

EFFECTS OF PROTEIN SUPPLEMENTATION DURING THE LAST THIRD OF
PREGNANCY ON BLOOD FLOW TO THE MAMMARY GLAND IN BEEF COWS

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ABSTRACT

Calves born to cows fed a protein supplement during late pregnancy have greater production characteristics compared to calves from cows not supplemented, despite similar birth weights. Our hypothesis was that cows receiving protein supplement during late gestation would have greater mammary gland blood flow allowing for enhanced lactation. Mammary gland hemodynamics were recorded during late gestation. While protein supplementation did not alter the blood flow to the mammary gland, this data demonstrates that mammary gland blood flow can be monitored during late pregnancy.

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TABLE OF CONTENTS

ABSTRACT	iii
ACKNOWLEDGMENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF APPENDIX FIGURES.....	ix
CHAPTER 1. LITERATURE REVIEW	1
Introduction.....	1
Mammary Development in Beef Cattle	2
Nutritional Impacts on Mammary Growth during Pregnancy	3
Gestational Protein Impacts on Lactation Performance.....	4
Blood Flow to Mammary Gland	6
Factors That Influence Mammary Gland Blood Flow	6
The Theory of Doppler	7
Statement of the Problem.....	10
References.....	11
CHAPTER 2. SUPPLEMENTATION OF DDGS DURING LATE GESTATION IN BEEF COWS CONSUMING LOW QUALITY FORAGE DOES NOT IMPACT MAMMARY GLAND BLOOD FLOW	15
Abstract	15
Introduction.....	15
Materials and Methods.....	16
Animal Procedures.....	16
Nutrient Analysis	17
Mammary Artery Ultrasonography Measurements	17
Parturition Procedure	18

Blood Serum Analysis	18
Statistical Analysis.....	19
Results.....	19
Discussion.....	20
References.....	22
CHAPTER 3. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS.....	26
References.....	28
APPENDIX. NUTRITIONAL IMPACTS ON MAMMARY GLAND HEMODYNAMICS	29

LIST OF TABLES

<u>Table</u>	<u>Page</u>
2.1. Nutrient composition of feedstuffs fed to cows receiving or not receiving DDGS supplementation during late gestation	17
2.2. Maternal body weights and hemodynamic measurements obtained from the mammary gland on days 190, 204 and 218 of gestation	20

LIST OF FIGURES

<u>Table</u>	<u>Page</u>
1.1. Example of arterial waveform from the bovine. Arrows indicate peak systolic velocity and end diastolic velocity	9
2.1. Non-esterified fatty acids (NEFA; A) and blood urea nitrogen (BUN; B) concentrations for cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP)	20

LIST OF APPENDIX FIGURES

<u>Table</u>	<u>Page</u>
A.1. Heart rate for cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP)	29
A.2. Pulsatility index of the right mammary artery in cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).....	29
A.3. Pulsatility index of the left mammary artery in cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).....	30
A.4. Resistance index of the right mammary artery of cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP)	30
A.5. Resistance index of the left mammary artery of cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).....	31
A.6. Blood flow through the right mammary gland artery of cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP)	31
A.7. Blood flow through the left mammary gland artery of cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP)	32
A.8. Total mammary blood flow for cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).....	32

CHAPTER 1. LITERATURE REVIEW

Introduction

Lactation is the secretion of milk by the mammary gland and is important for neonatal nutrition in mammalian animals. In beef cattle, because colostrum is a complete diet for offspring, lactation is a critical component of reproductive success of the dam (Stelwagen et al., 2009). In the period immediately after parturition, the dam has the ability to consume necessary nutrients, converting them into milk that is rich in nutrients for the offspring (Stelwagen et al., 2009). Colostrum, the first milk produced by the dam, provides protein, fat, vitamins and minerals, and perhaps most importantly, colostrum provides immunoglobulins (Hurley et al., 2011). These immunoglobulins provide passive immunity for the offspring until its active immunity is efficient enough to take on immunological challenges (Stelwagen et al., 2009). Passive immunity is critical for the long-term health and production of the offspring (Larson et al., 1980). Throughout lactation, the quantity and quality of milk provided by the dam is also important, especially in ruminant offspring that initially do not have the ability to digest forages efficiently until their stomach fully develops (Huber, 1969). Most recently, it has been determined that suckling in pigs not only aids in passive immunity and neonatal growth, but can influence adult performance of the offspring (lactocrine hypothesis; Bartol et al., 2009). It is known that quality of milk production by the dam has a positive effect on a beef calf's adjusted 205-day weaning weight and calves from heavier milking dams require less days to finish weight, due to the heavier weaning weight (Clutter and Nielson, 1987). Additionally, dairy heifer calves that consume more milk early in development produce more milk as cows (Bach, 2011). Having adequate nutrition during gestation lays the foundation for the dam's ability to provide proper nutrition for the offspring. The dam requires proper nutrition during the rest of her

