SUPPLEMENTATION OF CORN DRIED DISTILLER'S GRAINS PLUS SOLUBLES TO GESTATING BEEF COWS FED LOW-QUALITY FORAGE

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Title

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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

MASTER OF SCIENCE

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ABSTRACT

To investigate the effects of corn dried distiller's grains plus solubles (**DDGS**) supplementation to cows during late gestation, 27 multiparous beef cows were divided randomly into 2 treatment groups (**CON**; n = 15; **SUP** = DDGS at 0.3% of BW; n = 12). Supplemented cows gained BW ($P < 0.01$) while CON cows tended to lose BW ($P = 0.06$). A main effect of treatment ($P = 0.02$) and day ($P < 0.01$) was observed for total uterine blood flow (**BF**). Calves born to SUP cows tended to be heavier than calves born to CON cows ($P = 0.06$). Both groups gained ($P < 0.01$) BW with advancing lactation. Supplementing DDGS altered voluntary feed intake, cow body maintenance, uterine and mammary blood flow, and calf weaning weights. Results suggest an influence on overall maternal metabolism and nutrient flux to the fetus.

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"Well, now that we have seen each other," said the unicorn,
"if you'll believe in me, I'll believe in you."
Lewis Carrol, *Alice's Adventures in Wonderland and Through the Looking Glass*

"It is true that those we meet can change us, sometimes so profoundly that we are not the same afterwards, even unto our names."
Yann Martel, *Life of Pi*

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CHAPTER 1. LITERATURE REVIEW

Introduction

Developmental programming is the notion that a stimulus or insult to the developing offspring has long-term effects on productivity and consequently lifelong health. From a biological basis, developmental programming is possible due to "developmental plasticity," (Barker, 2004) which is the cultivation of a phenotype in anticipation of surviving the demands of the environment being experienced in utero, rather than simply the production of the same phenotypes regardless of environmental influences or stressors. More concisely, it is a form of short-term generational adaptability. Various fields of research ranging from human epidemiological studies to animal models such as rats, sheep and cattle have built support for the concept of developmental programming. While various influences during gestation can be responsible for their downstream effects, alterations in maternal nutrition have dramatic consequences for fetal development and lifelong health.

Maternal nutrition is essential to fetal and placental development, which can influence the lifetime performance of our livestock species, including cattle (Funston et al., 2010). Understanding the role of altered maternal nutrition during pregnancy in changing the maternal and fetal environment perinatally is of great value to producers for planning feeding strategies to optimize potential of the offspring.

Uteroplacental vasculature is a key component of transplacental exchange and thusly vital to fetal development (Reynolds and Redmer, 1995). One way to quantify nutrient delivery to the fetus is to measure uterine arterial blood flow (BF; Ferrell, 1991). Doppler ultrasonography offers the possibility of measuring uterine BF in a noninvasive, reliable and repeatable manner, with the added benefit of taking observations in the same animal over time.

Modification of uterine BF and nutrient transfer capacity enables increased oxygen and nutrient delivery to the growing fetus (Vonnahme and Lemley, 2012). In addition, maternal nutrient intake can also alter circulating vasoactive steroids, specifically estradiol-17β (E2) and progesterone (P4), which may influence uterine BF and/or nutrient flux to the conceptus. Therefore, measurement of production and clearance of those steroids can help explain how altered maternal nutrition influences uterine arterial BF.

Mammary gland BF is a vital component for milk synthesis and, therefore, nutrient delivery to the offspring post-partum. Mammary BF is also strongly correlated with milk yield (Götze et al, 2010). Once again, Doppler ultrasonography can provide an accurate, reproducible, and less invasive tool for measuring BF to the mammary gland. However, little work has been done to characterize mammary BF in beef cattle and its influences on calf postnatal performance.

In relation to mammary BF, maternal nutrient intake during gestation can also alter systemic BF via changes in circulating hormones and growth factors during pregnancy that facilitate nutrient delivery to the still developing mammary gland in anticipation of colostrum and milk production (Svennersten-Sjaunja and Olsson, 2005). Additionally, differing nutritional protein and energy planes can influence milk production to varying degrees depending on timing of differences in gestational diet (Sullivan et al., 2009, McSweeney et al., 1993).

Previous research has demonstrated benefits of supplementing dams fed low-quality forage with dried distiller's grains plus solubles (DDGS; as a protein supplement) such as increased percentage calves weaned, weaning weights, and average daily gain (Stalker et al., 2006), increased growth and reproductive success of heifer calves (Martin et al., 2007), and improved quality grade of steer calves (Larson et al., 2009). However, investigation of the

mechanisms behind those phenotypic benefits is warranted; perhaps arterial BF to the uterus and/or mammary glands play a role in observed advantages.

This literature review will discuss: 1) the concept of developmental programming; 2) maternal nutrition during gestation, particularly in regards to protein supplementation; 3) uterine BF during gestation and its influence on nutrient delivery to the fetus; 4) early lactation in the beef cow including preparation of the mammary gland for lactation, milk production and arterial BF to the mammary glands; and 5) conclude with the rationale for the experimental objectives of the study addressed in this dissertation. When possible, examples from research in cattle will be preferentially discussed: however, relevant studies to foundational knowledge across species will be included as needed. Following this literature review, the main experimental objectives of this thesis dissertation will be discussed in their own chapters, followed by a general discussion and reflection on future directions.

Developmental Programming

To begin with, the large majority of research concerning pregnancy conducted in the current scientific climate, especially in our livestock species, cannot be excluded from the concept of developmental programming. Developmental programming is the notion that a stimulus or insult to the developing offspring has long-term effects on productivity and consequently lifelong health. The concept has had so much support that in 1990 Dr. Barker called for more research directed towards the intrauterine environment (rather than the environment later in childhood), considering it the new model for adult degenerative disease (Barker, 1990). From a biological basis, developmental programming is the result of "developmental plasticity," (Barker, 2004) which essentially enables the phenotype of a fetus to be more adapted to the environment it will be born into, rather than production of the same

phenotype regardless of environment. Research lending its support to the concept has spanned various fields and species, ranging from human epidemiological studies (Barker, 1990) to livestock production (Reynolds and Caton, 2012) and the use of large animal models to develop therapeutics during pregnancy across species (Reynolds et al., 2010).

The concept of developmental programing first came to light in the context of human epidemiological studies, most famously those dealing with the concept of the "thrifty genotype" (Neel, 1962), the "Dutch Hunger Winter" (G. P. Ravelli and Z. A. Stein, 1976; Schulz, 2010), and the establishment of connections between low birth weight and coronary heart disease, termed the "Barker Hypothesis" (Barker, 1995).

Neel (1962) describes the "thrifty genotype" as an individual who is predisposed from birth to be "exceptionally efficient in the intake and/or utilization of food," which was likely beneficial to our hunter-gatherer ancestors. However, in current times it has become less of an asset and instead may be a major contributor to increased incidence of diabetes mellitus, which Neel even dubs a "disease of civilization." Although our knowledge of causes of diabetes has changed dramatically since the publication of that paper in 1962 (Neel, 1962), the idea of the "thrifty genotype" is still relevant in the spectrum of developmental programming.

A related example is the plethora of research related to the "Dutch Hunger Winter" of 1944-45. Of the conclusions drawn from observations on children gestated during that period of famine, two important concepts come to the forefront: identification of critical periods in development (i.e., when and how long in utero the fetus was exposed to famine), and the extent of the influence of birth weight on adult disease (Schulz, 2010). "The Dutch Hunger Winter" highlighted that although intrauterine exposure to stress (famine) can have long term effects on adult health, they do not always result in or are dependent on differences in birth weight; some

children had significantly reduced birth weights while others had normal birth weights, but those with normal birth weights that were exposed to famine during early gestation had the greatest incidence of adult obesity. The second important observation from the "Dutch Hunger Winter" demonstrated the importance of timing, or the existence of critical windows, to developmental programming. Those exposed to the famine only during late gestation were born small and remained small through adulthood, but those exposed to famine earlier in gestation had significantly higher rates of obesity, altered lipid profiles, and cardiovascular disease in adult life, as well as impaired selective attention when they were older (56 to 59 yr; Schulz, 2010). However, more recent human and animal studies have demonstrated that outside of famine, fetal undernutrition late in gestation is more commonly a result of inadequate maternoplacental (i.e., uteroplacental) supply capacity created earlier in gestation (Godfrey and Barker, 2000).

It is impossible to discuss research in the area of developmental programming without mention of Dr. David Barker, the reason why this concept is often referred to as the "Barker Hypothesis." His original hypothesis proposed that "alterations in fetal nutrition and endocrine status result in developmental adaptations that permanently change structure, physiology and metabolism, thereby predisposing individuals to cardiovascular, metabolic and endocrine disease in adult life" (Barker, 1995). Barker's observations on associations of low birth weights with high death rates from coronary heart disease in adult life showed for the first time that low rates of fetal growth were associated with the disease (findings have been replicated; Godfrey and Barker, 2000). Thusly the concept of developmental programming has become reflective of a general principle in developmental biology.

As previously mentioned, in addition to human epidemiological studies, research in animals, from rats to larger models such as sheep and cattle, also support the concept and helped

define many of the basic principles in developmental programming (Nijland et al., 2008). Within the realm of factors contributing to the influences of developmental programming lies the impact of the maternal system, and as many of the studies mentioned above have demonstrated, fetal growth is limited by the nutrients it receives. Barker (2004) explains the relationship between the fetal response to undernutriton and adult disease concisely:

> "..increased allocation of energy to the development of one trait, such as brain growth, necessarily reduces its allocation to one or more other traits, such as tissue repair processes. Smaller babies, who have had a lesser allocation of energy, much incur higher costs, and these it seems include disease in later life."

It is clear that despite the broad spectrum of potential influences on fetal growth, the maternal environment is of vital importance to fetal growth throughout gestation. In fact, birth weight is a product of both the fetus's trajectory of growth as well as the maternoplacental (i.e. uteroplacental) capacity to supply sufficient nutrients to maintain that trajectory (Godfrey and Barker, 2000). With this in mind, it is important to first focus on the impact of maternal nutrition during pregnancy in a livestock setting (specifically cows when possible) before honing in on the mechanisms of nutrient delivery to the fetus.

Maternal Nutrition During Gestation

The balance of macronutrients (protein, fat, carbohydrates, macrominerals and water) in the maternal diet can have significant short- and long-term effects on the offspring, as demonstrated by various studies in rats (Godfrey and Barker, 2000). In livestock, maternal nutrition is known to be essential to fetal and placental development, which consequently influences the lifetime performance of that animal (calves in this study; Funston et al., 2010).

This is important chiefly because suboptimal nutrition during gestation remains a significant problem for many livestock species (Wu et al., 2004), but offspring born at above average body weight have a greater chance of survival compared to their underweight peers in livestock studies (Funston et al., 2010). Conversely, an embryo transfer study in horses showed that foals born heavier than control foals exhibited early insulin resistance (Peugnet et al., 2014). In an attempt to mitigate the negative effects of suboptimal nutrition, supplementation of various macronurtients offer a potential solution for helping dams maintain body weight and composition during gestation, as well as foster more beneficial phenotypes in offspring. Increased birth weights in our livestock species can be either advantageous or deleterious depending on production environment, thusly it is important to always recognize the demand environment places an animal's genetics (Jenkins and Ferrell, 2006).

There is a growing body of literature in regards to altered nutrition during gestation, however, the focus of this review is to provide background for an experiment concerned with protein supplementation, consequently the benefits of added protein will be the focus of this section. In cattle and the majority of mammals, most fetal growth occurs during the latter part of pregnancy; this "window" has been the focus for investigating dietary supplementation and resulting altered fetal phenotypes. In regards to protein supplementation in beef cattle, the most recent observations come from data collected in winter grazing systems with promising results for cow weight and body condition (Larson, et al., 2009) as well as performance of female (Martin et al., 2007) and male (Stalker et al., 2006) progeny.

Cows receiving a protein supplement during the last trimester of gestation had increased body weight and greater body condition than those who only grazed winter range un-supplemented (Larson et al., 2009). Similar results were found in another study, where cows

grazing winter range with protein supplementation maintained body weight and condition better than their un-supplemented peers (Stalker et al., 2006). Weaning weight of calves from protein supplemented dams in winter grazing systems was not affected in the two studies (Stalker et al., 2006; Martin et al., 2007; same cattle). These results precede an observed tendency for increased weaning weights (Larson et al., 2009), but are likely confounded by the previous treatments. However, two of those studies both demonstrated an increase in cow body condition score prepartum was related to increased birth weights in calves (Stalker et al., 2007; Larson et al., 2009). Finally, weaning weights (actual and adjusted) were greater in calves from protein-supplemented dams (Larson et al., 2009).

Heifers from protein-supplemented dams were heavier at weaning, pre-breeding, first pregnancy diagnosis and before the second breeding season in addition to having greater pregnancy rates than those from un-supplemented dams (Martin et al., 2007). However, in another study (Funston et al., 2010), although more heifers from supplemented cows were pubertal before breeding, they did not demonstrate the same advantage in pregnancy rates.

Steers from those same studies had increased weaning weights when born to supplemented dams, which was coupled with heavier carcass weights (Stalker et al., 2006; Larson et al., 2009). Those carcasses also had greater intramuscular fat content, adding value and, in the later study (Larson et al., 2009), increased percentage of choice quality grade.

This body of research clearly demonstrates an effect of fetal programming from maternal diet, specifically protein supplementation. Cows benefitted from protein supplementation in maintenance of body weight and condition through gestation. The altered phenotypes of the offspring not only created advantages at birth, but also persisted through weaning and slaughter

or rebreeding. In steers, protein supplementation improved economic values at weaning and slaughter while heifers experienced improved pregnancy rates.

In addition to maintenance of body weight and condition, maternal nutrition is interrelated with subsequent reproduction, particularly rebreeding. Weight loss during late gestation has been linked to decreased fertility in successive breeding of cows and first calf heifers (Randel, 1990). More specifically, prepartum nutrition has been found to be more important than postpartum nutrition in shaping the length of the postpartum interval, but energy and protein intake in both periods can alter subsequent pregnancy rates (Randel, 1990).

Finally, although little research has been conducted with pregnant beef cows in the area, intake behavior logically impacts maternal body weight and condition, and when coupled with the nutritional challenge of pregnancy, can certainly be an influential part of production of the altered offspring phenotypes mentioned above. Feeding behavior can add insight to how diets are affecting animals' intake, which is influenced by a variety of physiological factors. In quantifying feeding behavior, Forbes (1995) suggests focusing on intake in relation to meals (eating periods that may be separated by short breaks), feeding bouts (short intake periods within a meal), and intermeal intervals (the time between meals). However, he points out housing situations may alter behavior, such as in situations where there are not enough feeders to accommodate all animals at the same time. In those cases, hierarchy will play a heavy role in who is eating when and for how long. Physiological state, such as pregnancy, lactation, and weaning, markedly affects feeding behavior, so one may reasonably postulate that late gestation beef cows will have differing daily intake behavior than open cows. In most livestock species there will be a noticeable decrease in daily intake during late pregnancy, especially in the last month where animals may even tend to spend less time eating. In contrast, during lactation,

especially immediately after calving, cows will rapidly increase intake due to the increased nutrient requirements needed just for maintenance during lactation (Forbes, 1995). In addition to physiological state, cows follow circadian rhythms in their eating patterns (Forbes, 1995), often eating when fresh feed is first available, around sunset (which has been observed changes with season), and around midnight (especially animals lower on the social hierarchy). Finally, the physiological nature of the ruminant gastrointestinal tract dictates feeding behavior. Due to the long period of food storage (for microbial fermentation) the physical capacity of the "stomachs" (Forbes, 1995), principally the rumen, offer a potential limiting factor to intake rates and behavior, especially when accounting for rates of digestions, breakdown, and passage of food particles. Confounding the issue of rumen capacity are the presence of mechano- and chemoreceptors, both of which are properties of the same neurons that communicate with the CNS. These receptors communicate information related to rumen fill, pH, VFA concentration and osmolality, all of which can influence feeding behavior but the effects of which are difficult to study individually. Despite the complexity of explaining the mechanisms behind ruminant feeding behavior, especially in pregnant and lactating animals, there still is a confirmed positive relationship between digestibility of a forage and the level of voluntary intake due to sheer physical limitation of the gastrointestinal tract; this relationship can certainly aid in the interpretation of voluntary feeding behavior (Forbes, 1995).

Now that the importance of maternal nutrition, from a nutrient intake as well as behavioral intake point of view, has been well established, illuminating upon how those nutrients are delivered to the conceptus during gestation is the next step in completing the picture of how alterations in maternal diets directly affect fetal development.

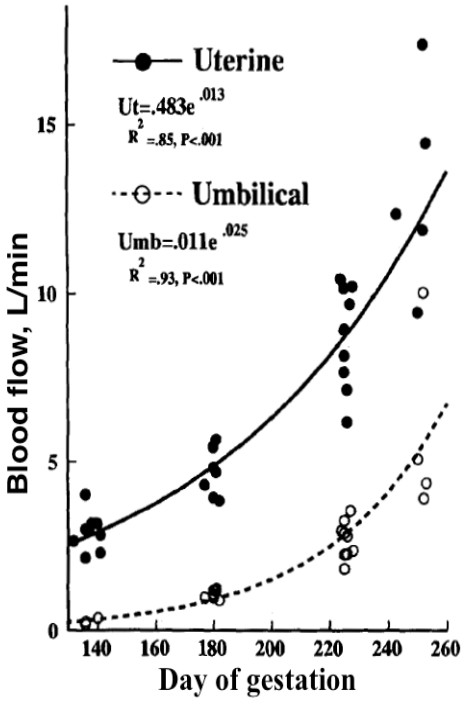

Figure 1.1. Regression of uterine blood flow in cows

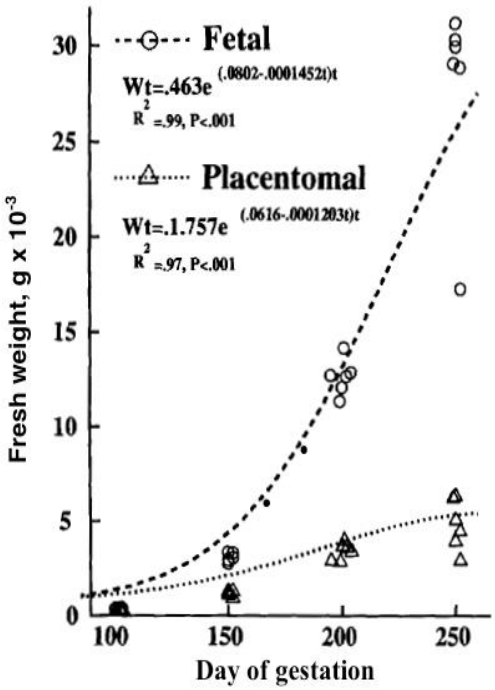

Figure 1.2. Regression of fetal and placentomal weight in cows
(Reynolds and Redmer, 1995).

Uterine Blood Flow during Gestation and Nutrient Delivery to the Fetus

Uteroplacental vasculature is an essential component of transplacental exchange, and thusly vital to fetal development (Reynolds and Redmer, 1995; Reynolds et al., 2010). The term "uteroplacental" here and throughout this dissertation refers to and emphasizes the intimacy of the maternal and fetal components of the placenta and their contribution to placental function, as well as the involvement and, therefore, impossibility of separating the rest of the gravid uterus from its support of a successful pregnancy (Reynolds and Redmer, 1995). The function of the uteroplacental tissues include transmission of water, gases and nutrients to the fetus, excretion of waste products of fetal metabolism, and production of hormones or hormone precursors modifying maternal metabolism to meet the needs of the growing fetus (Ferrel, 1991). As a

result, perfusion of those tissues is a primary component of maternal constraint on fetal growth. Therefore, one way to quantify nutrient flux to the fetus is to measure BF to the uterus via the uterine arteries, which, therefore, supply blood to the uteroplacental system (Ferrell, 1991). In pregnant cows (and many other mammals) uterine BF increases exponentially with advancing gestation (Figure 1.1), becoming highest in the last third of pregnancy, which is also when the majority of fetal growth occurs (Figure 1.2; Reynolds and Redmer, 1995; Reynolds et al., 2010). Reynolds et al. (2010) also explains placental transport capacity (or placental uptake) in relation to the Fick principle:

$$\text{Uptake} = \text{blood flow} \times [A - V]$$

where [A – V] is the difference in arteriovenous concentration. Thus, increased BF to the uterus is critical to increasing transplacental exchange. Modification of uterine BF, and therefore placental transport capacity, enables increased nutrient delivery to the growing fetus (Vonnahme and Lemley, 2011).

Various methods have been used to measure uterine BF in the pregnant cow, including electromagnetic BF transducers (Ford and Christensen, 1979; Ford, 1982) and infusion of deuterium water (Ferrell, 1991). Although these methods have provided the groundwork for knowledge of the important changes in uterine BF during pregnancy, they are very invasive and often times provide data limited only to specific windows of gestation.

Use of color Doppler ultrasonography provides the opportunity to measure uterine BF hemodynamics in a noninvasive, reliable and repeatable manner. Bollwein et al. (2002) demonstrated that color Doppler ultrasonography is a suitable technique for measuring uterine BF throughout gestation in the same animal. Dr. Ginther wonderfully describes how Doppler ultrasonography works (Ginther, 2007) as well as how ultrasound technologies have

revolutionized research in reproduction in large animals (Ginther, 2014). Doppler ultrasonography is based on Doppler-shift frequencies echoes produced by moving red blood cells as they move through vessels towards or away from the transducer (the probe), creating a similar effect to the Doppler effect of sound, wherein the sound frequency changes as the source moves towards or away from the listener. Color Doppler ultrasonography (color-flow mode) applies color to the direction of BF relative to the transducer, which is valuable to the operator in identification of specific blood vessels or even quantifying a percentage of tissue coloration (vascularization), which the computer can calculate.

