therefore was removed from all model statements. Appropriate (minimize information criterion) covariance structures were used. Linear and quadratic coefficients for day effects were constructed for unequally spaced orthogonal polynomial contrasts for dependent variables. Regression coefficient solutions for significant polynomials were generated using day as a continuous variable (removing day as a classification variable) and is presented in figures.

Results

Cow BW and BCS

During Period 1 (restriction), there was no treatment × day interaction (*P* = 0.68; Figure 2.2 panel A) for maternal BW. However, there was a treatment effect (*P* = 0.04), where RES cows were lighter than CON cows during the restriction period. Also, there was a linear day (*P* < 0.01) response, where BW decreased linearly regardless of nutritional treatment during Period 1. During Period 2, there was no treatment × day interaction (*P* = 0.79) for maternal BW. Similar to Period 1 there was a treatment effect (*P* = 0.05), where RES cow were lighter compared to CON cows during Period 2. Though, there was a linear day (*P* < 0.01) response, where both treatment groups had a linear increase in BW during Period 2. When BW was expressed as percentage BW, there was a treatment × linear day (*P* = 0.04; Figure 2.2 panel B) response, where both treatment groups had a linear decrease in percentage BW change however RES cows had a greater rate of percentage BW change compared to CON cows. During Period 2, there was no treatment × day interaction (*P* = 0.93) or treatment effect (*P* = 0.13). But there was a linear day (*P* = 0.02) response, where both treatment groups had a linear increase in percentage BW change during Period 2. Maternal BCS during Period 1 showed a treatment × linear day (*P* < 0.01; Figure 2.2 panel C) response. Cows from the RES group had a greater linear decrease in BCS compared to CON cows during Period 1. During Period 2, there was no treatment × day interaction (*P* = 0.69) or day effect (*P* = 0.20). However, there was a treatment effect (*P* = 0.02), where RES cows continued to have lower BCS compared to RES cows during Period 2.

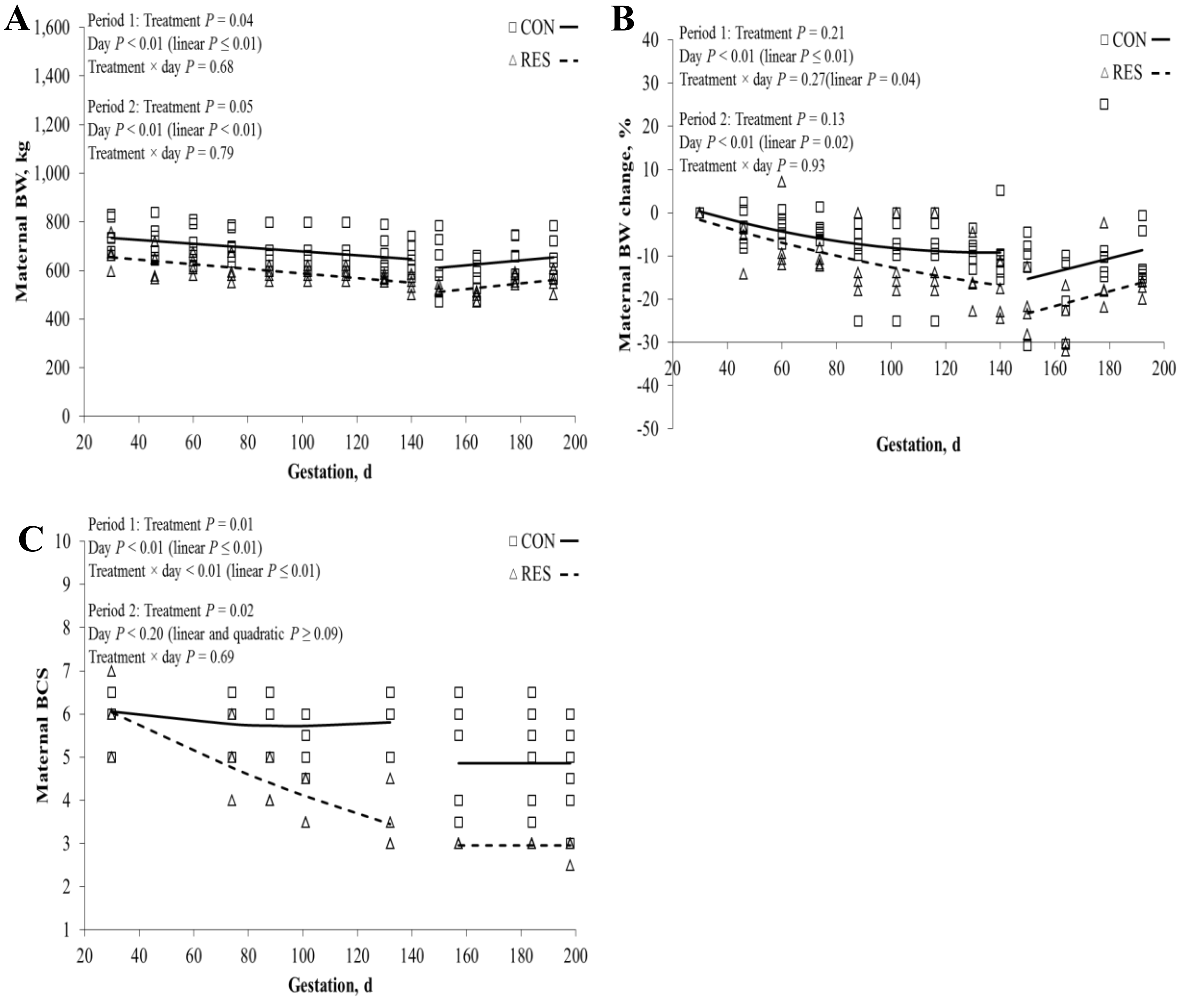

Figure 2.2. Maternal BW (panel A), percentage BW change (panel B), and BCS (panel C) from d 30 to 198 of gestation. Individual cows received either control (100% NRC; CON □) or nutrient restricted diet (60% of NRC from CON; RES △) from d 30 until 140 (Period 1) and thereafter being realimented until d 198 of gestation (Period 2). Data were analyzed within period. During Period 1, best fit line for CON (−) was BW = 0.76 (± 0.02) – 0.0008 (± 0.0001) × d and RES (--) was BW = 0.68 (± 0.03) – 0.0009 (± 0.0001) × d. CON percentage BW change= -0.006 (± 0.004) – 0.00023 (± 0.00009) × d + 0.0000008 (± 0.0000005) × d^2 and RES percentage BW change = -0.004 (± 0.005) – 0.00022 (± 0.00012) × d + 0.0000005 (± 0.0000006) × d^2. CON BCS = 0.0064 (± 0.0004) – 0.000014 (± 0.000009) × d + 0.00000007 (± 0.00000005) × d^2 and RES BCS = 0.0071 (± 0.0005) – 0.000036 (± 0.0000011) × d + 0.00000006 (± 0.00000007) × d^2. During Period 2, CON BW = 0.46 (± 0.10) – 0.0010 (± 0.0006) × d and RES BW = 0.34 (± 0.12) – 0.0012 (± 0.0007) × d. CON percentage BW change = -0.04 (± 0.02) + 0.00016 (± 0.00010) × d and RES percentage BW change = -0.05 (± 0.02) + 0.00017 (± 0.00012) × d. CON BCS = 0.0049 (± 0.0004) and RES BCS = 0.0030 (± 0.0005).

Blood Flow and Resistance Indices

There was no treatment × day interaction (*P* = 0.98; Figure 2.3 panel A) or treatment effect (*P* = 0.81) during Period 1 for ipsilateral uterine artery BF. However, there was a linear and quadratic day response ($P \leq 0.01$), where ipsilateral uterine BF increased during the restriction period. During Period 2, there was no treatment × day interaction (*P* = 0.86). However, there was a treatment effect (*P* = 0.05), where RES cows had greater ipsilateral uterine BF compared to CON after being realimented. There was also a linear day response ($P < 0.01$) for ipsilateral uterine artery BF. Both treatment groups had a linear increase in BF during Period 2. There was no treatment × day interaction (*P* = 0.60; Figure 2.3 panel B) but there was a treatment effect (*P* = 0.04) for ipsilateral uterine artery CSA during Period 1. The RES cows had bigger CSA compared to CON cows. In addition, there was a linear and quadratic response ($P \leq 0.01$) for ipsilateral uterine artery CSA during the restriction period, where CSA increased quadratically, regardless of treatment. Similar to Period 1, there was no treatment × day interaction (*P* = 0.98) but there was a treatment effect (*P* = 0.01) for ipsilateral uterine artery CSA during Period 2. Cows from RES group continued to have greater CSA compared to CON after being realimented. Ipsilateral uterine artery CSA increased linearly ($P < 0.01$) during Period 2 in both treatments. Ipsilateral uterine artery PI during Period 1 did not show a treatment × day interaction (*P* = 0.83; Figure 2.3 panel C) or treatment effect (*P* = 0.38). However, there was a linear day response ($P < 0.01$) for ipsilateral uterine artery PI.

Both treatment groups decreased linearly during the restriction period. During Period 2, there was no interaction or main effect of treatment and day ($P \geq 0.35$) for ipsilateral uterine artery PI. Ipsilateral uterine RI responded similar to PI. There was no treatment × day interaction (*P* = 0.74) or treatment effect for ipsilateral RI (*P* = 0.13; Figure 2.3 panel D) during Period 1.

But there was a linear day (*P* < 0.01) response, where both treatment groups RI decreased linearly during the restriction period. In Period 2, there was no treatment × day, treatment, or day effect (*P* ≥ 0.34) for ipsilateral uterine artery RI.

Contralateral uterine BF is illustrated in Figure 2.4 (panel A). There was no treatment × day interaction (*P* = 0.10) but there was a tendency (*P* = 0.09) for a treatment effect during Period 1. Cows from CON group tended to have greater contralateral uterine BF compared to RES cows. There was a linear day response (*P* < 0.01), where both treatment groups had a linear increase in contralateral uterine BF during the restriction period. In addition, there were treatment × linear and quadratic day responses (*P* ≤ 0.02), where contralateral uterine BF from CON cows increased, whereas RES cows did not change during Period 1. During Period 2, there was no treatment × day interaction (*P* = 0.42). However, there was a tendency (*P* = 0.07) for a treatment effect, where CON cows continued to have greater contralateral uterine BF compared to RES cows after realimentation. There was also a linear day response (*P* = 0.04) for contralateral uterine BF. Cows from CON and RES groups had a linear increase in contralateral uterine BF during Period 2. There was no treatment × day interaction (*P* = 0.60; Figure 2.4 panel B) but there was a treatment effect tendency (*P* = 0.06) for contralateral uterine artery CSA during Period 1. The CON cows had bigger CSA compared to RES cows. There was no day effect (*P* = 0.72) for contralateral uterine artery CSA during the restriction period. Similarly, during Period 2, there was no treatment × day interaction (*P* = 0.86) but there was a treatment effect (*P* < 0.01) for contralateral uterine artery CSA. Cows from CON group continued to have greater CSA compared to RES cows during Period 2. There was no day effect (*P* = 0.72) for contralateral uterine artery CSA. Contralateral uterine artery PI is illustrated in Figure 2.4 (panel C). During Period 1, contralateral uterine PI did not show a treatment × day interaction (*P* =

0.25) or treatment effect (*P* = 0.19). However, there was a linear day response (*P* < 0.01) for contralateral uterine PI. Both treatment groups decreased linearly during the restriction period. During Period 2, there was no interaction or main effect of treatment and day (*P* ≥ 0.15) for contralateral uterine PI. Contralateral uterine RI during Period 1 did not show a treatment × day interaction (*P* = 0.78; Figure 2.4 panel D). However, there was a treatment effect (*P* = 0.05) for contralateral uterine RI during Period 2, where RES cows had increased RI compared to CON cows. Also, there was a linear and quadratic day (*P* ≤ 0.01) response, where RI decreased quadratically during Period 1 in both groups. During Period 2, there was no treatment × day interaction (*P* = 0.87) or day effect (*P* = 0.75) for contralateral uterine RI. However, there was a treatment effect (*P* = 0.04) for contralateral uterine RI where RES cows continued to have increased RI compared to CON cows.

Total uterine BF is illustrated in Figure 2.5 (panel A). During Period 1, there was no treatment × day interaction (*P* = 0.80) or treatment effect (*P* = 0.60) for total BF. However, there was a linear and quadratic day (*P* ≤ 0.01) response for total uterine BF. In both treatment groups total BF increase quadratically during Period 1. Also, there was no treatment × day interaction (*P* = 0.96) or treatment effect (*P* = 0.24) for total uterine artery BF during Period 2. But there was a linear day (*P* < 0.01) response for total BF, where total BF increased linearly during Period 2 regardless of treatment. Maternal HR showed a treatment × quadratic day quadratic (*P* = 0.02; Figure 2.5 panel B) response during Period 1. In addition, there was a linear and quadratic day (*P* ≤ 0.03) response for maternal HR. During Period 2, there was no treatment or day effect (*P* ≥ 0.20) for maternal HR. however, there was a treatment × quadratic day quadratic (*P* = 0.03) response.

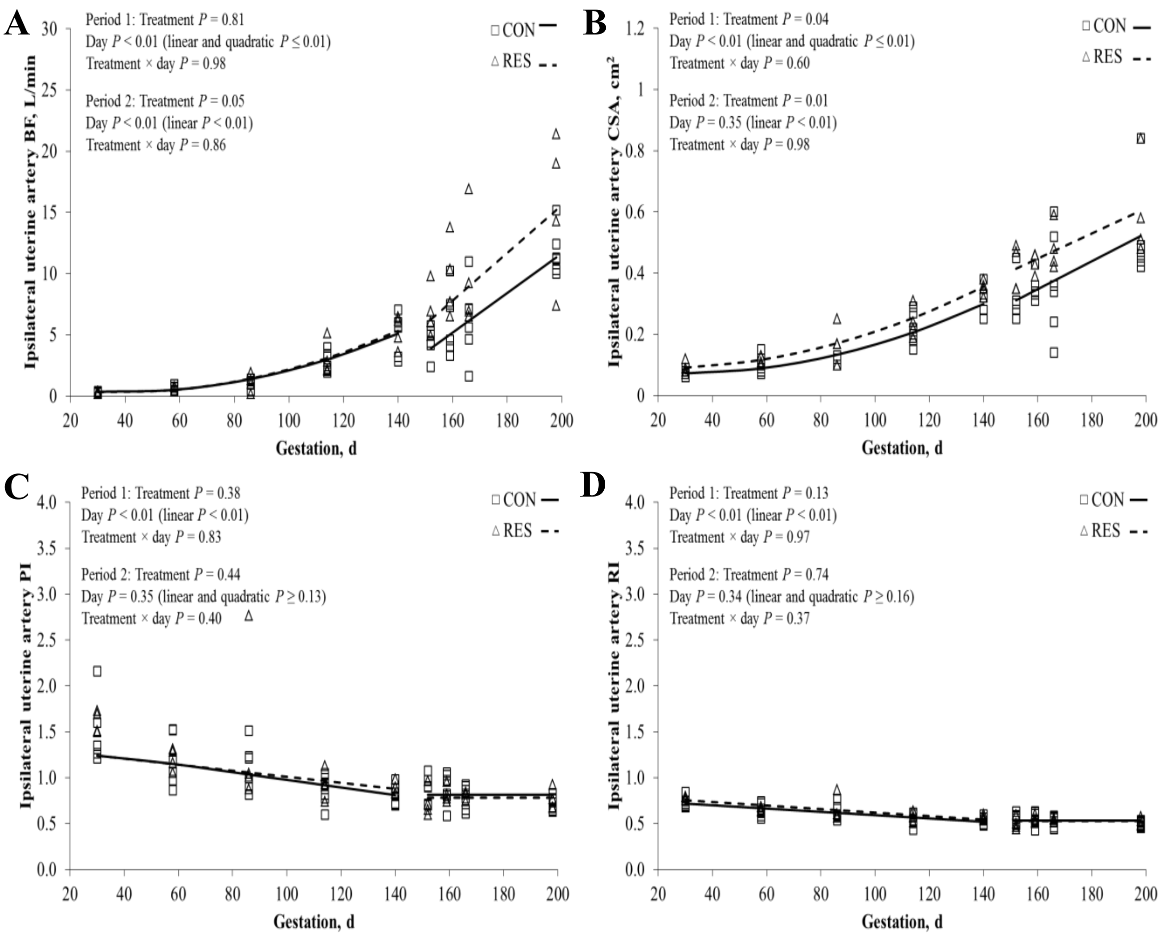

Figure 2.3. Ipsilateral uterine artery blood flow (BF; panel A), cross-sectional area (CSA; panel B), pulsatility index (PI; panel C), and resistance index (RI; panel D) from d 30 until d 198 of gestation. Individual cows received either control (100% NRC; CON ☐) or nutrient restricted diet (60% of NRC from CON; RES △) from d 30 until 140 (Period 1) and thereafter being realimented until d 198 of gestation (Period 2). Data was analyzed within period. During Period 1, best fit line for CON (−) was BF = 0.98 (± 0.18) – 0.035 (± 0.007) + 0.00046 (± 0.00006) and RES (--) was BF = 0.96 (± 0.23) – 0.035 (± 0.008) + 0.00048 (± 0.00007). CON CSA = 0.086 (± 0.024) – 0.0010 (± 0.0008) – 0.000018 (± 0.000004) and RES CSA = 0.094 (± 0.029) - 0.0006 (± 0.0009) + 0.000018 (± 0.000005). PI = 1.39 (± 0.13) – 0.0041 (± 0.0010) and RES PI = 1.33 (± 0.16) – 0.0033 (± 0.0012). CON RI = 0.773 (± 0.030) - 0.00180 (± 0.00032) and RES RI = 0.813 (± 0.037) – 0.00192 (± 0.00039). During Period 2, CON BF = -20.7 (± 4.7) + 0.162 (± 0.032) and RES BF = -23.3 (± 5.8) + 0.195 (± 0.039). CON CSA = -0.38 (± 0.21) + 0.0046 (± 0.0013) and RES CSA = -0.21 (± 0.26) + 0.0041 (± 0.0015). CON PI = 0.815 (± 0.027) and RES PI = 0.782 (± 0.033). CON RI = 0.534 (± 0.011) and RES RI = 0.528 (± 0.014).

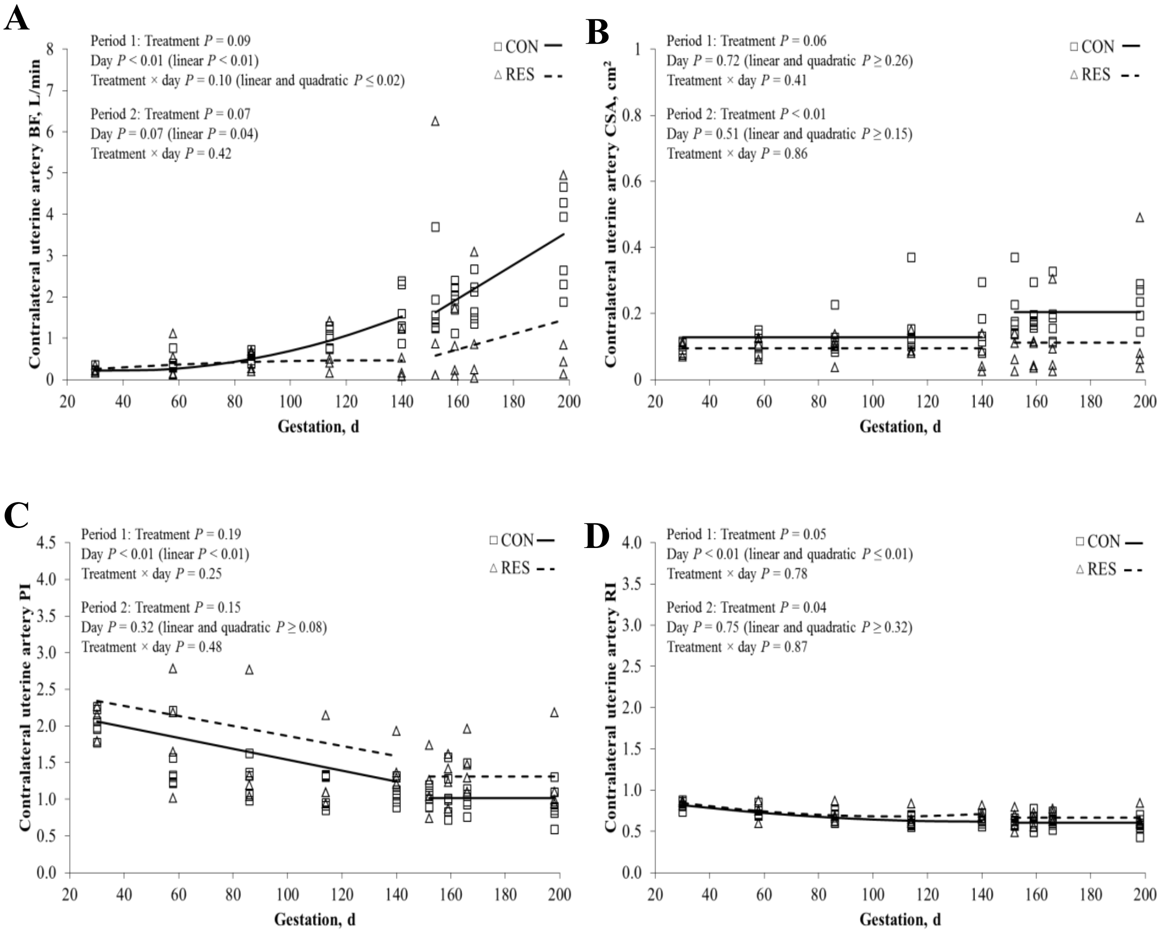

Figure 2.4. Contralateral uterine blood flow (BF; panel A), cross-sectional area (CSA; panel B), pulsatility index (PI; panel C), and resistance index (RI; panel D) from d 30 until d 198 of gestation. Individual cows received either control (100% NRC; CON ☐) or nutrient restricted diet (60% of NRC from CON; RES △) from d 30 until 140 (Period 1) and thereafter being realimented until d 198 of gestation (Period 2). Data was analyzed within period. During Period 1, best fit line for CON (−) was BF = 0.39 (± 0.11) – 0.0096 (± 0.0045) + 0.000127 (± 0.000034) and RES (--) was BF = 0.11 (± 0.14) + 0.0055 (± 0.0055) – 0.000021 (± 0.000042). CON CSA = 0.128 (± 0.010) and RES CSA = 0.095 (± 0.013). CON PI = 2.285 (± 0.056) – 0.00745 (± 0.00030) and RES PI = 2.547 (± 0.077) – 0.00683 (± 0.00042). CON RI = 0.944 (± 0.067) – 0.0047 (± 0.0018) + 0.000017 (± 0.000010) and RES RI = 0.993 (± 0.091) – 0.0058 (± 0.0023) + 0.000027 (±0.000013). During Period 2, CON BF = -4.64 (± 1.61) + 0.041 (± 0.011) and RES BF = -2.27 (± 1.97) + 0.019 (± 0.014). CON CSA = 0.204 (± 0.19) and RES CSA = 0.112 (± 0.023). CON PI = 1.02 (± 0.12) and RES PI = 1.31 (± 0.14). CON RI = 0.605 (± 0.017) and RES RI = 0.666 (± 0.021).