production periods in order to maintain production throughout her lactation. In this literature review, I will review mammary development in beef cattle, nutritional impacts on mammary gland growth during pregnancy, gestational protein impacts on lactation performance, blood flow to mammary gland, factors that alter blood flow to the mammary gland, and the theory of Doppler.

Mammary Development in Beef Cattle

Mammogenesis, or the development of the mammary gland, begins in the developing embryo. Mammary ridges begin to develop from thickened ridges of epidermis (Schmidt, 1971). These ridges form along two lateral lines that run from the axillary region to the inguinal region on the ventral surface of the developing conceptus. In cows, mammary glands develop in the inguinal region of that mammary ridge (Schmidt, 1971).

From the mammary ridge the primary mammary bud is developed (Schmidt, 1971). The primary mammary bud grows into the underlying dermis. Branches from the primary mammary bud continue to push into the dermis, forming secondary mammary buds (Schmidt, 1971). These branches continue to extend and lengthen to form canals. Canalization creates the duct system of the gland, with each bud becoming a duct (Schmidt, 1971).

Growth and development of the mammary gland continues through puberty and full differentiation of the gland will not become complete until parturition (Schmidt, 1971). Between birth and puberty, the mammary gland grows at the same rate as other tissue, referred to as isometric growth. However, at the onset of puberty the mammary gland will go through a period of allometric growth, or a period of relatively faster growth when compared to other tissues of the body (Folley, 1952, Sinha and Tucker, 1969, Schmidt, 1971).

During her initial estrous cycles, further development of the mammary gland is occurring under the influence of estrogen, progesterone, prolactin, and growth hormone (Sinha and Tucker, 1969, Schmidt, 1971). Estrogen causes the ducts to branch and increase in diameter; this is enhanced with the presence of prolactin and growth hormone (Sinha and Tucker, 1969, Schmidt, 1971). Alveoli, the functional secretory part of the mammary gland, are formed during the luteal phase of the estrous cycle under the influence of progesterone (Sinha and Tucker, 1969, Schmidt, 1971).

Final development of the mammary gland takes place during the last third of pregnancy. During this time, alveoli develop into lobules, or milk producing cells of the mammary gland. Immediately preceding parturition, the hormones prolactin, adrenal cortical hormones, and placental lactogen allow milk synthesis to take place, thus lactogenesis (Hammond, 1927, Schmidt, 1971, Senger and Senger, 2012).

Nutritional Impacts On Mammary Growth During Pregnancy

While there is a great amount of information showing the impact of nutrition on the mammary gland of prepubertal and pubertal dairy heifers, this topic is beyond the scope of the current thesis. It is interesting to note that while gestational diets have been reported to impact mammary gland development in dairy cattle and sheep, there has been very little research regarding the growth of the mammary gland in response to nutritional changes in beef cattle. In sheep and dairy, gestational nutrition has direct effects on mammary gland development, colostrum quantity and quality, and milk production (Banchero et al., 2006; Swanson et al., 2008; Dessauge et al., 2011). This information is more critical when one considers that late gestation is also the period of most rapid fetal development (Ferrell, 1991) and is also a time of reduced feed intake by the dam (Grummer, 1995). This implies that during late gestation limited

nutrient intake must provide for the dam's maintenance requirements as well as proper mammary gland and conceptus growth. In first parity ewe lambs, mammary gland weight after a 21-day lactation was reduced in ewes experiencing nutrient restriction during the last two-thirds of gestation compared to adequately fed ewes (Swanson et al., 2008, Neville et al., 2012). Neville et al. (2012) went on further to demonstrate that under-fed pregnant ewes had fewer alveolar numbers at day 20 of lactation compared to overfed ewes. It was hypothesized by the authors that the reduced alveolar numbers resulted in the decreased milk yield of those same ewes (Meyer et al., 2011; Neville et al., 2012). In multiparous dairy cows, maternal nutrition affects not only milk yield and milk components such as milk fat, milk protein and lactose, restricting nutrients in late pregnancy and early lactation negatively impacts mammary gland weight (Dessaugue et al., 2011).