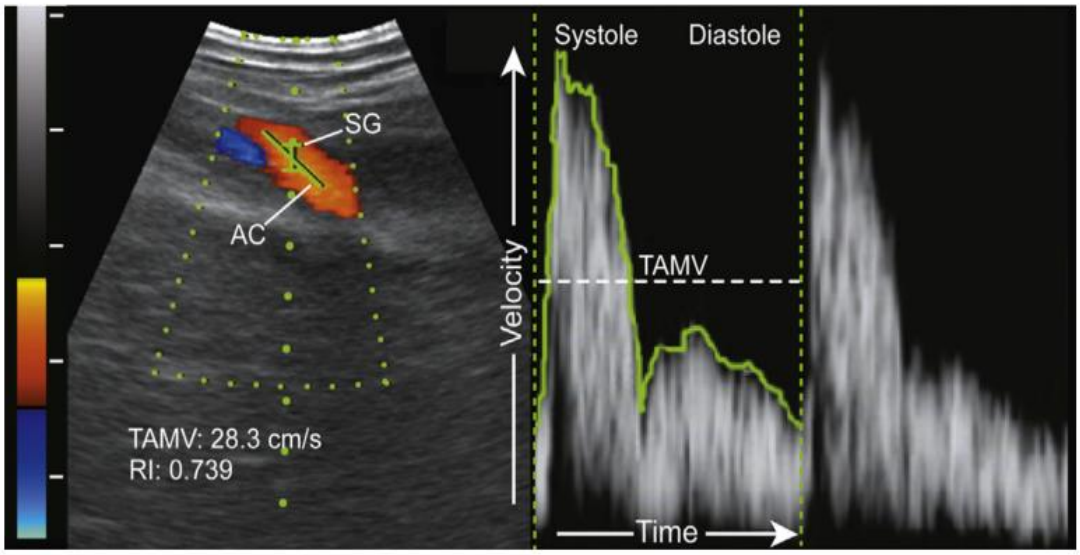

Figure 1.3. Color-Doppler ultrasound of an artery during an individual cardiac pulse.
SG = sample gate, delineating the small focus of the artery used for calculating velocities in the selected cardiac cycle; AC = angle cursor, which is placed by the operator to indicate angle of blood flow; blood velocities of the systolic and diastolic segments are depicted, with average velocity (time-averaged maximum velocity; TAMV) in dashed lines; RI = resistance index

For quantification of BF, numerical velocities (BF volume) are calculated by the computer program (in relation to the Doppler shift frequency of the moving blood cells and the cosine of the Doppler angle) for a selected cardiac cycle. Once a cardiac cycle is selected, a

tracing of the maximal velocity values over time is done (Figure 1.3). The maximum point along the traced outline represents peak systolic velocity (PSV), which is also the maximum Doppler shift frequency. The maximum value at the lowest point before the next systolic increase represents end diastolic velocity (EDV). Finally, an average of the maximum velocity values over the time of the selected cardiac wave is called time-averaged maximum velocity (TAMV).

Doppler indices (resistance index and pulsatility index) are also calculated by the computer program and use the velocity Doppler measurements. The resistance index (RI) is calculated as: (PSV – EDV) / PSV. Therefore, the higher the RI, the lower the perfusion since the vessel being measured is experiencing resistance downstream. Pulsatility index (PI) is calculated as: (PSV-EDV) / TAMV. Therefore PI is an expression of the extent of the difference between PSV and EDV of the blood pulse in the vessel at the level of the arterial examination (Ginther, 2007). The Doppler indices are very useful for intertwining arteries of the reproductive tract because they are calculated independent of the angle of the transducer to the angle of BF and instead represent hemodynamics (as they are ratios of velocity measurements) of the tissue supplied by the artery downstream to where the sample gate is measuring. They give the operator the opportunity to make inferences about vascular perfusion surrounding the area they are measuring.

Color-Doppler ultrasonic imaging studies in cattle have given us valuable information regarding hemodynamics of the reproductive system, including support for increased uterine artery BF volume as well as establishing the decrease in RI during advancing pregnancy (Figures 1.4 and 1.5); in both of those measures the artery ipsilateral to the conceptus experiences a greater effect (Bollwein et al., 2002).

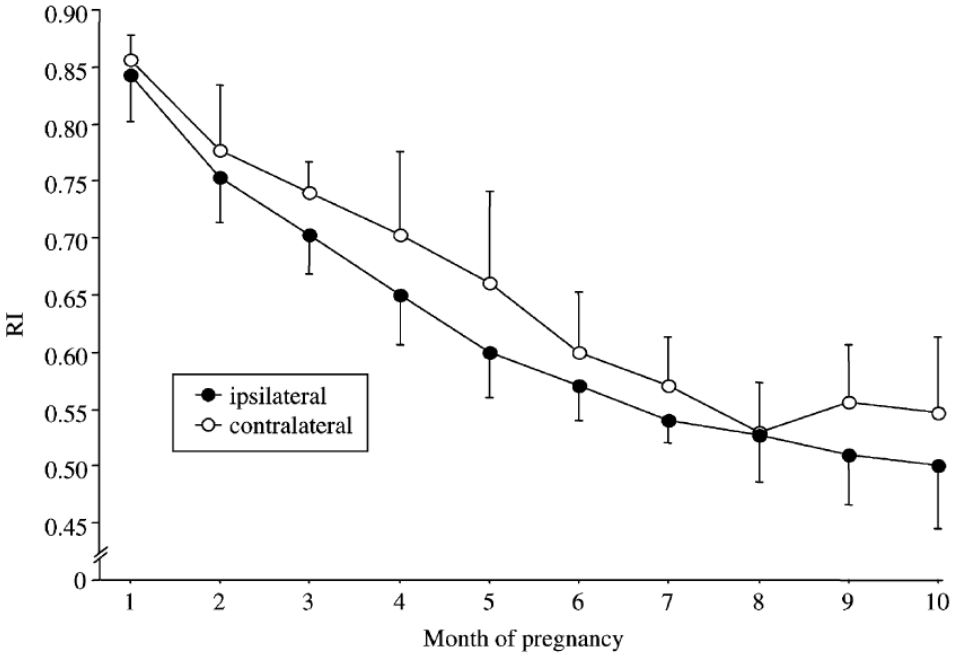

Figure 1.4. Resistance index (RI) in the uterine arteries ipsi- and contralateral to the conceptus
(Bollwein et al., 2002).

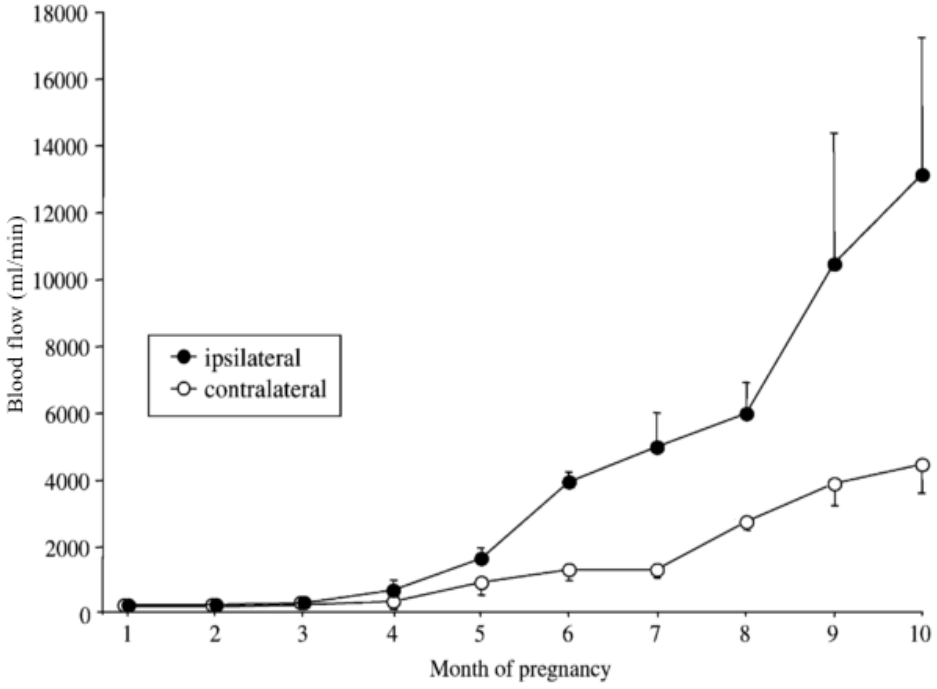

Figure 1.5. Volume of blood flow in the uterine arteries ipsi- and contralateral to the conceptus
(Bollwein et al., 2002).

In order to better understand the whole picture of nutrient delivery to the fetus as a function of maternal nutrition and circulation, factors that affect vasoactivity must first be discussed. It has already been reviewed that the uteroplacental vasculature must go through various changes to accommodate its responsibilities in the transmission of water, gases and nutrients to the fetus, excretion of waste products of fetal metabolism, and production of hormones or hormone precursors modifying maternal metabolism to meet the needs of the growing fetus (Ferrell, 1991). The maternal system makes those changes via reduction in system vascular resistance, increased cardiac output, heart rate, stroke volume and blood volume as well as modifying normal endothelial function (Ford, 1982; Ford, 1995; Valdes et al., 2009). To facilitate this, various vasodilator systems alter uteroplacental vasculature via the renin-angiotensin system (RAS), the kallikrein-kinin system, prostacyclin, nitric oxide and vascular endothelia growth factor (VEGF). During normal pregnancy, the predominating effect is a decrease in uterine arterial vascular smooth muscle tone, which results in progressive increase in arterial diameter and thus baseline BF (Ford, 1995). Endothelial cells control vascular tone through the release of nitric oxide and prostacyclin, whose effects are intertwined with the influence of RAS, particularly the action of the stimulatory factor angiotensin (1-7), and stimulation from bradykinin and L-arginine (Valdes et al., 2009). It should be noted that majority of the research base for these systems comes from studies in rodents, with some support from ovine studies; however, this further demonstrates the need for research elucidating their influences in larger mammals such as cattle (Ford, 1982; Ford, 1995; Valdes et al., 2009).

In addition to the above-mentioned vasoactive factors, circulating steroids, specifically estrogens (e.g. estradiol-17β, E2) and progesterone (P4), also affect uterine arterial vasodilation to aid in the need for increased BF during pregnancy. Progressive decrease of uterine arterial

tone seems to be a result of E2 metabolism into vasoactive catechol forms, while progesterone maintains the phasic contractility of the uterine arterial smooth muscles (Ford, 1995). As maternal nutrient intake can alter circulating concentrations of those steroids via altered liver metabolism of E2 and P4 (Sangsritavong et al., 2002), hepatic steroid metabolizing enzyme activity could have an important role in the mechanisms behind altered maternal nutrition influencing nutrient flux during gestation as well. Altered metabolic clearance rates (MCR) of these important hormones could affect pregnancy retention and embryonic development as well as reproductive performance during early lactation (Lemley et al., 2009).

Few studies have been done to characterize hepatic enzymes that contribute to steroid MCR in cattle (for recent work, see Hart et al.,2014), therefore much of the knowledge discussed here comes from in vitro or ovine studies. The enzymes of the cytochrome P450 (CYP) superfamily are part of various pathways that include endogenous vitamin D3 activation, metabolism of cholesterol to bile acids, metabolism of all major classes of steroid hormones and xenobiotic metabolism (Lemley and Wilson, 2010). In sheep, CYP2C and CYP3A metabolize P4 to 21-hydroxyprogesterone and 6β-hydroxyprogesterone (respectively) via the addition of hydroxyl groups to the steroid nucleus (Murray, 1991; Murray, 1992). Aldo-keto reductase (AKR) enzymes reduce glucose, metabolize prostaglandin, generate bile acids and reduce steroids containing aldehyde or ketone groups. More specifically, AKR1C converts P4 to 3α-hydroxyprogesterone or 20α-hydroxyprogesterone (Penning et al., 2000). The third group of enzymes involved in steroid metabolism are uridine diphosphate-glucuronosyltransferase (UGT) enzymes, which conjugate inactive hydroxysteroid metabolites with glucuronic acid.

Progesterone metabolism in hepatocytes occurs in two phases (I and II), with Phase I utilizing CYP2C, CYP3A and AKR1C to add hydroxyl groups to the steroid nucleus for

production of hydroxyprogesterone metabolites. Phase II uses UGT enzymes to conjugate the metabolites with glucuronic acid, creating a hydroxyprogesterone-glucuronide metabolite (Lemley and Wilson, 2010). Various studies have been performed to investigate protein expression and activity of CYP, AKR and UGT enzymes in the liver of mice, pigs, cattle, goats and sheep, with interspecies differences found for all species tested, further demonstrating the need for more research characterizing hepatic enzyme activity in our research animals of different breeds (such as dairy vs. beef cattle) and species, as well as across physiological states (Lemley, 2010).

Activity of these enzymes can also be altered by nutrition—liver mass in beef cattle increases in response to altered nutritional plane (Camacho et al., 2014). Increasing level of feed intake has also been shown to increase the relative proportion of visceral organs to body mass in sheep, where changes in liver weight in response to level of nutrition were of a greater degree than any other organ (Burrin et al., 1990). The same study also found that those changes in organ size contributed to an altered whole-body metabolic rate. It has also been shown in dairy cows that a continuous high plane of nutrition may chronically elevate liver BF and metabolic clearance rate of P4 and E2 (Sangsritavong et al., 2002).

Early Lactation

This portion of the literature review will describe the influence of maternal nutrition during late gestation on lactation in relation to mammary gland development during late gestation in preparation for lactation, BF to the mammary gland, colostrum and milk production during early lactation, and offspring postnatal performance as a result of those influences.

Maternal nutrition during gestation affects lactation because the mammary gland is still developing, with emphasis on proliferation of the mammary epithelium for mammogenesis; this

process is dependent on the synergy of E2, P4, prolactin, growth hormone and placental lactogen (Tucker, 2000; Neville et al., 2002). Progesterone is critical for lobulo-alveolar development and its withdrawal helps trigger lactogenesis, while estrogen stimulates secretion of IGF-1 (insulin-like growth factor) causing growth of epithelial cells as well as mediation of signals essential for ductal morphogenesis (Svennersten-Sjaunja and Olsson, 2005; Neville et al., 2002). Prolactin is essential for the proliferative phase of alveologenesis; growth hormone has a role in ductal morphogenesis; placental lactogen is present in high levels during secretory differentiation (Tucker, 1981; Tucker, 2000; Neville et al., 2002).

Whole body metabolism also changes at the end of pregnancy and becomes anabolic in order to meet increased nutritional demands of oncoming lactation (Svennersten-Sjaunja and Olsson, 2005). Differing nutritional protein and energy planes during gestation can influences milk production to varying degrees depending on timing of the changes (Sullivan et al., 2009); prepartum protein supplementation in particular has resulted in increased milk yields in beef heifers (McSweeney et al., 1993).

In cows, mammary gland BF is a vital component for milk synthesis and, therefore, nutrient delivery to the offspring. At parturition, the increased BF to the uteroplacental tissues is redirected to the mammary glands (Svennersten-Sjaunja and Olsson, 2005). Additionally, the same hormones and growth factors are needed for mammogenesis to stimulate vessel development. Blood flow to the mammary glands comes through the pudendoepigastric trunk, from which branches the caudal epigastric and external pudendal arteries (Budras et al., 2011). Mammary BF is strongly correlated with milk yield (Götze et al, 2010). In 2010, Götze and collegues utilized Doppler ultrasonography to quantify mammary BF in dairy cows and confirmed that using the pudendoepigastric trunk was equally as effective as measuring the

external pudendal artery. However, little work has been done to characterize mammary BF in beef cattle and its influences on calf postnatal performance; currently there is a dearth of data for ultrasonographic mammary BF in beef cows.

Prepartum energy and protein supplementation can also influence body weight and condition scores of cows during lactation; supplementation during late gestation resulted in increased BCS (Sullivan et al., 2009). Winter protein supplementation to beef cows fed low quality forages linearly increased body weight and condition over gestation and postpartum with increasing supplementation; unsupplemented cows lost weight and condition leading up to parturition, but when provided supplement during lactation gained weight to achieve a positive energy balance before rebreeding (Winterholler et al., 2012). Milk yields in dairy cows increased when ruminanlly undegradable protein was supplemented during the dry period (Moorby et al., 2010).

The same winter protein supplementation study(Winterholler et al., 2012) also increased milk yield (with increasing supplement) and subsequent calf birth weights as well as a tendency for greater weaning weights in beef cows. However, as Winterholler and collegues noted, prepartum supplementation has produced mixed results on calf birth weights. Milk yield affects the profit potential of the beef producer via increased meat yields from heavier calves at weaning and prepartum protein supplementation in the last month of gestation did increase milk yields (Sullivan, 2009). However, simliar to the lack of data for mammay BF in beef cattle, there are few studies focusing on shifts in lactation for beef cows, so data specifically regarding milk production in beef cattle is sparce. Nevertheless, inferences can be made from research in dairy cattle and, combined with known benefits of maternal nutrition on offspring performance (as

mentioned earlier in this literature review), the value of further research interests in this area is obvious.

Statement of the Problem & Experimental Objectives

Research in the area of developmental programing has demonstrated the significant influence of the maternal system on fetal development and offspring potential as well as growth trajectories and even development of disease in adult life (Barker, 1990). Maternal nutrition is essential to fetal and placental development, which can influence the lifetime performance of the calf (Funston et al., 2010). Uteroplacental vasculature is a key component of transplacental exchange and thusly vital to fetal development (Reynolds and Redmer, 1995). One way to quantify nutrient delivery to the fetus is to measure uterine arterial BF (Ferrell, 1991) and Doppler ultrasonography offers the possibility of measuring BF in a noninvasive, reliable and repeatable manner, with the added benefit of taking observations in the same animal over time. Modification of uterine BF and nutrient transfer capacity enables increased oxygen and nutrient delivery to the growing fetus (Vonnahme and Lemley, 2012). Maternal nutrient intake alters circulating vasoactive steroids, specifically E2 and P4, which may influence uterine BF and/or nutrient flux to the conceptus. Therefore, measurement of steroid production and clearance can explain how altered maternal nutrition influences uterine arterial BF.

In addition to uterine BF, mammary gland BF is a vital component for milk synthesis and therefore nutrient delivery to the offspring post-partum. Mammary BF is strongly correlated with milk yield (Götze et al., 2010) and Doppler ultrasonography can provide an accurate, reproducible, and less invasive tool for measuring BF to the mammary gland compared with other methods. In 2010, Götze et al. utilized Doppler ultrasonography to quantify mammary BF in dairy cows and confirmed that using the pudendoepigastric trunk was equally as effective as

measuring the external pudendal artery. However, little work has been done to characterize mammary BF in beef cattle and its influences on calf postnatal performance. In fact, there is a dearth of data for ultrasonographic mammary BF in beef cows.

In regards to mammary BF, maternal nutrient intake during gestation can alter systemic BF via changes in circulating hormones (as mentioned above) and growth factors during pregnancy that facilitate nutrient delivery to the still developing mammary gland in anticipation of colostrum and milk production (Svennersten-Sjaunja and Olsson, 2005). Additionally, differing nutritional protein and energy planes can influence milk production to varying degrees depending on timing of differences in gestational diet (Sullivan et al., 2009, McSweeney et al., 1993).

Dramatic increases in corn production in North Dakota have resulted in more corn production byproducts, such as corn stover, available to producers to use for winter feed (Winterholler, 2012). Additionally, last year an estimated 13.3 gigaliters of ethanol was produced in the United States, with over 98% derived from corn (Renewable Fuels Association, 2014). The byproducts of corn-based ethanol production, particularly dried distiller's grains plus solubles (DDGS), can provide an important supplemental energy and protein source for livestock (Klopfenstein et al, 2008). Making use of these byproducts for pregnant beef cows during the winter offers economic benefits to cow-calf operations (Kim et al., 2008), but research is needed to elucidate the nutritional benefits of using DDGS as a supplemental source of nutrients for cows fed low-quality forages such as corn stover.

Numerous studies have investigated effects of protein supplementation as well the use of DDGS, but focusing on supplementation during late gestation (when most of the fetal growth occurs) has also been investigated with promising results. Previous research has demonstrated

benefits of supplementing dams fed low-quality forage with DDGS such as increased percentage calves weaned, weaning weights, and ADG (Stalker et al., 2006), increased growth and reproductive success of heifer calves (Martin et al., 2007), and improved quality grade of steer calves (Larson et al., 2009a).

With this in mind, we hypothesized that supplementation of DDGS to beef cows fed a low quality forage during late gestation would increase uterine BF and thusly nutrient flow to the fetus. Altered BF could be a result of increased circulating E2, likely from an increase in placental secretion or decreased activity of respective hepatic metabolizing enzymes. DDGS supplementation may also increase BF to the mammary glands and therefore colostrum and milk production, ultimately resulting in an advantage in calf weight gain during early lactation and at weaning. Finally, we predicted that supplementation will alter maternal voluntary feed intake, resulting in altered maintenance of body weight and condition through late gestation as well as early lactation.

Ergo, the objectives of this study are to investigate the effects of supplementing DDGS to cornstover during late gestation on 1) feeding behavior (in relation to intake of a low quality forage) and resulting maintenance of cow body condition and weight during late gestation as well as subsequent early lactation; 2) uterine BF, circulating concentrations of E2, P4 and their corresponding metabolizing enzymes; 3) arterial BF to the mammary glands during late gestation and early lactation; colostrum and milk production; and calf weight gain during early lactation and at weaning.

The following chapters of this dissertation will be divided by these experimental objectives, beginning with feeding behavior in Chapter 2, uterine BF, circulating steroids, hepatic enzymes and measurements at parturition in Chapter 3, mammary BF, colostrum and

milk production in Chapter 4, and finally conclude with a general discussion and future directions in Chapter 5.