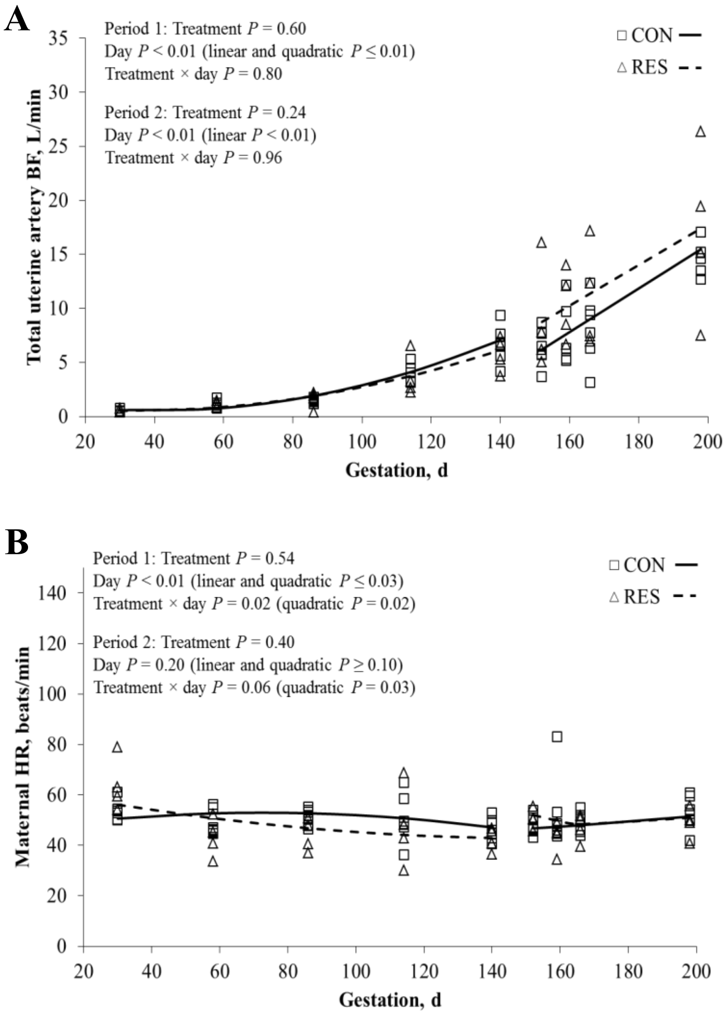

Figure 2.5. Total uterine artery blood flow (BF; panel A) and maternal heart rate (HR; panel B) from d 30 until d 198 of gestation. Individual cows received either control (100% NRC; CON □) or nutrient restricted diet (60% of NRC from CON; RES △) from d 30 until 140 (Period 1) and thereafter being realimented until d 198 of gestation (Period 2). Data was analyzed within period. During Period 1, best fit line for CON (−) was BF = 1.56 (± 0.30) – 0.052 (± 0.011) + 0.00065 (± 0.00009) and RES (--) was BF = 1.01 (± 0.37) – 0.031 (± 0.014) + 0.00048 (± 0.00011). CON HR = 46.2 (± 5.8) + 0.19 (± 0.13) – 0.00128 (± 0.00070) and RES HR = 63.3 (± 0.71) – 0.27 (± 0.16) + 0.00087 (±0.00085). During Period 2, CON BF = -24.50 (± 5.02) + 0.202 (± 0.032) and RES BF = -19.86 (± 6.15) + 0.188 (± 0.039). CON HR = 42.3 (± 67.1) – 0.03 (± 0.78) + 0.0004 (± 0.0022) and RES HR = 275.1 (± 82.2) – 2.58 (± 0.95) + 0.0073 (±0.0027).

Discussion

We reject our hypothesis that during nutrient restriction total uterine BF would be reduced. Moreover, while total uterine BF was similar after realimentation, ipsilateral uterine BF was enhanced in cows that were previously restricted. In many sheep models investigated to date (reviewed in Reynolds et al., 2006; Lemley et al., 2012), nutrient restriction results in reduced uterine and/or umbilical BF. This could be innate species differences, or also due to parity or age of the dam. Regardless, until more beef cattle work is performed to confirm our results, caution should be used when comparing data acquired in sheep as it may not be directly applicable to beef cattle.

To our knowledge this is the first experiment to examine uterine BF during early to mid-gestation in nutrient restricted pregnant cows followed by realimentation during late gestation using color Doppler ultrasonography. Foundational uterine BF work in beef cattle has been conducted using more invasive techniques such as electromagnetic blood flow transducers (Ford and Christensen, 1979) or infusion of deuterium-labeled water (Reynolds and Ferrell, 1987). By using color Doppler ultrasonography to assess uterine BF and vascular resistance throughout gestation in pregnant beef cows, we were able to examine the same animal continuously throughout gestation with no surgical preparation and with minimal interference to dam. Similarly, Bollwein et al. (2000) measured uterine BF in cows during the estrous cycle. They suggested that Doppler ultrasonography was a reliable method to determine uterine BF and did not require use of blood flow probes and/or chronically catheterized animals. Moreover, findings from this study and others suggest that the use of Doppler ultrasonography as the technique to investigate uterine BF during pregnancy may also constitute a reliable method. In several breeds of cattle, when Doppler ultrasonography assessed uterine hemodynamics throughout gestation (i.e., d 30 to 270) RI decreased and uterine BF increased exponentially with increased BF in ipsilateral vs. contralateral horns (Bollwein et al., 2002; Panarace et al., 2006). Moreover, RI was negatively correlated to uterine BF (Bollwein et al., 2002).

The current study suggests that nutrient restriction during early to mid-gestation followed by realimentation impacts uterine BF in pregnant beef cows. Restriction did not alter uterine BF in the horn that carried the fetus (i.e., ipsilateral side); however, upon realimentation, those cows that were previously restricted had increased BF compared to CON cows. Interestingly, resistance indices (RI and PI) were not affected by dietary treatment. The contralateral side responded differently to restriction and realimentation, with CON cows having increased uterine BF compared to RES. Similar to the ipsilateral uterine artery, contralateral PI was not affected by dietary treatment; however, contralateral RI was increased in RES cows compared to CON. Moreover, we observed a concomitant increase in uterine BF with decreasing resistance indices as gestation progressed in both uterine arteries. However, resistance indices were not affected by treatment even though uterine BF was increased. The CSA of both uterine arteries increased in size as gestation progressed, with a more dramatic increase in the ipsilateral (7-fold increase) uterine artery compared to the contralateral (2-fold increase). According to Poiseulle's law, the major determinant impacting flow is the diameter of the artery as diameter is elevated to the 4th power, suggesting that small changes in vessel diameter will have big changes in BF. So while the changes in velocities (i.e., peak systolic, end diastolic, or mean; data not shown) were not major contributors (i.e., there were not major differences of treatment in our RI and PI measurements) BF was still impacted. It is interesting to think why the ipsilateral and contralateral BF responded so differently to nutrient availability. We hypothesize that nutrient restriction may have altered the growth trajectory of the placenta. While the majority of the placenta is surely housed in the side where the fetus is located, we cannot predict the placental occupation in the contralateral side, which may impact BF to the contralateral uterine horn. Perhaps the variation in CSA and BF observed during later gestation in the contralateral horn is due to the variation in placental size. Future studies are necessary to determine how nutrient restriction and realimentation impact the bovine placenta.

Regardless of dietary treatment total uterine BF increased 30-fold from d 30 to d 198 of gestation. In addition, both resistance indices decreased. Research using Doppler technology to assess changes during pregnancy in women suggests that in order to have better placentation and a greater birth weight, uterine artery resistance needs to decrease early for better fetal and maternal health (Carbillon et al., 2004; Gomez et al., 2006). For example, in human pregnancies, when uterine artery resistance remains high or does not decrease during the last third of gestation, there is an association with deficiency in nutrient supply. This also has been related with preeclampsia, intrauterine growth restriction, or, in more severe cases, fetal death (Tamura et al., 2008). Our results in general followed similar uterine BF patterns compared to those reported by Bollwein et al. (2002) and Panarace et al. (2006) when using Doppler ultrasonography. In addition, Reynolds and Ferrell (1987) measured uterine BF at different stages of pregnancy in cows using a steady-state diffusion method. This study showed an exponential increase in uterine BF from d 137 to d 250 of gestation, whereas we calculate a linear increase in the current study during Period 2. The differences between Reynolds and Ferrell’s study and the current study can be attributed to differences in timing for data collection and techniques utilized. Regardless if there is an exponential or linear increase in uterine BF, pregnancy is associated with increases in cardiac output and uterine BF and a reduction in systemic vascular resistance. This increase in uterine BF supports fetal growth during the last trimester of gestation and provides adequate oxygen and nutrient delivery. In sheep during early gestation, the caruncular vascular bed only receives 27% of the total uterine BF. However, of the overall increase in uterine BF during late gestation, more than 85% is directed toward the caruncular vascular beds, which transfer oxygen and nutrients to the placenta and fetus through the adjacent cotyledonary vasculature (Rosenfeld and Fixler, 1977).

The placenta plays an important role in providing physiological exchange between the maternal and fetal systems (Reynolds and Redmer, 1995). During placentation, angiogenesis and vascularization at the fetal-maternal interface is extensive and, subsequently, a rapid increase in uterine and umbilical BF results (Reynolds and Redmer, 1995). In order to support the growth of the developing fetus during late gestation, even though the placenta is not growing as much as early gestation, placental function increases dramatically after mid-gestation (Reynolds et al., 1986). Vonnahme et al. (2007) demonstrated that nutrient restriction during early to mid-gestation (from d 30 to 125 of gestation) did not affect vasculature of the bovine placenta. However, after nutrient restricted cows were realimented, placental vasculature was altered near term, indicating the placenta compensated after restriction. In the current experiment, ipsilateral uterine BF was increased in RES compared to CON cows in late pregnancy, but total uterine BF was not affected by dietary treatment suggesting that the conceptus might be driving the local effect observed in BF. Perhaps placentomes in closer proximity to the fetus function to either produce more vasodilatory or less vasoconstrictive factors that enhance local BF. The ability for the bovine placenta to adapt to nutritional changes warrants further investigation into placentome vascularity and vascular function.

When nutrient availability during pregnancy is reduced, the dam might go through a series of metabolic and physiologic adaptations to protect her body stores from depletion by the conceptus (Rosso and Streeter, 1979). In the current study, cow BCS was decreased through gestation in RES compared to CON cows even after realimentation. However, BW was not affected by nutrient restriction and all cows, regardless of dietary treatment, lost BW from the beginning of the experiment and started gaining BW after realimentation. Control diets were designed to meet or exceed NE requirements; however, CON cows lost BW during early gestation, suggesting that cows had greater nutrient requirements than those estimated or feed energy values were overestimated. Nutrient requirements could be greater than estimated because of several factors which could include differences in environmental conditions, genetics, lactation, or fetal growth (NRC, 2000). Predicting energy values of feeds is difficult and using approaches to predict TDN (Weiss et al., 1992) from diet analysis along with converting TDN values to NE values (NRC, 2000) may overvalue the energy value of lower quality forage (Weiss, 1998) and, therefore, provide less NE than predicted. Previous research has shown that when beef cows are nutrient restricted during pregnancy, realimentation results in increased BW and similar BW to CON are achieved by d 245 of gestation (Meyer et al., 2010). However, Meyer et al. (2010) restricted cows to 68% of NE recommendations and realimented cows above 100% of nutrient requirements to achieve a similar BW to CON animals by d 220 of gestation.

In summary, nutrient restriction from early to mid-gestation does not alter total uterine BF. However, upon realimentation, there is enhanced uterine BF, but only to the horn where the majority of the conceptus is housed. However, it was unknown how much of the conceptus was present in the contralateral horn. It also appears that cattle may respond differently to inadequate nutrition during early to mid-gestation compared to sheep. In addition, results from this experiment suggest that the bovine placenta may be programmed to function differently after a period of nutrient restriction. Perhaps, timely management strategies applied during gestation might enhance conceptus development. Even though more research is necessary, opportunities may be available to intervene during times of poor nutrition. Moreover, further research needs to be done to determine how realimentation at different critical time points impacts uterine BF and conceptus development, during late gestation.

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CHAPTER 3. EFFECTS OF MATERNAL NUTRIENT RESTRICTION FOLLOWED BY REALIMENTATION DURING EARLY AND MID-GESTATION ON BEEF COWS. I. MATERNAL PERFORMANCE AND ORGAN WEIGHTS AT DIFFERENT STAGES OF GESTATION

Abstract

The objectives were to evaluate the effects of nutrient restriction during early to mid-gestation followed by realimentation on maternal performance and organ mass in pregnant beef cows. On d 30 of pregnancy, multiparous, non-lactating cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) were assigned to 1 of 3 dietary treatments: control (C; 100% NRC; n = 18) and restricted (R; 60% NRC; n = 30). On d 85, cows were slaughtered (C, n = 6; R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12), or were realimented to control (RC; n = 11). On d 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254, all remaining cows were slaughtered. Cows were weighed prior to slaughter and all maternal organs were dissected and weighed. The diet consisted of grass hay to meet 100% or 60% NEm recommendations for fetal growth and to meet or exceed recommendations for other nutrients. At d 85 slaughters, BW and empty BW (EBW) were not affected ($P \geq 0.84$) by maternal nutrition. However back fat was decreased ($P = 0.05$) in R vs. C cows. Large intestine and abomasum mass were increased ($P \leq 0.05$) in R cows vs. C. Organ masses and masses relative to EBW for all other tissues collected were similar ($P \geq 0.07$) across treatments on d 85. At d 140, BW was decreased ($P = 0.05$) and EBW was ($P = 0.10$) similar across treatments. Liver mass was decreased ($P = 0.02$) in RR vs. CC with RC being intermediate. Ruminal mass was decreased ($P = 0.003$) in RR vs. CC and RC. Organ masses and masses relative to EBW for all other tissues collected were similar ($P \geq 0.07$)

across treatments. At d 254, BW, EBW, and organ masses were similar ($P \geq 0.08$) across treatments. We observed partial changes in maternal weight and organ masses due to different lengths of maternal nutrient restriction followed by realimentation. It appears that the dam undergoes some adaptations during an early to mid-gestation nutrient restriction and becomes more efficient in the use of nutrients after being realimented and as gestation advances.

Keywords: beef cows, nutrient restriction, organ mass, realimentation

Introduction

Beef cows are commonly managed in grazing systems where forage quality varies according to regional growing conditions. Forage quality or availability is often poor, affecting nutritional and physiological status of the animal (Funston et al., 2010). During this period of reduced nutrient availability, the dam will undergo a series of metabolic and physiologic adaptations to protect some of her body stores from depletion and to supply nutrients to the growing conceptus, which has increased demands as gestation advances (Rosso and Streeter, 1979). Proper placental development allows for regulation of nutrient metabolism and supports fetal growth, while still maintaining maternal homeostasis, through hormone secretion and ability to transfer nutrients (King, 2000). Energy expenditure from liver and gastrointestinal tract accounts for close to 50% of the total energy utilized by ruminants (Ferrell, 1988). Previous research in mature cows has demonstrated an adaptation in energy utilization during nutrient restriction and realimentation (non-pregnant cows, Freetly and Nienaber, 1998; pregnant cows, Freetly et al., 2008). Meyer et al. (2010) reported that visceral organ masses were responsive to both nutrient restriction during early to mid-gestation and realimentation in mid- to late gestation in beef cows. However, little is known about changes in maternal organ mass during different lengths of nutrient restriction, or the effect of realimentation, particularly during early to mid-gestation. Therefore, we hypothesized that maternal organs in pregnant beef cows would be responsive to nutrient restriction from early to mid-gestation, and changes would remain even after nutrient realimentation. Our objectives were to evaluate the effects of nutrient restriction during early to mid-gestation followed by realimentation on maternal BW, BW change, BCS, carcass composition, and organ mass in pregnant beef cows.

Materials and Methods

All procedures involving animals were approved by the North Dakota State University (**NDSU**) Animal Care and Use Committee (#A10001).

Animals, Diets, and Breeding

A total of 54 non-lactating, multiparous crossbred beef cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) predominately of Angus breeding were synchronized using a Select Synch plus progesterone insert (CIDR; Pfizer Animal Health, New York, NY) and fixed-time AI (TAI) protocol. At the NDSU Beef Research and Teaching Unit (Fargo, ND), cows were assigned to 1 of 6 breeding groups (n = 4 to 11 cows per breeding group with all treatments being represented in each breeding group) with breeding dates ranging from July 13 to October 24, 2011. Cows received GnRH (100 µg as 2 mL of Factrel i.m.; Fort Dodge Animal Health, Fort Dodge, IA) and a CIDR on d 0. On d 7 CIDR devices were removed, and cows were given an injection of PGF2α (25 mg as 5 mL of Lutalyse i.m., Pharmacia & Upjohn Co., Kalamazoo, MI). Estrotect Heat Detectors (Rockway Inc., Spring Valley, WI) were used to monitor estrous behavior at least twice daily at 0700 and 1900 for a minimum of 72 h. Artificial insemination was performed utilizing the AM/PM rule 12 h after the first detected estrus (Gonzalez et al., 1985). All cows were bred using semen from one Angus bull (Select Sires, Plain City, OH). Cows not detected in estrus after 72 h received a second GnRH injection and TAI was performed. Cows were fed a hay-based diet during the pre-breeding period. Inseminated cows were transported to the Animal

Nutrition and Physiology Center (ANPC; Fargo, ND; temperature controlled building) within 3 d post-insemination. From arrival at ANPC until confirmed pregnant, cows were grouped in pens (4.87 m^2; n = 4 to 5/pen) and trained to use the Calan gate feeding system. At this time, all cows were fed chopped grass/legume hay [8.0% CP, 69.2% NDF, 41.5% ADF, and 57.9% TDN (DM basis); containing predominately cool season grasses with small amounts of alfalfa] to pass a 15.24-cm screen and a mineral and vitamin supplement (details provided below) to meet NEm recommendations and fetal growth and to meet or exceed recommendations for MP, minerals, and vitamins (NRC, 2000) until pregnancy was confirmed. Hay NEm concentration was predicted using equations described by Weiss (1993) and NRC (2000). Water was available for ad libitum intake.

On d 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). Non-pregnant cows restarted the same breeding protocol; cows were only subjected to AI twice during the experiment; if not pregnant after the second AI, cows were not utilized for the experiment. On d 30 of pregnancy, cows were randomly assigned to dietary treatments (n = 4 to 5/pen with greater than 1 dietary treatment per pen): control (C; 100% NRC; n = 18; Fig. 1) and nutrient restriction (R; 60% NRC; n = 30). On d 85 cows were slaughtered (C, n = 6 and R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12) treatments, or were realimented to control (RC; n = 11). On d 140 cows were slaughtered (CC, n = 6; RR, n = 6; RC, n =Fig. 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254 all remaining cows were slaughtered (CCC, n = 6; RCC, n = 5; RRC, n = 6). An animal from the RC group was removed from the study due to early embryonic loss and a second cow was removed from the RCC group due to a twin pregnancy.

The control diet consisted of grass hay (Table 1) fed to meet 100% NE recommendations for maintenance and fetal growth (NRC, 2000) and to meet or exceed MP, mineral, and vitamin recommendations. Nutrient restricted cows received 60% of the same control hay diet. Cows were individually fed once daily in a Calan gate system at 1000 h and had free access to water. The mineral and vitamin supplement (Trouw Dairy VTM with Optimins; Trouw Nutrition International, Highland, IL; 10% Ca, 5% Mg, 5% K, 2.7% Mn, 2.7% Zn, 1,565,610 IU/kg vitamin A, 158,371 IU/kg vitamin D 3 and 2,715 IU/kg vitamin E) was top-dressed 3 times per week at a rate of 0.18% of hay DMI to meet or exceed mineral and vitamin requirements relative to dietary NE intake (NRC 2000). Cows were weighed weekly at approximately 0800 h throughout the experiment. Initial and final BW were taken on 2 consecutive days. Dietary intake was adjusted relative to BW weekly and to NE requirements for the specific period of gestation (average requirements for periods from d 30 to 85, d 86 to 140, 141 to 197, and d 198 to 254).

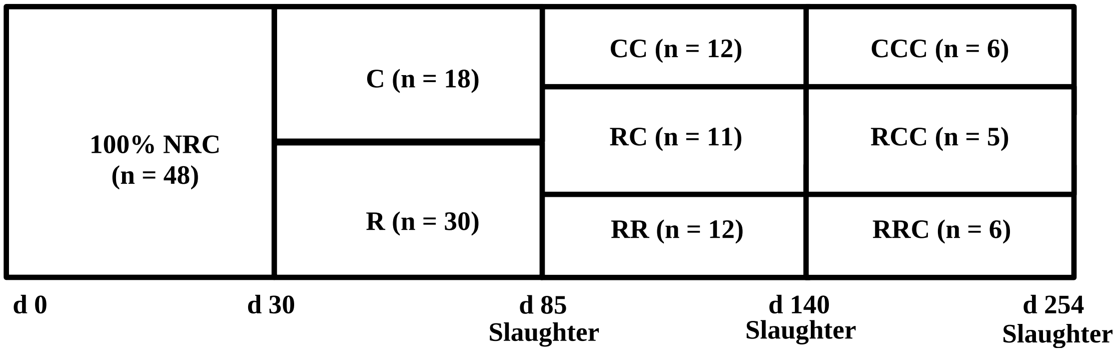

Figure 3.1. Diagram of experimental design. Multiparous, non-lactating beef cows (n = 46) were bred and fed similar diets until d 30 of gestation. On d 30 of pregnancy, cows were randomly assigned to dietary treatments: control (C; 100% NRC; n = 18) and nutrient restriction (R; 60% NRC; n= 30). On d 85 cows were either slaughtered (C; n = 6 and R; n = 6), or remained on control (CC; n = 12), restricted (RR; n = 12) or were realimented to control (RC; n =11). On d 140 cows were ether slaughtered (CC; n = 6; RR; n = 6; RC; n = 5) or remained on control (CCC; n = 6; RCC; n = 5) or realimented to control (RRC; n = 6). On d 254 all remaining cows were slaughtered. An animal from the RC group was removed from the study because early embryonic loss and a second cow was removed from RCC group because the presence of twins. Diets consisted of grass hay and mineral and vitamin supplement at either 100% NRC recommendations for NE for maintenance and fetal growth (NRC, 2000). Nutrient restricted cows received 60% of the same control diet.

Table 3.1. Nutrient analysis of grass/legume[1] hay.

Item, % DM basis	
Ash	11.8
CP	8.1
NDF	69.2
ADF	41.5

[1] Containing predominately cool season grasses with small amounts of alfalfa.

Body Condition Score and Carcass Ultrasonography

Body condition score was obtained using a 1 to 9 scale (with 1 = emaciated and 9 = obese; Wagner et al., 1988) by 4 trained technicians on d 30, 57, 83, 112, 138, 167, 195, and 254 of gestation and their scores were averaged. Carcass ultrasonography measurements were also taken on d 30, 57, 83, 112, 138, 167, 195, and 254 of gestation. Briefly, at the 12th rib and rump, hair was clipped (< 1.27 cm), vegetable oil was applied to the area where the probe was placed, and measurements for back fat thickness at the 12th rib, LM area at the 12th rib, and rump fat thickness were determined using a model 500-SSV ultrasound machine (Aloka, Tokyo, Japan) with a 5 MHz linear transducer probe.

Slaughter Procedure and Tissue Collection

Cows were weighed the day before and the morning of slaughter to obtain a 2 d average live BW (d 85, 140, and 254 ± 2 d SD). All weights were obtained prior to morning feeding and cows were not fed on the morning of slaughter. Cows were transported from ANPC to the NDSU Meat Laboratory approximately 30 min prior to slaughter. No more than 2 cows were slaughtered per day due to Meat Laboratory capacity and time constraints of sample collection (slaughters ranged from November 2011 until April 2012). On the day of slaughter, cows were stunned with a captive bolt and exsanguinated. The gravid uterus was immediately removed and weighed, then the hide was removed and cows were eviscerated. A total viscera weight (including digestive tract contents) was then obtained. The liver, spleen, and pancreas were dissected from the mesentery and associated tissues and were weighed. The digestive tract was stripped of fat and digesta, and the stomach complex and small and large intestines were dissected and weighed. The stomach complex was then divided into the reticulum, rumen, omasum, and abomasum, and each component was weighed. The heart, lungs, kidneys, perirenal fat, and adrenals were dissected from the carcass and weighed. The carcass was split at the 12th and 13th rib, and LM muscle area (ribeye area; **REA**) was determined using a USDA beef measuring grid. A fat thickness measurement was made at a point three-fourths of the way up the length of the LM from the split chine bone. Average LM area and fat thickness was determined from both left and right sides of each carcass to determine an average.

Calculations

Percentage of BW change was calculated as (final BW - initial BW) divided by initial BW times 100, where initial BW was BW at d 30 of gestation. Initial BW was an average of a 2 d weight. Digesta weight was calculated by difference (total full viscera weight − visceral tissues after stripping of digesta contents). Empty BW (**EBW**) was determined by subtracting digesta weight and gravid uterus from the final BW obtained before slaughter. Stomach complex weight was calculated as the sum of the empty rumen, reticulum, omasum, and abomasum weights.

Statistical Analysis

Organ weight data were analyzed as a completely randomized block (breeding group) design within slaughter day (d 85, 140, or 254 of gestation) using a mixed model (SAS Inst. Inc., Cary, NC). In addition, effects of maternal nutrition on BW, % BW change, BCS, and carcass ultrasonography measurements were examined using repeated measures analysis of the mixed procedure. Factors included in the repeated measures model were treatment, day, and the treatment × day interaction. Means were separated using the PDIFF option of the LSMEANS statement of SAS and were considered significant when $P \leq 0.05$. In the absence of interactions ($P > 0.05$) for the repeated measures model, significant main effects are reported; otherwise, interactive means are discussed.