Gestational Protein Impacts on Lactation Performance

While there are limited data on the impact protein supplementation during late gestation has on milk production and quality in beef cattle, research linking gestational nutrition with milk production in dairy cows, ewes, and sows is available. Dairy cows fed 80% of NRC protein recommendations during gestation have decreased milk yields and decreased total solids by about 15% (Chew et al., 1984). Although the authors did not report mammary gland development in this experiment, they attributed these responses to prepartum mammary gland development resulting from the altered crude protein concentrations.

In sheep, reduced nutrient intake by pregnant ewes reduced colostrum yields (Mellor and Murray, 1985; Swanson et al., 2008). Interestingly both nutrient restricted and overfed ewes had decreased colostrum quality as colostrum contained less fat, protein, and lactose (Swanson et al., 2008) compared to controls. Rate of blood flow to the mammary gland has a great effect on the

quantity and quality of the colostrum and milk produced by the dam. Nutrient composition of the milk are related to the nutrients that are ingested during lactation (Chew et al., 1984). Moreover, greater blood flow to the mammary gland results in greater milk production (Prosser and Davis, 1992). In sheep, nutritional plane during mid to late gestation alters the vascularity to the mammary gland (Vonnahme et al., 2008). This additional vascularity may be a reason why ewes fed a higher nutritional plane during gestation have greater number of alveoli and more milk production throughout their lactation period (Swanson et al., 2008; Meyer et al., 2011; Neville et al., 2012).

Although adequate lactation diets may be able to offset poor gestational nutrition in sows, without the aid of the adequate diet during lactation, litters born to sows that were fed less protein during late gestation had poorer average litter weaning weights (Mahan and Mangan, 1975). We assume that the additional litter performance was due to the availability of either greater quantity or higher quality milk from the dam, indicating that gestational nutrition impacted milk production quantity and quality. A similar result was demonstrated in primiparous ewes, where nutrient restriction during gestation decreased mammary gland weight at parturition (Neville et al., 2012). However, those differences were eliminated after 20 days of common lactation diet (Neville et al., 2012).

It has also been demonstrated in sows that greater protein content of gestational diets affects mammary development and production in primiparous sows more so than in multiparous sows. From their results the authors hypothesized that as first parity gilts are still growing their muscle mass at the same time as their mammary gland was developing, an elevated requirement for protein, or at minimum certain amino acids (e.g. lysine) is needed (Mahan, 1998).

Blood Flow to Mammary Gland

Currently there is no data to confirm that there is a positive correlation of milk production and mammary gland blood flow in beef cows. In dairy cows however, Kronfeld et al. (1968) demonstrated a positive correlation of mammary blood flow during lactation and milk production. However, it remains unknown in any species if mammary gland blood flow prior to lactation influences the ability milk production after parturition. Moreover, if pre-partum mammary gland blood flow could predict future lactation performance, the specific days of gestation when one could obtain that information is unknown.

Blood supply to the mammary gland begins with the posterior dorsal aorta. The posterior dorsal aorta splits into the right and left iliac arteries, and then splits into the internal and external iliac arteries (Frandsen et al., 2009). The external pudendic (also referred to as the mammary artery) develops from the external iliac. Just behind the rear teats, the mammary arteries enter each half of the udder and diverge into the anterior and posterior mammary arteries (Frandsen et al., 2009). These arteries then supply both fore and rear quarters by branching into arterioles. The alveoli are supplied by capillaries that derived from the anterior and posterior mammary arteries that have branched and spread through the parenchyma of each of the udder quarters (Frandsen et al., 2009).

Factors That Influence Mammary Gland Blood Flow

Many things can influence the rate of blood flow to the mammary gland. Some items affecting the mammary blood flow include hormones, weather, suckling stimulus, and mastitis. Insulin plays a role in regulating blood flow to the mammary system. Mammary gland blood flow increased by 42% in goats subjected to an insulin clamp (i.e. having greater levels of circulating insulin). The authors hypothesized that the insulin clamp may have also caused

insulin-like growth factor-1 (IGF-1) to increase causing the elevated blood flow (Bequette et al., 2001). Administration of somatotropin to dairy cows enhances milk production by stimulating hepatic IGF-1 (Bauman, 1999). The increased milk production is due to an increased blood flow to the mammary gland (Bauman, 1999).