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CHAPTER 2. SUPPLEMENTATION OF CORN DRIED DISTILLER'S GRAINS PLUS SOLUBLES TO GESTATING BEEF COWS FED LOW-QUALITY FORAGE: ALTERED INTAKE BEHAVIOR, BODY CONDITION AND REPRODUCTION

Abstract

To investigate the effects of corn dried distiller's grains plus solubles (DDGS) supplementation to cows fed corn stover and silage during late gestation, 27 multiparous beef cows (674 ± 17 kg) were divided randomly into 2 pens equipped with Insentec feeders. For 10 wk, both groups were fed the basal diet for ad libitum intake while one group was supplemented (**SUP**; n = 12) with DDGS at 0.3% of BW (DM basis). Following parturition, all cows received the same diet for an additional 8 wk. During gestation, SUP cows gained boyd weight (BW; $P < 0.01$) and there was no change in body condition score (BCS; $P = 0.79$). CON cows tended to lose BW ($P = 0.06$) and lost BCS ($P < 0.01$). SUP cows consumed more forage ($P < 0.01$) and total feed than non-supplemented cows (**CON**). An interaction of treatment and day was observed for time spent consuming forage ($P < 0.01$), and SUP cows also consumed forage faster than CON ($P \leq 0.01$). Control cows ate more meals than SUP cows ($P = 0.06$) from d 201 to 218 of gestation. SUP cows tended to consume larger meals than CON cows ($P = 0.09$) and spent more time eating than CON cows around d 240 of gestation ($P < 0.01$). Calves born to SUP cows tended to be heavier than calves born to CON cows ($P = 0.06$). During lactation, both groups gained ($P < 0.01$) BW with advancing lactation, with CON cows appearing to exhibit compensatory gain despite still having less body condition than SUP cows ($P < 0.01$) at the end of the study. Dry matter intake increased ($P < 0.01$) over time but was not influenced by treatment. SUP cows spent more time eating than CON cows ($P < 0.01$) after wk 4 of lactation. SUP cows ate faster than CON cows until wk 3 of lactation and CON cows ate faster than SUP

cows from wk 6 onward of lactation (P = 0.01). Number of meals increased with advancing lactation (P < 0.01) and CON cows averaged more meals than SUP cows (P = 0.01). Conversely, meal size decreased as lactation advanced (P < 0.01) and SUP cows consumed larger meals than CON cows (P = 0.05). Supplementation with DDGS during gestation influenced intake behavior during gestation and lactation as well as maintenance of maternal BW and BCS, and calf birth BW.

Introduction

Dramatic increases in corn production in North Dakota have resulted in more corn production byproducts, such as corn stover, available to producers to use for winter feed (Winterholler et al., 2012). Additionally, last year an estimated 13.3 gigaliters of ethanol was produced in the United States, with over 98% derived from corn (Renewable Fuels Association, 2014). The byproducts of corn-based ethanol production, particularly dried distiller's grains plus solubles (DDGS), can provide an important supplemental energy and protein source for producers (Klopfenstein et al, 2008). Making use of these byproducts for pregnant beef cows during the winter offers economic benefits to cow-calf operations (Kim et al., 2008), but research is needed to elucidate the nutritional benefits of using DDGS as a supplemental source of nutrients for cows fed low-quality forages such as corn stover.

Accompanying the need for more information on the benefits of supplementing DDGS to cows fed corn stover is the need for further understanding of the influence of maternal nutrition during gestation on fetal and postnatal development. Developmental programming is the concept that a stimulus or insult to the developing offspring has long-term effects on productivity and, consequently, lifelong health (Neel, 1962; Barker, 1995; Barker, 2004). Maternal nutrition is essential to fetal and placental development, which is thought to influence a calf's lifetime

performance (Funston et al., 2010a). Numerous studies have investigated effects of protein supplementation as well the use of DDGS, but focusing on supplementation during late gestation (when most of the fetal growth occurs) has also been investigated with promising results. Previous research has demonstrated benefits of supplementing dams fed low-quality forage with DDGS such as increased percentage calves weaned, weaning weights, and ADG (Stalker et al., 2006), increased growth and reproductive success of heifer calves (Martin et al., 2007), and improved quality grade of steer calves (Larson et al., 2009a).

The objective of this study was to investigate the effects of supplementing DDGS during late gestation on feeding behavior as well as the use of corn stover as a source of forage; and maintenance of cow body condition and weight.

Materials and Methods

Experimental Design, Cows and Dietary Treatments

All procedures were approved by the North Dakota State University Animal Care and Use Committee (IACUC #A14007). Twenty-seven multiparous beef cows (Angus or Angus x Simmental) were divided randomly into a control group (CON; n = 15) and a treatment group (SUP; n = 12); cows weighed 674 ± 17 kg on average and were 6 ± 5 yr old at the start of the study. Cows were housed at the NDSU Beef Cattle Research Complex in two adjacent pens, one for the CON group and one for the SUP group. Following a 3-week acclimation period, intake was monitored and controlled via RIC feeders (Insentec, B.V., Marknesse, Netherlands) beginning on d 201 of gestation for 10 wk. A basal diet of 90% corn stover and 10% corn silage (5.0% CP on a DM basis, marginally deficient in NE, rumen degradable protein deficient) was fed for ad libitum intake to both groups, with the SUP group supplemented with dried distiller's grains plus solubles (DDGS) at 0.3% of BW (DM basis). Corn silage inclusion was increased to

20% on d 246 of gestation (gestational diet 2; 4.7% CP on a DM basis) to meet increased NE demands during pregnancy, but supplementation regimes remained the same. Corn silage inclusion was again increased to 30% on d 260 of gestation to meet increasing NE requirements (gestational diet 3; 5.5% CP on a DM basis). Each pen contained 8 feeders, with all 8 feeders in the CON pen containing the basal diet. In the SUP pen, 6 feeders contained the basal diet and 2 contained the DDGS supplement. Both pens had free access to water and trace-mineralized salt blocks (95.5 to 98.5% NaCl, 3,500 mg of Zn/kg, 2,000 mg of Fe/kg, 1,800 mg of Mn/kg, 280 to 420 mg of Cu/kg, 100 mg of I/kg, 60 mg of Co/kg).

All cows were fitted with radio-frequency identification tags to facilitate monitoring of intake and feeding behavior. Feeding behavior measurements were characterized as described by Islas et al. (2014) and defined as: events (number of bunk visits and meals daily); eating time (min per visit, per meal, and per day); and feed intake (g per visit, per meal, and per minute), with data averaged for each cow over 1-wk periods. A visit was defined as each time the Insentec system detected a cow at a bunk. A meal was defined as a distinct eating period, which could include short breaks separated by intervals no longer than 7 min (Forbes, 1995). Each feeding behavior measurement is reported for forage (corn stover and corn silage mixture), DDGS, and total intake averaged over a week (10 wk of gestation). Intake will be discussed in reference to the gestational day midway through each week.

On d 270 of gestation, close to expected parturition, all cows were fed the same diet (48% corn stover, 30% corn silage, 22% DDGS; DM basis; 10.8% CP; Table 2.1) for ad libitum intake for a period of 10 weeks; DDGS supplementation ceased.

Lactational dry matter intake (DMI) and feeding behavior were defined in the same manner as gestational intake and feeding behavior; however, data will be discussed in reference

to week of lactation (as calculated per individual cow). Data for cows are still reported relative to their treatment groups during gestation (as CON or SUP), and are defined as the interval between calving and d 56 of lactation for each cow (an 8-wk period).

Body Weight ,Condition and Rebreeding

During gestation, cows were weighed mid-day (between feed delivery, which occurred twice daily at 0730 and 1630) every 2 wk from initiation of the project until d 242 of gestation and a blood sample was collected via jugular venipuncture. Cows were also weighed on d 180, 216, and 246 (± 5 d) of pregnancy and body condition was scored by three technicians. At parturition, cows and calves were weighed at 0 and 24 hr. Gestation length was calculated for each cow.

During lactation, cows were weighed relative to their individual parturition date, noted as d 14, 28, 42, 44 and 56 of lactation. Body condition score was also recorded on d 44 of lactation and upon leaving the facility at the end of the study (d 61 of lactation, ± 8 d).

Following the study, cows we rebred with artificial insemination (AI), followed by the use of a clean up bull for any cows that were not confirmed pregnant with ultrasound. Pregnancy status was confirmed using ultrasound at 35 days (for AI status) and 98 days (for clean-up bull status) after initial breeding. Rebreeding success rates were thusly recorded as follows for data analysis: 1= pregnant after AI, 2 = pregnant after rebreed with bull, 3 = remained open.

Feed Analysis

Samples of diets fed during gestation and lactation, and DDGS were collected weekly and analyzed. All feed samples were analyzed for ash, CP, NDF, ADF, EE, Ca, and P (Table 2.1). Forage and DDGS samples (approximately 500 g) were collected weekly. Forage samples were dried in a 55oC oven for at least 48 h and ground to pass a 1-mm screen. Hay and DDGS

samples were analyzed for DM, ash, N (Kjehldahl method), Ca, P, and ether extract (EE) by standard procedures (AOAC, 1990) and for NDF (using heat stable amylase and sodium sulfite and expressed inclusive of residual ash) and ADF (expressed inclusive of residual ash) concentration sequentially by the methods of Robertson and Van Soest (1981) using a fiber analyzer (Ankom Technology Corp., Fairport, NY). Crude protein was calculated by multiplying N concentration × 6.25.

Table 2.1. Analyzed composition of cow diets derived from corn silage and dried distillers grains (DDGS) during late gestation and early lactation.

Diet	% of DM								
	Stover	Silage	Ash	CP	NDF	ADF	Fat	Ca	Phos
Gestation Diet #1 (d 201 - 245)	90	10	14.65	4.95	66.98	38.28	0.55	0.46	0.23
Gestation Diet #2 (d246 - 259)	80	20	11.57	4.73	60.32	40.4	0.65	0.45	0.93
Gestation Diet #3 (d260 - 269)	70	30	19.7	5.46	50.54	30.01	0.51	0.52	0.16
Lactation Diet (d270 through lactation; 20%DDGS)	48	30	11.88	10.83	56.18	30.44	2.46	0.36	0.32
DDGS			5.64	31.55	47.52	14.66	8.16	0.08	0.9

Statisical Analysis

Data were analyzed as repeated measures with the mixed procedure of SAS (SAS Institute Inc., Cary, N.C.). A regression solution was used to estimate the linear slope of BW and BCS over time. The class statements included cow, maternal diet (SUP vs. CON), day of gestation or day of lactation, and the interaction of day and maternal diet. Two cows were not included in the analysis for early lactation intake behavior due to the death of one cow and the

death of the other cow's calf, which resulted in her being removed from the experiment. The model statement tested all feeding behavior measurements described above as dependent variables. For rebreeding data, the Somer's D test of SAS was used to more accurately estimate differences in rebreeding success based on pregnancy statuses from AI, bull breeding or remaining open after both breeding attempts.

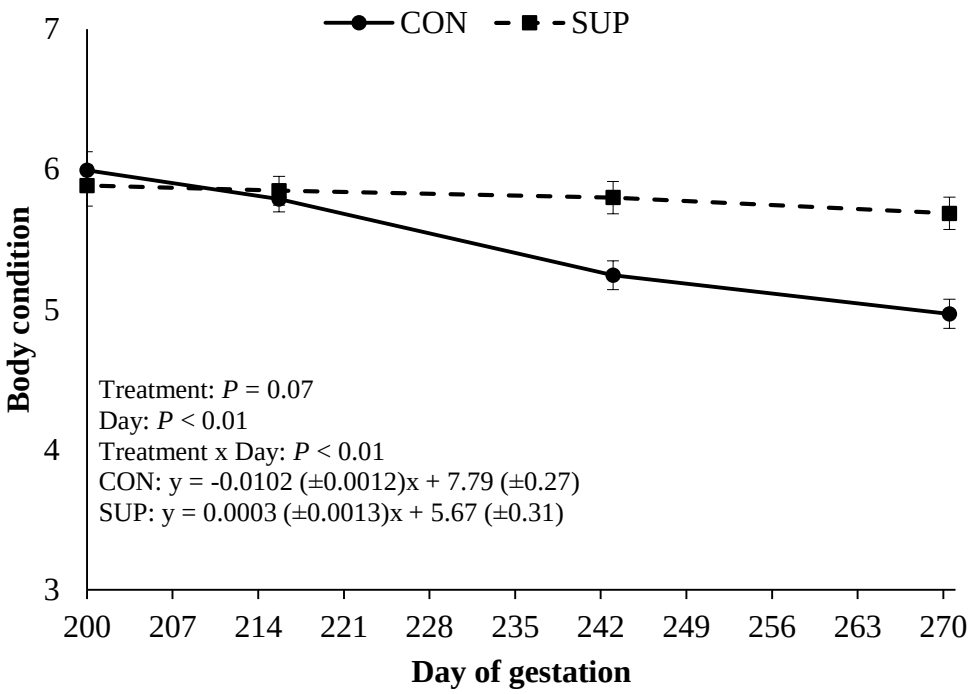

Figure 2.1. Body condition of cows fed control or control plus supplement during late gestation.

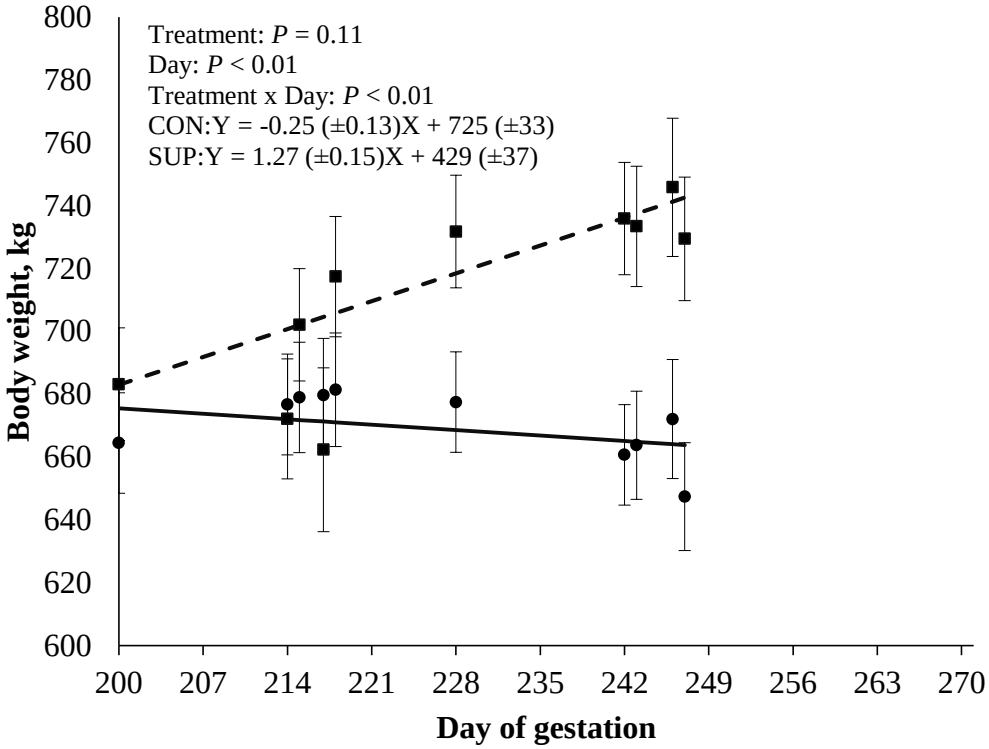

Figure 2.2. Body weight of cows fed control or control plus supplement during late gestation.

Results

Late Gestation

Body condition scores of CON cows decreased (P < 0.01), whereas BCS of SUP cows did not change (P = 0.79; Figure 2.1). Control cows began the experiment with an average BCS of 5.7 and by calving had an average BCS of 4.9, whereas SUP cows had a BCS of 5.7 at calving. Supplemented cows gained BW (P <0.01) at an average rate of 1.27 kg/d and CON cows had a tendency to lose BW (P = 0.06) at an average rate of 0.23 kg/d (Figure 2.2). Finally, calves born to SUP cows had a tendency (P = 0.06) to be heavier than calves born to CON cows, with SUP calves weighing an average of 43.3 kg and CON calves averaging 40.5 kg (± 0.9 kg).

Dry matter (DM) intake of DDGS for supplemented cows averaged 2.32 kg/d throughout late gestation and was not affected by day (P = 0.40; Figure 2.3). An interaction of treatment and

day (P = 0.01) was observed for forage DM intake, where SUP cows consumed more per day than CON cows and both groups, after an initial decrease in consumption, increased their daily intake for the rest of gestation (Figure 2.3). Time spent consuming forage per day was also affected by the interaction of treatment and day (P < 0.01), with CON cows initially spending more time eating forage than SUP cows, followed by SUP cows spending more time eating around d 240 to d 246 (Figure 2.4). An interaction of treatment by day was also observed for intake rate of forage (g/min; P < 0.01), with SUP cows consuming forage faster than CON cows early in the experiment, followed by similar rates of consumption with advancing gestation (Figure 2.5). A main effect of treatment (P = 0.03) and day (P < 0.01) influenced size of forage meals; SUP cows tended to consume larger meals than CON cows (1.84 vs. 1.36 ± 0.15 kg on average) as both groups increased the size of their meals over time after the first week (P = 0.09; Figure 2.6). An interaction of treatment and day was observed for number of forage meals consumed daily (P = 0.06; Figure 2.7) as well as time spent per meal (P < 0.01; Figure 2.8). An interaction of treatment and day was also observed for daily number of visits (P < 0.01; Figure 2.9), forage intake per visit (P < 0.01; Figure 2.10), and time spent consuming forage per visit (P < 0.01; Figure 2.11).

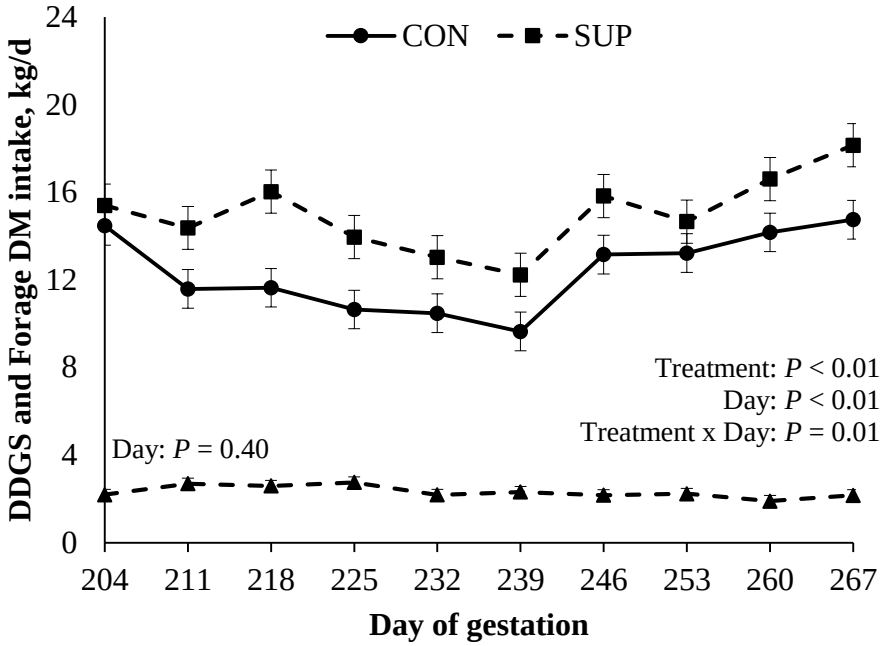

Figure 2.3. DM Intake of forage and dried distiller's grains plus solubles (DDGS) of cows fed control or control plus supplement from d 201 to d 270 of gestation

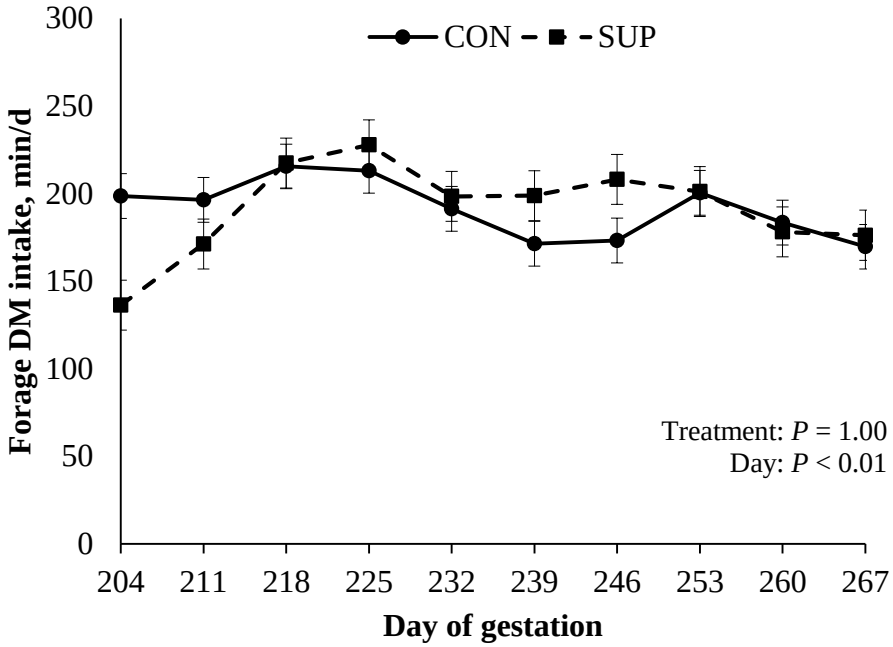

Figure 2.4. Time spent consuming forage daily of cows fed control or control plus supplement from d 201 to d 270 of gestation