Results and Discussion

Cow BW and BW Change

At d 85 there was no treatment × day interaction ($P = 0.12$; Figure 3.2 panel A) for cow BW. Regardless of nutritional treatment, all cows lost BW after initiation of the experiment until d 85 (day effect, $P < 0.001$). There was a treatment × day interaction ($P = 0.04$; Figure 3.2 panel B) for maternal BW change (%) for cows slaughtered at d 85. Cow BW change was similar ($P \geq 0.13$) from d 30 to 72 of gestation, but BW loss was greater ($P \leq 0.05$) in R than in C cows on d 79 and 85 of gestation.

There was a treatment × day interaction ($P = 0.02$; Figure 3.3 panel A) for cow BW in dams slaughtered at d 140. From d 30 to 100 of gestation, cow BW was similar ($P \geq 0.18$) among treatments. From d 107 to 140 RR cows had a decreased ($P \leq 0.04$) BW compared to CC, with RC being intermediate ($P \geq 0.13$). When cow BW was expressed as percentage change, there was also a treatment × day interaction ($P = 0.01$; Figure 3.3 panel B). Cow BW percentage change was similar ($P \geq 0.07$) among treatments from d 30 to 44 of gestation. From d 51 to 65 of gestation, the RR group had greater ($P \leq 0.03$) BW loss compared with CC and RC being intermediate ($P \geq 0.07$). From d 72 to d 93, the RR group tended to have greater ($P \leq 0.06$) BW loss compared with CC and RC cows. At d 100, RR group had greater ($P = 0.01$) BW loss compared with CC and RC being intermediate ($P \geq 0.21$). From d 107 to 135 of gestation RC and RR had greater ($P \leq 0.04$) BW loss compared with CC. On d 140, RR cows had the greatest

BW loss ($P < 0.05$), followed by RC, and CC had the least ($P \leq 0.04$) percentage BW change (RR = -18.78%; RC = -13.12%; CC = -6.68 %; SEM = 2.92%).

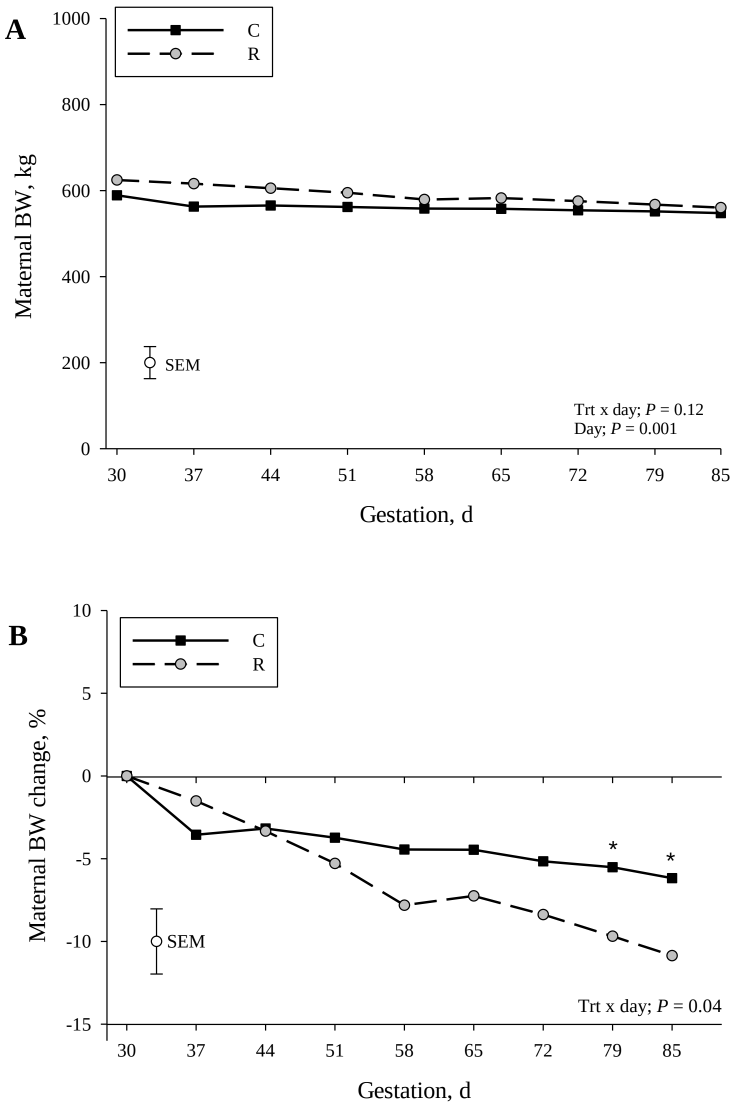

Figure 3.2. Cow BW (kg; panel A) and BW change (%; panel B) of cows slaughtered at d 85 of gestation. Cows received either control diet (100% NRC; C) or restricted from d 30 to 85 (60% NRC; R). There was no treatment × day interaction ($P = 0.12$) or treatment main effect ($P = 0.49$) for cow BW but there was a day effect ($P < 0.001$). There was a treatment × day interaction ($P = 0.04$) for cow BW change. *C cows different ($P < 0.05$) from R. The SEM is average of SEM for treatment × day interaction.

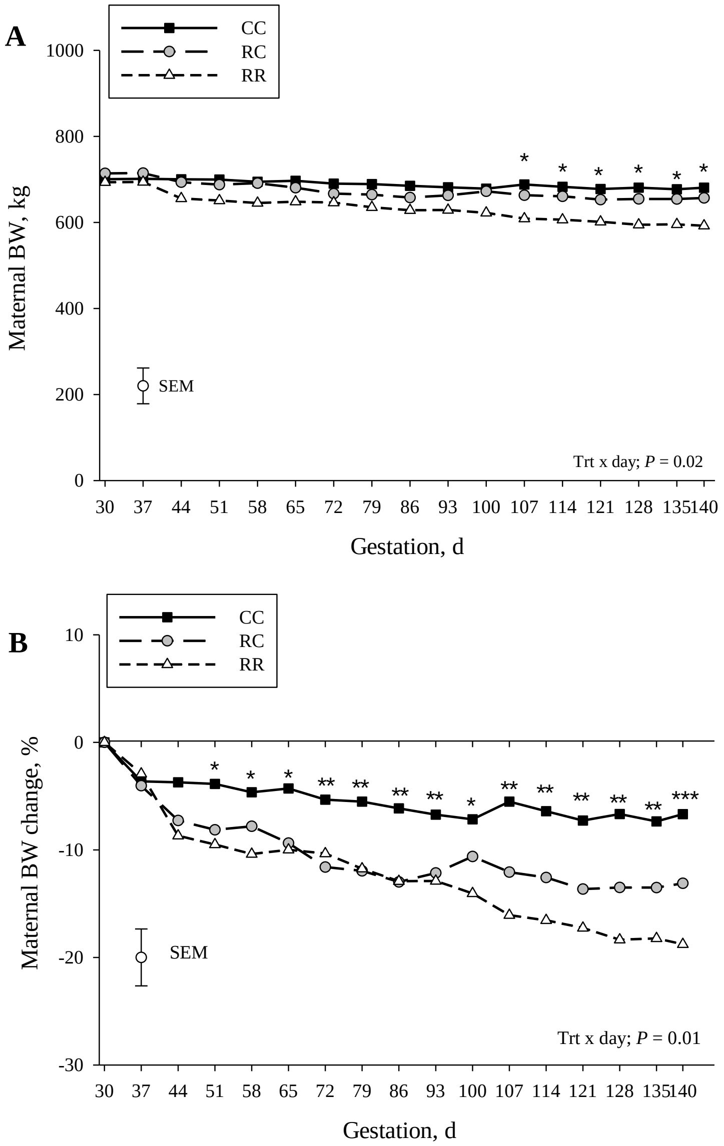

Figure 3.3. Cow BW (kg; panel A) and BW change (%; panel B) of cows slaughtered at d 140 of gestation. Cows received the control diet (100% NRC) from d 30 until 140 (CC), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 140 (RC), and restricted from d 30 to 140 (60% NRC; RR). There was a treatment × day interaction ($P \leq 0.02$) for cow BW and BW change. *CC cows significantly different ($P < 0.05$) from RR and RC being intermediate; **CC cows different ($P < 0.05$) from RR and RC; ***CC cows different ($P < 0.05$) from RC and RC different from RR. The SEM is average of SEM for treatment × day interaction.

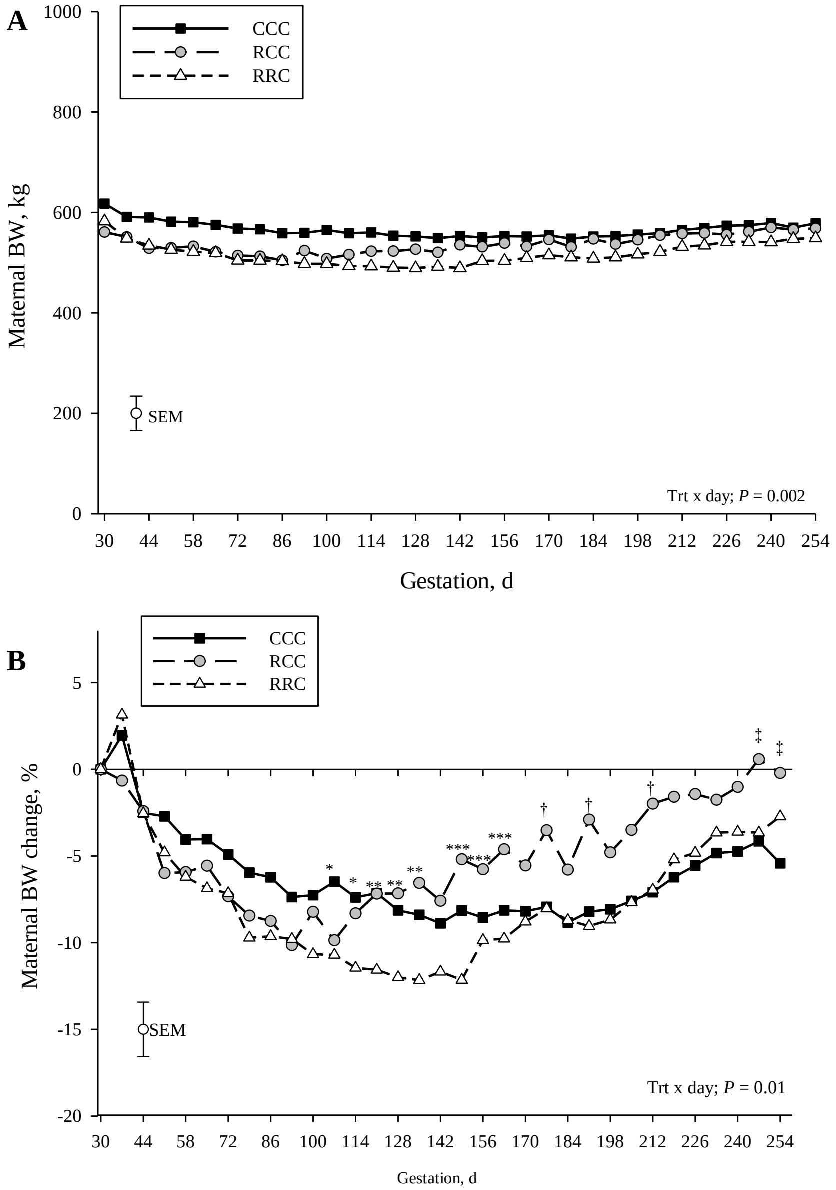

Figure 3.4. Cow BW (kg; panel A) and BW change (%; panel B) of cows slaughtered at d 254 of gestation. Cows received the control diet (100% NRC) from d 30 until 254 (CCC), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RCC), and restricted from d 30 to 140 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RRC). There was a treatment × day interaction ($P \leq 0.01$) for cow BW and BW change. *CCC cows different ($P < 0.05$) from RRC and RCC being intermediate; **CCC and RCC cows different ($P < 0.05$) from RRC; ***RCC cows different ($P < 0.05$) from RRC and CCC being intermediate; †RCC cows different ($P < 0.05$) from CCC and RRC; ‡CCC cows different ($P < 0.05$) from RCC and RRC being intermediate. The SEM is average of SEM for treatment × day interaction.

There was a treatment × day interaction (*P* = 0.002; Figure 3.4 panel A) for BW on cows slaughtered at d 254 of gestation. However, upon further means separation no significant differences existed among groups. There was also a treatment × day interaction (*P* = 0.01; Figure 3.4 panel B) for percentage BW change of cows slaughter at d 254. From d 30 to 100 of gestation, maternal percentage BW change was not affected ($P \geq 0.14$) by treatment. From d 107 to 114 of gestation, RRC cows had decreased ($P < 0.05$) percentage BW change compared to CCC cows and RCC being intermediate ($P \geq 0.13$). From d 121 to 135 RRC cows had greater ($P \leq 0.05$) BW loss than CCC and RCC cows. At d 142 there was no difference ($P \geq 0.19$) among treatments. From d 149 until 163 RRC cows had increased ($P < 0.05$) BW loss than RCC, with CCC being intermediate ($P \geq 0.12$). At d 170, percentage BW change was similar (*P* = 0.15) among treatments and by d 177 RCC cows had increased (*P* = 0.05) percentage BW change compared to CCC and RRC. All treatment groups were similar ($P \geq 0.18$) at d 184. By d 191 RCC had greater (*P* = 0.02) percentage BW change compared to RRC and CCC. From d 198 to 205 there was no difference ($P \geq 0.07$) among treatments. At d 212 RCC had greater ($P \leq 0.03$) percentage BW change compared to RRC and CCC. From d 219 to 240 treatment groups did not differ ($P \geq 0.10$). By d 247 until 254, RCC cows had greater ($P < 0.03$) increased percentage BW change compared to CCC cows and RRC being intermediate ($P \geq 0.09$).

Nutrient restriction at d 85 of gestation did not affect BW; however, percentage BW loss was greater towards the end of nutrient restriction compared to control cows. At d 140 of gestation cows that were restricted for a longer period of time lost more BW and percentage BW change was also greater compared to other treatments. At d 254 of gestation, longer nutrient restriction (restriction from d 30 to 140 of gestation) lead to greater loss in percentage BW change, and those cows that were realimented during early gestation had the greatest increase in

BW change compared with other treatments. Control diets were calculated to meet or exceed NE requirements; however, control cows lost BW, especially during early gestation, suggesting that cows had greater nutrient requirements than estimated. This could be due to several factors including differences in genetics or fetal growth (NRC, 2000); however, we had similar cattle and they were bred to the same bull. Previous research in beef cows has shown a decrease in BW during nutrient restriction from d 30 to 125; however, these cows had increased BW after realimentation and were similar in BW to controls by d 245 of gestation (Meyer et al., 2010). However, Meyer et al. (2010) restricted animals to a predicted 68.1% of NE recommendations and realimented cows above NRC NE recommendations to accomplish this.

Cow BCS and Carcass Ultrasonography Measurements

No treatment × day interaction ($P \geq 0.48$) or main effect of treatment ($P \geq 0.37$) was observed for BCS for cows slaughtered at d 85, 140, or 254 of gestation. For cows slaughtered at d 85 of gestation, there was a day effect ($P = 0.02$), where the average BCS for all cows was 4.4 ± 0.6 at d 30 of gestation, decreased to ($P = 0.02$) to 4.1 ± 0.6 by d 57 and then remained similar ($P = 0.77$) until d 85 of gestation. No interactions or main effects of treatment or day ($P \geq 0.18$; data not shown) for carcass ultrasound traits (12th rib back fat thickness and REA) were observed for the group slaughtered on d 85. Average 12th rib back fat thickness at d 30 was 0.72 ± 0.23 cm and 0.65 ± 0.23 cm at d 85 and REA at d 30 was 79.9 ± 7.13 cm^2 and 76.7 ± 7.1 cm^2 at d 85 of gestation. Cows slaughtered at d 140 of gestation also showed a day effect ($P < 0.001$) where the average BCS was 5.1 ± 0.1 at d 30, and decreased to 4.5 ± 0.1 by d 140 of gestation. There was no treatment × day interaction or main effect of treatment ($P \geq 0.13$; data not shown) on 12th rib back fat thickness and REA for groups slaughtered at d 140. Initial 12th rib back fat thickness and REA were 0.60 ± 0.12 cm and 80.8 ± 5.3 cm^2 respectively. At d 140, 12th rib back fat thickness and REA were 0.45 ± 0.08 cm and 71.4 ± 4.1 cm^2 respectively. However, there was a day effect ($P = 0.04$) for 12th rib back fat thickness and REA for cows slaughtered at d 140, where both measures decreased as gestation advanced.

In addition, there was no day effect ($P = 0.07$) for BCS on cows slaughtered at d 254. The average BCS for all the cows at d 30 was 4.8 ± 0.2 and 4.9 ± 0.2 prior to slaughter at d 254. There was no treatment × day interaction or main effect of treatment ($P \geq 0.13$; data not shown) for 12th rib back fat thickness and REA for groups slaughtered at d 254. However, cows slaughtered at d 254 showed a day effect ($P < 0.001$; average of 0.80 ± 0.16 cm at d 30 and 0.42 ± 0.07 cm at d 254) for 12th rib back fat thickness and day effect for REA ($P < 0.0001$; average 92.4 ± 4.3 cm^2 at d 30 and 60.5 ± 4.3 cm^2 at d 254). Both ultrasonography measurements decreased across time during gestation (from d 30 to 254). Even though BCS did not change through gestation for cows slaughtered at d 254 of gestation, carcass 12th rib back fat and REA measured by ultrasonography decreased from early to late gestation regardless of dietary treatment. Perhaps cows were mobilizing muscle and fat stores in order to keep up with nutrient demands from the growing conceptus.

The observed change in BW and percentage BW change in our current study was not accompanied by a change in BCS by nutrient restriction. Camacho et al. (2013) showed a decrease in BCS in cows that were nutrient restricted from d 30 until d 140 compared to controls, and BCS continued to decrease in restricted cows even after nutrient realimentation until d 198 of gestation. The differences between these 2 studies could be attributed to breed, environmental conditions and/or lactation (Camacho et al., 2013 used Simmental multiparous lactating cows that were in an outdoor facility). Interestingly, all cows slaughtered at d 85 and 140 of gestation started losing BCS around d 57 of gestation until their slaughter day. However, cows slaughtered at d 254 of gestation had similar BCS regardless of treatment, which may have been due to the realimentation. Cows slaughtered at d 254 were realimented for a longer period of time compared with cows that were slaughtered on d 140. Miller et al. (2004) reported a decreased in BCS by d 45 in cows that were nutrient restricted from d 30 to d 140 of gestation compared with controls. However, BW in those cows followed a similar pattern as BCS. In addition, Miller et al. (2004) realimented cows to achieve a similar BCS by d 220 of gestation; thus, it is difficult to compare the effects of nutrient realimentation with our study.

Cow Composition and Organ Mass at Slaughter

Cow BW and EBW at d 85 slaughter were not affected ($P \geq 0.84$; Table 3.2) by maternal nutrient restriction. Carcass REA did not differ ($P = 0.97$) between treatments, whereas 12th rib back fat thickness was decreased ($P = 0.05$) in R cows compared with C cows. While the majority of organ weights were similar ($P \geq 0.10$) between C and R cows, weights of the abomasum and large intestine were altered. Abomasum and large intestinal weights were greater ($P = 0.05$) in R cows compared with C at d 85. Similarly, when expressed relative to EBW, R cows tended ($P = 0.07$) to have greater abomasum and large intestinal weights compared with C cows.

Body weight, EBW, and REA were not affected by maternal nutrient restriction when cows were slaughtered at d 85. At this time point, gestating cows were restricted for only 55 d compared to C cows. However, 12th rib back fat was decreased in R cows compared to C, therefore, R cows might be mobilizing fat in order to compensate for the nutrient restriction. The only organ masses that were impacted by this 55 d restriction included abomasum and large intestine.

Table 3.2. The effects of nutrient restriction on maternal organ weight in beef cows at d 85 of gestation

	Nutritional treatments[1]			
Item	C	R	SEM	*P*-value
BW, kg	556.5	550.3	36.9	0.88
EBW[2], kg	419.4	426.8	28.7	0.84
REA, cm^2	64.5	69.7	4.32	0.42
Back fat at 12th rib, cm	0.66	0.33	0.23	0.05
Gravid uterus, kg	2.51	2.80	0.20	0.19
Heart, kg	1.87	1.88	0.09	0.96
g/kg EBW	4.47	4.44	0.32	0.95
Lung, kg	3.12	3.90	0.39	0.10
g/kg EBW	7.61	9.05	1.02	0.15
Adrenals, kg	0.03	0.03	0.01	0.37
g/kg EBW	0.07	0.08	0.01	0.52
Kidneys, kg	1.10	1.13	0.05	0.67
g/kg EBW	2.67	2.67	0.14	0.98
Perirenal fat, kg	1.64	1.12	0.69	0.43
g/kg EBW	3.71	2.46	1.45	0.40
Stomach complex, kg	15.8	15.8	0.75	0.99
g/kg EBW	38.0	37.4	1.39	0.77
Abomasum, kg	1.85	2.16	0.09	0.05
g/kg EBW	4.48	5.13	0.22	0.07
Omasum, kg	5.75	6.02	0.83	0.71
g/kg EBW	13.8	13.7	1.24	0.96
Reticulum, kg	1.25	1.22	0.06	0.73
g/kg EBW	2.99	2.89	0.13	0.58
Rumen, kg	6.85	6.78	0.51	0.88
g/kg EBW	16.5	15.4	1.84	0.46
Small intestine, kg	4.12	4.43	0.17	0.24
g/kg EBW	10.0	10.5	0.62	0.58
Large intestine, kg	3.43	4.40	0.20	0.01
g/kg EBW	8.45	10.11	0.83	0.07
Spleen, kg	0.57	0.67	0.07	0.19
g/kg EBW	1.40	1.55	0.18	0.40
Liver, kg	3.50	4.05	0.53	0.46
g/kg EBW	8.71	9.55	1.45	0.66

[1] Cows received either control (100% NRC) diet (C; n = 6) or restricted (60% NRC) from d 30 to 85 (R; n = 6).

[2] EBW: empty BW = final BW – (gravid uterus + digesta).

[3] REA: LM muscle area (ribeye area).

Table 3.2. The effects of nutrient restriction on maternal organ weight in beef cows at d 85 of gestation (continued)

Item	Nutritional treatments[1]			
	C	R	SEM	*P*-value
Pancreas, kg	0.39	0.44	0.04	0.39
g/kg EBW	0.94	1.04	0.07	0.37
Omental and mesenteric fat, kg	11.2	10.6	2.41	0.85
g/kg EBW	28.22	25.58	7.19	0.74

[1] Cows received either control (100% NRC) diet (C; n = 6) or restricted (60% NRC) from d 30 to 85 (R; n = 6).

[2] EBW: empty BW = final BW – (gravid uterus + digesta).

[3] REA: LM muscle area (ribeye area).

Similarly, Meyer et al. (2010) found greater relative abomasum weight (g/kg of EBW) in cows that were restricted from d 30 until d 125 compared to controls. However, they did not observe differences in large intestine weight. In ewe lambs that were nutrient restricted during gestation, a decrease in large intestine mass was observed (Reed et al., 2007). Scheaffer et al. (2001) did not find differences in large intestine weight as pregnancy progressed in growing heifers suggesting that this organ is not affected by stage of pregnancy or by the demands of the growing fetus. The lack of differences in organ masses in the current study is not surprising as BW was similar between treatments when cows were slaughtered at d 85. Perhaps, mature cows, as used in the current study, is a less sensitive model to nutrient restriction during early gestation than using young ewes. While percentage BW change started decreasing towards the end of the restriction period this was not due to observable changes in organ mass. During nutrient restriction an important adaptation that has been observed in sheep is the reduction in visceral organ masses (Burrin et al., 1990; Reed et al., 2007). This might be an adaptation to the reduced nutrient intake in order for the animal to survive (Ferrell and Jenkins, 1985, Reed et al., 2007).

When cows were slaughtered at d 140, BW was decreased (*P* = 0.05; Table 3) and EBW tended to decrease (*P* = 0.10) in RR compared with CC cows (*P* = 0.04), with RC being intermediate (*P* ≥ 0.15; Table 3.3). Carcass REA and back fat at the 12th rib were not affected (*P* ≥ 0.62; Table 3) by treatment. A greater number of organ masses were influenced by maternal diet at d 140 than was observed at d 85. While abomasal and large intestinal masses were not different by d 140 (Table 3), there were differences in liver and rumen weights, with tendencies (*P* ≤ 0.10) observed in small intestinal, total stomach complex, and reticulum weights. Liver weight (kg) was decreased (*P* = 0.02) in RR cows compared with CC (*P* = 0.006) and RC being intermediate (*P* ≥ 0.20). However, when expressed relative to EBW, liver weight (g/kg) was not affected (*P* = 0.55) by treatment at d 140. At d 140 of gestation, rumen weight was decreased (*P* = 0.003) in RR compared with CC and RC cows.