Temperature can also impact mammary gland blood flow. When goats are placed in an environment that is below their thermoneutral zone, mammary blood flow decreases, resulting in depressed milk production (Thompson and Thompson, 1977).

Other external factors seem to play a role in mammary blood flow regulation. It has been demonstrated many times that the length of milking interval affects the rate of blood flow to the mammary gland (Delamaire and Guinard-Flament, 2006; Prosser and Davis, 1992; Guinard-Flament and Rulquin, 2001). As the interval between milk letdown is increased, mammary gland blood flow is decreased (Prosser and Davis, 1992). This process ultimately results in decreased milk production and involution of the mammary gland.

The Theory of Doppler

Christian Doppler presented a paper entitled “On the Colored Light of the Double Stars and Certain Other Stars of the Heavens” in May of 1842. This paper explained the Doppler Effect with light and sound (reviewed by Maulik, 2005). The Doppler Effect is the observed changes in the frequency of transmitted waves when relative motion exists between the source of the wave and an observer (reviewed by Maulik, 2005). The frequency increases when the source and the observer move closer and decreases when they move apart. It was later determined that the portions of this paper that dealt with light were incorrect; however, the Doppler effect in relation to sound has had many lasting impacts in research (reviewed by Maulik, 2005). One of the first uses for the Doppler principle was sonar, which used ultrasonic waves to detect

submarines during World War I and World War II. The first medical use of Doppler sonography came during the 1950s when Shigeo Satomura was able to record cardiac valvular movements. In the 1980s real-time ultrasonography was used in animals (Maulik, 2005).

Doppler ultrasonography in its simplest description is the measurement of sound waves interacting with matter. Sound waves occur when something vibrates in a medium of solid, liquid, or gas. As the source moves forward, it causes an increase in pressure (compression) and the opposite occurs when the source moves away (rarefaction). One cycle, or hertz (Hz), of compression and rarefaction makes up a wavelength; frequency is the number of wavelengths in 1 second. Typically, frequencies from 10 Hz to 20 kHz are audible to humans, frequencies over 20 kHz are inaudible and are called ultrasound (Nelson and Pretorius, 1998).

The angle of insonation, also known as the Doppler angle, is important for determining rate of blood flow. The beam insonation angle is the angle from which the transducer is transmitting sound waves relative to the vessel being measured. A 90-degree angle of insonation makes it virtually impossible to calculate the flow rate of the red blood cells that are to be measured. This is due to almost total disappearance of the Doppler shift. An ideal angle of insonation is between 30 and 60 degrees but a proper measurement of that angle is the most critical. It is known that the greater the angle of insonation, the greater the error in measurement of flow (Nelson and Pretorius, 1998).

The waveforms displayed from sampling arterial circulation represents one cardiac cycle (Figure 1.2). The leftmost part of the wave depicts the systole whereas the right side represents the diastole. In the two-dimensional Doppler sonogram, only time and frequency are quantitatively measured. Maternal heart rate (HR), pulsatility index (PI), resistance index (RI), and blood flow (BF) were calculated by software where $PI = (\text{peak systolic velocity} - \text{end}$

diastolic velocity)/ mean velocity; $RI = (\text{peak systolic velocity} - \text{end diastolic velocity}) / \text{peak systolic velocity}$; and $BF \text{ (mL/ min)} = \text{mean velocity (cm/ s)} \times (\pi/ 4) \times \text{cross-sectional diameter (cm}^2) \times 60 \text{ s/min}$.