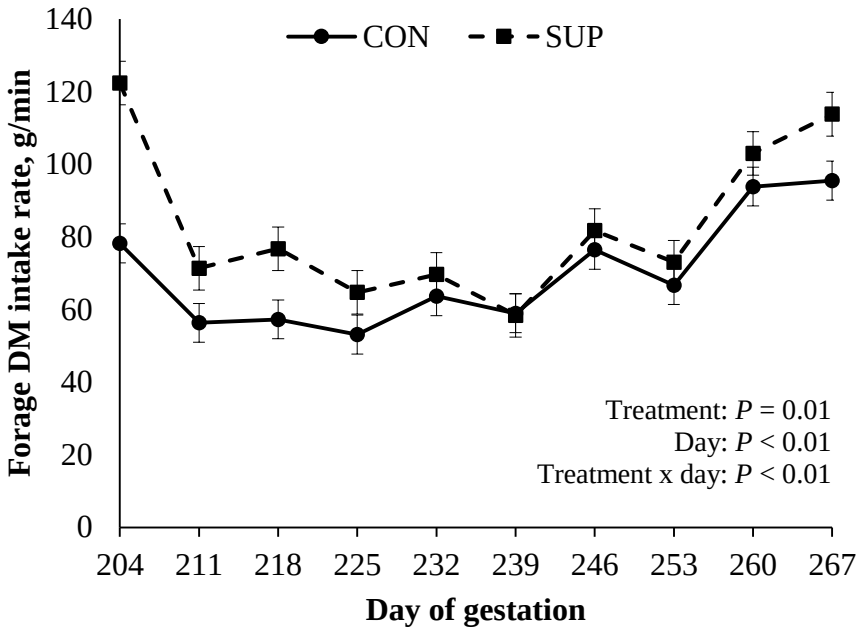

Figure 2.5. DM Forage intake rate of cows fed control or control plus supplement from d 201 to d 270 of gestation

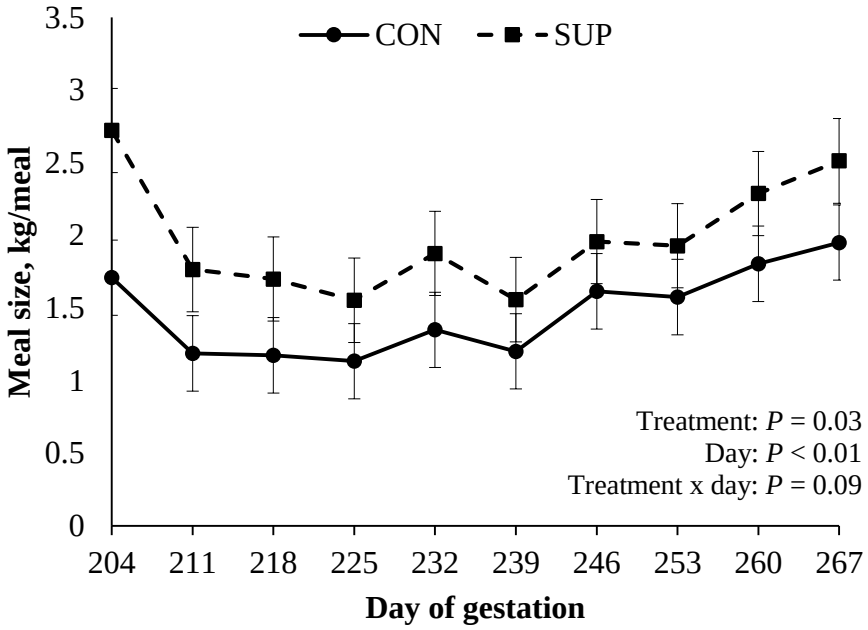

Figure 2.6. Size of forage meals consumed by cows fed control or control plus supplement from d 201 to d 270 of gestation

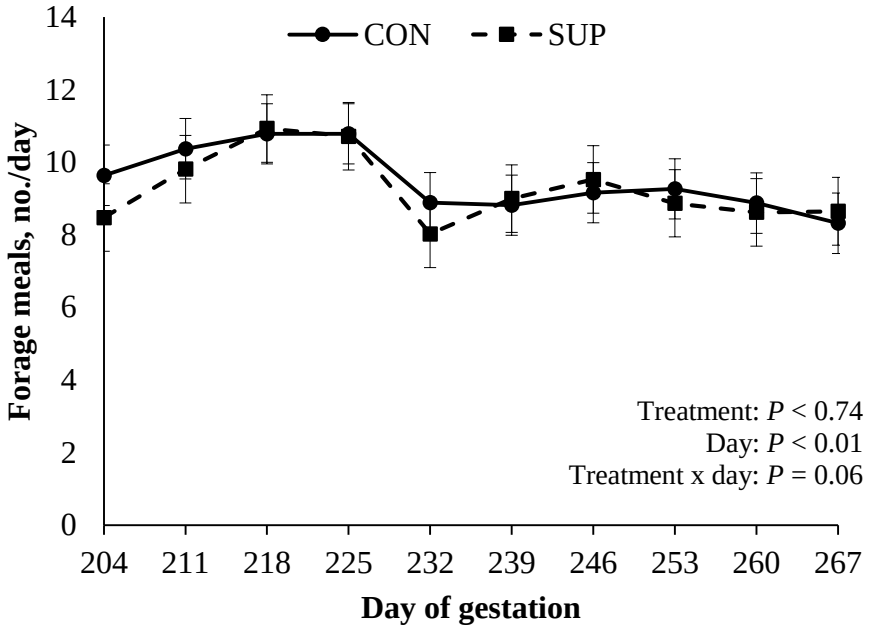

Figure 2.7. Number of forage meals consumed daily of cows fed control or control plus supplement from d 201 to d 270 of gestation

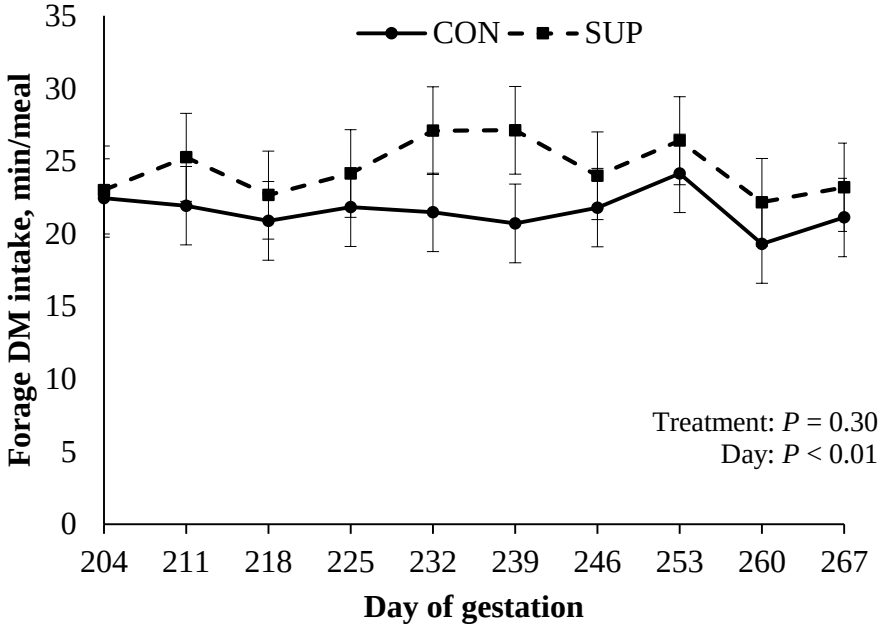

Figure 2.8. Time spent consuming forage meals of cows fed control or control plus supplement from d 201 to d 270 of gestation

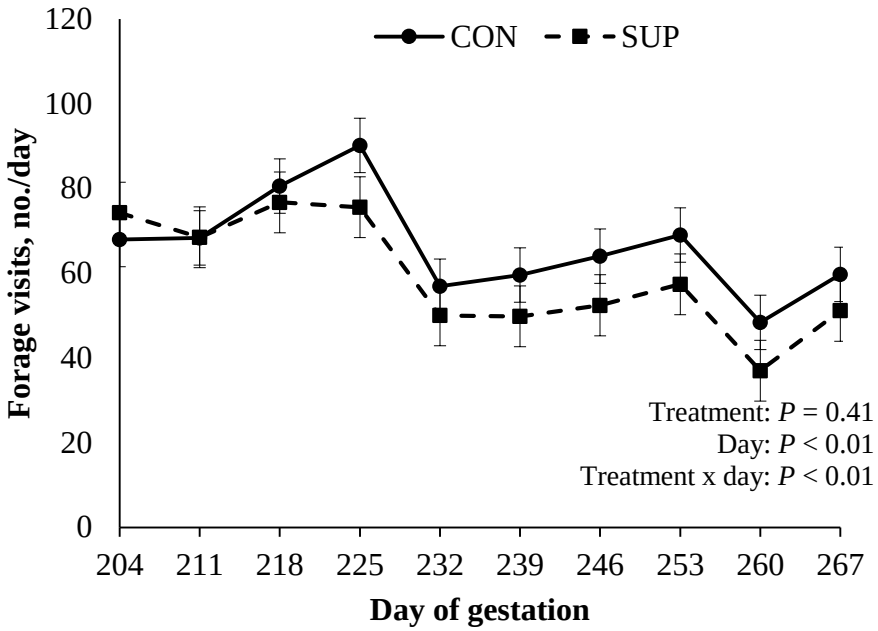

Figure 2.9. Daily visits to feed bunk of cows fed control or control plus supplement from d 201 to d 270 of gestation

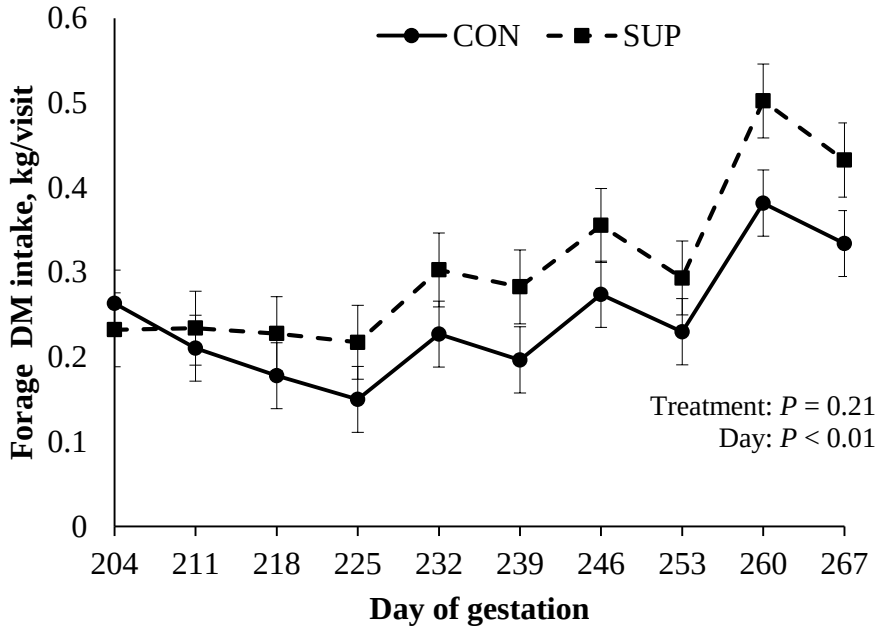

Figure 2.10. DM Intake per visit of cows fed control or control plus supplement from d 201 to d 270 of gestation

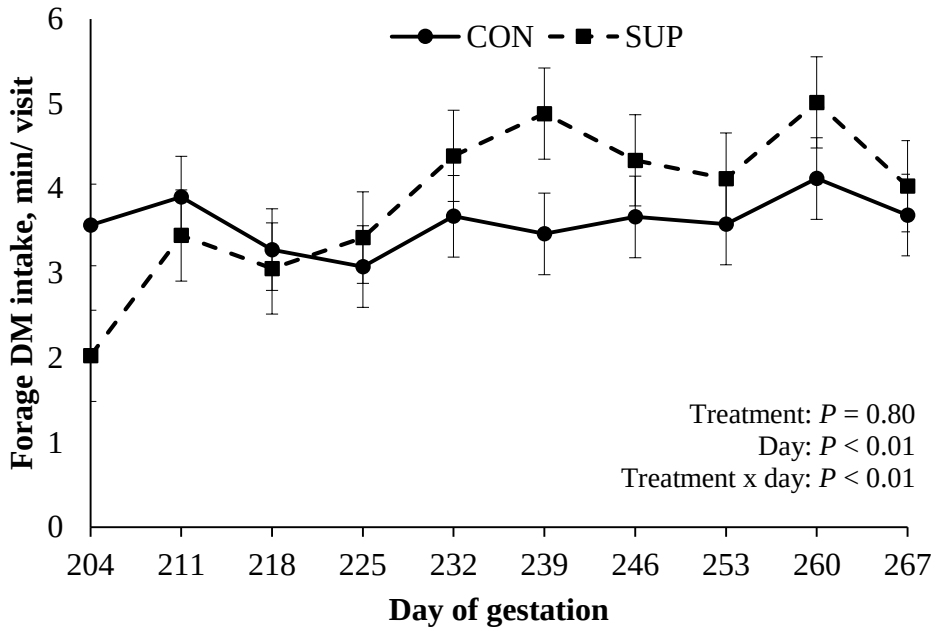

Figure 2.11. Time spent consuming forage per visit of cows fed control or control plus supplement from d 201 to d 270 of gestation

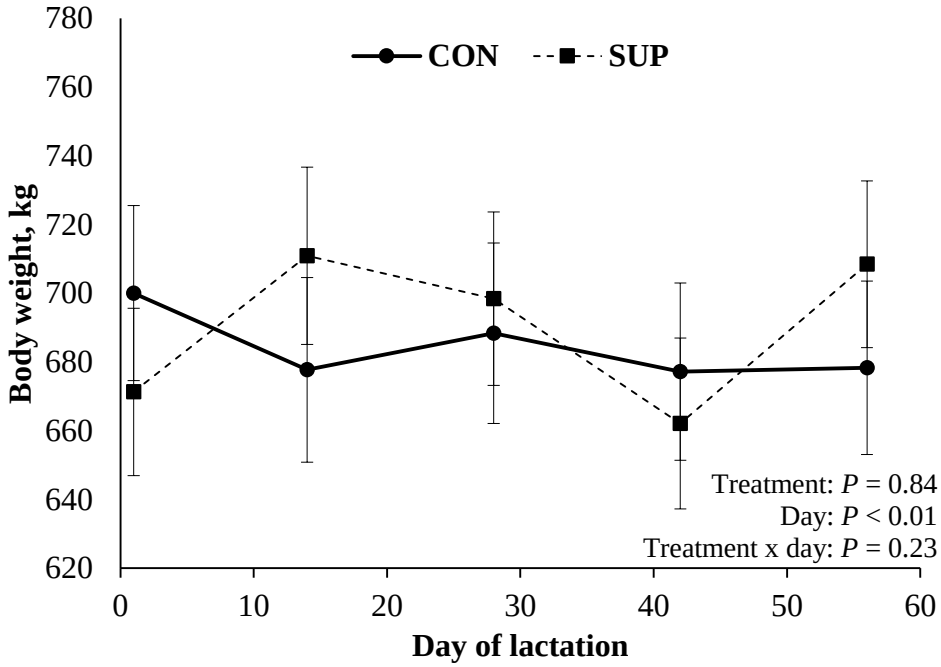

Figure 2.12. Body weight during early lactation of cows fed control or control plus supplement from d 201 to d 270 of gestation

Early Lactation

There was no effect of gestational dietary treatment on BW during the early lactation period for either group (P = 0.84), but there was a main effect of day (P <0.01; Figure 2.12). Control group cows entered early lactation with an average BW of 700.0 ± 26.9 kg and finished the project on day 56 of lactation with an average BW of 678 ± 28 kg, while SUP cows began early lactation with an average BW of 671.3 ± 25.8 kg and left project with an average BW of 708 ± 27 kg; both groups gained weight with advancing early lactation. Upon leaving the research facility (d 61± 8 d of lactation), body condition of the CON group was still less than that of SUP cows (4.7 vs. 5.5, ± 0.4; P <0.01).

During early lactation, DMI increased over time (P < 0.01; Figure 2.13) but no difference was observed between treatment groups. There was an interaction of treatment and day (P < 0.01; Figure 2.14) on time spent consuming feed daily, with SUP cows spending more time eating than CON cows from week 4 of lactation onward. A treatment by day interaction (P = 0.01) was also observed for rate of feed consumption, with SUP cows consuming more forage per minute than CON cows until week 3 of lactation followed by CON cows consuming at a faster rate than SUP cows during week 7 onward (Figure 2.15). Size of meals was influenced by a main effect of treatment (P = 0.05), with SUP cows consuming larger meals on average than CON cows (0.6 vs. 0.9 ± 0.1 kg; Figure 2.16), and a main effect of day (P < 0.01), where meals decreased in size with advancing lactation. A main effect of treatment (P ≤ 0.02) and day (P ≤ 0.01) was observed for number of meals (Figure 2.17) daily; CON cows averaged more meals per day than SUP cows (52.3 vs. 34.9 ± 4.6 meals) as both groups consumed more meals daily with increasing lactation. Time spent per meal increased until around week 5 of lactation then gradually decreased until the end of the experiment (P = 0.02; Figure 2.18). A main effect of

treatment (P ≤ 0.02) and day (P ≤ 0.01) was observed for number of visits daily (Figure 2.19), which followed similar patterns to that of daily meals. A main effect of day (P < 0.01) was observed for intake per visit, which decreased with advancing lactation as well as a main effect of treatment, with SUP cows consistently consuming more per visit than CON cows (P = 0.04; Figure 2.20). Finally time spent per visit (Figure 2.21) was affected by day (P < 0.01 and P = 0.02, respectively), with increased amount of time spent per visits up to week 5 of lactation followed by a gradual decrease to the end of the lactation period.

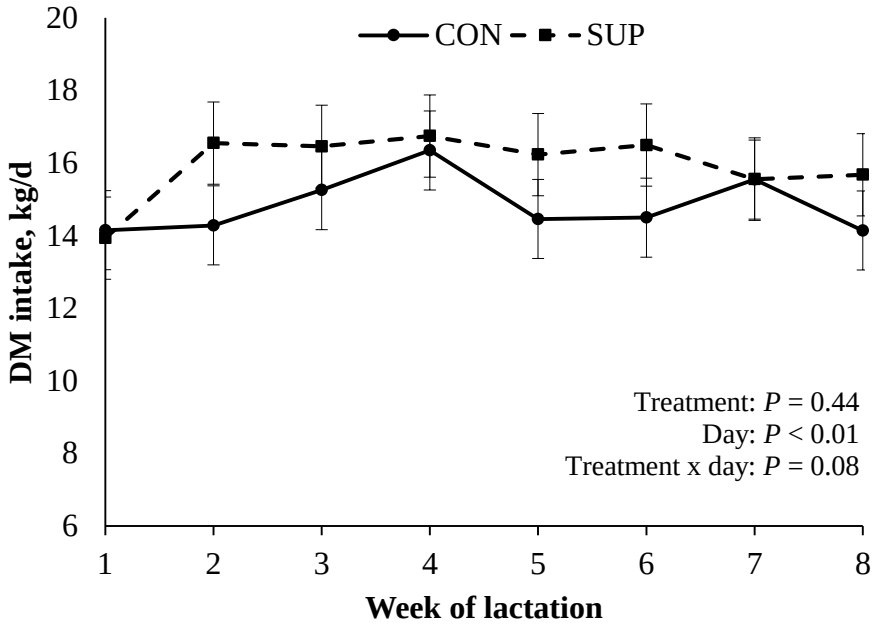

Figure 2.13. DM Intake during early lactation of cows fed control or control plus supplement from d 201 to d 270 of gestation

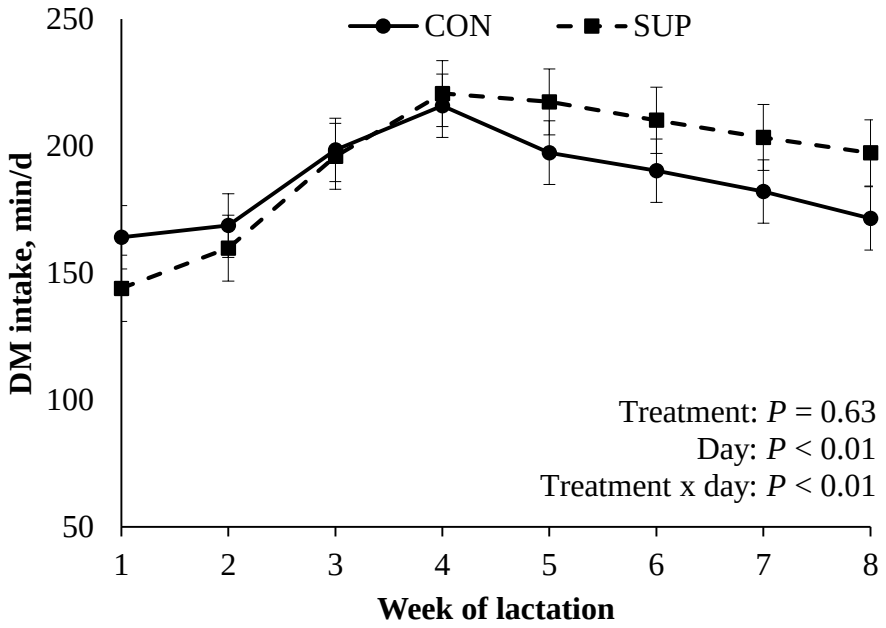

Figure 2.14. Time spent consuming feed during early lactation of cows fed control or control plus supplement from d 201 to d 270 of gestation