When rumen was expressed relative to EBW, RR cows tended (*P* = 0.10) to have greater relative weights than CC and RC at d 140 of gestation. Reticulum and total stomach complex weights (kg) tended (*P* = 0.09) to decrease in RR cows compared with CC (*P* = 0.03), with RC being intermediate (*P* ≥ 0.12). However, when expressed relative to EBW, reticulum or total stomach complex weights were not affected (*P* = 0.50) by treatment at d 140 of gestation.

Longer nutrient restriction decreased BW compared to control cows that were slaughtered at d 140 of gestation. We observed decreased 12th rib back fat in R cows at d 85 slaughter; however, when a similar length of restriction (55 d) was followed by realimentation there was no difference in 12th rib back fat at d 140 slaughter. Surprisingly, carcass REA and 12th rib back fat were not affected in cows that were nutrient restricted for 110 d. There was also a tendency for EBW to follow a similar pattern as BW.

Table 3.3. The effects of realimentation after nutrient restriction on maternal organ weight in beef cows at d 140 of gestation

	Nutritional treatments[1]				
Item	CC	RC	RR	SEM	*P*-value
BW, kg	609.2[a]	584.2[ab]	527.4[b]	28.6	0.05
EBW[2], kg	450.5	439.8	399.6	24.5	0.10
REA[3], cm^2	64.3	63.1	64.6	4.45	0.97
Back fat at 12th rib, cm	0.43	0.28	0.15	0.20	0.21
Gravid uterus, kg	11.0	11.3	11.7	0.50	0.86
Heart, kg	2.08	2.06	2.00	0.10	0.84
g/kg EBW	4.69	4.83	5.01	0.28	0.70
Lung, kg	3.54	3.40	3.67	0.20	0.66
g/kg EBW	7.95	8.00	9.18	0.62	0.21
Adrenals, kg	0.04	0.05	0.03	0.01	0.51
g/kg EBW	0.08	0.12	0.08	0.03	0.54
Kidneys, kg	1.20	1.07	1.11	0.06	0.32
g/kg EBW	2.70	2.52	2.79	0.18	0.60
Perirenal fat, kg	1.81	1.90	1.25	0.53	0.66
g/kg EBW	4.04	4.72	2.95	1.28	0.48
Stomach complex, kg	17.4	15.4	14.8	0.77	0.09
g/kg EBW	39.0	36.2	37.0	1.90	0.50
Abomasum, kg	2.01	1.88	2.81	0.59	0.52
g/kg EBW	4.51	4.49	6.51	1.05	0.34
Omasum, kg	5.58	4.69	4.80	0.58	0.49
g/kg EBW	12.3	10.5	12.4	1.63	0.55
Reticulum, kg	1.47	1.35	1.18	0.08	0.09
g/kg EBW	3.32	3.18	2.94	0.19	0.39
Rumen, kg	8.29[a]	7.47[a]	6.05[b]	0.34	0.003
g/kg EBW	18.7	17.8	15.2	1.07	0.10

[a,b] Means without a common superscript letter differ, $P < 0.05$.

[1] Cows received the control diet (100% NRC) from d 30 until 140 (CC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 140 (RC; n = 5), and restricted from d 30 to 140 (60% NRC; RR; n = 6).

[2] EBW: empty BW = final BW – (gravid uterus + digesta).

[3] REA: LM muscle area (ribeye area).

Table 3.3. The effects of realimentation after nutrient restriction on maternal organ weight in beef cows at d 140 of gestation (continued)

	Nutritional treatments[1]				
Item	CC	RC	RR	SEM	*P*-value
Small intestine, kg	4.56	4.30	3.85	0.26	0.10
g/kg EBW	10.2	10.1	9.66	0.59	0.81
Large intestine, kg	3.52	3.71	3.35	0.29	0.61
g/kg EBW	7.93	8.75	8.26	0.60	0.65
Spleen, kg	0.71	0.59	0.70	0.06	0.41
g/kg EBW	1.06	0.89	0.98	0.06	0.21
Liver, kg	4.37^{a}	4.01^{ab}	3.74^{b}	0.15	0.02
g/kg EBW	9.79	9.40	9.34	0.36	0.55
Pancreas, kg	0.47	0.38	0.40	0.03	0.17
g/kg EBW	1.06	0.89	0.98	0.06	0.21
Omental and mesenteric fat, kg	11.2	10.6	7.47	3.10	0.16
g/kg EBW	23.9	23.1	18.4	5.96	0.40

[a,b] Means without a common superscript letter differ, $P < 0.05$.

[1] Cows received the control diet (100% NRC) from d 30 until 140 (CC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 140 (RC; n = 5), and restricted from d 30 to 140 (60% NRC; RR; n = 6).

[2] EBW: empty BW = final BW – (gravid uterus + digesta).

[3] REA: LM muscle area (ribeye area).

Differences observed in abomasum and large intestine weight when cows were slaughtered at d 85 were not present when cows were restricted from d 30 until d 85 and then realimented and slaughtered at d 140 of gestation. It is difficult to explain why nutrient restriction for 55 d increased mass of these organs but restriction for 110 d did not. Perhaps after a certain period of nutrient restriction, the dam no longer has large enough nutrient reserves (from body energy and protein mobilization) to draw from to increase visceral organ mass in an attempt to maintain fetal tissue development. The liver and rumen were the only organs for which weight was affected by nutrient restriction at d 140, where both organ masses were decreased in RR cows compared to CC and RC being intermediate. Similar to our data, Meyer et al. (2010) reported decreased liver and rumen weight in nutrient restricted cows from d 30 to 125 of gestation. Scheaffer et al. (2001) reported no differences in liver weight in pregnant heifers suggesting that the liver might not increase due to stage of pregnancy. Previous research in sheep indicated that liver mass increases in pregnant mature ewes compared to non-pregnant ewes (Scheaffer et al., 2004). In vitro oxygen consumption studies have shown that hepatic oxygen consumption decreases as feed intake is reduced (Burrin et al., 1989). However, hepatic oxygen consumption has been shown to increase in mature ewes as gestation progresses (Freetly and Ferrell, 1997). In addition, previous researchers have demonstrated that the decrease in oxygen consumption is partially driven by a decrease in tissue weight (Ferrell and Koong, 1985). It has been shown that pregnant beef heifers have decreased oxygen consumption in the ileum and decreased energy use in ileum and total small intestine due to pregnancy (Scheaffer et al., 2003). This might suggest an adaptation from the dam to conserve energy during gestation.

All cows were fed to meet 100% of NE requirements from d 140 to 254. By d 254, maternal BW and EBW were not affected ($P \geq 0.78$; Table 3.4) by treatment. Also, carcass REA and back fat at 12th rib were similar ($P \geq 0.72$) among treatments. There were no impacts of maternal diet on organ weights; however, there was a tendency ($P = 0.08$) for kidney and pancreas ($P = 0.10$) weight to be altered. Kidney weight (kg) tended to be greater ($P = 0.08$) in RCC cows compared with CCC, with RRC being intermediate. However, when expressed relative to EBW, kidney weight did not differ ($P = 0.55$) among treatments. Pancreas weight tended to be smaller ($P = 0.08$) in RRC cows compared with RCC, with CCC being intermediate. However, when expressed relative to EBW, pancreas weight did not differ ($P = 0.21$) among treatments.

When cows were slaughtered at d 254 of gestation, BW, EBW, and carcass ultrasonography measurements were not different among treatments. This could be because the mature cows used in this experiment were less sensitive to nutrient restriction and/or compensatory BW gain after nutrient realimentation compared to control cows. In addition, the few differences previously observed due to restriction at d 85 and 140 slaughters disappeared. This is probably due to realimentation occurring either at d 85 or 140 of gestation for both treatments that previously were nutrient restricted. Perhaps cows were able to recover from the different periods of nutrient restriction after nutrient realimentation. When nutrient restricted cows were allowed approximately 125 d of realimentation, Meyer et al. (2010) reported no differences in organ weights of cows. Even though the realimentation protocol in our experiment was designed to meet NRC NE requirements, whereas the realimentation protocol of Meyer et al. (2010) allowed for cows to be realimented above NRC recommendations to achieve a similar BCS to control animals, we observed similar results by d 254. In a sheep model of nutrient restriction during mid-gestation followed by a realimentation period (control; 100% NRC recommendations) in late gestation, restricted ewe lambs had greater relative liver, pancreas, reticulum, stomach, small intestine, and large intestine weight than the control ewes during late pregnancy (Carlson et al., 2009). The differences between this study and ours could be due to species differences, time of restriction and/or age. Carlson et al. (2009) used gestating ewe lambs which were still growing and at the same time providing nutrients to the growing fetus.

Energy expenditure from liver and gastrointestinal tract accounts for close to 50% of the total energy utilized by ruminants (Ferrell, 1988). In addition, during pregnancy maternal oxygen consumption changes reflecting the energy requirements needed for tissue accretion and fetal and maternal metabolism (Stock and Metcalfe, 1994). After nutrient restriction, maintenance requirements for the whole animal have been shown to decrease and a time interval is required to reach a steady-state again (Koong and Nienaber, 1985). In addition, nutritional plane has been shown to have an effect on maintenance energy requirements and is highly correlated with changes of visceral organs weights (Ferrell et al., 1986).

Table 3.4. The effects of realimentation after nutrient restriction on maternal organ weight in beef cows at d 254 of gestation

	Nutritional treatments[1]				
Item	CCC	RCC	RRC	SEM	*P*-value
BW, kg	624.2	622.0	605.8	31.2	0.85
EBW[2], kg	420.9	419.3	402.3	22.0	0.78
REA[3], cm^2	56.2	53.4	55.5	6.06	0.92
Back fat at 12th rib, cm	0.11	0.08	0.18	0.07	0.62
Gravid uterus, kg	59.1	59.5	65.4	3.40	0.37
Heart, kg	2.06	1.93	1.82	0.12	0.30
g/kg EBW	4.85	4.68	4.50	0.23	0.57
Lung, kg	3.66	2.99	3.17	0.31	0.13
g/kg EBW	8.43	7.28	7.82	0.63	0.48
Adrenals, kg	0.03	0.32	0.04	0.19	0.51
g/kg EBW	0.08	1.10	0.11	0.66	0.51
Kidneys, kg	1.27	1.13	1.23	0.06	0.08
g/kg EBW	3.00	2.78	3.03	0.17	0.55
Perirenal fat, kg	1.27	1.80	0.99	0.35	0.24
g/kg EBW	3.02	4.08	2.50	0.77	0.31
Stomach complex, kg	18.1	17.3	17.0	0.65	0.49
g/kg EBW	43.7	42.0	42.3	1.93	0.80
Abomasum, kg	2.14	2.23	2.19	0.13	0.86
g/kg EBW	5.09	5.49	5.42	0.39	0.76
Omasum, kg	5.56	5.04	5.03	0.28	0.35
g/kg EBW	13.5	12.2	12.6	0.76	0.54
Reticulum, kg	1.72	1.38	1.34	0.17	0.25
g/kg EBW	4.21	3.36	3.35	0.45	0.35
Rumen, kg	8.68	8.63	8.42	0.32	0.83
g/kg EBW	21.0	20.9	21.0	0.73	0.99

[a,b] Means without a common superscript letter differ, $P < 0.05$.

[1] Cows received the control diet (100% NRC) from d 30 until 254 (CCC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RCC; n = 5), and restricted from d 30 to 140 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RRC; n = 6).

[2] EBW: empty BW = final BW – (gravid uterus + digesta).

[3] REA: LM muscle area (ribeye area).

Table 3.4. The effects of realimentation after nutrient restriction on maternal organ weight in beef cows at d 254 of gestation (continued)

	Nutritional treatments[1]				
Item	CCC	RCC	RRC	SEM	*P*-value
Small intestine, kg	4.68	5.01	4.59	0.15	0.19
g/kg EBW	11.1	12.4	11.5	0.85	0.56
Large intestine, kg	4.35	4.43	3.81	0.21	0.11
g/kg EBW	10.5	10.9	9.48	0.80	0.44
Spleen, kg	0.60	0.58	0.58	0.05	0.94
g/kg EBW	1.41	1.39	1.43	0.08	0.94
Liver, kg	4.31	4.33	4.54	0.19	0.67
g/kg EBW	10.3	10.4	11.3	0.46	0.20
Pancreas, kg	0.48	0.52	0.39	0.04	0.10
g/kg EBW	1.13	1.24	0.98	0.09	0.21
Omental and mesenteric fat, kg	10.68	9.49	7.92	1.61	0.49
g/kg EBW	25.2	21.9	19.7	3.18	0.49

[a,b] Means without a common superscript letter differ, $P < 0.05$.

[1] Cows received the control diet (100% NRC) from d 30 until 254 (CCC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RCC; n = 5), and restricted from d 30 to 140 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RRC; n = 6).

[2] EBW: empty BW = final BW – (gravid uterus + digesta).

[3] REA: LM muscle area (ribeye area).

A very important adaptation during pregnancy is the increase in blood volume (Robson et al., 1989; Thornburg et al., 2006), cardiac output (Stock and Metcalfe, 1994), and decreased total peripheral resistance in the pregnant female (Thornburg et al., 2006). Pregnancy also requires an increase in organ workload, particularly in the gastrointestinal tract in order to keep up with the increase in nutrient demands by the conceptus (Ferrell, 1988; Thornburg et al., 2006). Some adaptations by the gastrointestinal tract are increases in organ mass or functional capacity (Stock and Metcalfe, 1994). This increase in the workload of the maternal organs, results in increased maternal energy requirements (Ferrell, 1988). Perhaps a restricted diet during early gestation programs the dam to be more efficient with the resources that it may or may not have as gestation continues. In our particular experiment, realimentation appeared to supply the dam with sufficient nutrients for the developing conceptus, as gravid uterine weight was similar across treatments near term. So in other words, even though the demands of the early conceptus for nutrients from the dam may be minimal, perhaps we are programming the way the maternal system will utilize its available nutrients throughout the remainder of gestation. This may be a reason why we observed few changes in organ weights (especially after realimentation) in the current study.

In summary, nutrient restriction from early to mid-gestation followed by realimentation in pregnant beef cows impacts maternal BW and organ masses. Our observations differ from those previously reported using pregnant sheep as a model. Therefore, cows might adapt differently to nutrient restriction than ewes, perhaps being less sensitive to reduced nutrient supply. Further investigations on maternal metabolic changes and effects of nutrient restriction followed by realimentation during early to mid-gestation on conceptus development are necessary.

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CHAPTER 4. EFFECTS OF MATERNAL NUTRIENT RESTRICTION FOLLOWED BY REALIMENTATION DURING EARLY AND MID-GESTATION ON BEEF COWS. II. PLACENTAL DEVELOPMENT AT DIFFERENT STAGES OF GESTATION AND UTERINE BLOOD FLOW DURING LATE GESTATION

Abstract

The objectives were to examine the effects of maternal nutrient restriction followed by realimentation during early to mid-gestation on placental development and uterine and umbilical blood flow (BF). On d 30 of pregnancy, multiparous, non-lactating cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) were assigned to 1 of 3 dietary treatments: control (C; 100% NRC; n = 18) and restricted (R; 60% NRC; n = 30). On d 85, cows were slaughtered (C, n = 6; R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12), or were realimented to control (RC; n = 11). On d 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254, all remaining cows were slaughtered. Experiment 1: Umbilical and uterine hemodynamics were determined via Doppler ultrasonography in a subset of cows (CCC, RCC, and RRC). Measurements included uterine BF, resistance index (**RI**), and pulsatility index (**PI**). Experiment 2: Prior to slaughter umbilical hemodynamics were assessed (in C, R, CC, RC, and RR cows) and placental tissues were collected at slaughter (d 85, 140, and 245). Experiment 1, there was no treatment by day interaction ($P > 0.20$) for umbilical BF and RI measured from d 60 to 140 of gestation; but, there was a treatment effect for umbilical PI ($P = 0.05$) where RRC cows had decreased PI vs. RCC and CCC being intermediate. During late gestation, RCC cows had greater ($P = 0.02$) ipsilateral and total uterine BF vs. CCC and RRC. Experiment 2, at d 85 slaughter R cows had more and heavier ($P \leq 0.02$) placentomes vs. C cows. Umbilical BF relative to fetal

BW was also decreased ($P = 0.03$) in R vs. C cows. At d 140 slaughter, there were more placentomes ($P = 0.03$) in RR vs. CC and RC cows, but placentome weight was not affected ($P = 0.18$) by maternal dietary treatment. Fetal HR, umbilical PI, and BF were not different ($P > 0.21$) among treatments, but RI tended (P = 0.06) to increase in RR vs. CC and RC cows. Maternal nutrient restriction during early to mid-gestation increased fetal growth and placentome weight, however continued restriction did not alter fetal weight. Realimentation appears to enhance uterine blood flow and may compensate for reduced nutrients early in gestation

Keywords: maternal restriction, placenta, umbilical blood flow, uterine blood flow

Introduction

Intrauterine growth restriction is associated with altered fetal organ development and subsequent performance of offspring (Godfrey and Barker, 2000; Wu et al., 2006). As postnatal growth is largely dependent upon fetal growth and development in utero, it is important to determine how management decisions could impact the growth trajectory of the bovine fetus. Even during the time of early embryonic development, when nutrient requirements appear trivial for conceptus growth, maternal nutrition can alter organogenesis and establishment of the placenta, which are imperative for proper prenatal growth and development (Robinson et al., 1999).

The placenta plays a major role in the regulation of fetal growth. Placental nutrient transport efficiency is directly related to utero-placenta blood flow (Reynolds and Redmer, 1995). Gases, nutrients, and metabolic end products are exchanged between maternal and fetal circulation via the placenta (Bleul et al., 2007; Reynolds and Redmer, 1995; Reynolds and Redmer, 2001). Increases in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on growth of the placenta during early pregnancy followed by dramatic development and reorganization of the uteroplacental vasculature during the last half of gestation (Meschia, 1983; Reynolds and Redmer, 1995). If an insult (i.e. maternal nutrient restriction) during early gestation impairs placental development, calf development during later pregnancy could be altered. We have shown (Chapter 3) that the dam undergoes adaptations during an early to mid-gestation nutrient restriction. It appears that the multiparous cow becomes more efficient in the utilization of nutrients after being realimented and as gestation advances providing sufficient nutrients for the developing conceptus (Chapter 3).

We have previously reported (Chapter 2) that nutrient restriction from d 30 to 140 of gestation does not alter total uterine BF. However, upon realimentation, ipsilateral uterine BF is enhanced until d 198 of gestation. It is still unclear how realimentation after various lengths of restriction would impact uterine blood flow during late gestation. Results from our previous experiment suggest that the bovine uteroplacenta may compensate during the nutrient restriction and be programmed to function differently after the insult. Therefore, we hypothesized that nutrient restriction during early to mid-gestation in beef cows would impact placental development throughout gestation. Moreover, we further hypothesized that upon nutrient realimentation the placenta would become more efficient and uterine BF would surpass the BF from control animals. The specific objective was to examine the effect of maternal nutrient restriction followed by realimentation during early to mid-gestation on umbilical and uterine artery hemodynamics as well as placental development.

Materials and Methods

Animals and Management

All procedures involving animals were approved by the North Dakota State University (**NDSU**) Animal Care and Use Committee (#A10001).

Animals, Diets, and Breeding

A total of 54 non-lactating, multiparous crossbred beef cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) of similar genetic background were synchronized using a Select Synch plus progesterone insert (CIDR; Pfizer Animal Health, New York, NY) and fixed-time AI (TAI). Breeding protocol has been previously published (Chapter 3). Inseminated cows were transported to the Animal Nutrition and Physiology Center (ANPC; Fargo, ND) within 3 d post-insemination. On d 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). Non-pregnant cows restarted the same breeding protocol; cows were only subjected to AI twice during the experiment; if not pregnant after the second AI, cows were not utilized for the experiment. On d 30 of pregnancy, cows were randomly assigned to dietary treatments: control (C; 100% NRC; n = 18; Figure 1) and nutrient restriction (R; 60% NRC; n = 30). On d 85 cows were slaughtered (C, n = 6 and R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12) treatments, or were realimented to control (RC; n = 11). On d 140 cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254 all remaining cows were slaughtered (CCC, n = 6; RCC, n = 5; RRC, n = 6). An animal from the RC group was removed from the study due to early embryonic loss and a second cow was removed from the RCC group due to a twin pregnancy.

The control diet consisted of chopped grass/legume hay [8.0% CP, 69.2% NDF, 41.5% ADF, and 57.9% TDN (DM basis); containing predominately cool season grasses with small amounts of alfalfa] to pass a 15.24-cm to meet 100% NEm recommendations and fetal growth (NRC, 2000) and to meet or exceed MP, mineral, and vitamin recommendations. Hay NEm concentration was predicted using equations described by Weiss (1993) and NRC (2000). Nutrient restricted cows received 60% of the same control hay diet. Cows were individually fed once daily in a Calan gate system at 1000 h and had free access to water. The mineral and vitamin supplement (Trouw Dairy VTM with Optimins; Trouw Nutrition International, Highland, IL; 10% Ca, 5% Mg, 5% K, 2.7% Mn, 2.7% Zn, 1,565,610 IU/kg vitamin A, 158,371 IU/kg vitamin D 3 and 2,715 IU/kg vitamin E) was top-dressed 3 times per week at a rate of 0.18% of hay DMI to meet or exceed mineral and vitamin requirements relative to dietary NE intake (NRC 2000). Cows were weighed weekly at approximately 0800 h throughout the experiment and dietary intake was adjusted relative to BW and NE requirements for the specific period of gestation (average requirements for periods from d 30 – 85, d 86 – 140, 141 – 197, and d 198 - 254).

Experiment 1

Ultrasonography Evaluation. Fetal and placental characteristics, as well as uterine and umbilical hemodynamic measurements were evaluated by ultrasonography in a subset of cows that were on study (i.e. all the CCC, RCC, and RRC cows). Fetal measurements were obtained on d 50, 60, 85, 95, and 105. Placental measurements were obtained on d 60, 85, 95, and 105. . Briefly, average placentome diameter was determined by selecting 10 placentomes randomly and. the diameter at the largest position was recorded. After d 105 of gestation, measurements were stopped because the sizes of the placentomes exceeded the visible view of the monitor.

Fetal growth parameters were measured at d 50, 60, 85, 95, and 105. Because of the depth of the uterus and the size of the fetus, measurements were not taken after d 105. Fetal abdominal diameter and biparietal distance were recorded.

Umbilical hemodynamics were obtained via color-Doppler ultrasonography (Aloka SSD-3500; Aloka America, Wallingford, CT, USA) at 0700 h from at d 60, 85, 95, 105, 140, and 150 for cows slaughtered at d 254. Briefly, a transrectal-finger probe (~5 x 2 cm; Aloka UST-672; 7.5 MHz) was inserted into the rectum and the umbilical cord was located. In B-mode using the linear transducer, a longitudinal section of the umbilical cord was visualized by manually turning the transducer of the probe. The probe was aligned with the umbilical cord at an average angle of insonation of 58 ± 3 degrees. Once an adequate portion of umbilical cord was identified, the sample gate cursor was placed over the umbilical artery whereas at the same time pulsatile waves in D mode (Doppler spectrum) were recorded (average time of ultrasonography examination was 12.0 ± 0.7 min).

To examine uterine BF, the probe was inserted through the rectum and the aorta was located (Chapter 2). In B mode using the finger probe, the origin of the external iliac, ipsilateral to the gravid uterine horn, was located and the transducer was moved caudally to locate the internal iliac artery. The maternal umbilical artery begins as a major branch of the internal iliac, and gives rise to the uterine artery (Bollwein et al., 2000). After the uterine artery was identified as a movable and pulsating artery, a longitudinal section was visualized by manually turning the transducer of the probe. The probe was aligned to the uterine artery at an average angle of insonation of 79 ± 0.2 degrees and uterine artery hemodynamic measurements were collected.

For uterine artery BF, 3 similar cardiac cycle waveforms from 3 separate ultrasonography evaluations from each side (ipsilateral and contralateral uterine artery) and 3 separate ultrasonography evaluations for umbilical artery were obtained with spectral Doppler and averaged per cow within a gestational day (i.e. 9 measurements per side/artery per sampling day). Maternal and fetal heart rate (**HR**), pulsatility index (**PI**), resistance index (**RI**), and uterine and umbilical artery blood flow (**BF**) were calculated by pre-programmed Doppler software where PI = (peak systolic velocity - end diastolic velocity)/mean velocity; RI = (peak systolic velocity - end diastolic velocity)/ peak systolic velocity; and BF (mL/min) = mean velocity (cm/s) x (π/4) x cross-sectional diameter (cm^2) x 60 s. Total BF was calculated as the sum of ipsilateral and contralateral uterine artery BF.