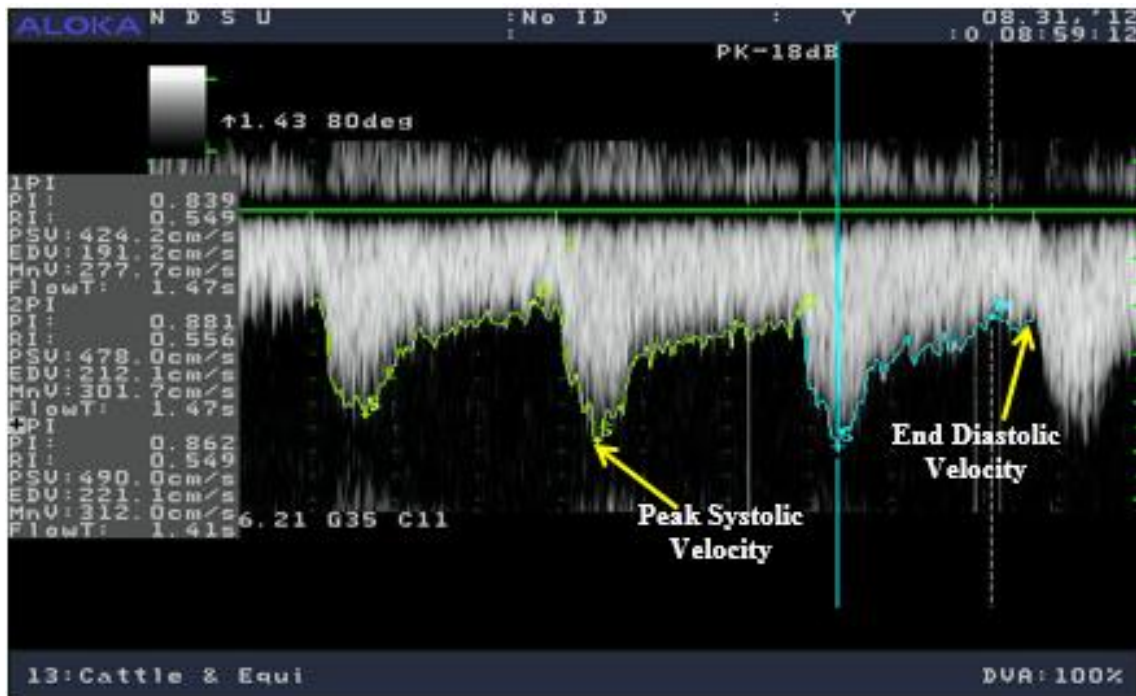


Figure 1.1. Example of arterial waveform from the bovine. Arrows indicate peak systolic velocity and end diastolic velocity.

Other methods have been used for monitoring blood flow in livestock, such as heart rate volumetric flow probes. Most of these other methods require surgeries or other invasive procedures. One of the great advantages of Doppler ultrasonography is that it is relatively non-invasive, allowing one to obtain blood flow measurements from an unsedated, non-surgically instrumented animal over a period of time. As with all techniques there are downsides to Doppler ultrasonography and it is important to recognize these shortcomings. Doppler sonography only estimates blood velocity but does not actually measure flow (Nelson and Pretorius, 1998). Although the hemodynamic information generated from Doppler ultrasonography is an estimate

based on accepted scientific principles and backed by experimental data, the information produced is still an estimate. One of the most important estimates is the angle of insonation (Nelson and Pretorius, 1998). If the technician estimating the angle of insonation is not properly trained it could have serious impacts on the outcomes of the Doppler readings. It is important to note that the angle of insonation is of utmost importance. The other important determinant of an accurate blood flow reading is the diameter of the vessel, which is being read. Poiseuille's law states that the velocity of the steady flow of a fluid through a narrow tube (as a blood vessel or catheter) varies directly as the pressure and the fourth power of the radius of the tube and inversely as the length of the tube and the coefficient of viscosity (Sutera and Skalak, 1993). Therefore, the diameter, or radius, which is measured by use of ultrasonography, has the greatest impact on the flow data recorded, and therefore could cause the greatest error.

Statement of the Problem

The last third of pregnancy for beef cows has the highest nutritional requirements due to rapid fetal growth. Additionally, in the upper Midwest in many beef operations late gestation coincides with parts or all of the coldest months of the year (January and February). Due to the gestational and environmental draws on nutrients from the cow, feeding only low or medium quality forages may not be able to provide for the cow's maintenance and gestational requirements.

Feeding more protein during pregnancy improves many production areas in beef cattle. Some of these areas are improved fertility in daughter offspring of supplemented cows, improved pre-weaning growth as well as improved carcass quality (Martin et al., 2007, Stalker et al., 2006, Larson et al., 2009)

It is unknown what causes improved production in the offspring of protein supplemented cows. As mentioned above, birth weights were similar between protein supplemented and non-supplemented cows. We hypothesized that the enhanced calf production traits due to increased maternal protein during gestation is due to increased mammary blood flow and increased mammary gland tissue development. Due to a greater alveolar development, protein supplemented dams may have greater milk production.