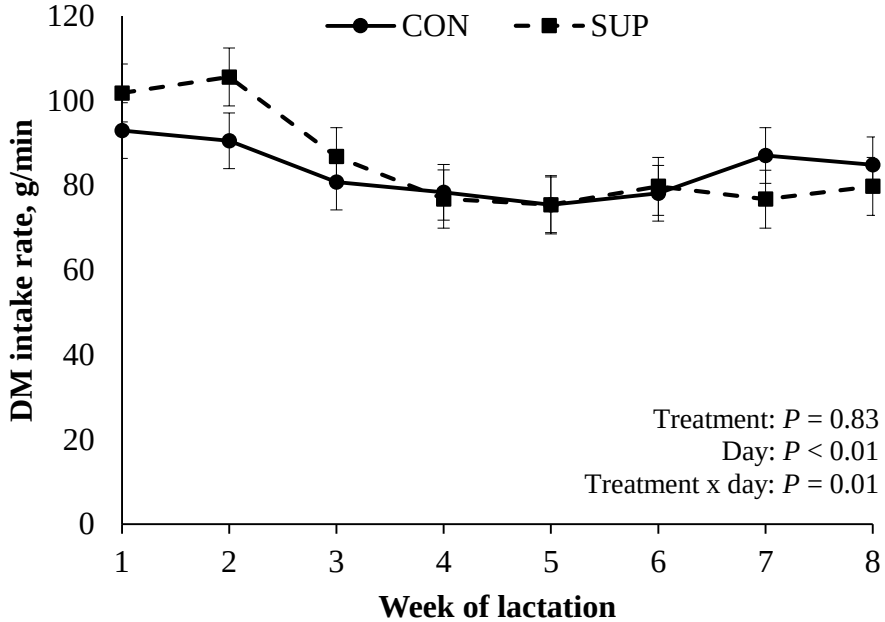

Figure 2.15. DM Intake rate during early lactation of cows fed control or control plus supplement from d 201 to d 270 of gestation

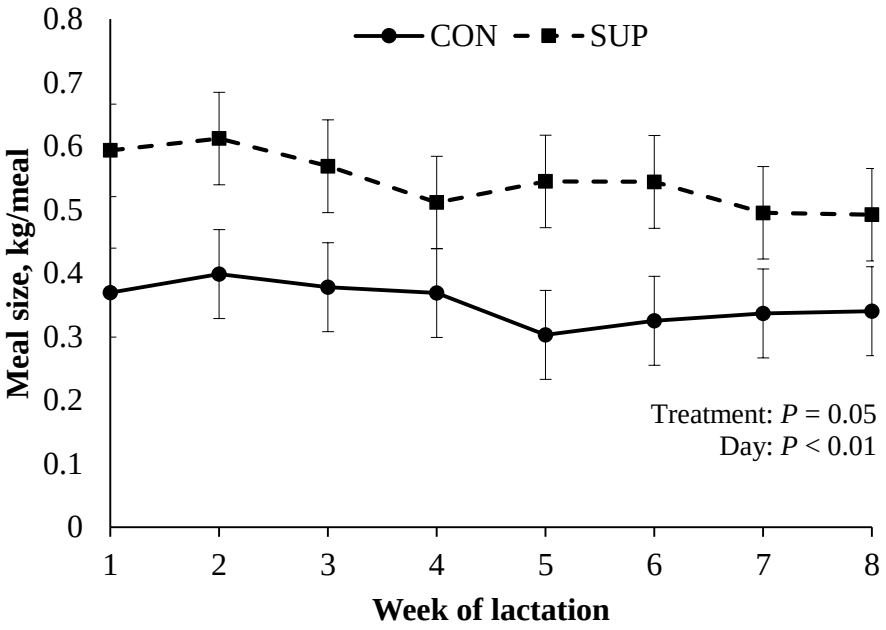

Figure 2.16. Size of meals during early lactation of cows fed control or control plus supplement from d 201 to d 270 of gestation

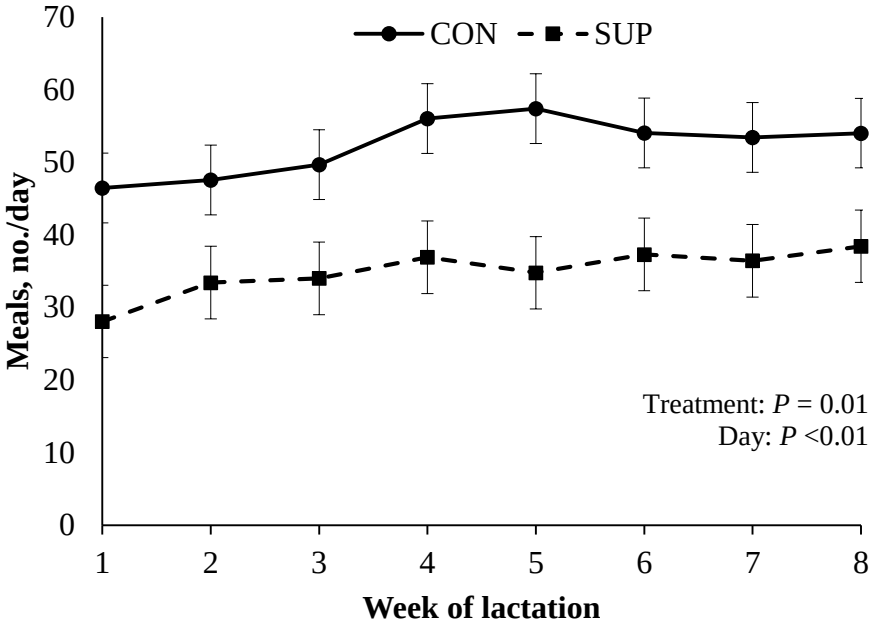

Figure 2.17. Number of meals consumed daily during early lactation of cows fed control or control plus supplement from d 201 to d 270 of gestation.

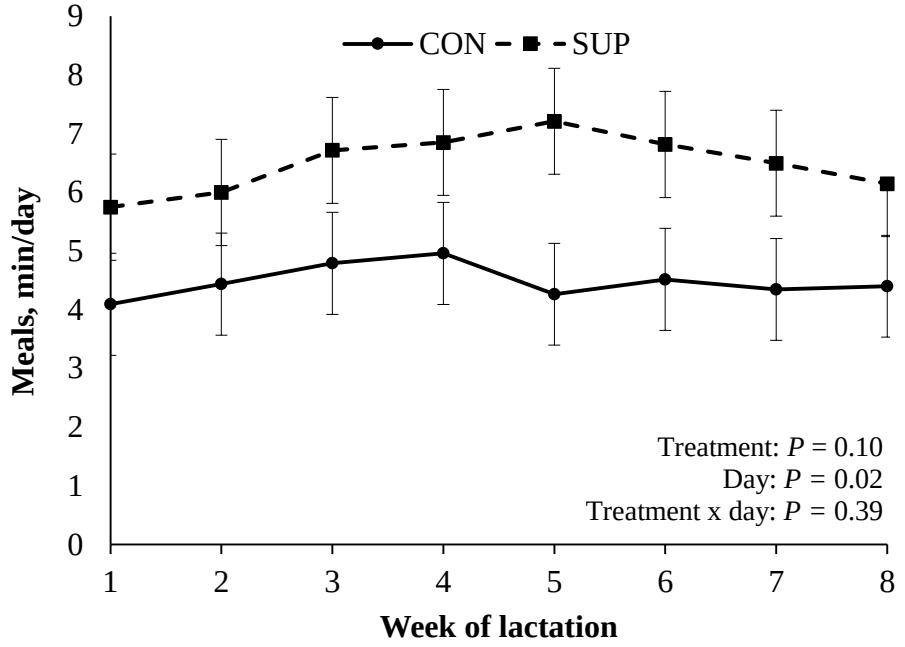

Figure 2.18. Time spent consuming meals during early lactation of cows fed control or control plus supplement from d 201 to d 270 of gestation.

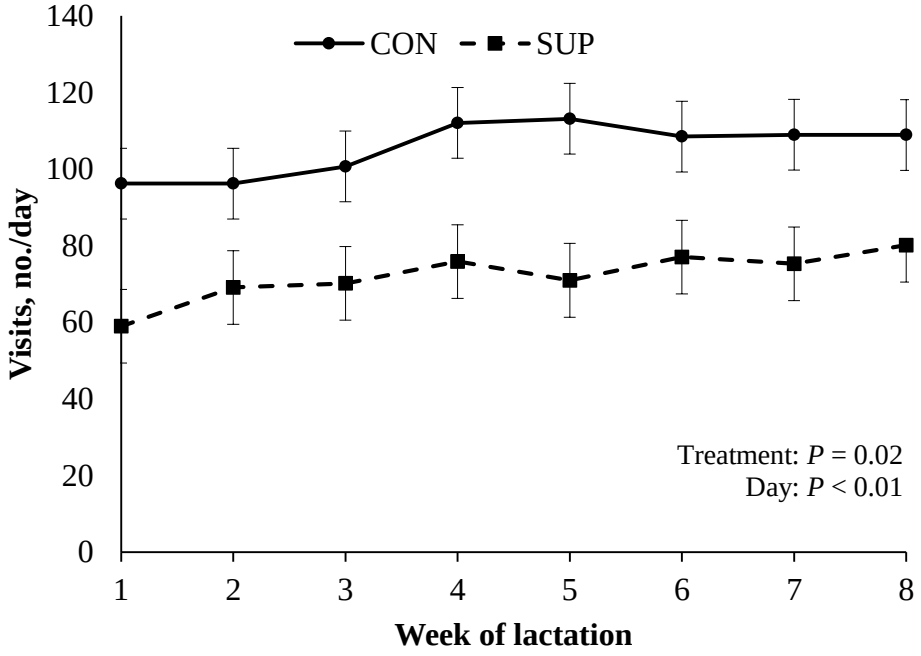

Figure 2.19. Daily visits to feed bunk during early lactation of cows fed control or control plus supplement from d 201 to d 270 of gestation.

Rebreeding

Of the 12 supplemented cows that we rebred in the spring, 8 (66.7%) became pregnant from AI, 3 from bull breeding (25%) and 1 remained open (8.3%). There were 14 cows from the CON group that were bred; 7 became pregnant from AI (50.0 %), 6 from bull breeding (42.9%) and 1 remained open (7.1%). However there were no differences (P= 0.15) in pregnancy rates regardless of AI vs. bull bred, as a result of supplementation during gestation.

Discussion

We accept our hypothesis that supplementation of DDGS to cows fed a low-quality forage diet altered intake behavior during late gestation, which impacted maintenance of maternal body weight and condition as a function of increased total metabolizable protein (MP) and net energy (NE) consumed (when provided the supplement), in addition to the tendency for increased calf birth weights. The influences of treatment during gestation also predisposed feeding behavior during early lactation, during which maternal body weight and condition were altered.

Following analysis using the NRC (1996), the aim of creating a basal forage diet deficient in rumen degradable protein (RDP) and thus a distinction between treatment groups during gestation was confirmed. Supplemented cows had increased RDP (estimated at 430 vs. 866 g/d, respectively) and MP (estimated at 654 vs. 1245 g/d, respectively) supply due to DDGS supplementation The control diet was also deficient in NE throughout the treatment period but was sufficient by supplementation (14 Mcal/d, compared to 22 Mcal/d when supplemented). As a result, feeding only the basal diet resulted in decreased body condition and a tendency for weight loss in CON cows while SUP cows maintained body condition and gained weight, at a time when fetal growth is increasing exponentially, so maternal weight was decreased in CON

cows. Additionally, the differences observed in body weight and condition are supported by the marked differences in intake behavior during late gestation between treatment groups, where SUP cows had increased responses in nearly all variables measured. Specifically, DDGS increased total daily DMI, intake rate and size of meals. Shifts in feeding behaviors are related to physiological state of the cow (late gestation vs. early lactation), diet quality, factors that impact satiety or rumen fill, and microbial activity (Forbes, 1995). This is reflected in the CON cows feeding behavior, where despite eating the low quality basal diet more often, cows presumably reached rumen fill without obtaining sufficient nutrition for maintaining body weight and condition (especially to meet the demands of pregnancy). Increased ruminal fermentation in the SUP group likely increased overall digestion, which could therefore alter daily intake behavior. It is also probable that the greater amount of RDP provided in the supplemented diet increased microbial activity and therefore improved nutrient utilization to the SUP group.

It should also be noted that during the treatment period, average temperature was -10 °C and wind speed was 18.3 km/hr (min. 16 km/hr, max. 21 km/hr) which created very cold conditions for cows and certainly could have contributed to weight and body condition loss. The influence of these weather conditions was also difficult to estimate for the NRC model.

During early lactation, when both groups were consuming the same diet (which was of higher nutritional value due to increased corn silage and DDGS inclusion), only a few of the differences in feeding behavior carried over from gestational dietary treatments. Previously supplemented cows still spent more time eating larger meals than the CON group, however CON cows consumed more meals per day than SUP cows. The lack of an overall treatment effect on body weight during early lactation may be reflective of the large changes in BW the CON group experienced compared to the relatively stable state of the SUP group. Control cows gained BW

to quickly reach similar weights to that of the SUP group, clearly exhibiting some level of compensatory weight gain, but their body condition did not recover as well.

In conclusion, supplementation of DDGS to cows fed corn stover and silage during late gestation altered feeding behavior and allowed for maintenance of body condition and BW gain. This research can contribute to new approaches for optimizing fetal development and subsequent beef cattle productivity with the use of corn byproducts (corn stover and DDGS). In particular, this work will further acquaint producers with the value of supplementing cows during late pregnancy, notably via the use of DDGS as a beneficial energy and protein source that could subsequently alter intake behavior.

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CHAPTER 3. SUPPLEMENTATION OF CORN DRIED DISTILLER'S GRAINS PLUS SOLUBLES TO GESTATING BEEF COWS FED LOW-QUALITY FORAGE: IMPACTS ON UTERINE BLOOD FLOW, CIRCULATING ESTRADIOL- 17β AND PROGESTERONE AND HEPATIC STEROID METABOLIZING ENZYME ACTIVITY

Abstract

The objective of this study was to investigate the effects of supplementing DDGS during late gestation on uterine blood flow (BF), circulating steroid hormones and hepatic steroid metabolizing enzymes, calf, and placental weights. Multiparous beef cows were divided randomly into a control group (CON; n = 15) consuming a diet containing 90% corn stover and 10% corn silage (DM basis) ad libitum and a treatment group (SUP; n = 12) consuming the same diet and DDGS (0.3% of BW). Corn silage inclusion was increased to 30% as gestation progressed to meet increasing caloric requirements. Ipsilateral and contralateral uterine BF and cross sectional area (CSA) of each uterine artery was measured by Doppler ultrasonography on d 180, 216, and 246 of pregnancy. Contralateral BF and CSA increased ($P < 0.01$) as gestation advanced. Ipsilateral BF and CSA was affected by a treatment and day of gestation interaction ($P < 0.05$). A main effect of treatment ($P = 0.02$) and day ($P < 0.01$) was observed for total BF; BF increased over time and SUP cows had greater BF than CON cows. Circulating concentrations of both progesterone (P4) and estradiol-17β (E2) were affected by an interaction of treatment and day ($P <0.01$). Concentrations of circulating E2 increased steadily throughout the study were higher in CON cows than SUP cows by d 242. Concentrations of P4 also increased over time; P4 of CON cows was greater than SUP cows by d 242. Uridine 5'-diphospho-glucuronosyltransferase (UGT) and cytochrome P450 (CYP) 1A activity increased with advancing gestation ($P < 0.01$). There was greater UGT activity ($P < 0.05$) and a trend for greater

CYP1A activity (P =0.06) in SUP vs CON cows. Activity of CYP3A was greater (P <0.01) in SUP cows and decreased (P < 0.05) with advancing gestation. Supplementing DDGS to low quality forage during late gestation increased uterine BF, but decreased circulating E2 and P4 concentrations and hepatic steroid metabolizing enzyme activity. It was anticipated that enzyme activity would reflect circulating hormone levels, however our data suggests the observed increases in BF are not driven by alterations in hormone concentration, therefore further research is warranted to elucidate the underlying mechanisms.

Introduction

Maternal nutrition is essential to fetal and placental development, which can influence the lifetime performance of the calf (Funston et al., 2010). One way to quantify nutrient delivery to the fetus is to measure uterine arterial BF (Ferrell, 1991). Uteroplacental vasculature is a key component of transplacental exchange and thusly vital to fetal development (Reynolds and Redmer, 1995). Modification of uterine BF and nutrient transfer capacity enables increased oxygen and nutrient delivery to the growing fetus (Vonnahme and Lemley, 2012). Maternal nutrient intake alters circulating vasoactive steroids, specifically E2 and P4, which may influence uterine BF and/or nutrient flux to the conceptus. Measurement of steroid production and clearance can, therefore, explain how altered maternal nutrition influences uterine arterial BF.

Dramatic increases in corn production in the U.S. have resulted in more corn production byproducts, such as cornstalks, available to producers. A byproduct of corn-based ethanol production, DDGS, provides supplemental energy and protein in addition to the basal diet. Feeding corn byproducts to pregnant beef cows during winter offers economic benefits to cow-calf operations (Kim et al., 2008), but research is needed to elucidate the nutritional benefits of using DDGS as a supplement to cows fed low-quality forages (i.e. cornstover). Numerous studies

exploring the effects of DDGS supplementation during late gestation produced promising results (Stalker et al., 2006; Martin et al., 2007; Larson et al., 2009).

We hypothesized that supplementation of DDGS to a low quality forage would increase uterine BF via increased circulating E2 and therefore increase nutrient flow to the fetus. Therefore the objective of this study was to investigate the effects of supplementing DDGS to cornstalks during late gestation on uterine BF, circulating concentrations of E2 and P4 as well as their corresponding metabolizing enzymes

Materials and Methods

Experimental design, cows and dietary treatments

All procedures were approved by the North Dakota State (N DSU) Animal Care and Use Committee (IACUC #A14007). Treatments applied to animals have been previously described (Chapter 2). Briefly, 27 multiparous beef cows (Angus or Angus x Simmental; 674 ± 17 kg; 6 ± 5 yr) were divided randomly into a control group (CON; n = 15) and a treatment group (SUP; n = 12) and housed at the Beef Cattle Research Complex. Following a 3-week acclimation period, intake was monitored and controlled via Insentec roughage feeders (Insentec, B.V., Marknesse, Netherlands) beginning on day 201 of gestation for 10 weeks. A basal diet of 90% corn stover and 10% corn silage (5.0% CP DM basis, marginally NE deficient, RDP deficient) was fed for ad libitum intake to both groups, with the SUP group supplemented DDGS at 0.3% of BW (DM basis). Corn silage inclusion was increased to 20% on day 246 of gestation (gestational diet 2) to meet increased nutritional demands during pregnancy, but supplementation regimes remained the same. Both pens had free access to water and trace-mineralized salt blocks (95.5- 98.5% NaCl, 3,500ppm Zn, 2,000ppm Fe, 1,800ppm Mn, 280-420ppm Cu, 100ppm I, 60ppm Co). Following parturition, gestation length was calculated for each cow.

Body weights, BCS and blood collection

During gestation, cows were weighed every two weeks at midday (between feedings) from initiation of the project until day 242 of gestation, as reported in the previous chapter (Chapter 2). On each weigh date, a blood sample was collected via jugular venipuncture. Samples were centrifuged for 20 min at 1,380 x g to separate serum, which was then pipetted into cryo-vials and frozen at 0°C for later analysis of circulating E2 and P4.

Uterine hemodynamic measurements

To measure uterine hemodynamics, color Doppler ultrasonography was employed. Ipsilateral and contralateral uterine BF cross-sectional area and pulsatility indices of each uterine artery were measured by Doppler ultrasonography on d 180, 216, and 246 (± 5 d) of pregnancy. A 7.5 MHz finger probe was insert into the rectum and the bifurcation of the internal and external iliac arteries was identified. By following the former, the uterine artery was identified descending toward the uterus. This was confirmed by its Doppler coloration and as well as maneuverability relative to the iliac artery or caudal aorta. The probe was placed just below the branch point to ensure measurements were taken at the same spot in each cow on each ultrasound day. Three separate cardiac cycle waveforms were measured from 2 to 3 separate ultrasound evaluations for data collection and averaged (i.e., 6 to 9 measurements per artery per cow). Resistance index (RI), pulsatility index (PI), peak systolic velocity, end diastolic velocity, flow time, maternal heart rate (HR), mean velocity, BF volume, cross-sectional area (CSA) and cross-sectional diameter were all recorded. The Doppler software was preprogrammed to calculate 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) × cross-

sectional area (cm2) × 60 s. Finally, total BF was calculated as the sum of ipsilateral and contralateral uterine artery BF late for statistical analyses.

Liver biopsy procedure and sample processing

Liver biopsies were performed on d 187 (± 1 day) and d 221 or 222 of gestation, which will be referred to as "pre-" and "post-treatment" biopsies, respectively. Biopsies were performed in a squeeze chute. For reference, an imaginary line was drawn from the point of the tuber coxae to the olecranon and the location of the liver was estimated between the 10th and 11th rib on the right side of the animal. Hair was removed from the surgical site using clippers. The liver was scanned using Doppler ultrasonography to confirm its location and ensure the point of biopsy needle insertion was devoid of major hepatic vessels. The skin was then scrubbed twice with betadine. A local anesthetic (10 ml of 2 % lidocaine hydrochloride) was administered at the tenth intercostal space and the skin punctured using a scalpel. A biopsy tool was inserted until it passed the liver capsule, the biopsy needle deployed, and then biopsy tool withdrawn. The biopsy needle collected 0.5 to 1.0 g of liver tissue, which was then placed in a cryo-vial and snap frozen in liquid nitrogen. Sterility was maintained throughout the biopsy procedure in order to safely sample multiple cows in one day. Immediately following liver sample collection the skin was stapled and treated with Blu-Kote (H.W. Naylor Co. Inc., Morris, N.Y.) antiseptic spray. Finally, feed intake and behavior following surgery. Any changes warranted a call to the attending veterinarian, however no animals experienced any complications. Skin staples were removed 7 to 10 d post-surgery.

Parturition

During calving, cows were allowed to remain in their pens with the group until signs of labor were observed. If it was possible to move the cow inside the barn without causing undue

stress, she was brought inside and put in an individual pen for calving, otherwise she was allowed to calve outside with the herd and the pair was immediately brought inside using a sled for the newborn calf. Time of birth and calving ease was recorded. Calving ease was scored on a 1 – 5 scale, with 1 = no assistance and 5 = Caesarian section. Time to standing was recorded and after which calves were removed from dams and dried with a blow drier. Body weight, sex, crown rump measurements, and heart girth measurements were recorded.