***Statistical Analysis*.** For placental and fetal growth parameters via ultrasonography and umbilical and uterine BF the repeated measures analysis of the MIXED procedure of SAS (SAS software version 9.2, SAS Inst., Cary, NC) was used. The model included treatment, day, and treatment by day interaction. Breeding group was used as a block and appropriate covariance structures were selected.

Experiment 2

***Ultrasonography Evaluation*.** In addition to the umbilical hemodynamics obtained as described above, additional data were collected via color-Doppler ultrasonography at 0700 h on the day prior to d 85 and 140 slaughters. Umbilical measurements on d 254 were not recorded as it was not logistically possible to obtain an image of the umbilical cord due to the location and size of the fetus. All methods are similar as Experiment 1, and the average angle of insonation of 58 ± 3 degrees. Umbilical BF obtained prior to d 85 and 140 slaughters was divided by fetal, total placentome, and cotyledonary weight to obtain measurements to reflect indices of placental function. These calculations are important because they reflect umbilical BF relative to fetal and placental weight and with this estimation we have a better idea how much BF is being received per gram of tissue (fetal and placental).

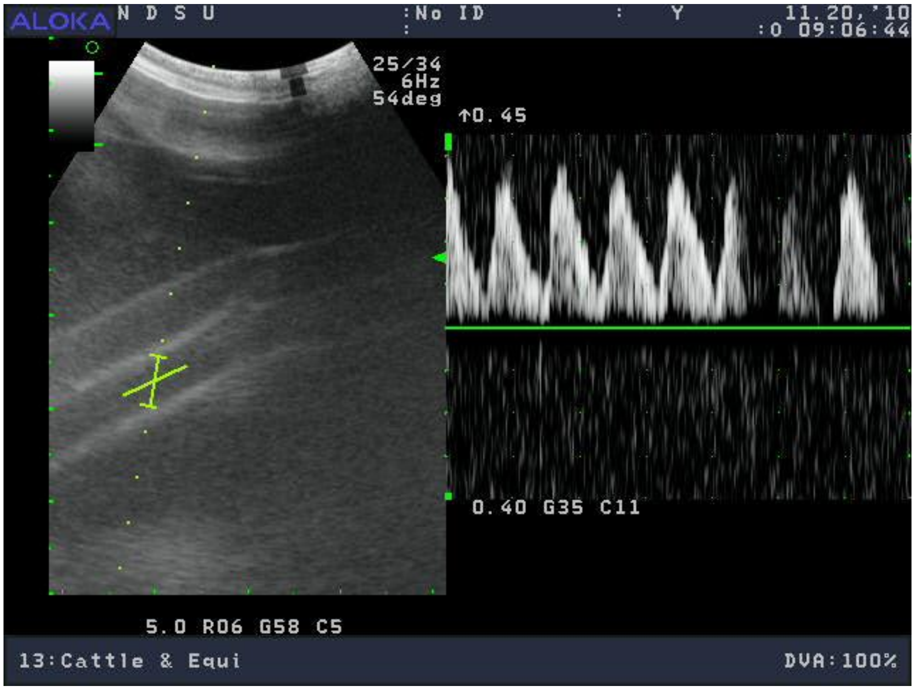

Figure 4.1. Image of umbilical artery blood flow obtained via Doppler ultrasonography at d 140 of gestation.

Slaughters and Tissue Collection. A subset of pregnant cows from each breeding group was randomly selected for slaughter at d 85, 140, and 254 (± 2 d SD). Cows were transported from ANPC to the NDSU Meat Laboratory approximately 30 min prior to slaughter. No more than 2 cows were slaughtered per day due to time constraints of sample collection (slaughters ranged from November 2011 until April 2012). On the day of slaughter, cows were stunned with a captive-bolt gun and exsanguinated. The gravid uterus was immediately collected and weighed. The fetus was immediately removed from the placenta at the umbilicus and weighed.

Chorioallantoic and amniotic fluids were combined and volume was recorded. After each individual placentome was weighed, placentome dimensions [length **(l)**, width **(w)**, depth **(d)**] were measured using digital calipers and were separated manually into cotyledonary and caruncular portions. The mass of total cotyledonary tissue and total caruncular tissue was recorded. Average placentome density (g/cm^3) was calculated as placentome weight divided by placentome volume (l × w × d). Placental efficiency was calculated as fetal weight divided by total placentome weight. After all placentomes and fetal membranes were removed, the uterus was reweighed to obtain an empty uterine weight.

***Statistical Analysis*.** Placental and fetal measurements and umbilical BF at slaughter were analyzed using the MIXED procedure of SAS (SAS software version 9.2, SAS Inst., Cary, NC). The model statement included treatment and fetal sex. When a significant treatment effect was detected ($P \leq 0.05$), treatment differences were separated using the PDIFF option of the LSMEANS statement.

Results

Experiment 1

Placental and Fetal Growth Parameters via Ultrasonography. There was a treatment × day interaction ($P = 0.04$; Figure 4.2) for placentome diameter obtained via Doppler ultrasonography from d 60 to 105 of gestation. From d 60 to d 95, placentome diameter was similar ($P \geq 0.39$) among treatment groups. But at d 105 of gestation, RRC cows had greater ($P \leq 0.05$) placentome diameter compared to CCC and RCC cows. In addition, there was a day effect ($P < 0.01$) where regardless of maternal dietary treatment placentome diameter was increasing as gestation progressed.

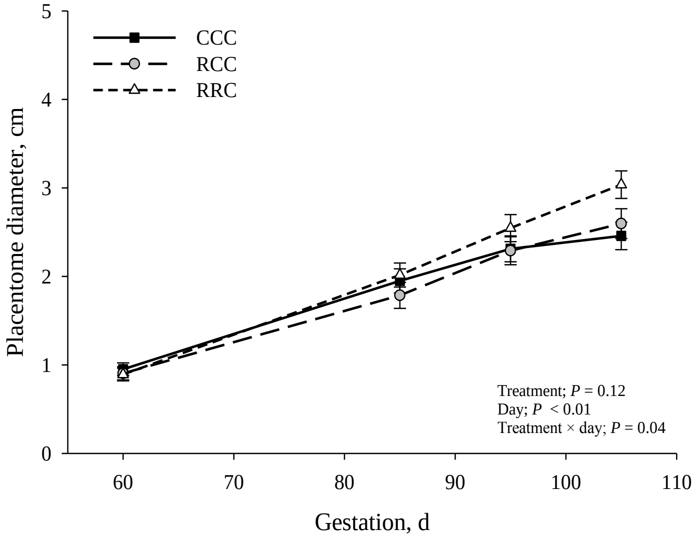

Figure 4.2. Placentome diameter via Doppler ultrasonography during d 60 to 105 of gestation. The CCC cows (n = 6) received 100% NRC from d 30 to 105; RCC cows (n = 5) received 60% NRC from d 30 to 85 and 100% NRC to d 105; RRC cows (n = 6) received 60% NRC from d 30 to 105.

There was no treatment × day interaction (*P* = 0.89; data not shown) for fetal abdominal diameter obtained via Doppler ultrasonography from d 50 to 105 of gestation. In addition, there was no treatment effect (*P* = 0.19) for abdominal diameter. However, there was a day effect (*P* < 0.01) where fetuses from all treatment groups were larger as gestation progressed. Average biparetal distance in fetuses at d 50 and 105 of gestation were 1.15 ± 0.02 and 4.58 ± 0.09 cm, respectively.

For fetal biparietal distance there was no treatment × day interaction (*P* = 0.32; data not shown). In addition, treatment did not influence (*P* = 0.21) biparietal distance. However, there was a day effect (*P* < 0.01) biparietal distance where fetuses from all treatment groups were increased as gestation progressed. Fetal average biparietal distance at d 50 and 105 of gestation was 1.02 ± 0.03 and 3.25 ± 0.09 cm, respectively.

Umbilical BF from d 60 to 150 of Gestation. There was no treatment × day interaction (*P* = 0.36; data not shown) for umbilical artery PI from d 60 to 140 of gestation. However, there was a treatment effect (*P* = 0.05) where RRC cows had decreased PI compared to RCC cows and CCC being intermediate. There was also a day effect (*P* < 0.01) for umbilical artery PI where fetuses had decreased umbilical artery PI as gestation progressed from d 60 (average PI = 1.62 ± 0.05) to d 140 (average PI = 1.21 ± 0.05). There was no treatment × day interaction (*P* = 0.52; data not shown) or treatment effect (*P* = 0.15) for umbilical artery RI from d 60 to 140 of gestation. However, there was a day effect (*P* = 0.01) for umbilical artery RI. Umbilical artery RI was 0.70 ± 0.01 at d 60, 0.75 ± 0.01 at d 105, and 0.72 ± 0.01 at d 150 of gestation.

Fetal HR was measured from d 85 to d 150 of gestation. There was no treatment × day interaction (*P* = 0.20; data not shown) or treatment effect (*P* = 0.94) for fetal HR. However, there was a day effect (*P* < 0.01) where fetal HR was 175 ± 3 beats/min at d 85 and 144 ± 3 beats/min at d 140 of gestation. Umbilical artery BF was measured from d 85 to d 140 on cows that were slaughtered at d 254 of gestation. There was no treatment × day interaction (*P* = 0.74; data not shown) or treatment effect (*P* = 0.88) for umbilical artery BF. However, there was a day effect (*P* < 0.01) where umbilical artery BF increased from d 85 (average umbilical BF = 40.4 ± 4.3 ml/min) to d 140 (average umbilical BF = 232.0 ± 40.6 ml/min) of gestation.

Late Gestation Uterine BF. There was no treatment × day interaction (*P* = 0.36; Figure 4.3 panel A) for ipsilateral uterine artery BF in cows that were slaughter at d 254. However, there was a treatment (*P* = 0.02) and a day effect (*P* = 0.01). Cows from the RCC treatment had greater ipsilateral uterine artery BF compared to CCC and RRC cows. In addition, ipsilateral uterine BF increased in all treatment groups during late gestation. For ipsilateral uterine artery CSA, there was no treatment × day interaction (*P* = 0.96; Figure 4.3 panel B) or main effect of treatment (*P* = 0.70) but there was a day effect (*P* < 0.01). Ipsilateral uterine artery CSA increased during late gestation regardless dietary treatment. For ipsilateral uterine artery PI, there was no treatment × day interaction (*P* = 0.79; Figure 4.3 panel C) or main effect of day (*P* = 0.22). However, there was a tendency for ipsilateral uterine artery PI to be decreased (*P* = 0.09) in RCC and RRC cows compared to CCC cows. Similarly, ipsilateral uterine artery RI did not display a treatment × day interaction (*P* = 0.60; Figure 4.3 panel D) or main effect of day (*P* = 0.36). However, ipsilateral uterine artery PI decreased (*P* = 0.03) in RCC and RRC cows compared to CCC cows.

There was no treatment × day interaction (*P* = 0.92; Figure 4.4 panel A) or treatment effect (*P* = 0.33) for contralateral uterine artery BF. However, there was a day effect (*P* = 0.01) where contralateral uterine BF increased in all treatment groups during late gestation. For contralateral uterine artery CSA, there was no treatment × day interaction (*P* = 0.97; Figure 4.4 panel B) or main effect of day (*P* = 0.14) but there was a tendency (*P* = 0.09) for a treatment effect. Contralateral uterine artery CSA tended to be decreased in RCC cows compared to CCC and RRC during late gestation. For contralateral uterine artery PI, there was no treatment × day interaction (*P* = 0.84; Figure 4.4 panel C) or main effects of treatment (*P* = 0.65) or day (*P* = 0.20). Similarly, for contralateral uterine artery RI there was no treatment × day interaction (*P* = 0.98; Figure 4.4 panel D) or main effects of treatment (*P* = 0.26) or day (*P* = 0.84).

There was no treatment × day interaction (*P* = 0.34; Figure 4.5) for total (ipsilateral plus contralateral BF) uterine artery BF. But there was a main effect of treatment (*P* = 0.05) where total uterine artery BF was increased in RCC cows compared to CCC and RRC cows. In addition, there was a day effect (*P* < 0.01) where all cows had increased total uterine artery BF during late gestation. For maternal HR, there was no treatment × day interaction (*P* = 0.88; data not shown) or main effects of treatment (*P* = 0.65) or day (*P* = 0.15). The average maternal HR during late gestation was 63 beats/min (± 1 SEM).

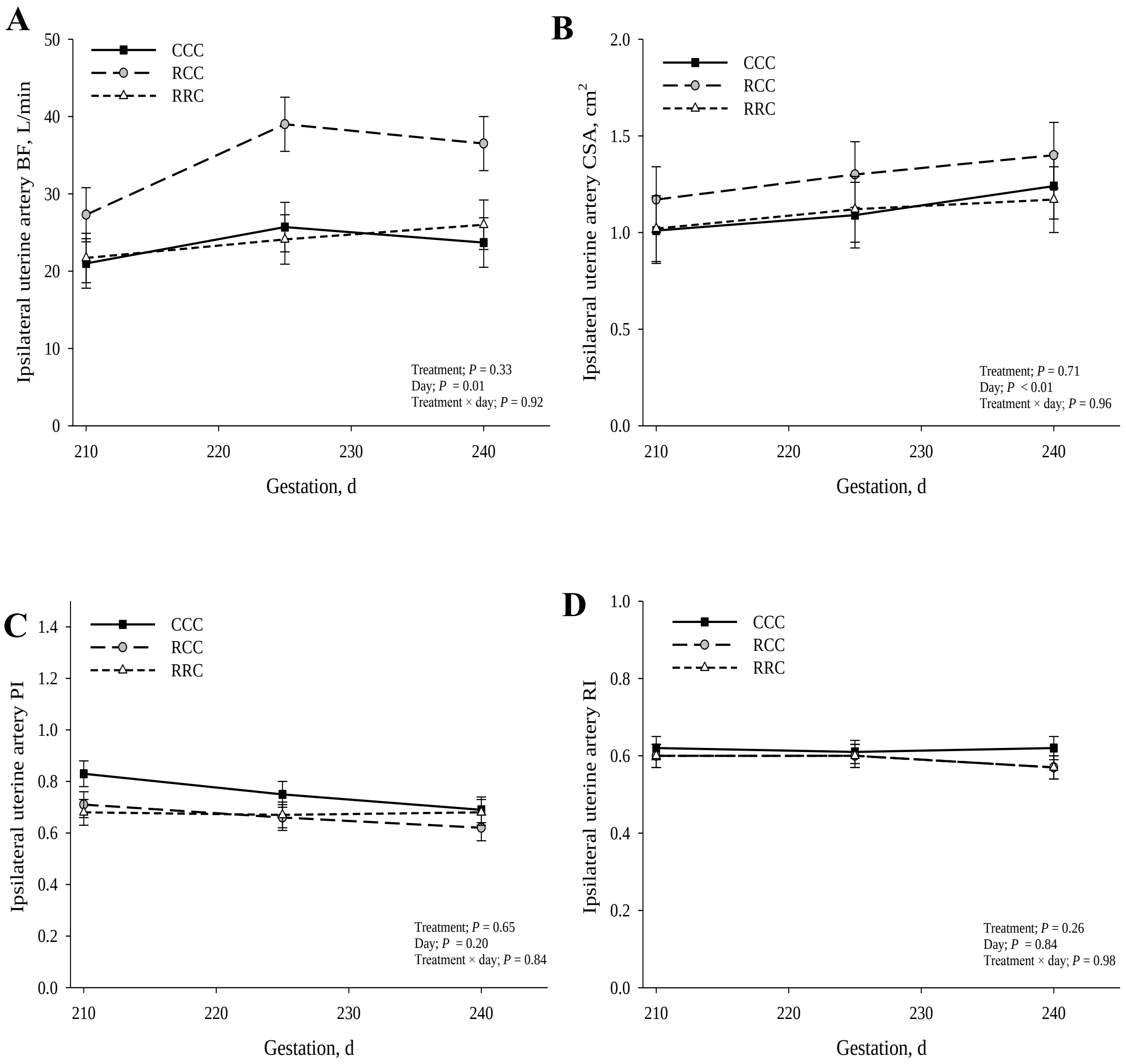

Figure 4.3. Ipsilateral uterine artery blood flow (BF; panel A), cross-sectional area (CSA; panel B), pulsatility index (PI; panel C), and resistance index (RI; panel D) during late gestation. On d 30 of gestation CCC cows (n = 6) received 100% NRC from d 30 to 254; RCC cows (n = 5) received 60% NRC from d 30 to 85 and 100% NRC to d 254; RRC cows (n = 6) received 60% NRC from d 30 to 140 and 100% NRC to d 254.

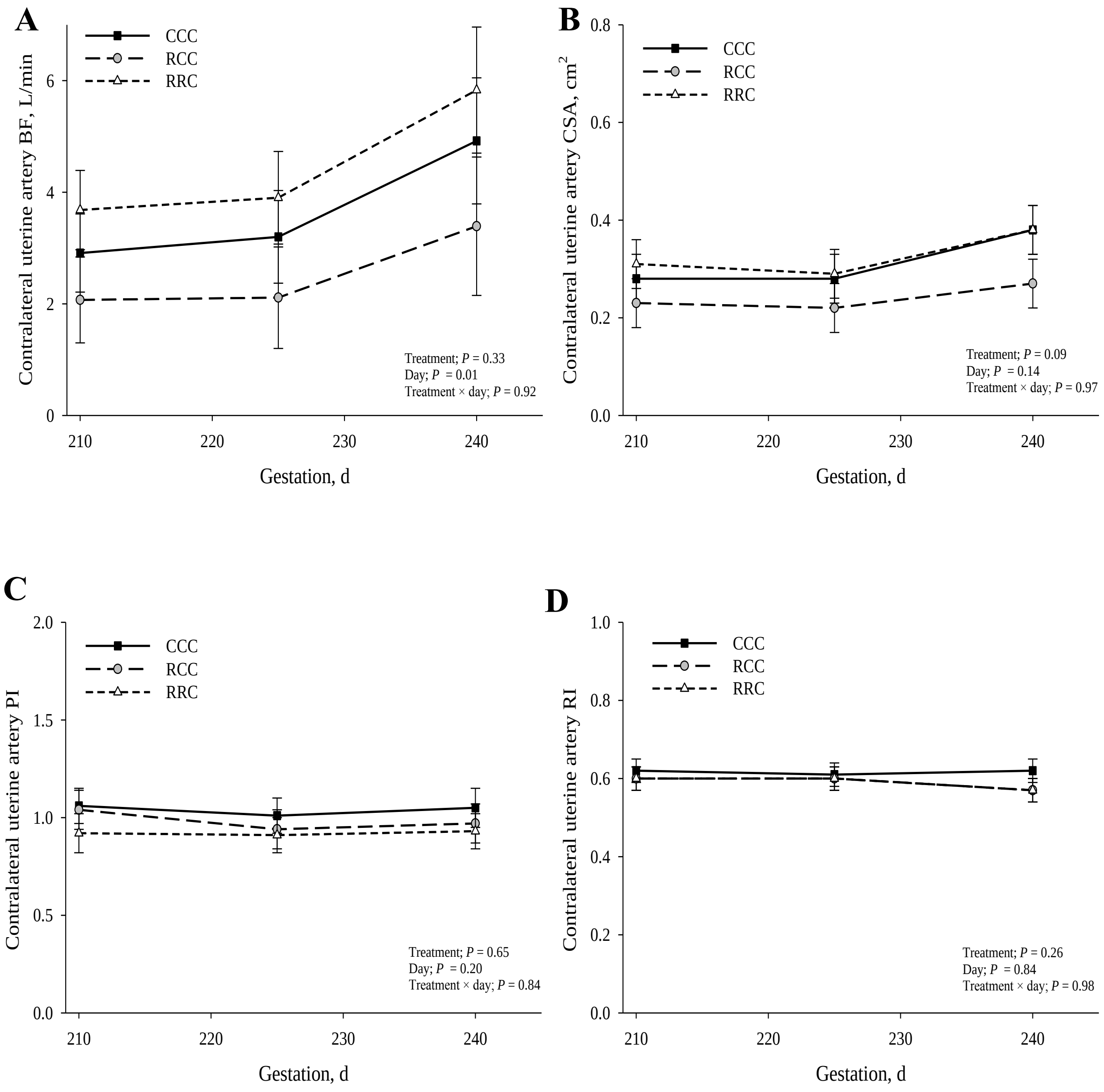

Figure 4.4. Contralateral uterine artery blood flow (BF; panel A), cross-sectional area (CSA; panel B), pulsatility index (PI; panel C), and resistance index (RI; panel D) during late gestation. On d 30 of gestation cows received On d 30 of gestation CCC cows (n = 6) received 100% NRC from d 30 to 254; RCC cows (n = 5) received 60% NRC from d 30 to 85 and 100% NRC to d 254; RRC cows (n = 6) received 60% NRC from d 30 to 140 and 100% NRC to d 254.

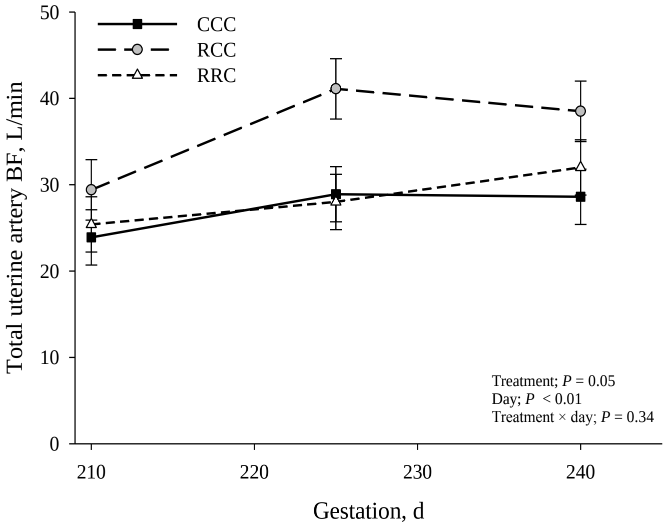

Figure 4.5. Total uterine BF during late gestation in cows slaughtered at d 254 of gestation. On d 30 of gestation cows received On d 30 of gestation CCC cows (n = 6) received 100% NRC from d 30 to 254; RCC cows (n = 5) received 60% NRC from d 30 to 85 and 100% NRC to d 254; RRC cows (n = 6) received 60% NRC from d 30 to 140 and 100% NRC to d 254.

Experiment 2

Umbilical BF prior to Slaughter at d 85.Umbilical PI and RI were not affected ($P \geq 0.25$; Table 4.1) by maternal nutrient restriction. Fetal HR was decreased ($P < 0.01$) in R compared to C fetuses (185 vs. 173 ± 2 beats/min, respectively). Umbilical artery BF, umbilical artery BF per total placentome weight, and umbilical artery BF relative to total cotyledon weight were not affected ($P \geq 0.13$) by maternal nutrient restriction. However, umbilical artery BF relative to fetal weight was decreased ($P = 0.03$) in R compared to C cows.

Placental and Fetal Measurements at d 85. Fetal weight tended to be increased ($P = 0.07$; Table 4.1) in R cows vs. C cows. Gravid uterine weight was not affected ($P = 0.19$) by treatment at d 85. However, empty uterine weight tended to be greater in R cows compared to C cows. Fetal membrane weight and chorioallantoic and amniotic fluid volume were similar ($P \geq$

0.45) between treatments. There were more (*P* = 0.02) placentomes in R compared to C cows, and therefore the total mass of the placentomes was greater (*P* < 0.01) in R compared to C cows. Total cotyledon weight and total caruncle weight were not affected (*P* ≥ 27) by maternal dietary treatment. Average placentome weight and average placentome volume and density were similar (*P* ≥ 0.51) between treatments. Placental efficiency was similar (*P* = 0.13) between treatments.

Umbilical BF prior to Slaughter at d 140. Umbilical PI was not affected (*P* = 0.21; Table 4.2) by maternal dietary treatment. However, there was a tendency for a treatment effect (*P* = 0.06) where RR cows had the greatest RI compared to CC and RC, which did not differ. Fetal HR was similar (*P* = 0.41) among treatments (average = 152 ± 2 beats/min). Umbilical artery BF, umbilical artery BF relative to total placentome weight, umbilical artery BF relative to total cotyledon weight, and umbilical artery BF relative to fetal weight were not affected (*P* ≥ 0.21) by maternal dietary treatment.