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CHAPTER 2. SUPPLEMENTATION OF DDGS DURING LATE GESTATION IN BEEF COWS CONSUMING LOW QUALITY FORAGE DOES NOT IMPACT MAMMARY GLAND BLOOD FLOW¹

Abstract

Positive effects have been observed in offspring from beef cows supplemented with corn dried distiller's grain with solubles (DDGS) during late gestation. The hypothesis of the current study was that late gestational DDGS supplementation to beef cows would increase blood flow (BF) to the mammary gland thus enhancing milk production. Mammary gland BF in multiparous cows during late pregnancy was determined. Beef cows were fed a control (CON) diet of low quality hay (n = 5) or a supplement diet (SUP) of low quality hay with DDGS (1.7 g/kg of BW; n = 6). There were no impacts of diet or day on mammary gland hemodynamics. While mammary gland BF during late gestation was not impacted, studies are necessary to determine how lactational performance may be altered.

Introduction

Several studies have demonstrated that nutrition during gestation impacts both fetal and postnatal growth (Barker et al., 1995; Wu et al., 2006). This is due to the impacts of nutrients and pregnancy altering nutrient transferring tissues such as the gastrointestinal tract, placenta, and mammary gland (Vonnahme et al., 2015). Gestational feeding either directly impacts postnatal performance of the offspring via placental nutrient transport of nutrients, or indirectly through impacts of gestational feeding on mammary gland development. Alveolar development within the mammary gland is reduced in ewes nutrient restricted during mid to late pregnancy (Neville

¹ The material in this chapter was co-authored by Dr. Kimberly Vonnahme and Dr. Marc Bauer. Craig Zimprich had primary responsibility for collecting data and data analysis. Craig Zimprich also drafted and revised all versions of this chapter. Dr. Kimberly Vonnahme and Dr. Marc Bauer served as editors, proofreaders and confirmed the statistical analysis.

et al., 2013) and milk production is reduced, even though adequate nutrients are provided during lactation (Meyer et al., 2011). Placental production of several hormones drives mammary gland development, one of which is progesterone, which serves to enhance glandular development of the mammary gland (Tucker, 1985). During late gestation, nutrient restriction delays the progesterone decline prior to parturition (Mellor et al., 1987). Nutrient restriction has been shown to increase circulatory concentrations of progesterone as well as non-esterified fatty acids (Luther et al., 2007; Lekatz et al., 2010a; Lekatz et al., 2011; Vonnahme et al., 2013).

Dietary protein supplemented during gestation positively alters bovine offspring growth and performance (Stalker et al., 2006; Larson et al., 2009; Funston et al., 2010), while impacts on lactational performance of the dam is highly variable, increasing (Winterholler et al., 2012), decreasing (Sullivan et al., 2009b), or not changing (Larson et al., 2009). Increasing dried distillers' grains plus solubles (DDGS) supplementation three weeks prior to parturition linearly increased calf birth weight and body weights at days 60 and 90 of age (Winterholler et al., 2012). The hypothesis of the current study is that supplementation with DDGS during late gestation in beef cows will enhance blood flow to the mammary gland in preparation for lactation positively impacting offspring after birth.

Materials and Methods

Procedures for the animal experimentation were approved by the North Dakota State University Animal Care and Use Committee (#A0926).

Animal Procedures

Multiparous crossbred (primarily Angus) cows ($n = 11$; 4.8 ± 0.8 (SD) years of age; 3.0 ± 1.0 (SD) parities; 747 ± 127 kg (SD) of body weight) were bred using artificial insemination to the same sire and were transported from the North Dakota State University Beef Unit to the Animal Nutrition Physiology Center (an indoor facility) at ~180 d of gestation. Cows were

trained to using electronic gates (American Calan, Northwood, NH) so that individual feed intake could be determined. During the training period, cows received 2% of BW daily of low quality hay (6.83% CP). On day 190 of gestation, cows were randomly assigned to either receive no additional supplementation (CON; $n = 5$) or were supplemented (SUP) with 1.7 g/ kg of BW daily with DDGS. Supplementation began on day 190 of gestation after initial blood flow measurements were taken (see below). Feed refusals were weighed twice weekly. Cows were weighed on day 204 and 218 of gestation and intake was adjusted.

Nutrient Analysis

Each time hay was ground, samples were collected and composited for nutrient analysis. Only one lot of DDGS was used. All samples were analyzed for DM, ash, and CP (AOAC, 2010; methods 934.01, 942.05, and 2001.11, respectively); NDF and ADF (ANKOM2000 Fiber Analyzer, Macedon, NY); and CP associated with NDF and ADF.

Table 2.1. Nutrient composition of feedstuffs fed to cows receiving or not receiving DDGS supplementation during late gestation.