While calves were being processed, the cow was weighed (in the same room and within sight of the calf). Cows and calves were returned together to their individual pen where they were monitored for general calf health, and a calf vigor score was recorded. Calf vigor was scored on a 1 – 5 scale, with 1= normal and 5 = stillborn. Cow-calf pairs remained in individual pens for 24 h before returning outside to the group.

Time to placenta expulsion was recorded for each cow and placentas were collected immediately. Each placenta was processed by recording whole weight, dissecting cotyledons and weighing them in total, weighing the remaining intercotyledonary tissue, and weighing the largest and smallest cotyledons. Finally, gestation length was calculated for each cow.

Preparation of liver samples for enzyme activity assays

Samples of liver (100 mg/2 ml potassium phosphate buffer) were homogenized using a glass Duounce homogenizer at 4 C. Homogenate was then centrifuged at 10,000 x g for 10 min at 4°C. The resulting supernatant (S9 fraction) was then aliquoted into separate tubes to prevent additional freeze-thaw cycles between enzymatic activity assays. All S9 fractions were then stored at -80°C. Protein concentrations of the S9 fractions were analyzed using a Coomassie Plus (Bradford) protein assay in accordance with the manufacturer's protocol (Thermo Scientific, Rockford, IL, USA). All enzyme activity was determined using the assays described below and

will be expressed relative to maternal liver protein (RLU/min/mg of protein), to grams of hepatic tissue (RLU/min/g) and to maternal BW (RLU/min/kg of BW); RLU refers to relative light units. All enzyme activity assays were performed similar to methods reported by Hart et al. (2014).

Cytochrome P450 activity assays

The CYP1A, CYP2C, and CYP3A assays kits as well as the NADPH regeneration system (Promega Corporation, Madison, WI, USA) were used following manufacturer's instructions. First, reconstitution buffer was added to the luciferin detection reagent. Luciferin CEE (CYP1A), luciferin H (CYP2C) and luciferin IPA (CYP3A) substrates were diluted in phosphate buffer. The NADPH regeneration system was prepared following manufacturer's instructions. The liver fractions and enzyme-specific luciferin substrates were pipetted in duplicate to 96-well opaque white plates and pre-incubated for either 10 min (for CYP1A and CYP3A assays) or 20 min (for the CYP2C assay) at 37°C. Following incubation, luciferin detection reagent was added to each well and the plate was again incubated at room temperature for 20 min while protected from light. Finally, all plates were read using a plate reader (Promega Multi-Plus, Madison, WI, USA) using luminescence detection. A linear relationship was determined between the rate of luminescence versus activity of hepatic protein.

Aldo-keto Reductase 1C

Activity of AKR1C was analyzed using the methods of Lemley and Wilson **(**2010). In brief, concentrations of AKR1C in the fractions were determined using the specific substrate 1-acenapthenol (Pfaltz & Bauer, Waterbury, CT, USA). Reactions were carried out in 96-well clear plates, with each well containing 150 μg of cytoplasmic protein, 250 μM 1-acenapthenol, and 500 μM NADP. Reduction of NADP by 1-acenapthenol was standardized by the amount of cytoplasmic protein used. NADP reduction was measured by quantifying absorbance at 340 nm

for 10 min using a plate reader (Spectra Max Plus, Sunnyvale, CA, USA). To calculate the rate of reduced NADP in pmol per min per mg of protein, the molar absorption coefficient of NADPH was used (6,220 L/mol x cm).

Uridine 5'-diphospho-glucuronosyltransferase

Activity of UGT was analyzed using an assay kit from Promega Corporations (Madison, WI, USA) following the manufacturer's instructions; however, incubation time was reduced per recommendation from Hart et al. (2014). A 96-well opaque white plate was used to carry out the reaction, with uridine diphosphoglucoronic acid (UDPGA) added to half the wells and water added to the other half to serve as controls. The UGT multienzyme substrate reaction mixture was then added to all wells. The liver fraction samples were then added in duplicate, with a control of phosphate buffer at the end of the plate. The plate was then covered with parafilm and pre-incubated at 37°C for 10 min. Finally, the detection reagent was added to each well and the plate was incubated at room temperature for 20 min protected from light. The plate was read using a luminescent plate reader and activity of UGT was calculated by subtracting the RLU in duplicate samples of reaction wells and control wells.

Estradiol- 17β and Progesterone analysis

Serum E2 concentrations were evaluated according to manufacturer's instructions for solid-phase ^{125}I radioimmunoassay (Siemens Healthcare Diagnostics Inc., Los Angeles, CA, USA), except that 500 μL of serum from samples was used instead of the recommended 100 μL. Standards and unknowns were pipetted in duplicate to E2 antibody-coated tubes. Next, 1 mL of E2 tracer (iodinated synthetic E2) was added to all tubes, which were then covered with parafilm, vortexed, and incubated at room temperature for 3 h. After incubation, all liquid in all tubes was aspirated and discarded. Finally, tubes were read in a Packard gamma counter

(Meriden, CT, USA). The average intra-assay coefficient of variation for the E2 assay was 5.00%.

Progesterone serum concentrations were evaluated according to manufacturer's instructions for solid-phase ^{125}I radioimmunoassay (Siemens Healthcare Diagnostics Inc., Los Angeles, CA, USA). Standards and unknowns were pipetted in duplicate to progesterone antibody-coated tubes. Next, 1 mL of P4 tracer (iodinated P4) was added to all tubes, which were then covered with parafilm, vortexed, and incubated at room temperature for 3 h. After incubation, all liquid in all tubes was aspirated and discarded. Finally, tubes were then read in a Packard gamma counter (Meriden, CT, USA). The average intra-assay coefficient of variation for the P4 assay was 3.17%.

Statistical Analysis

Data analysis utilized the mixed procedure of SAS (SAS Institute Inc., Cary N.C.) with day as the repeated measure. The class statements included cow, maternal diet (SUP vs. CON), day of gestation, and the interaction of day and maternal diet. The model statement tested the dependent variables of ultrasound indices, hormone and liver enzyme levels, calving data and placental measurements. To further elucidate changes in calf and cow weights from 0 h to 24 h postpartum, hormone levels and enzyme activity, percentage change was also included in the model. Differences between least square means were determined using the least significant difference method.

Results

As mentioned previously (Chapter 2), there was an effect of diet on maintenance of maternal body weight and BCS; SUP cows gained weight at an average rate of 1.27 kg/day ($P < 0.01$) and CON cows tended to lose weight ($P = 0.06$) while losing BCS ($P < 0.01$).

Uterine Blood Flow and Maternal Heart Rate

A treatment by day of gestation interaction (P = 0.05) was observed for both CSA and uterine BF for the ipsilateral uterine artery. Cross sectional area spanned 0.5 to 1.1 (± 0.07 cm2) and was similar (P = 0.30) between treatments on d 181, but greater in SUP vs, CON cows on d 216 and 246 (Figure 3.1). Ipsilateral uterine BF was also similar on d 181 in SUP cows compared to CON cows but was greater (P < 0.01) on d 216 and 246. Ipsilateral uterine BF was greater than contralateral BF, with an average minimum of 8.2 and maximum of 31.6 (± 1.9 L/min) overall (Figure 3.2).

There was no interaction (P > 0.31) for CSA or BF on the contralateral side. Only a main effect of day (P <0.01) was observed for CSA and BF on the contralateral side, with CSA spanning from 0.24 to 0.47 (± 0.05 cm2) and increasing from d 181 to 216 (Figure 3.3). Blood flow increased as gestation advanced and averaged from 2.5 to 7.7 (± 1.0 L/min; Figure 3.4).

When ipsilateral and contralateral BF were summed, there were main effects of treatment (P = 0.02) and day (P < 0.01) for total uterine BF. Uterine BF increased as gestation advanced, and SUP cows had greater BF than CON (Figure 3.5). There was a treatment by day interaction (P < 0.01) for maternal heart rate. While HR was similar (P = 0.37) on d 181, CON cows experienced a reduction in heart rate on d 221 and it was decreased (P = 0.02) compared to SUP cows. Heart rate in CON cows returned to d 181 rates by d 241, whereas SUP cows experienced an increase (P < 0.01) in heart rate (Figure 3.6).

In addition to uterine BF, altered hemodynamic indices were also seen as result of advancing gestation. On the contralateral side, an effect of day was observed for average PI, which decreased with day of gestation (P <0.01), however no main effects or interactoins were observed for RI. On the ipsilateral side, again there was a main effect of day on PI (P < 0.01),

which also decreased with advancing gestation. No main effects of observed for RI on the ipsilateral side.

Circulating E2 and P4

There was a treatment by day interaction ($P < 0.01$) for circulating E2 and P4. Concentrations of E2 were similar on d 181 in both groups. While E2 concentration remain relatively steady throughout the experiment in SUP cows, in CON cows E2 concentration increased from d 188 to d 200-244. By d 246, the greatest levels were observed within the CON cows (Figure 3.7). Concentrations of P4 were similar in both groups until d 246 where CON cows had greater P4 concentration compared to SUP cows (Figure 3.8).

Enzyme Activity

An interaction of treatment and day was observed for AKR activity as expressed per mg of protein ($P < 0.01$), per g of liver ($P = 0.01$), and per kg of maternal body weight ($P < 0.01$); all measures of AKR activity decreased post-initiation of treatment, with CON cows having higher pre-treatment values than SUP cows but no significant difference post-treatment (Figure 3.9). No interaction of maternal dietary treatment and day of gestation was observed for remaining steroid metabolizing enzyme activities, but main effects of day and treatment were seen (Table 3.1).

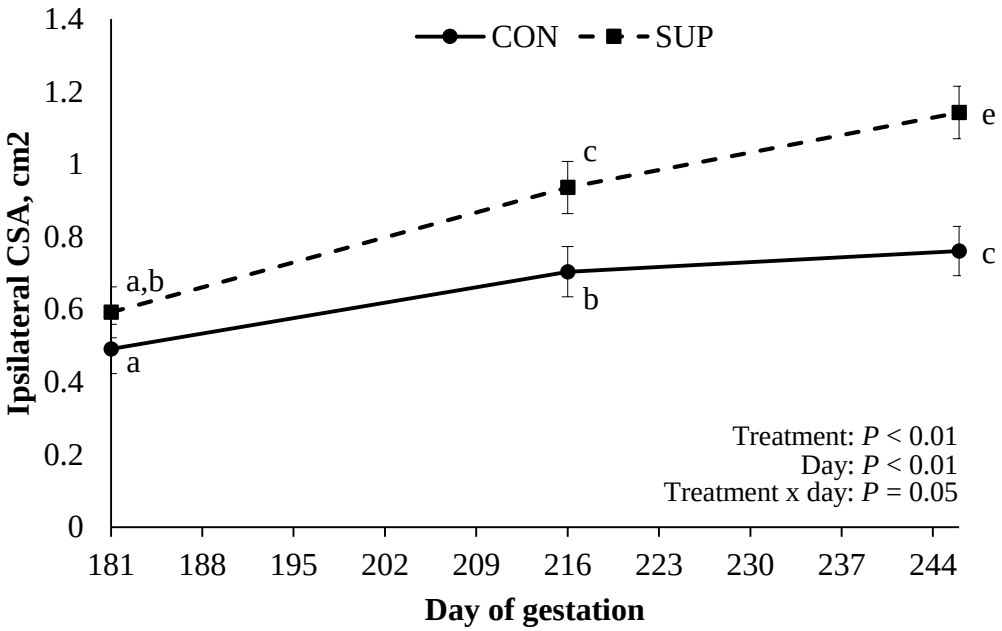

Figure 3.1. Ipsilateral uterine artery cross sectional area (CSA) of beef cows fed control or control plus supplement from d 201 to d 270 of gestation.

[a,b] Different superscripts differ at $P \leq 0.05$

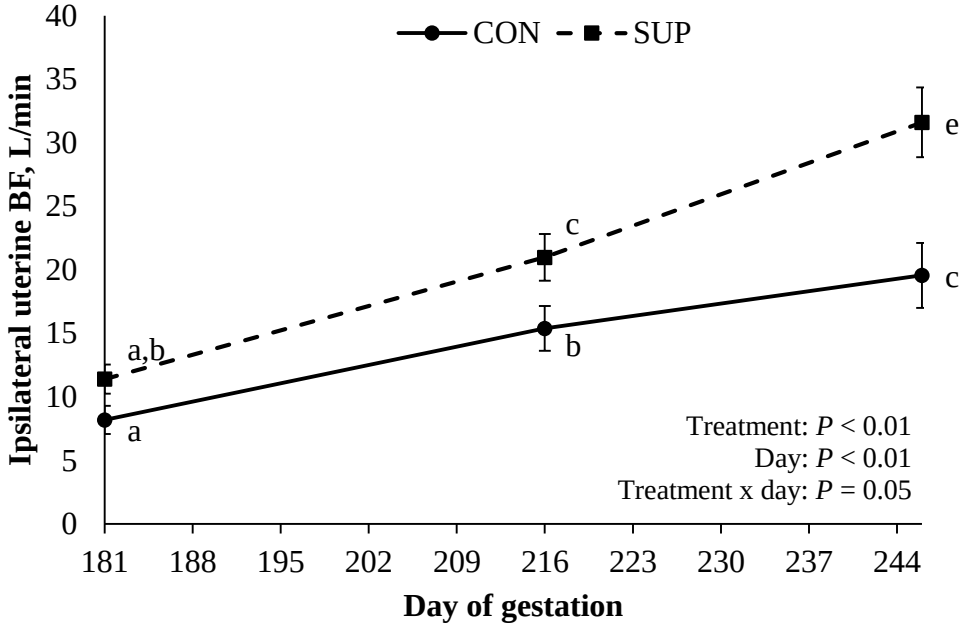

Figure 3.2. Ipsilateral uterine artery blood flow (BF) of beef cows fed control or control plus supplement from d 201 to d 270 of gestation.

[a,b] Different superscripts differ at $P \leq 0.05$

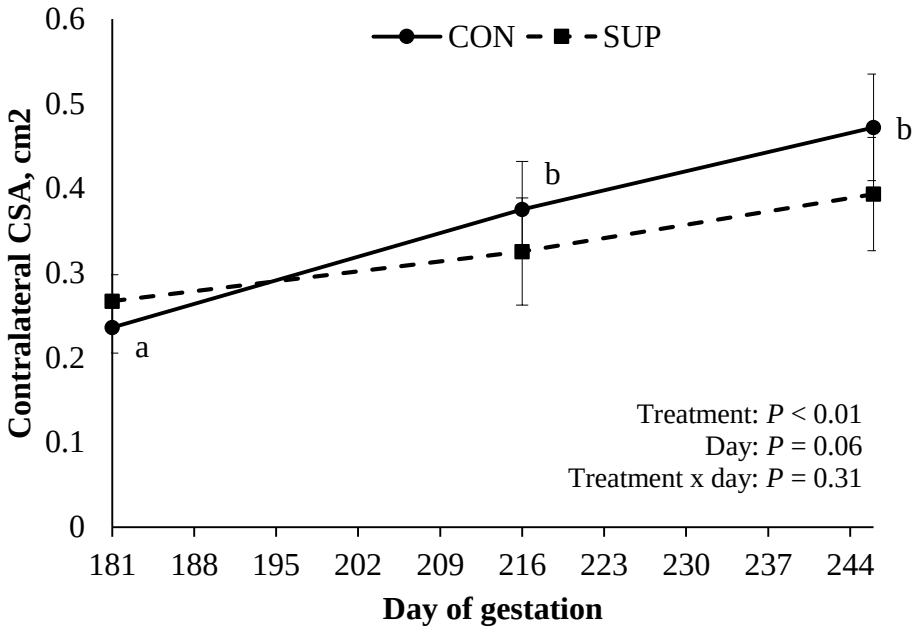

Figure 3.3. Contralateral uterine artery cross sectional area (CSA) of beef cows fed control or control plus supplement from d 201 to d 270 of gestation.

[a,b] Different superscripts differ at $P \leq 0.05$

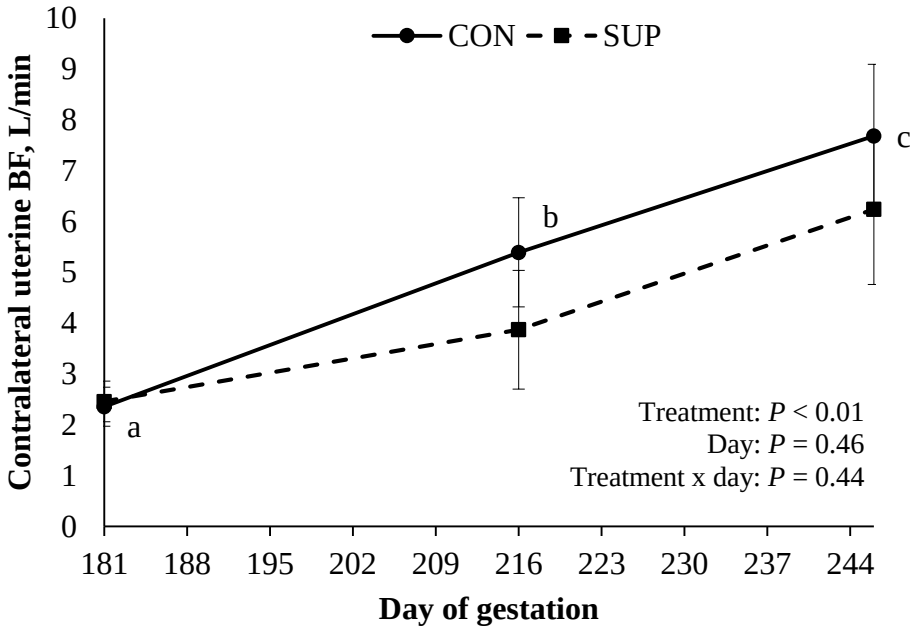

Figure 3.4. Contralateral uterine artery blood flow (BF) of beef cows fed control or control plus supplement from d 201 to d 270 of gestation.

[a,b] Different superscripts differ at $P \leq 0.05$

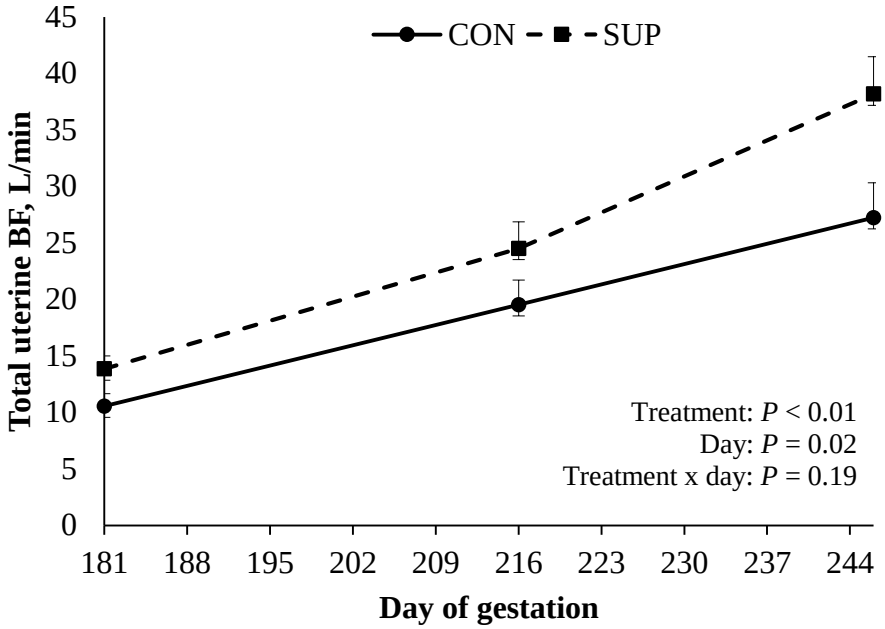

Figure 3.5. Total uterine blood flow (BF) of beef cows fed control or control plus supplement from d 201 to d 270 of gestation.

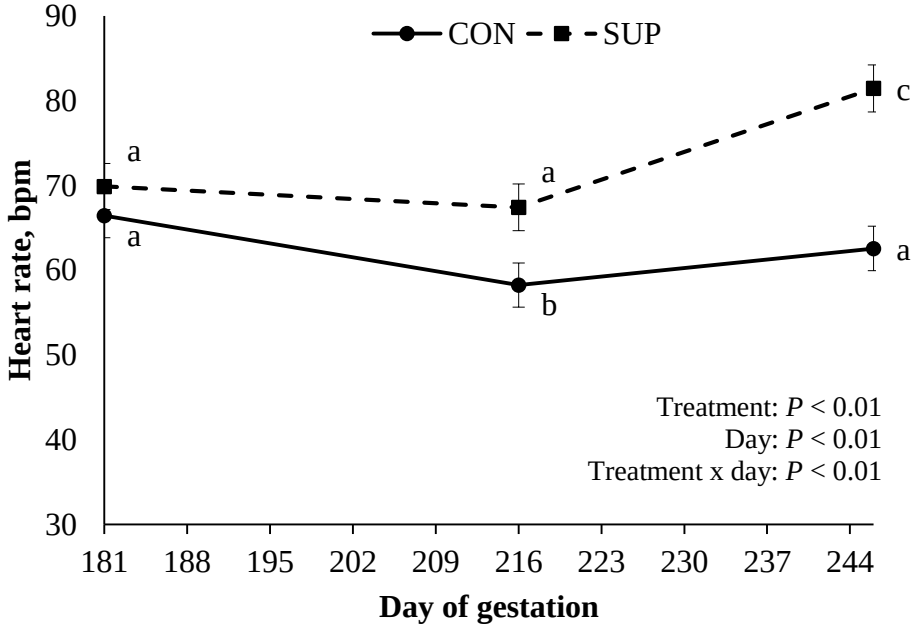

Figure 3.6. Maternal heart rate of beef cows fed control or control plus supplement from d 201 to d 270 of gestation.
[a,b] Different superscripts differ at $P \leq 0.05$

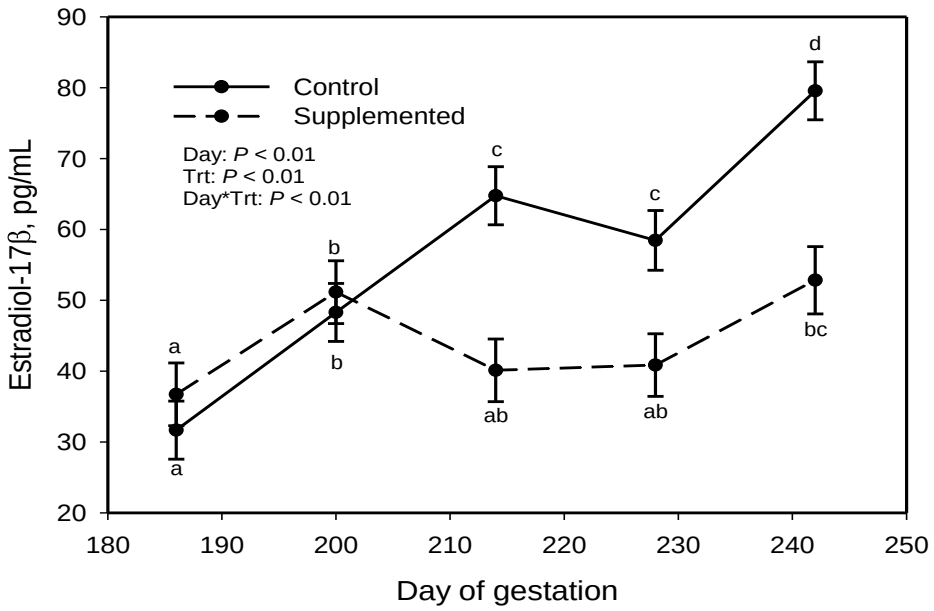

Figure 3.7. Circulating estradiol-17β concentrations beef cows that were fed control or control plus supplementation from d 201 to 270 of gestation.