Placental and Fetal Measurements at d 140. Fetal weight was similar (*P* = 0.54; Table 4.2) among treatments. Gravid and empty uterine weight were similar (*P* ≥ 0.86) among treatments. Fetal membrane weight and chorioallantoic and amniotic fluid volume were also similar (*P* ≥ 0.63) among treatments. Total number of placentomes was greater (*P* = 0.03) in RR cows compared to CC and RC cows; however, total weight of placentomes was not affected (*P* = 0.18) by maternal dietary treatment. In addition, total cotyledon weight and total caruncular weight were similar (*P* ≥ 0.51) among treatments. Average placentome weight, average placentome volume and density were similar (*P* ≥ 0.14) among treatments. Placental efficiency decreased (*P* = 0.02) in RR cows compared to CC and RC cows.

Table 4.1. The effects of nutrient restriction on placental measurements and umbilical hemodynamics in beef cows at d 85 of gestation

	Nutritional treatments[1]			
Item	C	R	SEM	*P*-value
Fetal BW, g	117.0	138.9	7.1	0.07
Uterus				
Gravid uterus[2], kg	2.5	2.8	0.2	0.19
Empty uterus, kg	1.0	1.3	0.1	0.09
Fetal membranes, g	188.3	166.4	29.2	0.58
Chorioallantoic and amniotic fluids, L	0.9	0.8	0.1	0.45
Total placentome				
Number	45.7	68.4	6.2	0.02
Weight, g	84.8	118.7	5.9	0.002
Caruncle, g	45.0	52.1	6.6	0.44
Cotyledon, g	36.5	46.2	6.2	0.27
Average placentome				
Weight, g	2.1	1.9	0.2	0.51
Volume, cm^3	1.9	2.0	0.2	0.65
Density, g/cm^3	1.3	1.3	0.2	0.96
Placental efficiency	1.4	1.2	0.1	0.10
Umbilical hemodynamics				
PI[3]	1.5	1.6	0.1	0.24
RI[4]	0.7	0.7	0.1	0.74
BF[5], ml/min	49.7	42.9	3.6	0.19
Umbilical BF relative to fetal weight, mL·min-1·kg-1	439.3	297.0	40.6	0.03
Umbilical BF relative to placental weight, mL·min-1·kg-1	183.3	164.2	30.6	0.64
Umbilical BF relative to cotyledon weight, mL·min-1·g-1	1.7	1.0	0.3	0.13

[1] Cows received either control (100% NRC) diet (C; n = 6) or restricted (60% NRC) from d 30 to 85 (R; n = 6).

[2] Gravid uterus weight previously reported by Camacho et al. (2013).

[3] PI = Pulsatility index.

[4] RI = Resistance index.

[5] BF = Blood flow.

Table 4.2. The effects of nutrient restriction followed by realimentation on placental measurements and umbilical hemodynamics in beef cows at d 140 of gestation

	Nutritional treatments[1]				
Item	CC	RC	RR	SEM	*P*-value
Fetal BW, kg	2.0	2.2	2.0	0.12	0.54
Uterus					
Gravid uterus,[2] kg	11.0	11.3	11.7	0.5	0.86
Empty uterus, kg	2.4	2.3	2.3	0.2	0.98
Fetal membranes, kg	0.3	0.3	0.3	0.1	0.63
Chorioallantoic and amniotic fluids , L	5.0	4.0	4.5	0.8	0.66
Total placentome					
Number	87.2^b	75.0^b	110.8^a	8.4	0.03
Weight, kg	1.0	1.0	1.2	0.1	0.18
Caruncle, kg	0.5	0.7	0.5	0.1	0.51
Cotyledon, kg	0.4	0.4	0.3	0.1	0.60
Average placentome					
Weight, g	12.0	14.2	10.5	1.1	0.14
Volume, cm^3	12.6	15.5	14.0	2.0	0.64
Density, g/cm^3	1.2	1.1	1.3	0.1	0.54
Placental efficiency	2.0^b	2.2^b	1.3^a	0.2	0.02
Umbilical hemodynamics					
PI[3]	1.19	1.26	1.37	0.08	0.23
RI[4]	0.71^a	0.72^a	0.79^b	0.03	0.06
BF[5], ml/min	217.5	346.6	130.3	95.0	0.21
Umbilical BF relative to fetal weight, mL·min-1·kg-1	126.0	152.1	100.1	40.4	0.60
Umbilical BF relative to placental weight, mL·min-1·kg-1	188.1	248.4	126.4	59.4	0.39
Umbilical BF relative to cotyledon weight, mL·min-1·g-1	0.5	0.9	1.2	0.5	0.64

[a,b] Means without a common superscript letter differ, $P < 0.05$.

[1] Cows received the control diet (100% NRC) from d 30 until 140 (CC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 140 (RC; n = 5), and restricted from d 30 to 140 (60% NRC; RR; n = 6).

[2] Gravid uterus weight previously reported in Chapter 3

[3] PI = Pulsatility index.

[4] RI = Resistance index.

[5] BF = Blood flow.

Placental and Fetal Measurements at d 254. Fetal weight was similar (*P* = 0.84; Table 4.3) among treatments. Gravid and empty uterine weight were similar ($P \geq 0.29$) among treatments. Fetal membrane weight increased (*P* = 0.04) in RRC cows compared to CCC and RCC cows. Chorioallantoic and amniotic fluid volume were also similar (*P* = 0.61) among treatments. Total number of placentomes and total weight were not affected ($P \geq 0.11$) by maternal dietary treatment. In addition, total cotyledon weight similar (*P* = 0.55) among treatments; however, total caruncle weight tended to be greater in RRC cows compared to CCC and RCC cows. Average placentome weight also tended to be greater in RRC cows compared to CCC and RCC cows. However, average placentome volume and density were similar ($P \geq 0.15$) among treatments. Placental efficiency was similar (*P* = 0.12) among treatment.

Discussion

We hypothesized that maternal nutrient restriction followed by realimentation from early to mid-gestation in beef cows would impair placental development, fetal hemodynamics, and late gestation BF. We observed a tendency for fetal growth to be increased at d 85 in R cows compared to C cows. Therefore, it appears that stunting of fetal growth was spared due to increased placental growth in restricted cows. Interestingly, there was a decrease in umbilical BF relative to fetal weight at d 85 in R fetuses compared to C. This might be the reason why when the dams were nutrient restricted and then realimented at d 140 and 254, they had similar fetal weights. However, when cows were realimented during early and mid-gestation we no longer observed treatment effects for placental or fetal weight. Previous research in beef cows, with a nutrient restriction to 50% of the requirements from d 30 to d 125 of gestation reduced caruncular and cotyledonary weight compared to control cows at the end of the restriction (Zhu et al., 2006).

Table 4.3. The effects of nutrient restriction followed by realimentation on placental measurements and umbilical hemodynamics in beef cows at d 254 of gestation

	Nutritional treatments[1]				
Item	CCC	RCC	RRC	SEM	*P*-value
Fetal BW, kg	30.3	29.8	31.0	2.4	0.84
Uterus					
Gravid uterus,[2] kg	59.15	59.52	65.37	3.36	0.37
Empty uterus, kg	6.36	5.87	6.67	0.32	0.29
Fetal membranes, kg	1.64^{b}	1.71^{b}	2.45^{a}	0.22	0.04
Chorioallantoic and amniotic fluids, L	9.0	11.1	9.8	1.9	0.61
Total placentome					
Number	102.5	97.8	86.7	11.1	0.59
Weight, kg	5.7	5.5	6.4	0.3	0.11
Caruncle, kg	3.3	3.0	4.0	0.2	0.08
Cotyledon, kg	2.2	2.2	2.5	0.5	0.55
Average placentome					
Weight, g	60.2	59.9	77.5	6.3	0.09
Volume, cm3	71.0	56.9	83.3	8.5	0.15
Density, g/cm3	1.1	1.2	1.2	0.1	0.83
Placental efficiency	6.0	6.5	5.8	0.2	0.12

[a,b] Means without a common superscript letter differ, $P < 0.05$.

[1] Cows received the control diet (100% NRC) from d 30 until 254 (CCC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RCC; n = 5), and restricted from d 30 to 140 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RRC; n = 6).

[2] Gravid uterus weight previously reported in Chapter 3.

However, when cows were realimented caruncular weight was similar between restricted and control cows at d 250 (Zhu et al., 2006). Vonnahme et al. (2007) demonstrated that restriction from d 30 to 125 did not affect placental capillary vascularity. Conversely, upon realimentation, placental capillary vascularity was altered near term, indicating that the placenta compensated after restriction. In this study, cows were slaughtered at d 85 of gestation following 55 d of maternal nutrient restriction (from d 30 to d 85 gestation). We observed differences in placentome weight and number where R cows had greater mass and greater number of placentomes than C cows by d 85 of gestation. Therefore, 55 d of maternal nutrient restriction during early gestation appears to lead to increased placental growth and development in R compared to C cows. However, at d 140 of gestation the only effect observed was for number of placentomes being increased in RR cows compared to CC and RC and at d 254 of gestation there were no differences in placentome numbers due to maternal dietary treatment. Vonnahme et al. (2007) observed dramatic differences in capillary vascularity after the realimentation period (from d 125 to 250), suggesting an alteration of placental development and function by early nutrient restriction. In a companion study, Reyaz et al. (2012) showed that cotyledonary arteries from R cows were more sensitive to bradykinin-induced vasorelaxation compared to cotyledonary arteries from C cows. Reynolds et al. (2006) summarized several studies that used sheep as a model of compromised pregnancies during late gestation (i.e. overfed and underfed dams, heat and hypoxia stress, multiple pregnancies) where most of the studies showed a decrease in umbilical BF and a decrease in fetal and placental weight. More specifically, nutrient restriction in sheep during early gestation (from d 28 to 78) resulted in intrauterine growth restriction in restricted vs. control fed ewes (Vonnahme et al., 2003). In human fetuses, abnormal umbilical artery BF during late gestation indicates intrauterine growth restriction, suggesting high risk for perinatal death (Kingdom et al., 1997). Currently, a paucity of research exists on umbilical hemodynamics in cattle; however, if we are able to determine abnormal umbilical wave forms during early gestation in cows we might be able to elucidate strategies to improve neonatal health. In this study we observed that d 85 fetuses from R cows tended to be heavier compared to fetuses from C cows suggesting an increased nutrient extraction from the nutrient restricted dams. As umbilical BF was similar between treatments we hypothesize that nutrient uptake by the placenta in nutrient restricted animals must be enhanced. However, when cows were realimented at d 85 and 140 of gestation fetuses had similar BW and also umbilical BF was similar among treatments.

Interestingly, during late gestation when all cows were receiving similar nutrition (100% of the NRC requirements), ipsilateral uterine BF and total BF were increased in cows that were nutrient restricted for 55 d and then realimented. Our laboratory has previously reported (Chapter 2) that nutrient restriction from d 30 to 140 followed by realimention in beef cows increases ipsilateral uterine artery BF, however, total uterine BF was not affected by nutrient restriction. The fact that we observed changes in total uterine BF during late gestation but not during mid-gestation due to nutrient restriction followed by realimentation might be due to the exponential increase of BF that occurs during late gestation. Late gestation is characterized by a well-known rapid increase in fetal growth and development and subsequent increase in nutrient demands of the fetus. Therefore, nutrient uptake by the gravid uterus and fetal energy deposition are the greatest during late gestation (Reynolds et al., 1986). In order to meet the nutrient demands, vascular smooth muscle tone of the uterine artery decreases causing an increase in diameter of the artery and subsequent increase in BF (Rosenfeld, 1984; Ford 1995).

In summary, nutrient restriction during early restriction tended to increase fetal growth and placental size. However, when cows were restricted longer and/or realimented during early and mid-gestation we no longer see treatment effects for placental or fetal growth. Suggesting that after longer restriction growth of those fetuses cached up with the early restricted cows and this might be due to nutrients transport capacity or placental function. It is known that pregnancy success and offspring health are dependent on nutrient intake and conceptus growth and development. A key player for proper conceptus development is the placenta as it plays an important role in providing physiological exchange between the maternal and fetal systems

(Reynolds and Redmer, 1995). During placentation, angiogenesis and vascularization at the fetal-maternal interface is extensive and, subsequently, a rapid increase in uterine and umbilical BF results (Reynolds and Redmer, 1995). In order to support the growth of the developing fetus during late gestation, even though the placenta is not growing as much as early gestation, placental function increases dramatically after mid-gestation (Reynolds et al., 1986). Therefore, more research is necessary looking at placental function during early to mid-gestation nutrient restriction and different realimentation times.

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CHAPTER 5. EFFECTS OF MATERNAL NUTRIENT RESTRICTION FOLLOWED BY REALIMENTATION DURING EARLY AND MID-GESTATION ON BEEF COWS. III. FETAL GROWTH AND DEVELOPMENT AT DIFFERENT STAGES OF GESTATION

Abstract

The objectives were to examine the effects of maternal nutrient restriction during early to mid-gestation followed by realimentation on fetal growth and development and maternal and fetal metabolite concentrations. On d 30 of pregnancy, multiparous, non-lactating cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) were assigned to 1 of 3 dietary treatments: control (C; 100% NRC; n = 18) and restricted (R; 60% NRC; n = 30). On d 85, cows were slaughtered (C, n = 6; R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12), or were realimented to control (RC; n = 11). On d 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254, all remaining cows were slaughtered. After the gravid uterus was excised, fetuses were removed and weighed. Fetal curved crown rump length (**CCR**), biparietal distance, and abdominal girth were recorded and fetal organ tissues were collected. Metabolites concentrations were analyzed in maternal and umbilical cord serum and amniotic fluid collected at slaughter. At d 85, eviscerated BW (**EvBW**) was similar ($P = 0.13$) between treatments. Fetal abdominal girth, ponderal index, and liver weight were increased ($P \leq 0.04$) and liver g/g of brain tended to be increased ($P = 0.09$) in R vs. C fetuses. Pancreas weight tended to be increased ($P = 0.06$) in R vs. C fetuses; but pancreas weight relative to brain weight was decreased increased ($P = 0.04$) in R vs. C fetuses. The rest of absolute organ weight were similar ($P \geq 0.13$) between treatments at d 85 of gestation. Fetal EvBW, growth parameters, and organ weights were similar ($P \geq 0.08$) among treatments at d 140; except for adrenals g and g/g of brain tended ($P \leq 0.09$) to be decrease and g/g of BW was decreased ($P = 0.04$) in RC and RR vs. CC. by d 254 all organ weight were similar ($P \leq 0.13$) among treatments. At d 85, maternal serum cholesterol and lactate were elevated ($P \leq 0.03$) in R cows vs. C. Maternal serum triglycerides, glucose, and blood urea nitrogen (**BUN**) were similar ($P \geq 0.28$) between treatments. Umbilical cord serum lactate was also greater ($P = 0.05$) and fructose and BUN tended to be greater ($P \leq 0.07$) in R fetuses vs. C. Umbilical cord cholesterol, triglycerides, glucose, were similar ($P \geq 0.12$) between treatments. Maternal and umbilical cord metabolites at d 140 and 254 of gestation were similar ($P \geq 0.13$) among treatments. The greater fetal weight previously observed in nutrient restricted cows might be due in part to the increased metabolites in fetal and maternal systems during early gestation.

Keywords: beef cows, fetal organs, nutrient restriction

Introduction

During early stages of embryo development, when nutrient requirements appear trivial for conceptus growth, maternal nutrient intake has an effect on prenatal growth and development (Robinson et al., 1999). An insult (e.g. improper maternal nutrition) during critical periods of fetal development can cause structural, metabolic and physiologic alterations in utero that have negative long lasting effects on the offspring (Godfrey and Barker, 2001). In livestock, intrauterine growth restriction (**IUGR**) has negative consequences in animal production such as decreased neonatal survival, decreased offspring birth weight, altered organ development and decreased postnatal growth and subsequent performance of the offspring (Wu et al., 2006; Neville et al., 2010; Reynolds et al., 2010; Vonnahme et al., 2010). Fetal reproductive organs, brain, heart, skeletal muscle, liver, small intestine, and mammary gland are some of the organs that have been shown to have abnormal development due to IUGR (Wu et al., 2006).

Pregnancy success and offspring health are dependent on nutrient intake and conceptus growth and development. Optimization of growth and development after birth is important for the profitability of the livestock industry. Maternal nutrition during pregnancy plays an important role in supporting adequate fetal and placental growth and development. We have shown (Chapter 3) that the dam undergoes adaptations during an early to mid-gestation nutrient restriction and becomes more efficient in the utilization of nutrients after being realimented and as gestation advances providing sufficient nutrients for the developing conceptus. Our laboratory has also demonstrated that nutrient restriction during early gestation in beef cows increases placental size and fetal weight tended to be bigger (Chapter 4). Therefore, we hypothesized that if placental and fetal size were altered, fetal composition would also be altered due to maternal nutrient restriction followed by realimentation. Moreover, we also hypothesized that the metabolites available for the fetus would be impacted by maternal nutrition. Our objectives were to determine the effects of maternal nutrient restriction during early to mid-gestation followed by realimentation fetal size and organ weights and metabolites concentrations in fetal and maternal systems in beef cattle.

Materials and Methods

Animals and Management

All procedures involving animals were approved by the North Dakota State University (**NDSU**) Animal Care and Use Committee (#A10001).

Animals, Diets, and Breeding

Fifty-four non-lactating, multiparous crossbred beef cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) of similar genetic background were synchronized using a Select Synch plus progesterone insert (CIDR; Pfizer Animal Health, New York, NY) and fixed-time AI (TAI).

Breeding protocol has been previously published (Chapter 3) Inseminated cows were transported to the Animal Nutrition and Physiology Center (ANPC; Fargo, ND) within 3 d post-insemination.

On d 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). Non-pregnant cows restarted the same breeding protocol; cows were subjected to AI no more than twice during the experiment. On d 30 of pregnancy, cows were randomly assigned to dietary treatments: control (C; 100% NRC; n = 18; Figure 1) and nutrient restriction (R; 60% NRC; n = 30). On d 85 cows were slaughtered (C, n = 6 and R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12) treatments, or were realimented to control (RC; n = 11). On d 140 cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254 all remaining cows were slaughtered (CCC, n = 6; RCC, n = 5; RRC, n = 6). An animal from the RC group was removed from the study due to early embryonic loss and a second cow was removed from the RCC group due to a twin pregnancy.

The control diet consisted of chopped grass/legume hay [8.0% CP, 69.2% NDF, 41.5% ADF, and 57.9% TDN (DM basis); containing predominately cool season grasses with small amounts of alfalfa] to pass a 15.24-cm to meet 100% NEm recommendations and fetal growth (NRC, 2000) and to meet or exceed MP, mineral, and vitamin recommendations. Hay NEm concentration was predicted using equations described by Weiss (1993) and NRC (2000). Nutrient restricted cows received 60% of the same control hay diet. Cows were individually fed once daily in a Calan gate system at 1000 h and had free access to water. The mineral and vitamin supplement (Trouw Dairy VTM with Optimins; Trouw Nutrition International, Highland, IL; 10% Ca, 5% Mg, 5% K, 2.7% Mn, 2.7% Zn, 1,565,610 IU/kg vitamin A, 158,371 IU/kg vitamin D 3 and 2,715 IU/kg vitamin E) was top-dressed 3 times per week at a rate of 0.18% of hay DMI to meet or exceed mineral and vitamin requirements relative to dietary NE intake (NRC 2000). Cows were weighed weekly at approximately 0800 h throughout the experiment and dietary intake was adjusted relative to BW and NE requirements for the specific period of gestation (average requirements for periods from d 30 – 85, d 86 – 140, 141 – 197, and d 198 - 254).

Slaughter Procedure and Tissue Collection

A subset of pregnant cows from each breeding group was randomly selected for slaughter at d 85, 140, and 254 (± 2 d). Cows were transported from ANPC to the NDSU Meat Laboratory approximately 30 min prior to slaughter. No more than 2 cows were slaughtered per day due to Meat Laboratory capacity and time constraints of sample collection (slaughters ranged from November 2011 until April 2012). On the day of slaughter, cows were stunned with a captive bolt and exsanguinated. The gravid uterus was immediately collected, and the fetus was removed from the placenta by tying off and then cutting the umbilical cord at the umbilicus and was then weighed to obtain a fetal BW. Fetal curved crown rump length (**CCR**), biparietal distance, and abdominal girth were recorded. Fetal ponderal index was calculated using the following equation: ponderal index = fetal weight (kg)/ CCR length (cm)^3. The fetal carcass was then processed similarly as previously described in sheep by our laboratory (Reed et al., 2007). Briefly, the full alimentary tract was weighed. The stomach complex (reticulum, rumen, omasum, abomasum) was removed at the entry of the esophagus and at the pyloric junction, drained of fluid, and weighed. Only for fetuses at d 254 of gestation, individual organs of the gastrointestinal tract were dissected, stripped of contents, and weighed. Omental and mesenteric fat were combine and weighed. The small intestine and large intestine were also stripped of contents and weighed. The kidney, adrenal glands, lungs, and perirenal fat were removed from the fetal carcass and weighed. Due to small fetuses at d 85 and 140 slaughters, individual organs from gastrointestinal tract were not dissected. Small and large intestine were combined at d 85 and then individually dissected and weights for d 140 and 254 slaughters. Heart was dissected and weighed. In addition, top, middle, and bottom measures of left and right ventricles of the heart were taken at d 140 and 254 slaughters. Also, reproductive organs were dissected and weighed. Fetal organ weight was divided by brain weight to assess asymmetric vs. symmetric growth restriction, as the brain is spared during time of maternal nutrient restriction used in this study (Anthony et al., 2003). For comparison, fetal organ weight was also divided by fetal BW.

Collection and Bioassays on Maternal Serum, Umbilical Serum, and Amniotic Fluid Samples

Prior to slaughter, jugular blood samples were collected by jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) and allowed to clot at room temperature for 15 min before being placed on ice. Samples were centrifuged at 4°C for 20 min at 2,000 × g and serum was stored at −20°C in plastic vials until assayed. Prior to dissection of the uterus amnionic fluid was collected, centrifuged at 2,000 x g for 20 min at 4˚C to precipitated solids that might be present. Then fluids were stored at -20°C until further analysis. The umbilical artery was located and blood samples were collected into serum separator tubes, placed on wet ice, and later centrifuged at 2,000 x g for 20 min at 4˚C. Serum was stored at -20°C until further analysis.

Plasma blood urea nitrogen (BUN) was measured using a urea N kit (Procedure No. 640, Sigma Diagnostics, St. Louis, MO). Glucose was measured using the Infinity Glucose Oxidase Liquid Stable Reagent according to manufacturer's instructions (Thermo Scientific, Middletown, Virginia). A sample volume of 2 μl and a reagent volume of 298 μl were used to accommodate a 96 well plate format. A 16.67 mM (300.5 mg/dL) glucose standard was made and utilized on every plate as a calibrator. Absorbance readings were collected on a BioTek (Winooski, Vermont) Epoch Microplate Spectrophotometer at 500nm. The concentration of glucose was calculated using the following formula: [Absorbance of Unknown/Absorbance of Calibrator] x Calibrator Value (in mM or mg/dL). The limit of detection for this assay was 0.06 mM or 1.15 mg/dL.

Cholesterol concentrations were measured using the Infinity Cholesterol Liquid Stable Reagent according to manufacturer's instructions (Thermo Scientific, Middletown, Virginia). A sample volume of 3 μl and a reagent volume of 297 μl were used to accommodate a 96 well plate format. A 5.17 mM (200 mg/dL) cholesterol standard was (Pointe Scientific, Inc., Canton, Michigan) utilized on every plate as a calibrator. Absorbance readings were collected on a BioTek (Winooski, Vermont) Epoch Microplate Spectrophotometer at 500 nm. The concentration of cholesterol was calculated using the same formula as was used to calculate the concentration of glucose. The limit of detection for this assay was 0.04 mM or 1.43 mg/dL. In addition, amniotic fluid concentrations of cholesterol were below the detection limit of the assay, suggesting very little to non-presence of cholesterol in the amniotic fluid at d 85, 140, and 254 of gestation.

Triglyceride concentrations were measured using the Infinity Triglycerides Liquid Stable Reagent according to manufacturer's instructions (Thermo Scientific, Middletown, Virginia). A sample volume of 3 μl and a reagent volume of 297 μl was used to accommodate a 96 well plate format. A 11.28 mM triglyceride standard was purchased (Cayman Chemical Company, Ann Arbor, Michigan). The standard was diluted 1:5 to 2.256 mM (200 mg/dL) and utilized on every plate as a calibrator. Absorbance readings were collected on a BioTek (Winooski, Vermont) Epoch Microplate Spectrophotometer at 500nm. The concentration of triglycerides was calculated using the same formula as was used to calculate the concentration of glucose. The limit of detection for this assay was 0.01 mM or 1 mg/dL.