% of dry matter	Hay	DDGS
CP	6.9	27.1
NDF	69.0	43 .7
ADF	42.0	11.4
Ash	12.3	8.3

*Hay fed from October 15, 2012 until December 12, 2012.

Mammary Artery Ultrasonography Measurements

Hemodynamic measurements were assessed using Doppler ultrasonography (model SSD-3500; Aloka UST-672 Wallingford, CT) equipped with a 7.5 MHz linear finger-probe transducer (Aloka) that was inserted into the rectum. The abdominal aorta was located and branches of the common iliac arteries were followed. Thereafter the probe was directed ventrally so that the external iliac artery was visualized and the external pudendal artery was identified. The external

pubdental artery, which branches through the inguinal canal and continues branching down to the udder, was measured and considered as representative of blood flow to the mammary gland (Götze et al., 2010; Potapow et al., 2010; Budras et al., 2011). Doppler mode was employed to confirm measurement of the artery and not surrounding veins.

For each arterial blood flow measurement, 3 similar cardiac cycle waveforms from 3 separate ultrasonography evaluations from each side were obtained with spectral Doppler and averaged per cow within a gestational day (i.e. 9 measurements per side per artery per sampling day). Maternal heart rate (HR), pulsatility index (PI), resistance index (RI), and mammary arterial BF were calculated by pre-programmed Doppler software where $PI = (\text{peak systolic velocity} - \text{end diastolic velocity}) / \text{mean velocity}$; $RI = (\text{peak systolic velocity} - \text{end diastolic velocity}) / \text{peak systolic velocity}$; and $\text{blood flow (mL/ min)} = \text{mean velocity (cm/ s)} \times (\pi/ 4) \times \text{cross-sectional diameter (cm}^2) \times 60 \text{ s/ min}$. Total blood flow was calculated as the sum of both mammary gland arteries. Mammary gland blood flow was determined on days 190, 204 and 218 of gestation. Each time a cow was in the chute, a blood sample was collected via the jugular vein and serum was removed and stored at -20°C until analysis.

Parturition Procedure

After their last ultrasonography scan, all cows were transported to the NDSU Beef Unit for calving. Birth weights were recorded. As ownership was not retained for all calves through weaning, weaning weights could not be obtained.

Blood Serum Analysis

Serum samples were analyzed for non-esterified fatty acids (NEFA) and urea by methods previously utilized in our laboratory (Lekatz et al., 2010b). Samples were run in a single assay, and the CV within those assays were 3.2% for urea and 5.6% for NEFA.

Statistical Analysis

All data were analyzed using generalized least squares procedure (mixed procedure, SAS Institute, Cary, NC). Ultrasound parameters, NEFA, urea, and body weight were analyzed with treatment, day of gestation, and the interaction of treatment by day of gestation in the model. The appropriate covariance structure was used that minimized the fit statistics. For the variables NEFA, BUN, heart rate, and PI on the left and right mammary artery the compound symmetry covariance structure was used. Unstructured covariance structure was used for RI and blood flow of the right mammary artery, while ante-dependence (1) was used for the RI and blood flow of the left mammary artery. Autoregressive (1) was used for total blood flow. Metabolic body weight was used as a covariate for all variables and was removed for all except total mammary BF, where it was < 0.20 .

Results

Dry matter intake of DDGS was 1.5 ± 0.1 kg/day in SUP cows. Dry matter intake of hay was similar ($p = 0.91$; 17.0 vs. 16.8 ± 1.1 kg/day) for CON vs SUP cows. Total dry matter intake (hay + DDGS) was similar ($p = 0.45$) for CON vs SUP (17.0 vs 18.3 ± 1.2 kg/day), but when expressed as a percentage of body weight, total dry matter intake was greater ($p = 0.05$) in SUP vs CON cows (2.46 vs. 2.28 ± 0.06 kg/day). There were no day by treatment interactions for NEFA ($p = 0.13$) or urea ($p = 0.50$; Figure 2.1). Day and treatment did not influence NEFA concentrations ($p \geq 0.10$; Figure 2.1A). There was a main effect of day ($p = 0.01$) and treatment ($p = 0.02$) for urea concentrations where concentration increased as gestation advance. Moreover, SUP cows had greater urea concentrations compared with CON (Figure 2.1B). There was no effect of day, treatment, or their interaction ($p > 0.23$) on maternal body weight or mammary gland hemodynamics (Table 2.2; Appendix).

Table 2.2. Maternal body weights and hemodynamic measurements obtained from the mammary gland on days 190, 204 and 218 of gestation.