[a,b] Different superscripts differ at $P \leq 0.05$

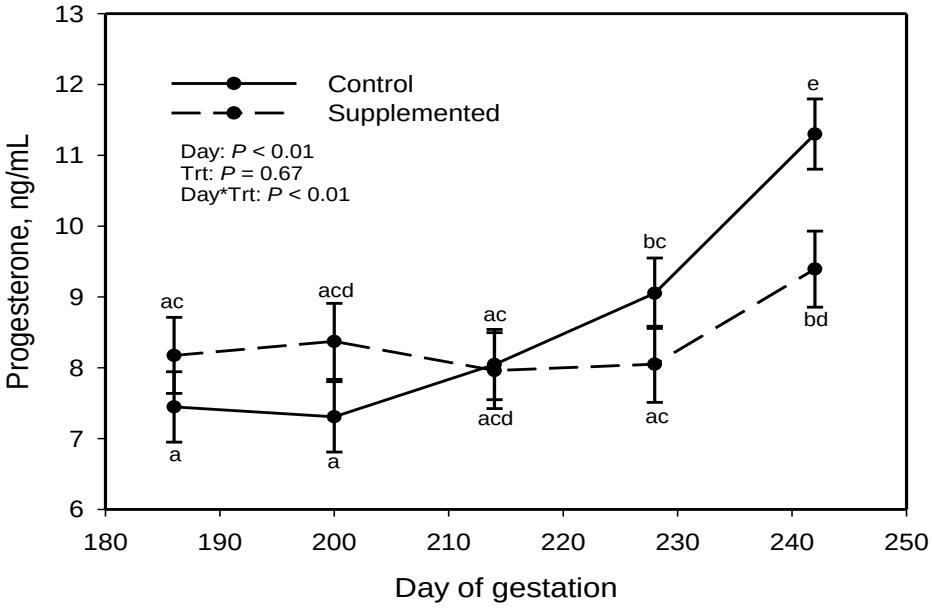

Figure 3.8. Circulating progesterone concentrations beef cows that were fed control or control plus supplementation from d 201 to 270 of gestation.

[a,b] Different superscripts differ at $P \leq 0.05$

A main effect of day was observed for UGT enzyme activity when expressed per mg of protein ($P < 0.01$), where enzyme activity was increased by the second liver biopsy. When expressed per g of liver, an effect of day ($P < 0.01$) and treatment ($P = 0.04$) was observed, with increased enzyme activity by the second liver biopsy; SUP cows had greater activity than CON cows (Table 1). Additionally, UGT enzyme activity when expressed per kg of maternal body weight increased by post-initiation of maternal dietary treatment ($P = 0.01$). A main effect of day was also seen for CYP1A activity as expressed per mg of protein ($P < 0.01$) and per kg of maternal body weight; both of which increased with advanced gestation. A tendency for an interaction of treatment and day was found for CYP1A activity per g of liver tissue ($P = 0.06$), with CON cows and SUP cows having similar activities at the first biopsy but significantly different activity levels by the second biopsy; by the second collection SUP cows had greater activity than CON cows. No effect of day or treatment was observed for CYP2C enzyme activity, although activity expressed per g of liver tissue tended to be greater by the second biopsy. CYP3A activity, as expressed per mg of protein, was influenced by treatment and day ($P = 0.04$ and $P = 0.03$, respectively), where concentrations decreased by the second biopsy and supplemented cows had higher average activity. When evaluated per g of liver sample, only a main effect of day was observed ($P = 0.02$), with supplemented cows averaging more activity. Finally, CYP3A activity per kg of maternal body weight was decreased by the second biopsy as an effect of day ($P = 0.02$).

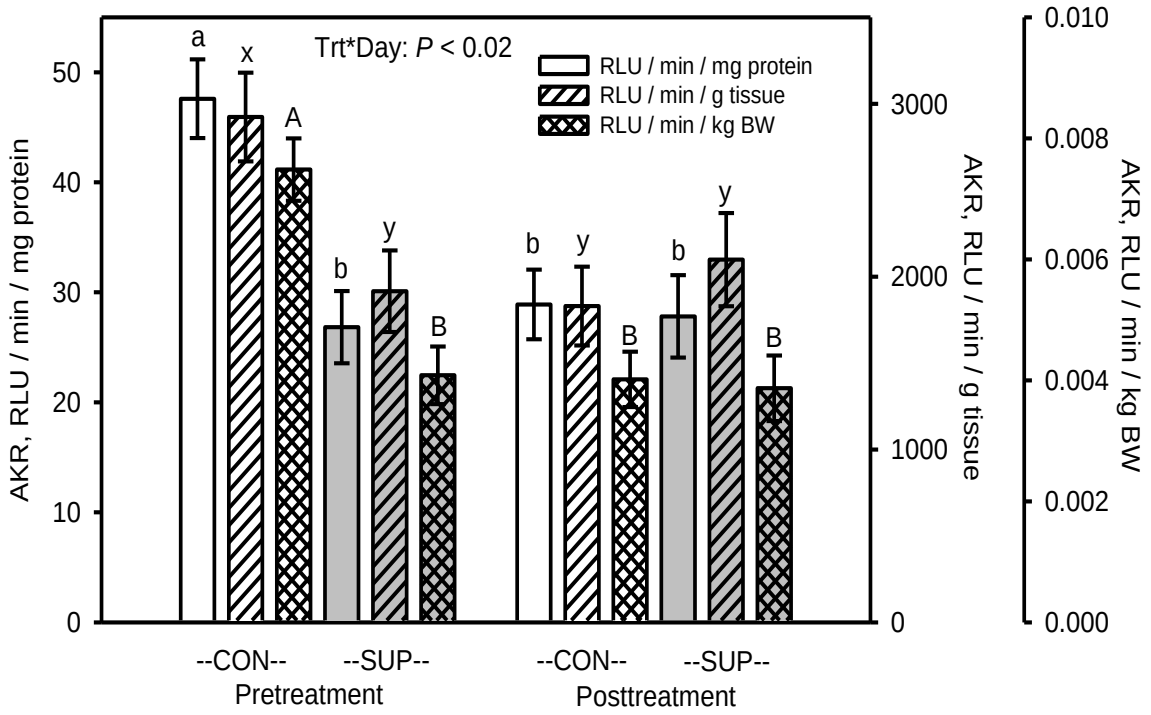

Figure 3.9. Hepatic aldo-keto reductase (AKR) 1C enzyme activity in beef cows that were fed control or control plus supplementation from d 201 to 270 of gestation.

[a,b] Different superscripts differ at $P \leq 0.05$

Table 3.1. Hepatic steroid metabolizing enzyme activity in cows "pre-" and "post-" initiation1 of dietary treatments

Dependent Variable		Dietary Treatment[2]			Day			*P*-values		
		CON	SUP	SEM	Pre	Post	SEM	Trt[4]	Day[5]	Trt*Day
UGT[5]										
protein	(RLU[4]/min/mg protein)×10^5	10.7	13.2	1.4	8.8	15.2	1.4	< **0.01**	0.2	0.84
gram	(RLU/min/gram)×10^6	68.2	95.8	9.4	57.8	106.2	9.3	< **0.01**	**0.04**	0.85
CYP1A[6]										
protein	(RLU/min/mg protein)×10^4	79.8	90.1	6.4	65.4	104.4	6.4	0.26	< **0.01**	0.27
gram	(RLU/min/gram)×10^6	48.4	66.5	4.3	43.6	71.3	4.3	< **0.01**	< **0.01**	**0.06**
CYP2C[7]										
protein	RLU/min/mg protein	3.0	3.0	0.2	2.8	3.2	0.2	0.96	0.15	0.38
gram	(RLU/min/gram)×10^4	18.5	21.8	1.4	18.3	22.0	1.4	0.11	**0.07**	0.83
CYP3A[8]										
protein	RLU/min/mg protein	569.0	685.1	39.2	690.3	563.8	39.2	**0.04**	**0.03**	0.64
gram	(RLU/min/gram)×10^6	35.8	50.6	3.2	46.8	39.7	3.2	< **0.01**	0.13	0.68

[1] Initiation of dietary treatments refers to d 201 of gestation; “pre-”: d 187 (± 1d); “post-”: d 221-222
[2] Maternal diets; CON (n = 15), control group consuming basal diet; SUP (n = 12), supplemented group consuming basal diet + DDGS at 0.3% of BW
[4] Day, gestational day
[5] Trt, dietary treatment
[4] RLU, relative light units
[5] UGT, uridine diphosphate-glucuronosyltransferase
[6] CYP1A, cytochrome P450 1A
[7] CYP2C, cytochrome P450 2C
[8] CYP3A, cytochrome P450 3A

Parturition

As previously reported (Chapter 2), there was a tendency (P = 0.06) for calves born to supplemented cows to be heavier, with SUP calves weighing 43.3 kg and CON calves averaging 40.5 kg (± 0.9 kg). There were no other impacts of maternal diet on any other birth or placental measurements (Tables 3.2 and 3.3).

Table 3.2. Calving measurements of offspring from beef cows that were fed control or control plus supplementation from d 201 to 270 of gestation.

	Means			
Variable	**CON[1]**	**SUP[1]**	**SEM**	***P*-value**
Gestation length, d	277.0	275.8	1.0	0.43
Calf birth wt, kg	40.5	43.3	0.9	0.06
Calving ease[2]	1.9	1.6	0.3	0.47
Time to stand, min	48.4	47.5	8.2	0.94
Heart girth[3], cm	82.1	84.1	1.2	0.22
Crown rump length[4], cm	84.7	83.1	2.0	0.57
Calf vigor score[5]	1.2	1.2	0.2	0.89
Calf wt, 24 hr BW, kg	43.8	44.0	2.6	0.96

[1] Maternal diets; CON (n = 15), control group consuming basal diet; SUP (n = 12), supplemented group consuming basal diet + DDGS at 0.3% of BW
[2]Calving ease scored on a 1-5 scale (1 = no assistance and 5 = Caesarian section)
[3]Heart girth was measured around calf's chest at withers
[4]Crown rump measured from point of skull to base of tail head
[5]Calf vigor scored on 1-5 scale (1= normal and 5 = stillborn)

Table 3.3. Placental measurements beef cows that were fed control or control plus supplementation from d 201 to 270 of gestation

	Means			
Variable	**CON[1]**	**SUP[1]**	**SEM**	***P*-value**
Placental delivery, min	226.8	286.3	54.5	0.45
Total placenta wt, g	5384.1	4721.4	365.9	0.22
Intercotyledonary wt[2], g	2700.2	4848.8	1545.0	0.34
Cotyledonary wt[3], g	2185.3	2168.2	163.7	0.94
Largest	90.1	91.7	9.0	0.90
Smallest	1.0	0.5	0.2	0.18

[1] Maternal diets; CON (n = 15), control group consuming basal diet; SUP (n = 12), supplemented group consuming basal diet + DDGS at 0.3% of BW
[2]Intercotyledonary wt measures all tissues excluding cotyledons
[3]Cotyledonary wt measures all dissected cotyledons

Discussion

Supplementation of DDGS, a source of much higher protein than the basal diet (as reported in Chapter 2), increased uterine BF during late gestation. This increase was also reflected in higher heart rates and CSA measurements in supplemented versus unsupplemented cows. A decrease in resistance indices with advancing gestation was also to be expected in order to accommodate the increases in total BF needed during pregnancy (Gómez et al., 2006) as the cow neared the end of term. Similarly, the increased CSA of both uterine arteries, particularly on the ipsilateral side where nearly a 4-fold increase was observed, adheres to the basic concept behind Poiseulle's law – that even small changes in the diameter of the artery (CSA) will result in dramatic changes in BF. In contrast to our hypothesized expectation, circulating concentrations of E2 were increased in the unsupplemented CON group and their respective metabolizing enzymes were increased in the SUP group. Similarly, concentrations of P4 differed between treatment groups, with CON cows having greater concenctrations than SUP cows. This

suggests that the prediction that elevated E2 and P4 would be associated with increased BF is inconsistent with our data and another possible mechanistic explanation is necessary.

In considering these results, what the changes in circulating hormone concentrations truly reflect, must be taken into account, considering they were collected from circulating venous blood. Since numerous metabolic enzyme activities coincided with the hormone concentrations observed – lower E2 levels were present with higher enzyme activity in the SUP group– perhaps the observed results are more understandable within the larger picture of how the altered nutrition is affecting the cow globally. With this in mind, we have analyzed those effects from a nutrient intake, feeding behavior and maternal performance standpoint (reference Chapter 2), where we observed increased intake in SUP cows coupled with maintenance of body condition compared to CON cows, who tended to lose weight during the treatment period. The differences in intake and feeding behavior, maternal performance during the treatment period and the following early lactation, as well as increased enzyme activity suggest a global effect of DDGS supplementation that may be altering overall metabolism via improved nutrient utilization. The increase in enzyme activity could also be a result of an increase in liver size; increased liver mass in response to altered nutritional planes has been seen in beef cattle (Camacho et al., 2014). Level of feed intake has also been shown to increase the relative proportion of visceral organs to body mass in sheep, where changes in liver weight in response to level of nutrition were of a greater degree than any other organ (Burrin et al., 1990). The same study also found that those changes in organ size contributed to an altered whole-body metabolic rate. It has also been shown in dairy cows that a continuous high plane of nutrition may chronically elevate liver BF and metabolic clearance rate of P4 and E2 (Sangsritavong et al., 2002). In our study, DDGS supplementation provided greater metabolizable protein in the diet and, coupled with the

increase in intake compared to the CON group, the SUP group's higher overall plane of nutrition throughout the treatment period could conceivably influence overall metabolism, including clearance of P4 and E2. This is supported by the elevated enzyme activity in the SUP group as well as their comparably lowered circulating E2 concentrations from d 214 onward and lowered circulating P4 concentrations from d 228 onward. However, since no measures of whole liver size were taken, it is not possible to say whether our diets increased liver mass and potentially total enzyme activity. We posit that the increased uterine BF in the SUP group may have been a contribution of a more localized mechanism pertaining to the uterus, which could be better defined in future experiments by measuring local (uterine vein) hormone concentrations, which could give us a more accurate measure of hormone production during pregnancy, compared to those circulating in the blood stream.

Protein supplementation in the form of DDGS to cows fed a basal diet consisting of low quality forage altered uterine hemodynamics , circulating E2 and P4 concentrations, and hepatic steroid metabolizing enzymes. Increased CSA coupled with decreased measures of resistance (despite no treatment effects) facilitated the large increase in blood flow volumes in SUP compared to CON cows. The tendency for calves born to SUP cows to be heavier than those of CON cows despite no difference in any placental weights may also be related to the increased blood flow in the uterine arteries and could be potentially reflective of increased placental efficiency. Regardless of the need for further investigating the mechanisms behind these changes, DDGS supplementation certainly modified some of the major contributors to a successful pregnancy in the beef cow, which clearly have downstream consequences for offspring growth in and out of the womb.

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CHAPTER 4. SUPPLEMENTATION OF CORN DRIED DISTILLER'S GRAINS PLUS SOLUBLES TO GESTATING BEEF COWS FED LOW-QUALITY FORAGE: IMPACTS ON MAMMARY GLAND BLOOD FLOW, COLOSTRUM AND MILK PRODUCTION, AND CALF WEIGHTS

Abstract

The objectives of the present study were to investigate the effects of distiller's grains plus solubles (DDGS) supplementation on blood flow (BF) to the mammary glands during late gestation and early lactation; colostrum and milk production; and calf weight gain during early lactation and at weaning. To test this, multiparous beef cows were divided randomly into a control group (CON; n = 15) consuming ad libitum a diet containing 90% corn stover and 10% corn silage (DM basis) and a treatment group (SUP; n = 12) consuming the same basal diet and DDGS (0.3% of BW). Corn silage inclusion was increased to 30% as gestation progressed to meet increasing requirements. Mammary gland BF ipsilateral and contralateral to the pregnant uterine horn was measured on d 245 of gestation and d 44 of lactation. At parturition, colostrum samples were collected. Milk production was assessed on d 44 of lactation. Calves were weighed every 2 wk from birth to d 56 and when weaned (d 205). Mammary gland BF contralateral to the conceptus (P = 0.85) and cross sectional area (CSA; P = 0.44) did not differ on d 245 of gestation. Ipsilateral BF of SUP cows was greater than CON cows (2.76 vs. 1.76 ± 0.30 L/min, respectively; P = 0.03). Calves from CON dams tended to have a greater loss in percentage of body weight after birth than those of SUP dams (-0.43 vs. -2.75 ± 0.92%, P = 0.09). Cows carrying heifers produced more colostrum (P < 0.01) than those carrying bulls. No effect of maternal diet was observed on total mammary BF (P = 0.33) or other measures on d 44 of lactation. The SUP cows tended to produce more milk on d 44 (2.78 vs. 2.13 ± 0.25 kg/5 h, P =

0.07). Calves gained weight from birth to d 56 ($P < 0.001$) and those from SUP cows were heavier ($P < 0.05$) and tended to have a heavier ($P = 0.06$) adjusted d 205 weight at weaning than those from CON cows (309.7 vs. 292.0 ± 6.0 kg; 288.4 vs. 274.0 ± 5.4 kg, respectively). In conclusion, we accept our hypothesis that DDGS supplementation during gestation influenced mammary BF, milk production and calf weights; underlying mechanisms need to be investigated.

Keywords: beef cow, lactation, mammary gland blood flow

Introduction

In cows, mammary gland BF is a vital component for milk synthesis and therefore nutrient delivery to the offspring. Blood flow to the mammary glands comes through the pudendoepigastric trunk, from which branches the caudal epigastric and external pudendal arteries (Budras et al., 2011). Mammary BF is strongly correlated with milk yield (Götze et al, 2010). Doppler ultrasonography provides an accurate, reproducible, and less invasive tool for measuring mammary BF compared with other methods. In 2010, Götze et al. utilized Doppler ultrasonography to quantify mammary BF in dairy cows and confirmed that using the pudendoepigastric trunk was equally as effective as measuring the external pudendal artery. However, little work has been done to characterize mammary BF in beef cattle and its influences on calf postnatal performance. In fact, there is a dearth of data for ultrasonographic mammary BF in beef cows.

Maternal nutrient intake during gestation can also alter systemic BF via changes in circulating hormones and growth factors during pregnancy that facilitate nutrient delivery to the still developing mammary gland in anticipation of colostrum and milk production (Svennersten-Sjaunja and Olsson, 2005). Additionally, differing nutritional protein and energy planes can influence milk production to varying degrees depending on timing of differences in gestational

diet (Sullivan et al., 2009, McSweeney et al., 1993). Numerous studies have explored the effects of protein supplementation as well as the use of dried distillers grains plus solubles (DDGS); focusing on supplementation of DDGS during late gestation has also been investigated with promising results (Stalker et al., 2006; Martin et al., 2007; Larson et al., 2009). Therefore, supplementation of DDGS during late gestation could positively influence arterial BF to the mammary glands, affecting colostrum and milk production and eventually calf growth trajectories.

We hypothesized that DDGS supplementation to cows fed a low quality forage would increase BF to the mammary glands and, therefore colostrum and milk production, ultimately resulting in an advantage in calf weight gain during early lactation and at weaning. The objectives of the present study were to investigate the effects of DDGS supplementation to arterial BF to the mammary glands during late gestation and early lactation; colostrum and milk production; and calf weight gain during early lactation and at weaning.

Materials and Methods

Experimental Design, Cows and Dietary Treatments

All procedures were approved by the North Dakota State University Animal Care and Use Committee (#A14007).