Concentrations of lactate were measured using a colorimetric assay kit from BioVision (Milpitas, CA) following manufacturer's instructions. For maternal serum samples, 4-8 μl were assayed, and for umbilical serum and amniotic fluid samples 4-5 μl were assayed. Absorbance readings were collected on a BioTek (Winooski, Vermont) Epoch microplate spectrophotometer at 570nm. The limit of detection for this assay was 0.001mM (or 1μM).

Fructose concentrations were determined using an assay from Abnova (Taipei City, Taiwan) that specifically measures fructose and does not detect other sugars such as glucose or galactose. No modifications were made to the assay. Maternal serum samples were assayed undiluted and the maximum volume (20 μL) allowed was used. Umbilical serum samples were diluted 1:6 and amniotic fluid samples were assayed at a 1:20 dilution. Absorbance readings were collected on a BioTek Synergy H1 multi-mode microplate reader at 565nm. The limit of detection for this assay was 12 μM. All samples were assayed in 1 d and the assay CV was 15% or less. In addition, maternal concentrations of fructose were below the detection limit of the assay, suggesting very little to non-presence of fructose in the maternal serum at d 85, 140, and 254 of gestation. Therefore concentrations of fructose in the maternal serum will not present in the result section and in the tables will be represented as non-detectable (**ND**).

Statistical Analysis

All data was analyzed using the MIXED procedure of SAS (SAS software version 9.2, SAS Institute, Cary, NC). Data was analyzed within gestational day (d 85, 140, and 254). The model statement included maternal treatment and fetal sex and blocked by breeding group as random effect. When fetal sex *P* > 0.30, it was removed from the model. Treatment means were separated using the PDIFF option of the LSMEANS statement

Results

Fetal Organ Weights at d 85

At d 85 slaughter, there was a tendency (*P* = 0.07) for maternal nutrition treatment effect on fetal weight, where fetuses from R cows had increased BW compared to C fetuses (Previously reported in Chapter 4; Table 5.1). However, there was no treatment effect (*P* = 0.13) when looking at eviscerated BW (**EvBW**). Abdominal girth and ponderal index ($P \leq 0.04$) were increased in R fetuses compared to C. However, biparietal distance, and CCR length were similar (*P* = 0.74) between treatments.

Table 5.1. The effects of nutrient restriction on fetal weight and size in beef cows at d 85 of gestation

	Nutritional treatments[1]			
Item	C	R	SEM	*P*-value
BW, g	117.0	138.9	7.08	0.07
EvBW[2], g	96.4	110.0	8.91	0.13
Abdominal girth, cm	10.3	10.8	0.2	0.04
Ponderal index, kg/cm[3]	21.1	27.3	1.2	0.01
Biparietal distance, cm	2.5	2.5	0.1	0.74
CCR length[4], cm	17.7	17.2	0.1	0.28

[1] Cows received either control (100% NRC) diet (C; n = 6) or restricted (60% NRC) from d 30 to 85 (R; n = 6).
[2] EvBW: eviscerated BW = fetal BW – organ weights.
[3] Ponderal index: fetal BW (kg)/ CCR length $(\text{cm})^3$.
[4] CCR length: curve crown rump length.

Brain weight (g) was similar (*P* = 0.52; Table 5.2) between treatments; however, when expressed relative to BW (g/g BW), fetuses from R cows had decreased (*P* = 0.02) brain weight compared to C fetuses. Lung and kidney weight (g and g/g of brain weight) were similar ($P \geq$

0.42) between treatments. When expressed as lung and kidney weight to BW ratio, there was a maternal nutrition tendency ($P \leq 0.06$), with fetuses from R cows having a decreased ratio compared to C fetuses. Stomach complex weight (g and g/g of BW) was similar ($P \geq 0.20$) between treatments. However, when expressed as stomach complex weight to brain ratio, fetuses from R cows tended to have an increased ($P = 0.08$) ratio compared to fetuses from C cows. Liver weight (g) was increased ($P = 0.04$) in R fetuses compared to C fetuses. Similarly, when expressed as liver weight to brain weight ratio, R fetuses tended to have greater ($P = 0.09$) ratio compared to C fetuses. But, when liver weight was expressed as g/g of BW, both treatments had similar ($P = 0.92$) ratios. Pancreas weight (g) tended ($P = 0.06$) to be bigger for R fetuses compared to C fetuses. In contrast, when pancreas weight was expressed as g/g of brain weight, R fetuses had decreased ratio ($P = 0.04$) compared to C fetuses. When pancreas was expressed as g/g of BW ratio, both groups had ($P = 0.18$) similar ratios. Heart weight (g and g/g of BW) were similar ($P \geq 0.13$) between treatment groups; however, when expressed as hearth weight relative to brain weight ratio, R fetuses had heavier ($P = 0.05$) hearts compared to C fetuses. Absolute fetal organ weight, organ weight relative to brain weight and organ weight relative to BW of adrenal glands, perirenal fat, stomach complex, small and large intestine, omental and mesenteric fat, and spleen were not affected ($P \geq 0.14$) by maternal nutrient restriction when cows were slaughter at d 85 of gestation.

Fetal reproductive organ weights are shown in Table 5.3. Uterus weight (g and g/g of brain weight) were not affected ($P \geq 0.10$) by maternal treatment, while uterus relative to fetal BW tended ($P = 0.08$) to be lighter for C female fetuses compared to C fetuses. Ovarian weight, weight relative to BW and brain weight tended ($P \leq 0.09$) to have a decrease weight in R females compared to C female fetuses. Mammary gland weight, weight relative to BW and brain weight were not affected ($P \geq 0.59$) by maternal nutrient restriction. There was only 1 male born from R cows and 5 males from C cows. Therefore the average testes weight across all treatments was 0.06 ± 0.01 g.

Table 5.2. The effects of nutrient restriction on fetal organ weight in beef cows at d 85 of gestation

	Nutritional treatments[1]			
Item	C	R	SEM	*P*-value
Brain, g	3.51	3.68	0.17	0.52
g/g BW	0.030	0.026	0.001	0.02
Heart, g	1.02	1.17	0.13	0.13
g/g BW	0.006	0.007	0.001	0.34
g/g Brain	0.223	0.273	0.023	0.05
Lung, g	4.12	4.27	0.24	0.69
g/g BW	0.034	0.030	0.002	0.06
g/g Brain	1.18	1.16	0.046	0.84
Adrenals, g	0.03	0.05	0.01	0.17
g/g BW	0.001	0.001	0.001	0.27
g/g Brain	0.010	0.014	0.002	0.14
Kidneys, g	0.71	0.85	0.11	0.42
g/g BW	0.006	0.005	0.001	0.0001
g/g Brain	0.200	0.215	0.035	0.76
Perirenal fat, g	0.33	0.33	0.04	0.98
g/g BW	0.001	0.001	0.001	0.66
g/g Brain	0.075	0.079	0.027	0.84
Stomach complex[2], g	2.14	2.56	0.20	0.20
g/g BW	0.018	0.018	0.001	0.98
g/g Brain	0.607	0.693	0.031	0.08
Small and large intestine, g	2.21	2.42	0.42	0.42
g/g BW	0.001	0.001	0.001	0.18
g/g Brain	0.629	0.654	0.025	0.49
Spleen, g	0.17	0.19	0.03	0.48
g/g BW	0.001	0.001	0.001	0.41
g/g Brain	0.040	0.048	0.011	0.23
Liver, g	4.29	5.11	0.23	0.04
g/g BW	0.037	0.037	0.001	0.92
g/g Brain	1.23	1.39	0.062	0.09
Pancreas, g	0.12	0.17	0.02	0.06
g/g BW	0.001	0.001	0.001	0.18
g/g Brain	0.030	0.012	0.003	0.04
Omental and mesenteric fat, g	1.01	1.06	0.22	0.31
g/g BW	0.007	0.008	0.001	0.40
g/g Brain	0.250	0.299	0.081	0.26

[1] Cows received either control (100% NRC) diet (C; n = 6) or restricted (60% NRC) from d 30 to 85 (R; n = 6).

[2] Stomach complex = (reticulum + rumen + omasum + abomasum) – digesta.

Table 5.3. The effects of nutrient restriction on fetal reproductive organ weight in beef cows at d 85 of gestation

	Nutritional treatments[1]			
Item[2]	C	R	SEM	*P*-value
Uterus, g	0.23	0.16	0.06	0.15
g/g BW	0.002	0.001	0.001	0.08
g/g Brain	0.062	0.037	0.016	0.10
Ovaries, g	0.08	0.04	0.01	0.09
g/g BW	0.0008	0.0002	0.0001	0.07
g/g Brain	0.026	0.010	0.006	0.09
Mammary gland, g	0.88	0.97	0.24	0.59
g/g BW	0.006	0.006	0.001	0.96
g/g Brain	0.207	0.227	0.069	0.61
Testes, g	---	---	----	----
g/g BW	---	---	----	----
g/g Brain	---	---	----	----

[1] Cows received either control (100% NRC) diet (C; n = 6) or restricted (60% NRC) from d 30 to 85 (R; n = 6).

[2] C = 3 males and 3 females; R= 1 male and 5 females.

Fetal Organ Weights at d 140

Fetal weight (Chapter 4) and fetal EvBW at d 140 slaughter were not affected ($P \geq 0.51$; Table 5.4) by maternal dietary treatment. In addition, abdominal girth, ponderal index, biparietal distance, and CCR length were similar ($P \geq 0.44$) among treatments. Reproductive organ weights (g, g/g of brain weight, and g/g of BW; Table 5.5) for males and females were not affected ($P \geq 0.11$) by maternal nutrition at d 140 of gestation. Females fetuses average weights were: uterine 1.20 ± 0.08 g, ovarian 0.12 ± 0.02 g, and mammary gland 8.41 ± 0.95 g. Adrenal weight (g and g/g of brain weight; Table 5.6) tended to be decreased in RC and RR fetuses compared to CC fetuses. Adrenal weight relative to BW was also decreased ($P = 0.04$) in RC and RR fetuses compared to CC fetuses. The rest of fetal organ weights (g, g/g of brain weight, and g/g of BW) at d 140 of gestation were not affected ($P \geq 0.15$) by maternal treatment. Heart weight (g, g/g of brain weight, and g/g of BW) was similar ($P \geq 0.16$) between treatments. When looking at the heart left and right ventricles thickness, we did not observe a maternal treatment effect ($P \geq 0.10$).

Table 5.4. The effects of nutrient restriction followed by realimentation on fetal weight and size in beef cows at d 140 of gestation

	Nutritional treatments[1]				
Item	CC	RC	RR	SEM	*P*-value
BW, g	1978.3	2171.3	2056.2	122.6	0.54
EvBW[2], g	1541.7	1709.3	1589.8	103.0	0.51
Abdominal girth, cm	27.2	28.1	27.6	0.5	0.48
Ponderal index, kg/cm^3	28.7	30.2	29.3	2.0	0.90
Biparietal distance, cm	5.3	5.6	5.4	0.2	0.44
CCR length[4], cm	41.1	41.6	41.4	1.1	0.95

[1] Cows received the control diet (100% NRC) from d 30 until 140 (CC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 140 (RC; n = 5), and restricted from d 30 to 140 (60% NRC; RR; n = 6).
[2] EvBW: eviscerated BW = fetal BW – organ weights.
[3] Ponderal index = fetal BW (kg)/ CCR length (cm)^3.
[4] CCR length: curve crown rump length.

Table 5.5. The effects of nutrient restriction followed by realimentation on fetal reproductive organ weight in beef cows at d 140 of gestation

	Nutritional treatments[1]				
Item[2]	CC	RC	RR	SEM	*P*-value
Uterus, g	1.29	1.23	1.39	0.09	0.27
g/g BW	0.0007	0.0006	0.0006	0.0001	0.60
g/g Brain	0.049	0.046	0.049	0.005	0.72
Ovaries, g	0.12	0.11	0.08	0.02	0.15
g/g BW	0.0001	0.0001	0.0001	0.0001	0.19
g/g Brain	0.004	0.004	0.002	0.001	0.11
Mammary gland, g	8.06	9.20	10.53	0.86	0.15
g/g BW	0.0045	0.0049	0.0049	0.0006	0.69
g/g Brain	0.314	0.349	0.370	0.032	0.26
Testes, g	0.52	0.55	0.36	0.09	0.35
g/g BW	0.0002	0.0002	0.0002	0.0001	0.38
g/g Brain	0.016	0.016	0.012	0.004	0.48

[1] Cows received the control diet (100% NRC) from d 30 until 140 (CC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 140 (RC; n = 5), and restricted from d 30 to 140 (60% NRC; RR; n = 6).
[2] CC = 4 males and 2 females; RC= 2 male and 3 females; RR= 5 male and 1 female.

Table 5.6. The effects of nutrient restriction followed by realimentation on fetal organ weight in beef cows at d 140 of gestation

	Nutritional treatments[1]				
Item	CC	RC	RR	SEM	*P*-value
Brain, g	29.5	31.5	30.2	2.25	0.58
g/g BW	0.159	0.154	0.152	0.0008	0.88
Heart, g	13.2	15.6	14.2	0.87	0.17
g/g BW	0.007	0.007	0.007	0.0002	0.16
g/g Brain	0.418	0.460	0.444	0.017	0.22
LV thickness[2], mm	3.26	3.03	3.84	0.27	0.14
RV thickness[3], mm	3.08	3.32	3.91	0.40	0.10
Lung, g	57.9	56.3	58.3	4.18	0.87
g/g BW	0.030	0.028	0.029	0.003	0.62
g/g Brain	2.09	1.95	2.05	0.168	0.53
Adrenals, g	0.62	0.51	0.51	0.07	0.09
g/g BW	0.0003[a]	0.0002[b]	0.0002[b]	0.0001	0.04
g/g Brain	0.022	0.018	0.018	0.003	0.06
Kidneys, g	13.1	15.6	14.7	1.60	0.25
g/g BW	0.007	0.007	0.007	0.0005	0.34
g/g Brain	0.444	0.491	0.490	0.027	0.36
Perirenal fat, g	8.87	8.32	9.10	1.39	0.80
g/g BW	0.0047	0.0040	0.0046	0.0005	0.36
g/g Brain	0.340	0.301	0.330	0.024	0.52
Stomach complex[4], g	27.8	31.4	27.7	2.86	0.31
g/g BW	0.0146	0.0151	0.0139	0.0011	0.54
g/g Brain	0.952	1.00	0.929	0.041	0.49
Small intestine, g	13.5	14.5	12.8	2.06	0.61
g/g BW	0.007	0.007	0.006	0.0009	0.73
g/g Brain	0.471	0.480	0.441	0.065	0.74
Large intestine, g	3.55	3.07	5.18	1.76	0.31
g/g BW	0.0019	0.0016	0.0025	0.0007	0.29
g/g Brain	0.137	0.113	0.176	0.045	0.25
Spleen, g	4.55	5.11	4.72	0.38	0.57
g/g BW	0.0023	0.0024	0.0023	0.0001	0.84
g/g Brain	0.144	0.150	0.148	0.007	0.84
Liver, g	67.1	77.1	70.1	7.11	0.36
g/g BW	0.034	0.035	0.034	0.0010	0.50
g/g Brain	2.13	2.27	2.21	0.120	0.68
Pancreas, g	1.19	1.42	1.28	0.33	0.70
g/g BW	0.0006	0.0006	0.0006	0.0001	0.93
g/g Brain	0.043	0.046	0.044	0.008	0.90
Omental and mesenteric fat, g	7.34	5.59	6.09	1.09	0.42
g/g BW	0.0038	0.0022	0.0031	0.0008	0.15
g/g Brain	0.231	0.171	0.190	0.031	0.30

[1] Cows received the control diet (100% NRC) from d 30 until 140 (CC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 140 (RC; n = 5), and restricted from d 30 to 140 (60% NRC; RR; n = 6).

[2] LV thickness: Left ventricle thickness.

[3] RV thickness: Right ventricle thickness.

[4] Stomach complex = (reticulum + rumen + omasum + abomasum) – digesta.

Fetal Organ Weights at d 254

Fetal weight (Chapter 4) and fetal EvBW at d 254 slaughter were not affected ($P \geq 0.51$; Table 5.7) by maternal treatment. In addition, abdominal girth, ponderal index, biparietal distance, and CCR length were similar ($P \geq 0.43$) among treatments. Adrenal glands tended ($P = 0.06$; Table 5.8) to be heavier in RRC vs. RCC fetuses and CCC fetuses being intermediate. However, when adrenal weight was expressed proportionally to brain weight and BW there was no maternal treatment effect ($P \geq 0.13$). The rest of fetal organ weights (g, g/g of brain weight, and g/g of BW) at d 140 of gestation were not affected ($P \geq 0.28$) by maternal treatment. Female reproductive organ weights were not affected ($P \geq 0.29$; Table 5.9) by maternal treatment and the average weights were: uterine 6.68 ± 0.68 g, ovarian 1.13 ± 0.74 g, and mammary gland 8.41 ± 0.95 g. Fetuses testes weight (g, g/g of brain weight, and g/g of BW) were increased ($P \leq 0.02$) in RRC males compared to CCC and RCC males. Heart weight (g, g/g of brain weight, and g/g of BW) was similar ($P \geq 0.58$) between treatments. When looking at the heart left and right ventricles thickness, we did not observe a maternal treatment effect ($P \geq 0.17$).

Table 5.7. The effects of nutrient restriction followed by realimentation on fetal weight and size in beef cows at d 254 of gestation

	Nutritional treatments[1]				
Item	CCC	RCC	RRC	SEM	*P*-value
BW, g	30337	29815	31007	2382.4	0.84
EvBW[2], g	20579	15413	18861	5716.7	0.51
Abdominal girth, cm	72.3	72.1	71.0	1.7	0.86
Ponderal index, kg/ cm[3]	35.5	36.8	31.1	1.8	0.81
Biparietal distance, cm	9.8	9.3	9.8	0.5	0.71
CCR length[4], cm	99.0	98.4	100.2	1.8	0.79

[1] Cows received the control diet (100% NRC) from d 30 until 254 (CCC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RCC; n = 5), and restricted from d 30 to 140 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RRC; n = 6).

[2] EvBW: eviscerated BW = fetal BW – organ weights.

[3] Ponderal index = fetal BW (kg)/ CCR length $(cm)^3$.

[4] CCR length: curve crown rump length.

Table 5.8. The effects of nutrient restriction followed by realimentation on fetal organ weight in beef cows at d 254 of gestation

	Nutritional treatments[1]				
Item	CCC	RCC	RRC	SEM	*P*-value
Brain, g	184.4	191.3	197.7	6.44	0.28
g/kg BW	5.90	6.14	6.09	0.364	0.75
Heart, g	196.1	194.4	208.9	16.4	0.58
g/kg BW	6.12	6.08	6.25	0.291	0.85
g/g Brain	1.09	1.05	1.10	0.07	0.84
LV thickness[2], mm	12.4	11.5	11.3	0.62	0.17
RV thickness[3], mm	11.2	11.3	11.3	1.21	0.99
Lung, g	709.9	721.1	752.1	52.0	0.79
g/kg BW	21.17	21.33	21.22	1.48	0.99
g/g Brain	3.85	3.78	3.81	0.28	0.98
Adrenals, g	2.00^{ab}	1.81^{a}	2.27^{b}	0.19	0.06
g/kg BW	0.063	0.058	0.068	0.003	0.13
g/g Brain	0.011	0.010	0.012	0.001	0.18
Kidneys, g	146.0	132.0	138.5	16.9	0.69
g/kg BW	4.70	4.35	4.40	0.241	0.51
g/g Brain	0.82	0.72	0.73	0.09	0.44
Perirenal fat, g	162.2	155.9	170.3	18.7	0.76
g/kg BW	4.96	4.73	4.93	0.302	0.84
g/g Brain	0.904	0.847	0.897	0.082	0.82
Stomach complex[4], g	259.1	344.0	583.9	215.4	0.44
g/kg BW	7.94	10.34	16.58	5.80	0.48
g/g Brain	1.49	1.84	3.00	1.04	0.48
Small intestine, g	219.1	218.8	219.9	21.4	0.99
g/g BW	6.92	7.01	6.76	0.358	0.86
g/g Brain	1.23	1.19	1.16	0.10	0.79
Large intestine, g	86.6	84.4	86.1	7.68	0.95
g/kg BW	2.75	2.73	2.67	0.16	0.92
g/g Brain	0.487	0.461	0.460	0.039	0.72
Spleen, g	66.4	61.1	59.5	14.0	0.83
g/kg BW	2.24	2.13	2.00	0.273	0.61
g/g Brain	0.378	0.330	0.310	0.070	0.52
Liver, g	507.0	528.1	5554.3	55.2	0.63
g/kg BW	16.92	17.71	17.98	0.62	0.40
g/g Brain	2.89	2.90	2.93	0.32	0.99
Pancreas, g	23.6	22.1	24.2	1.89	0.59
g/kg BW	0.704	0.662	0.690	0.039	0.73
g/g Brain	0.133	0.121	0.129	0.010	0.73
Omental and mesenteric fat, g	180.6	184.6	199.8	12.3	0.43
g/kg BW	5.42	5.56	5.75	0.421	0.72
g/g Brain	0.985	0.965	1.01	0.072	0.87

[1] Cows received the control diet (100% NRC) from d 30 until 254 (CCC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RCC; n = 5), and restricted from d 30 to 140 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RRC; n = 6).

[2] LV thickness: Left ventricle thickness.

[3] RV thickness: Right ventricle thickness.

[4] Stomach complex = (reticulum + rumen + omasum + abomasum) – digesta.

Table 5.9. The effects of nutrient restriction followed by realimentation on fetal reproductive organ weight in beef cows at d 254 of gestation

	Nutritional treatments[1]				
Item[2]	CCC	RCC	RRC	SEM	P-value
Uterus, g	6.44	7.09	6.85	0.75	0.68
g/g BW	0.19	0.17	0.19	0.014	0.58
g/g Brain	0.034	0.36	0.036	0.003	0.64
Ovaries, g	1.20	2.28	1.80	1.03	0.45
g/g BW	0.037	0.063	0.052	0.026	0.46
g/g Brain	0.006	0.012	0.009	0.005	0.41
Mammary gland, g	71.4	76.6	88.2	19.2	0.36
g/g BW	2.28	2.19	2.61	0.620	0.57
g/g Brain	0.391	0.421	0.478	0.092	0.29
Testes, g	3.56^a	3.59^a	5.33^b	0.36	0.01
g/g BW	0.120^a	0.128^a	0.174^b	0.014	0.008
g/g Brain	0.020^a	0.019^a	0.026^b	0.001	0.02

[1] Cows received the control diet (100% NRC) from d 30 until 254 (CCC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RCC; n = 5), and restricted from d 30 to 140 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RRC; n = 6).

[2] CCC = 2 males and 4 females; RCC= 4 male and 1 female; RRC= 3 male and 3 females.

Metabolites at d 85

Maternal cholesterol and lactate concentrations at d 85 slaughter were increased (P = 0.03) in R compared to C cows. However, maternal concentrations of triglyceride and glucose were similar ($P \geq 0.28$) between treatments. When looking at umbilical cord serum at d 85, cholesterol, triglycerides, and glucose were not affected ($P \geq 0.12$) by maternal dietary treatment. Umbilical cord fructose and BUN concentrations tended (P = 0.07) to be increased in fetuses from R cows compared to C fetuses and lactated concentrations (P = 0.05) were greater in fetuses from R cows compared to C fetuses. Amniotic fluid triglycerides, glucose, fructose, and lactate concentrations at d 85 were similar ($P \geq 0.38$) among treatments.

Table 5.10. Maternal serum, umbilical cord serum, and amniotic fluid metabolite concentrations at d 85 of gestation

	Nutritional treatments[1]			
Item	C	R	SEM	*P*-value
Maternal serum				
Cholesterol, mg/dL	72.97	95.13	6.23	0.03
Triglycerides, mg/dL	18.55	15.01	2.20	0.28
Glucose, mg/dL	93.37	99.50	4.43	0.35
Fructose[2], mg/dL	ND	ND	----	----
Lactate, mg/dL	6.39	10.00	0.99	0.03
BUN, mg/dL	7.59	7.33	0.47	0.70
Umbilical cord serum				
Cholesterol, mg/dL	41.94	41.76	2.30	0.96
Triglycerides, mg/dL	25.04	22.63	9.64	0.59
Glucose, mg/dL	4.44	12.29	9.61	0.12
Fructose, mg/dL	75.60	93.60	6.12	0.07
BUN, mg/dL	7.99	9.39	0.48	0.07
Lactate, mg/dL	6.30	6.57	0.08	0.05
Amniotic fluid				
Triglycerides, mg/dL	28.56	21.77	7.11	0.52
Glucose, mg/dL	39.44	35.57	12.16	0.63
Fructose, mg/dL	226.8	210.42	62.46	0.86
Lactate, mg/dL	20.27	16.57	1.62	0.14

[1] Cows received either control (100% NRC) diet (C; n = 6) or restricted (60% NRC) from d 30 to 85 (R; n = 6).