Animals and Management

Animal methods have been previously reported (Kennedy, Chapter 2). Briefly, 27 multiparous beef cows (Angus or Angus x Simmental; 674 ± 17 kg of BW; 6 ± 5 yr of age) were divided randomly into a control group (CON; n = 15) and a treatment group (SUP; n = 12). Cows were housed at the Beef Cattle Research Complex in two adjacent pens, one for each treatment group. Following a 3-week acclimation period, individual intake was monitored and controlled

via RIC feeders (Insentec, B.V., Marknesse, Nederlands) beginning on d 201 of gestation for 10 wk. All cows were fitted with radio frequency identification tags to facilitate monitoring of intake and feeding behavior. All cattle had free access to water and trace-mineralized salt blocks (97% NaCl, 3,500 mg/kg Zn, 2,000 mg/kg Fe, 1,800 mg/kg Mn, 350 mg/kg Cu, 100 mg/kg I, 60 mg/kg Co). A basal diet of 90% corn stover and 10% corn silage (5.0% CP of a DM basis, marginally NE deficient, rumen degradable protein deficient) was fed for ad libitum intake to both groups, with SUP group supplied DDGS at 0.3% of BW (DM basis). Corn silage inclusion was increased to 20% on d 245 of gestation and again on d 260 to 30% to meet increased nutritional demands during pregnancy, while DDGS supplementation remained the same. On d 270 of gestation, close to expected parturition, all cows were fed the same lactation diet (22% DDGS, 48% corn stover, 30% corn silage (DM basis; 11.0% CP) for ad libitum intake for a period of 10 wk; DDGS supplementation ceased.

Ultrasonography Evaluation

To measure mammary BF, color Doppler ultrasonography was employed. Ipsilateral and contralateral (as confirmed with uterine BF measurements) mammary BF, cross-sectional area (CSA) and pulsatility indices of each uterine artery were measured on d 245 (± 5 d) of pregnancy (determined from date of insemination) and d 44 of lactation (calculated from birth; ultrasound was performed following milk collection; see below). A 7.5 MHz finger probe was inserted through the rectum and used to identify the bifurcation of the internal and external iliac arteries. By following the latter, the external pudendal artery was identified. The external pudendal artery, which branches through the inguinal canal and continues branching down to the udder, was measured and considered as representative of BF to the mammary glands (Budras et al., 2011). Doppler mode was employed to confirm measurement of the artery and not surrounding veins.

Three separate cardiac cycle waveforms from 2 to 3 separate ultrasound evaluations were selected for data collection and averaged (i.e., 6 to 9 measurements per artery per cow). Resistance index (RI), pulsatility index (PI), peak systolic velocity, end diastolic velocity, flow time, maternal heart rate (HR), mean velocity, BF, cross-sectional area (CSA) and cross-sectional diameter (CSD) were recorded. The Doppler software was preprogrammed to calculate 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) × CSA (cm2) × 60 s. Finally, total mammary BF was calculated as the sum of ipsilateral and contralateral mammary BF.

Parturition, colostrum, calf weights

As previously reported (Chapter 2) during calving cows were allowed to remain in their pens with the group until signs of labor were observed. If it was possible to move the cow inside the barn without causing undue stress, she was brought inside and put in an individual pen for calving, otherwise she was allowed to calve outside with the group and the pair was then immediately brought inside using a sled for the newborn calf. Calves were weighed at parturition. Additionally, dams were separated briefly from their calves to collect a colostrum sample. For each cow, the right hind quarter was milked completely to collect a colostrum sample. Colostrum density was calculated based on volume and mass of the total sample. Cows and calves were returned to their individual pen where they were monitored for general health and remained for 24 hr before returning outside to the group. Finally, calves were weighed every 2 wk following calving to track weight gain. Weaning weights were also obtained in the subsequent autumn and adjusted for d 205 of age.

Milk Collection

On day 44 of lactation, each cow was milked completely using a single-cow portable milking machine (InterPuls, Albinea, IT) to determine milk production. Briefly, each cow was weighed, milked completely, kept separate from her calf in an individual pen for 5 hr with her normal diet and water, and then milked completely again for 5-hr milk production. Time required to completely milk each cow was recorded, total milk was weighed, and a sample was collected for DHIA milk component analysis (Dairy Lab Services, Inc., Dubuque, IA). Samples were analyzed for fat, true protein, somatic cell count, lactose, other solids, total solids, and milk urea.

Statistical Analysis

Data analysis utilized either the mixed procedure of SAS (SAS Institute Inc., Cary N.C.) with repeated measures or the general linear model procedure. Class statements included cow, maternal diet (SUP vs. CON), day of gestation or lactation, and the interaction of day and maternal diet. The effect of calf sex and the interaction of diet and calf sex were also used in separate class statements. Model statements tested the dependent variables of ultrasound indices, colostrum sample measures, milk collection measures, calf weights and placental weights. Differences between least square means were determined using the least significant difference method.

Results

On d 245 of gestation there was no effect of treatment on mammary gland BF ($P = 0.85$) or CSA ($P = 0.42$) measured contralateral to the conceptus (Table 4.1). Contralateral PI and RI were not influenced by treatment ($P = 0.16$). On the ipsilateral side, treatment affected ($P =$

0.03) mammary gland BF, where SUP cows had greater BF than CON cows. Average CSA did not differ (P = 0.71) between treatment groups. Ipsilateral PI was greater in CON cows than SUP cows (P = 0.01); however, RI was not affected by treatment (P = 0.11).

When totaled, mammary gland BF on d 245 of gestation (Table 4.1) did not differ (P = 0.12) between treatment groups but maternal HR did (P < 0.01), with SUP cows having greater heart rates than CON cows. PI and RI both contra and ipsilateral to the pregnant uterine horn also differed between treatment groups, with the CON group having greater values than the SUP group for both indices (P < 0.01 and P = 0.03, respectively).

Table 4.1. Mammary blood flow on d 245 of gestation in beef cows that were fed control or control plus supplementation from d 201 to 270 of gestation

	Means			
Variable	CON[1]	SUP[1]	SEM	*P*-value
Heart rate, bpm	63.7	75.4	2.40	< **0.01**
Total flow, L/min	2.3	2.9	0.30	0.12
CSA[2]	0.5	0.5	0.04	0.71
Average PI[3]	1.8	1.5	0.10	< **0.01**
Average RI[4]	0.8	0.7	0.01	**0.03**

[1] Maternal diets; CON (n = 15), control group consuming basal diet; SUP (n = 12), supplemented group consuming basal diet + DDGS at 0.3% of BW

[2]Cross sectional area, cm^2

[3]Pulsatility Index = (peak systolic velocity - end diastolic velocity)/mean velocity

[4]Resistance Index = (peak systolic velocity - end diastolic velocity)/peak systolic velocity

As previously reported, (Chapter 2), there was a tendency (P = 0.06) for calves born to SUP cows to be heavier than calves from CON cows despite no difference in gestation length (P = 0.43). Treatment did not affect (P = 0.15) colostrum weight; however cows carrying heifers produced greater (892.8 vs. 516.6 ± 90.3g ; P < 0.01) colostrum samples compared to cows carrying bull calves (CON: bulls n = 9, heifers n = 6; SUP: bulls n = 8, heifers n = 4).

On day 44 of lactation, no measurement of mammary arterial hemodynamics were altered (P = 0.34) by previous gestation diet (Table 4.2). Gestational diet did influence time required to milk cows, with SUP cows taking longer than CON cows to finish milking (P = 0.05). There was also a tendency for SUP cows to produce a heavier milk sample compared to CON cows (P = 0.07), resulting in a tendency for greater rate of milk production (P = 0.07). No other milk production parameters were affected by maternal diet (Table 4.3). Additionally, when included in the model, no main effect of calf sex was observed for milk production. Analysis of milk components revealed no differences between treatment groups (Table 4.4).

Table 4.2. Mammary blood flow on d 44 of lactation in beef cows that were previously fed control or control plus DDGS supplementation from d 201 to 270 of gestation.

	Means			
Variable	CON[1]	SUP[1]	SEM	*P*-value
Heart rate, bpm	76.1	75.0	1.79	0.67
Total flow volume[2], L/min	6.7	7.5	0.59	0.33
CSA[3]	0.7	0.8	0.04	0.57
Average PI[4]	1.0	1.0	0.04	0.25
Average RI[5]	0.6	0.6	0.01	0.41

[1] Maternal diets; CON (n = 15), control group consuming basal diet; SUP (n = 12), supplemented group consuming basal diet + DDGS at 0.3% of BW

[2]Cross sectional area, cm^2

[3]Pulsatility Index = (peak systolic velocity - end diastolic velocity)/mean velocity

[4]Resistance Index = (peak systolic velocity - end diastolic velocity)/peak systolic velocity

Table 4.3. Dam weight and milk measurements collected on d 44 of lactation in beef cows fed control or control plus DDGS supplementation during late gestation.

	Means			
Variable	CON[1]	SUP[1]	SEM	*P*-value
BW, kg	642	698	23	0.10
Total sample, kg	2.1	2.8	0.3	**0.07**
Milking time, min	10.7	12.9	0.8	**0.05**
Body condition score	4.8	5.1	0.1	0.17
Milk production rate, g/hr	425.6	562.7	50.7	**0.07**
Milk density, g/ml	1.0	1.0	0.001	0.48

[1] Maternal diets; CON (n = 15), control group consuming basal diet; SUP (n = 12), supplemented group consuming basal diet + DDGS at 0.3% of BW

Table 4.4. Analysis of milk components from samples collected on d 44 of lactation in beef cows fed control or control plus DDGS supplementation during late gestation.

	Means			
Variable	CON[1]	SUP[1]	SEM	*P*-value
Fat, %	4.1	4.2	0.3	0.83
Protein, %	3.1	3.0	0.1	0.33
Somatic Cell Count[2]	150.9	249.0	64.92	0.30
Lactose, %	5.1	5.1	0.1	0.64
Other Solids, %	6.0	6.0	0.1	0.69
Total Solids, %	13.2	13.2	0.3	0.95
Milk Urea Nitrogen, %	8.4	9.3	0.9	0.48

[1] Maternal diets; CON (n = 15), control group consuming basal diet; SUP (n = 12), supplemented group consuming basal diet + DDGS at 0.3% of BW
[2]Two outliers (cows likely with mastitis) were removed; when included in analysis means were as follows: CON = 16669.5, SUP = 428.5, ± 1127.4, *P* = 0.44

Calves gained weight (P < 0.01) from birth to d 56 of lactation with no difference (P = 0.68) due to maternal treatment (Figure 4.1). However, calves from SUP dams weighed more (P = 0.05) than those of the CON group (309.7 vs. 292.0 ± 6.0 kg) at weaning. Finally, adjusted d 205 weaning weights tended (P = 0.06) to be greater in calves from SUP vs. CON cows (288.4 vs. 274.0 ± 5.4 kg).

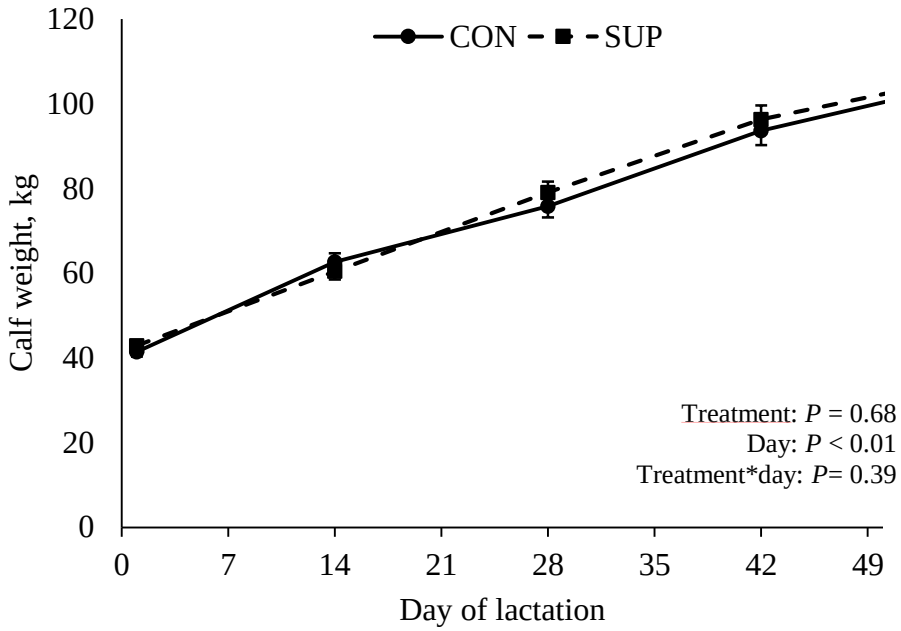

Figure 4.1. Weight gain during early lactation of calves born from beef cows that were fed control or control plus supplementation from d 201 to 270 of gestation.
CON calves n = 15, SUP calves n = 12

Discussion

While we failed to reach statistical significance in many of our results, likely due to a low number of observations, our findings are certainly of merit. During gestation, BF to the mammary gland on the ipsilateral side, but not the contralateral side, was increased with supplementation. These observations, along with notable decreases in measurements of resistance, still indicate a potential for increased BF and perfusion in supplemented dams. Additionally, uterine BF during late gestation was increased in SUP cows compared to CON cows, which could potentially be impacting the mammary gland locally. Growth of the udder parenchyma as well as vessels is occurring during the prepartum stage in preparation for lactation and does so under stimulus of the same vasoactive compounds that affect the uterus during pregnancy (Svennersten-Sjaunja and Olsson, 2005). In obtaining measurements of

mammary blood flow during late gestation, it is important to note that the presence of the near term fetus often caused difficulty in obtaining optimal angles of insonation (45°); however, we were consistently able to obtain similar angles on all measurements (average 80°). Traditional indirect measurements of milk production (i.e., weaning weight) were also enhanced in SUP cows. Perhaps, if we had measured mammary gland BF more frequently throughout gestation, or closer to calving, we would have different results. Moreover, the lack of difference in BF on d 44 of lactation may have been influenced by various factors including compensatory weight gain in CON cows (Chapter 2) or similar diets; more observations could have improved these results as well.

The observation that calves from SUP cows lost less weight during their first 24 h, indicates that either they consumed more colostrum, lost less body water, or both than calves from CON cows. While we did not measure a difference in colostral weight, we only took a sample from one quarter of the udder. Because viability of the calves did not differ as measured by time to stand and suckle (data not reported here), we assume that the ability for the calves to attain colostrum did not differ. We plan to investigate circulating hormones in neonatal blood as well; however, that is currently unknown. Perhaps we impacted metabolic and endocrine factors that influenced heat production of neonatal calves.

Interestingly, calf sex influenced colostral weight. This is similar to recent data reported in dairy cows (Hinde et al., 2014) where cows carrying heifers had greater colostral weight. Daughter-biased colostrum production may carry over to milk production throughout lactation, which could potentially have huge influences on weaning weight of those calves, especially considering the amount of time the calf remains with her dam in a typical cow-calf operation. A potential advantage simply as a result of greater milk production from birth until weaning could

result in greater economic gains from those animals. Our data supports this proposed benefit, as milk volume and rate of production tended to be enhanced in SUP cows. While this did not impact calf weight gain during the first 56 days of life, by weaning, this did, or tended to benefit those calves from SUP cows as they were heavier.

In conclusion, DDGS supplementation's influence on mammary BF, colostrum and milk production were not as significant as other parameters we investigated, such as maternal intake behavior or uterine BF (Chapters 2 and 3), however, these findings certainly warrant consideration as they still imply influences and may be strengthened by a greater number of observations in future experiments. Further investigations on how gestational diet impacts calf performance due to greater nutrient delivery, be it placental or mammary, warrant further investigation.

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CHAPTER 5. GENERAL DISCUSSION AND FUTURE DIRECTIONS

General Discussion

It is apparent that in the current environment of scientific and public interest, the concept of developmental programming will continue to be an area of curiosity for both human medicine and the livestock industry. The objectives of this thesis research have further demonstrated that maternal nutrition during gestation can have a global impact on the dam's feeding behavior, influence maintenance of her own body condition and weight, and consequently impact nutrient delivery to her offspring in and out of the womb.

Chapter two discussed the effects of DDGS supplementation on voluntary intake behavior during early gestation and early lactation as well as maintenance of cow body weight and condition. Briefly, supplementation of DDGS increased total daily DMI, intake rate, and size of meals during gestation, which was coupled with weight gain and maintenance of body condition. Lack of supplementation during gestation also influenced intake during early lactation, where CON cows increased their time spent consuming larger meals combined with compensatory weight gain but slow recovery of body condition. The outcomes observed are likely a result of a global shift in metabolism in SUP cows, a consequence of various interactions including increased MP and NE consumed, increased fermentation and microbial activity and therefore improved nutrient utilization. Despite interesting and significant results, it is difficult to pinpoint the mechanisms behind alterations in intake behavior; this is a question made more difficult by the complexity of a ruminant animal during gestation, a metabolically challenging physiological state.

Chapter three discussed impacts on uteroplacental vasculature as a key component of transplacental exchange and thusly fetal development, focusing specifically on uterine artery

hemodynamics, circulating concentrations of E2 and P4, and their associated hepatic enzymes. Supplementation of DDGS resulted in increased total uterine BF, which rose with advancing gestation. This increase was impacted by faster heart rates and larger arterial diameter, an expected adaptation to the growing demands of pregnancy on the maternal system. Circulating concentrations of E2 and P4 were elevated in CON cows and their hepatic metabolizing enzyme activities supported the observed difference between the treatment groups; SUP cows had greater or tended to have greater CYP1A and CYP3A activity. Increased enzyme activity in SUP cows and, therefore, lower circulating hormone levels could be a result of an increase in liver size in response to altered nutrient intake and whole body metabolic rate. Therefore, it is possible that while DDGS supplementation had a global effect on metabolism, and consequently enzyme metabolism of circulating hormones, a local effect more specific to the uterus could have created the elevation in total BF measured. The benefits of increased uterine blood flow were still evident in the tendency for calves from supplemented cows to be heavier than their peers.

Finally in chapter four the effect of DDGS supplementation on arterial blood flow to the mammary gland, colostrum and milk production as well as calf performance were discussed. While a significant increase in blood flow was only seen during gestation to the side ipsilateral to the pregnant uterine horn, a decrease in measures of resistance, which facilitates increased blood flow, was seen on both sides in SUP cows. No differences were found during early lactation, which was when cows were fed similar diets. Colostral weights differed not by treatment but by fetal sex. Milk volume and rate of production tended to be increased in SUP cows. Lastly, calves from SUP cows tended to have greater weaning weights than those from CON cows. These findings must be considered in light of the changing physiological states of cows in late gestation, when mammary gland development resumes in preparation for lactation. Changes in

mammary blood flow were also likely affected by increased uterine BF during late gestation, which could be reflective on an overall increase in BF to the reproductive system as a whole.

No animal study is without its flaws, and there are certainly areas for improving upon if this experiment were to be repeated. To begin, an obvious solution to obtaining more accurate results would be to increase the number of animals as well as observations – this would certainly aid in better understanding changes in blood flow during late gestation and early lactation. If possible, sampling blood closer to the uterus, such as from the uterine vein, and therefore measuring their concentrations in the female reproductive tract could help explain how local circulation of steroids are being affected by diet. Finally, the original objectives of this study included three treatment groups instead of two, with the aim of having a non-supplemented group (or control), and low and high supplemented groups, but facilities and animals prevented this from realization. Perhaps providing an excess of DDGS as a third treatment group would have yielded different results, such as overweight cows and inefficient metabolism.

This data further emphasizes the inescapable sway the maternal environment has on fetal development pre- and postpartum, as well as the clear impact relatively simple changes in her diet during the last part of gestation have on her physical and physiological state. This project has potential application both for future research as well a benefit to the livestock industry, particularly for those in cow-calf operations designing feeding regimes during the winter for their pregnant cows. Larger calves at birth nursing from cows that produce greater quantities of milk and have calves with the continued advantage of larger weaning weights will certainly be an economic benefit to the producer, especially in regions with harsh winters like North Dakota.

Future Directions

As was already addressed, there are obvious ways in which to improve upon the experiment that was conducted, but in pondering improvements it is even more apparent that this study has paved the way for future research.

The most obvious experiment to conduct next would be to further investigate the mechanisms behind how maternal diet is affecting the mammary gland during late gestation and into lactation. As previously mentioned, more ultrasounic measurements would bolster the observations from this study, especially if ultrasound was performed before resumption of mammary development during gestation. Functional differentiation of the mammary gland during pregnancy has been divided into three phases (based on rodent studies): the proliferative phase of early pregnancy; the secretory phase during the majority of pregnancy, which is when the gland gains the ability to secrete milk; and the secretory activation phase, around parturition (Neville et al., 2002). Measuring blood flow through the external pudendal artery during these phases, in addition to more measures during early lactation, would therefore be of great benefit.

While we saw a statistical influence of gestational diet on milk production, it is hard to identify the mechanism behind these results from the data at hand. One must consider the factors that act upon milk secretion: the two most important being prolactin and physical milk removal (Neville, 2006). Prolactin secretion is stimulated by suckling, is critically necessary for alveolar proliferation, survivial of mammary epithelial cells during lactation and synthesis of milk proteins (Neville, 2006). It can also be measured in blood plasma, but does not correlate with milk secretion and thefore quantification of secretion would have to pursued in another fashion (Neville, 2006)..Milk removal from the gland is also a cricital factor in stimulation of lactation and can even determine the volume of milk secretion, which is adjusted to the demands of the

offpsring (Neville, 2006). Monitoring calf behavior, such as how often calves nurse and performing more weigh-suckle-weigh measures could help to elucidate the impact calf nursing has on milk production in addition to affects from gestational diet.

Another consideration for future studies would be to include fetal sex in the statistical model for statistical analyses of measurements that we have already attributed the impressions of developmental programing to, such as our ultrasound measures of uterine and mammary blood flow. As Hinde and colleagues found (2014), daughter-biased milk synthesis could have significant impacts on business strategies of dairy farmers, especially with the increased availability of sexed semen. In our study, we noticed an influence of the female offspring on colostrum production. Perhaps it would be valuable to include fetal sex more often as a consideration in general when conducting research in the area of developmental programming, either by controlling for it with sexed-semen or fetal sexing with ultrasound. It would certainly be interesting to see if fetal sex affects milk production in the beef cow up until weaning, since the daughter-biased milk production highlighted by Dr. Hinde was in dairy cows, where calves would not benefit from the advantage.

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