[2] Fructose was non-detectable (ND) in maternal jugular serum

Metabolites at d 140

Maternal serum concentrations of cholesterol, triglycerides, and glucose at d 140 of gestation were similar ($P \geq 0.13$) among treatments. Maternal serum lactate tended ($P = 0.06$) to be decreased in RR cows compared to CC and RC being intermediate. Fetal umbilical cord serum concentrations of cholesterol, triglycerides, glucose, fructose, and lactate were not affected ($P \geq 0.27$) by maternal dietary treatment. Similarly, concentrations of triglycerides, glucose, fructose, and lactate were not affected ($P \geq 0.38$) by maternal dietary treatment in amniotic fluid at d 140 of gestation.

Table 5.11. Maternal serum, umbilical cord serum, and amniotic fluid metabolite concentrations at d 140 of gestation

	Nutritional treatments[1]				
Item	CC	RC	RR	SEM	*P*-value
Maternal serum					
Cholesterol, mg/dL	73.34	85.02	80.15	5.57	0.42
Triglycerides, mg/dL	18.83	18.30	15.80	1.92	0.52
Glucose, mg/dL	96.13	83.77	94.85	4.11	0.13
Fructose, mg/dL	ND	ND	ND	----	----
Lactate, mg/dL	12.25	11.98	9.19	0.99	0.06
BUN, mg/dL	6.21	6.69	6.95	0.60	0.64
Umbilical cord serum					
Cholesterol, mg/dL	38.41	41.01	40.61	4.24	0.90
Triglycerides, mg/dL	72.46	68.54	86.72	9.17	0.33
Glucose, mg/dL	88.91	103.03	98.45	22.18	0.90
Fructose, mg/dL	69.66	79.02	74.52	8.10	0.76
BUN, mg/dL	6.44	7.35	8.16	0.69	0.23
Lactate, mg/dL	7.29	7.93	7.48	0.27	0.27
Amniotic fluid					
Triglycerides, mg/dL	35.79	41.54	50.53	29.73	0.93
Glucose, mg/dL	17.25	22.58	18.9	2.68	0.46
Fructose, mg/dL	102.06	140.04	64.98	41.22	0.46
Lactate, mg/dL	12.25	12.34	11.98	2.16	0.99

[1] Cows received the control diet (100% NRC) from d 30 until 140 (CC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 140 (RC; n = 5), and restricted from d 30 to 140 (60% NRC; RR; n = 6).

[2] Fructose was non-detectable (ND) in maternal jugular serum

Metabolites at d 254

Serum concentrations of cholesterol, triglycerides, fructose, and lactate in maternal serum at d 254 of gestation were not affected ($P \geq 0.52$) by maternal dietary treatment. Fetal umbilical cord serum concentrations of cholesterol, triglycerides, glucose, fructose, and lactate were not affected ($P \geq 0.28$) by maternal dietary treatment. Also, amniotic fluid concentrations of triglycerides, glucose, fructose, and lactate were not affected ($P \geq 0.11$) by maternal dietary treatment at d 254 of gestation.

Table 5.12. Maternal serum, umbilical cord serum, and amniotic fluid metabolite concentrations at d 254 of gestation

	Nutritional treatments[1]				
Item	CCC	RCC	RRC	SEM	*P*-value
Maternal serum					
Cholesterol, mg/dL	93.13	97.30	98.31	7.54	0.86
Triglycerides, mg/dL	20.91	22.50	24.53	3.00	0.65
Glucose, mg/dL	90.76	88.62	85.45	4.89	0.70
Fructose, mg/dL	ND	ND	ND	----	----
Lactate, mg/dL	13.15	14.95	13.96	1.80	0.52
BUN, mg/dL	5.54	6.05	5.33	0.49	0.56
Umbilical cord serum					
Cholesterol, mg/dL	31.95	34.66	34.84	2.86	0.69
Triglycerides, mg/dL	15.8	21.86	25.46	5.98	0.47
Glucose, mg/dL	29.69	22.07	41.16	19.59	0.79
Fructose, mg/dL	68.76	60.66	71.1	7.38	0.62
BUN, mg/dL	6.07	6.10	6.35	0.32	0.77
Lactate, mg/dL	11.44	13.51	9.73	1.71	0.28
Amniotic fluid					
Triglycerides, mg/dL	4.38	3.91	6.24	1.67	0.33
Glucose, mg/dL	5.71	1.96	5.17	1.95	0.35
Fructose, mg/dL	178.56	154.98	184.86	19.26	0.51
Lactate, mg/dL	18.65	18.38	12.07	3.78	0.11

[1] Cows received the control diet (100% NRC) from d 30 until 254 (CCC; n = 6), restricted from d 30 to 85 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RCC; n = 5), and restricted from d 30 to 140 (60% NRC) and then realimented to 100% of the NRC requirements until d 254 (RRC; n = 6).

[2] Fructose was non-detectable (ND) in maternal jugular serum

Discussion

We hypothesized that maternal nutrient restriction during early to mid-gestation would reduce fetal weight and impair organ development. Moreover, we also hypothesized that upon nutrient realimentation the negative effects of nutrient restriction would be offset and fetal growth and development would be enhanced. However, during the first 55 d of maternal nutrient restriction we observed a tendency for bigger fetuses in R vs. C cows and when cows were nutrient restricted longer (110 d) there were no differences between treatments. Similarly after realimentation fetal weight was similar between treatments. In Chapter 4 we reported the placental growth and development for this particular project. Briefly, we observed bigger placentas when cows where nutrient restricted from d 30 to 85 of gestation compared to cows receiving control diet. However, when cows were realimented during early and mid-gestation we no longer observed treatment effects for placental or fetal growth. The placenta serves as interface between the dam and the fetus by supplying nutrients and oxygen to the fetus which are necessary for tissue accretion and energy metabolism (Fowden et al., 2008). Having bigger placentas at d 85 in R cows might helped with the increase in fetal growth we observed by transferring more nutrients.

We previously have shown (Chapter 3) that the dam undergoes adaptations during an early to mid-gestation nutrient restriction and becomes more efficient in the utilization of nutrients after being realimented and as gestation advances perhaps providing sufficient nutrients for the developing conceptus as we observed minimal differences after mid-gestation nutrient restriction in fetal growth and development. In nutrient restricted cows from d 30 to 125 of gestation fetuses had similar size and weight to controls, however, when calves where separated into IUGR-nutrient restricted (reduced fetal weight and asymmetrical growth) those calves were lighter and empty carcass weight was also reduced compared to control and non-IUGR fetuses (Long et al., 2009). Similarly to our findings, after nutrient realimentation fetuses from nutrient restricted cows has similar fetal weight compared to control at d 245 of gestation. Long et al. (2012) have shown that when cows were nutrient restricted to 70% of NRC requirements from d 45 to 185 of gestation and thereafter fed the control diet (100% of NRC) until parturition calves birth weight was similar between treatments.

Intrauterine growth restriction has been associated with abnormal organ development. In our experiment we observed heavier livers and a tendency for bigger pancreata in R fetuses compared to C fetuses at d 85 slaughters. However by d 140 and 254 we did not observed differences in absolute organ weights and weight relative to brain and fetal BW. Meyer et al. (2010) have shown that fetuses from cows that were nutrient restricted from d 30 to 125 of gestation have similar pancreas and liver weight. Similarly, after nutrient realimentation fetal pancreas and liver weight reminded the same (Meyer et al., 2010). In a model of IUGR in sheep, where ewes were nutrient restricted (60 % of NRC requirement from) d 50 to 130, fetuses from restricted dams had lower liver weight compared to controls; however, pancreas weight was similar between treatments (Lemley et al., 2011). In addition, when ewes were nutrient restricted from mid- to late gestation had fetal lambs with smaller pancreas and liver mass (Reed et al., 2007). It is important to note that the Lemley et al. (2011) and Reed et al. (2007) studies were conducted in sheep and at different time points of restriction, but, in general, an insult to the dam during fetal development has been shown to have negative consequences during organogenesis. Exponential growth of the bovine fetus occurs during the last trimester of gestation but fetal nutrient uptake becomes very important after mid-gestation and is reflected on nutrient requirements of the dam (Greenwood and Cafe, 2007). Although, most of the fetal growth occurs during late gestation, maternal insults during early gestation can impact fetal organogenesis. It has been reported in cattle that limb development occurs around d 25 of gestation and this is followed by other organs development including the pancreas, liver, brain, and kidneys (Hubbert et al., 1972). When the insult occurs at the organogenesis stage, changes in the tissue can be permanent and can stunt development (Fowden et al., 2006). In our study most of the fetal organs had similar weights regardless maternal nutrient restriction and/or different periods of realimentation which could be due to an alteration in those nutrient restricted fetuses to “catch-up” with the control fetuses. However, we do not know if the function of those organs was altered having negative consequences later in life.

When we looked at metabolites in the umbilical cord serum, we observed an increase in lactate in R fetuses compared to C fetuses. The rest of the metabolites measured in the umbilical cord serum were not affected by maternal nutrition at different stages of gestation. Lactate produced by the utero-placenta is used by the fetus and for amino acid metabolism which at the end will provide gluconeogenic amino acids for the fetus (Fowden et al., 2008). Freetly and Ferrell (1998) have shown in pregnant sheep that hepatic lactate increases throughout pregnancy. They also suggested that non pregnant and early pregnant ewes had a net release of lactate from splanchnic tissues; but during late pregnancy, there was a net uptake of lactate by splanchnic tissues. Sparks et al. (1982) have suggested that lactate in sheep does not diffuse from the fetus to the dam; instead, lactate is produced and distributed asymmetrically by the placenta. They also showed that the greatest lactate concentration was within the umbilical circulation. Also, it has been shown in cattle that the utero placenta has a net secretion of lactate and increases from d 137 until d 250 of gestation (Reynolds et al., 1986). In our study, R cows had greater lactate concentration in jugular vein compared to C cows at d 85 of gestation. When cows were slaughter on d 140, RR cows tended to have lower concentrations of lactate compared to other tissues; by d 254, lactate concentration sin jugular vein was similar among treatments. Freetly and Ferrell (1998) suggested that that increased lactate during sheep pregnancy is not related to diet since lactate released by the portal-drained viscera did not change throughout pregnancy in their study. Other metabolites apart from lactate are very important as source of nutrients for fetal development including glucose and amino acids (Sparks et al., 1982). In our study we did not observe a treatment effect during different stages of gestation on cholesterol, triglycerides, glucose, fructose, and BUN concentrations in maternal jugular vein serum, umbilical cord serum, and amniotic fluid. Previously, Rasby et al. (1990) reported decreased fructose in thin (BCS = 4) cows compared to moderate (BCS = 6) in amniotic fluid at d 256 of gestation; however concentrations of proteins was not affected by maternal nutritional status. It is important to mention that with our blood collection we just have a snap-shot of metabolite concentration, research still necessary on nutrient flux across the utero-placenta and fetus in beef cows during nutrient restriction and at different realimentation time points.

Even though we did not observe major changes in fetal growth and development due to maternal nutrient restriction we still do not know much about metabolic and endocrine impacts in this particular project and also how maternal realimentation after nutrient restriction will have an effect. Previously we reported that nutrient restriction during early to mid-gestation impacts the development of fetal muscle from cows of this particular project. After maternal nutrient realimentation, compensatory growth of the fetal muscle occurs (Gonzales et al., 2013). This is important for animal production as has an impact in meat quality. More research is necessary looking at fetal composition and metabolic function from fetuses born to dams that where nutrient restricted from early to mid-gestation followed by realimentation. In addition neonatal growth, reproductive function, and carcass quality warrants further investigation.

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CHAPTER 6. OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

General Conclusions

It is known that pregnancy success and offspring health are dependent on nutrient intake and conceptus growth and development. A key player for proper conceptus development is the placenta as it plays an important role in providing physiological exchange between the maternal and fetal systems (Reynolds and Redmer, 1995). To our knowledge, there is a paucity of information on how maternal realimentation in beef cows impacts placental and fetal development during early to mid-gestation. Two experiments were conducted in order to determine how maternal nutrient restriction followed by realimentation impacts conceptus development. In addition, we obtained data on maternal adaptations to the changes in nutrition at different stages of gestation.

Both experiments allowed us to adapt a reliable method of monitoring uterine and umbilical arteries BF and resistance indices utilizing Doppler ultrasonography. Doppler ultrasonography has been previously utilized to characterize uterine BF during gestation in beef and dairy ranging from as early as d 30 of gestation until approximately d 282 (Bollwein et al., 2002; Panarace et al., 2006; Herzog et al., 2011). Even though there are some limitations when using this technique in pregnant beef cows, we feel very confident in the results obtained from our experiments. In summary, nutrient restriction from d 30 until 140 of gestation did not alter total uterine BF. However, upon realimentation (from d 140 to 198 of gestation), there was enhanced ipsilateral uterine BF during mid-gestation. However, during late gestation (Chapter 4) when all cows were receiving similar nutrition (100% of the NRC requirements), ipsilateral uterine BF and total BF were increased in cows that were nutrient restricted from d 30 until d 85 of gestation and then realimented until d 254 of gestation. For umbilical artery BF, we did not observe any differences due to maternal nutrition. We have a limited window to obtained umbilical artery BF because the size of the fetus increases dramatically during late gestation. Therefore, we were not able to obtained umbilical BF measurements after d 150 of gestation making it difficult to examine what happened after the realimentation period. Therefore, results from both experiments suggest that the bovine placenta may be programmed to function differently after a period of nutrient restriction. Interestingly, in many sheep models investigated to date (reviewed in Reynolds et al., 2006; Lemley et al., 2012), nutrient restriction results in reduced uterine and/or umbilical BF. In swine, complete inanition did not alter uterine blood flow (Hard and Anderson, 1982). This could be innate species differences, or also due to parity or age of the dam. Regardless, until more beef cattle research is performed to confirm our results, caution should be used when comparing data acquired in sheep as it may not be directly applicable to beef cattle.

During our second experiment, we were able to slaughter cows at 3 different stages of gestation (d 85, 140, and 254). First we looked at the maternal performance and organ weights. We observed partial changes in maternal weight and organ masses due to different lengths of maternal nutrient restriction followed by realimentation. It appears that the dam undergoes some adaptations during an early to mid-gestation nutrient restriction to become more efficient in the utilization of nutrients after being realimented and as gestation advances. When we looked at placental and fetal growth and development in the same study we found that nutrient restriction during early restriction increased fetal growth and placental size. However, when cows were restricted longer and/or realimented during early and mid-gestation we no longer observe treatment effects for placental or fetal growth. Therefore, following extended restriction, growth of those fetuses caught up with the early restricted cows and this might be due to nutrient transport capacity or placental function.

From the results obtained in these 2 experiments we can conclude that maternal nutrient restriction during early gestation enhances fetal and placental growth. One potential mechanism is maternal adaptation to the nutrient restriction and the second mechanism is by enhancing uterine blood flow to the gravid uterus. Perhaps, timely management strategies applied during gestation might enhance conceptus development. Even though more research is necessary, opportunities may be available to intervene during times of poor nutrition in beef systems.

Future Directions

One vital observation from the current project is that future studies should not rely on sheep models in depicting cattle responses even though they are ruminants. Animal scientists at NDSU have developed a nutrient restricted model that works very well for sheep; however, after conducting 4 years of research utilizing the beef cow in a nutrient restricted model we have observed different results. One of the first limitations we encountered was that even though we utilized the NRC to calculate the nutrient requirements for pregnant beef cows, all cows lost weight, including the control (i.e. they received 100% of the NRC recommendations). We believe that cows have greater nutrient requirements than those estimated or feed energy values were overestimated. Nutrient requirements could be greater than estimated because of several factors which could include differences in environmental conditions, genetics, lactation, or fetal growth (NRC, 2000). The fact that cows were losing weight and the lack of appropriate facilities to handle late gestation cows and calving pens in our first experiment, we had to send the cows back to the NDSU Beef Unit. We were unable to continue monitoring feed intake and uterine BF during late gestation. Therefore, more research is necessary where we can follow uterine BF in both contralateral and ipsilateral arteries throughout gestation utilizing a similar experimental design but perhaps a better estimation and /or control of diets and cow weight with the upcoming and updated NRC.

We gathered an extensive amount of data from our second experiment including maternal, fetal and placental variables at different stages of gestation making it a significant contribution to the literature already available in beef cows. In order to obtain this information, we had to slaughter all of our experimental animals; therefore, the effects of nutrient restriction followed by realimentation on offspring growth and development are still largely unknown. It is well known that IUGR has negative consequences in animal production, such as decreased neonatal survival, decreased offspring birth weight, and decreased postnatal growth and subsequent performance of the offspring (reviewed by Wu et al., 2006). Interestingly, in a recent study (Mossa et al., 2013) where beef heifers were nutrient restricted 11 d prior to insemination until d 110 of gestation decreased ovarian reserve was observed in the female calves. The author suggests that this could be due to the observed increase in maternal circulating testosterone. However, female calves' growth parameters were similar to the control counterparts (Mossa et al., 2013). Therefore, experiments where we allow cows to calve and we can follow the offspring and measure growth and performance variables are necessary. In addition, studies following the female calves to measure reproductive parameters should be conducted and look at how they perform when heifers are bred and measure their uterine BF during gestation.

Focusing on the placenta, we observed an increase in ipsilateral uterine artery BF; however, we do not know how much of each horn the placenta occupied and how much variation exists between cows. In addition, we believe the conceptus might be secreting vasoactive factors which are related to the increase in uterine BF that was observed in both Chapters 2 and 4. From personal observations, when ultrasounding cows, most of the animals that had greater BF in the ipsilateral uterine artery had very low BF in their contralateral uterine artery (i.e. 20 L/min vs. 0.25 L/min, respectively). Therefore, more research is necessary to determine if the conceptus secretes vasodilatory factors and if this is the cause of the local increase in BF.

We were not able to perform surgeries because we wanted to salvage the carcasses from our cows thus all our animals were slaughtered at the NDSU Meat Laboratory. However, we were able to obtain jugular blood from dam and umbilical cord blood samples from the fetus. While we analyzed these samples for several metabolites, this is just a snapshot of what is going on at a specific time point and we were not able to separate venous from arterial blood. Conducting research where we can collect multiple samples from uterine and umbilical vein and artery thought catheters during early gestation and/or prior to slaughter will allow us to measure nutrient flux across the placenta.

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APPENDIX. BOVINE UTERINE BLOOD FLOW

Table A.1. Bovine blood flow gravid uterus: First trimester

Breed	Stage of pregnancy	Method	Response[1]	Additional Response[1]	Reference
Hereford	First 30 d	Electromagnetic BF probes	↔ UBF until d 13 2-3 fold ↑ ipsilateral UBF from d 14-18 and ↔ contralateral UBF	d 19 to 25 ↓ UBF ipsilateral UBF ↑ until d 30 and contralateral UBF ↓	Ford et al., 1979
Hereford	Approximately d 30 and 80	Electromagnetic BF probes	d 31-45 UBF 0.12 ± 0.02 L/min d 77-92 UBF 0.20 L/min		Ferrell and Ford, 1980
Simmental Brown Swiss	d 30, 60, and 90	Doppler	↔ both arteries d 30 avg. 0.10 L/min d 60 avg. 0.14 L/min d 90 avg. 0.30 L/min	↓ RI by d 60 ↔ diameter	Bollwein et al., 2002
Aberdeen angus	d 30 and 84	Doppler	d 30 Ipsilateral UBF 0.08 ± 0.01 L/min and contralateral UBF 0.04 ± 0.01 L/min d 84 Ipsilateral UBF 0.59 ± 0.05 L/min and contralateral UBF 0.17 ± 0.03 L/min		Panarace et al., 2006

[1] ↔: steady change; ↑: increased; ↓: decreased

Table A.2. Bovine blood flow gravid uterus: Second trimester

Breed	Stage of pregnancy	Method	Response[1]	Additional Response[1]	Reference
Hereford	Approximately d 140 and 180	Electromagnetic BF probes	d 139-155 2.0 ± 0.6 L/min d 178-199 3.2 ± 0.4 L/min		Ferrell and Ford, 1980
Hereford	163-166 d 173-176 d	Steady state diffusion; antipyrin solution	Mean UBF 5.9 ± 0.5 ml/min	Mean HR 68.6 ± 2.6 beats/min	Ferrell et al., 1983
Hereford	137-180 d	D2O (ipsi) infusion	d 137 UBF 2.9 L/min d 180 UBF 4.8 L/min		Reynolds et al., 1986 Reynolds and Ferrell, 1987
Simmental Brown swiss	d 120, 150, 180	Doppler	Ipsi d 120 0.7 L/min Contra d 120 0.4 L/min Ipsi d 150 1.7 L/min Contra d 150 0.9 L/min Ipsi d 180 4.0 L/min Contra d 180 1.3 L/min		Bollwein et al., 2002
Aberdeen angus	d 168	Doppler	Ipsi 5.5 ± 0.4 L/min Contra 0.6 ± 0.1 L/min		Panarace et al., 2006
Holsteins	d 147 and 175	Doppler	↑Total UBF in heavy vs. light cows: Light cow d 147 2.6 L/min heavy cow d 147 3.4 L/min Light cow d 175 4.2 L/min heavy cow d 175 5.4 L/min	At d 147 cows had similar total UBF when carrying heavy or light calves but at d 175 cows carrying heavy calves had ↑ BF vs. light calves	Herzog et al., 2011

[1] ↔: steady change; ↑: increased; ↓: decreased

Table A.3. Bovine blood flow gravid uterus: Last trimester

Breed	Stage of pregnancy	Method	Response[1]	Additional Response[1]	Reference
Jersey	Between 250 and 270 d	Diffusion equilibrium	330 ± 18.2 ml/kg/min		Comline and Silver, 1976
Hereford	Approximately d 210 and 240	Electromagnetic BF probes	d 202-224 4.0 ± 0.5 L/min d 230-258 3.1 ± 0.2 L/min		Ferrell and Ford, 1980
Hereford	226-250 d	D2O (ipsi) infusion	d 226 UBF 2.9 L/min d 250 UBF 13.1 L/min	↑ 4.5 fold in ipsilateral UBF from d 137 to 250 ↑ Maternal HR	Reynolds et al., 1986 Reynolds and Ferrell, 1987
Brahman Charolais	220 ± 0.4 d	D2O (ipsi) infusion	↑ipsi UBF in Charolais vs. Brahman	Charolais carrying Charolais fetuses ↑ BF vs. Charolais carrying Brahman fetuses	Ferrell, 1991
Charolais Hereford	190 ± 0.5 d	D2O (ipsi) infusion	↓ UBF Hereford 4.8 vs. Charolais 7.1 L/min	↓ in cows with twins 5.2 vs. single 6.7	Ferrell and Reynolds, 1992
Simmental Brown Swiss	d 210, 240, 270, and 282	Doppler	Ipsi d 210 5.0 L/min Contra d 210 1.3 L/min Ipsi d 240 6.0 L/min Contra d 240 2.8 L/min Ipsi d 270 10.5 L/min Contra d 270 3.9 L/min Ipsi d 282 13.1 L/min Contra d 282 4.5 L/min	↑UBF and diameter during gestation ↓ RI during first 8 months and ↔ after	Bollwein et al., 2002
Aberdeen angus	d 266	Doppler	Ipsi 14.1 ± 1.2 L/min Contra 2.0 ± 0.4 L/min	↑UBF 20-fold and diameter17-fold by d 270 ↔ Contralateral BF	Panarace et al., 2006

Table A.3. Bovine blood flow gravid uterus: Last trimester (continued)

Breed	Stage of pregnancy	Method	Response[1]	Additional Response[1]	Reference
Holsteins	d 203, 231, 259, and 273	Doppler	Light cow d 203 6.1 L/min heavy cow d 203 8.7 L/min Light cow d 231 10.5 L/min heavy cow d 231 13.1 L/min Light cow d 259 13.6 L/min heavy cow d 259 16.9 L/min Light cow d 273 14.1 L/min heavy cow d 273 19.2 L/min	Linear ↑ in total UBF 3.0 to 16.9 L/min ↑ total UBF in heavy cows vs. light Similar wt cows with heavy calves had ↑ total UBF vs. light	Herzog et al., 2011

[1] ↔: steady change; ↑: increased; ↓: decreased

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