	Day 190	Day 204	Day 218	SEM	p value
Weight, kg	745	747	752	44	0.45
Heart rate, bpm	60.2	63.9	63.2	2.4	0.29
Total blood flow, mL/min	2,550	4,165	4,664	912	0.97
Left side					
Pulsatility index	1.71	1.64	1.61	0.12	0.23
Resistance index	0.77	0.84	0.80	0.084	0.73
Blood flow, mL/min	1,333	2,005	2,290	491	0.91
Right side					
Pulsatility index	1.75	1.73	1.62	0.13	0.59
Resistance index	0.81	0.89	0.82	0.089	0.58
Blood flow, mL/min	1,491	2,526	2,691	887	0.71

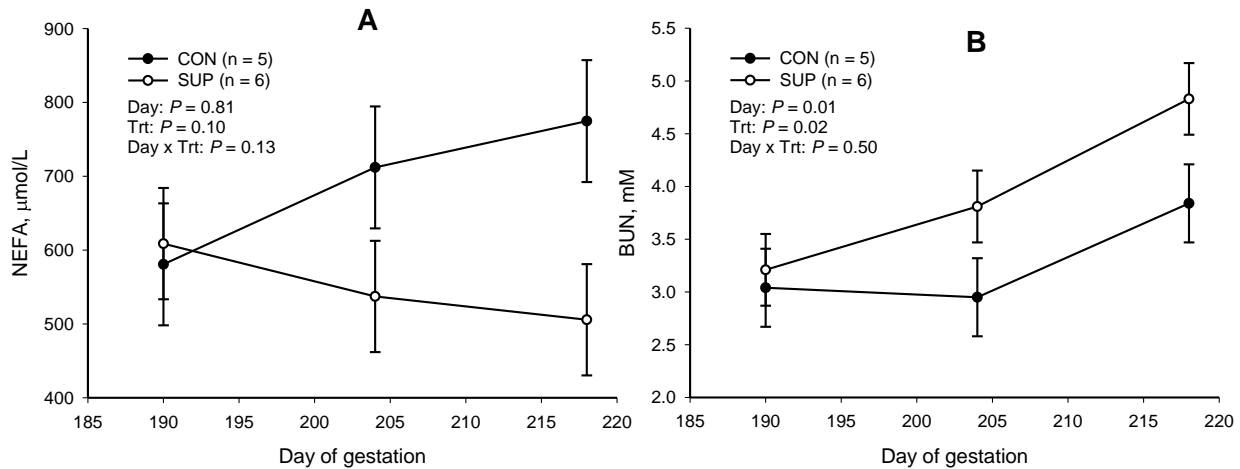


Figure 2.1. Non-esterified fatty acid (NEFA; A) and blood urea nitrogen (BUN; B) concentrations for cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).

Discussion

Contrary to our hypothesis, it appears that supplementation with DDGS during late gestation did not alter mammary gland BF. There is limited information on the use of how gestational diets impact mammary gland BF during late gestation. Most of the research utilizing Doppler ultrasonography has been directed towards responses of mammary gland BF to phase of

lactation (Kensinger et al., 1983; Neilsen et al., 1990), variability in milk production (Götze et al., 2010), mammary gland infection (Potapow et al., 2010), and milk vein hemodynamics (Braun et al., 2008, 2012). In both experiments, supplementation did not influence BF to the mammary gland throughout late gestation or between the two sides of the cow. Findings by Potapow et al. (2010) and Götze et al. (2010) have also demonstrated that side does not impact BF. Larson et al. (2009) reported DDGS supplementation does not affect milk production in beef cows in the period prior to the grazing season or at weaning. In contrast, Sullivan et al. (2009b) reported that beef heifers receiving a high amount of protein and energy supplement in the first trimester of gestation have a decrease in milk production. While milk yield and mammary BF are correlated, milk yield was not measured in the current study and, therefore, we cannot confidently predict if milk production would be altered in the cows of the current study.

The findings of this study do not necessarily rule out the possibility that positive effects in offspring evidenced by other studies are not due to mammary gland benefits. In other words, perhaps colostrum and milk production are enhanced, aiding to negate any harmful effects that were experienced during late gestation *in utero*. It is possible that the window of timing in the present study has not captured the period of mammary gland preparation for lactation. In the present study, our last ultrasounds were at least 40 days before calving. It is likely that the increase in arterial supply to the mammary gland necessary for lactation preparation occurred after our mammary gland data was collected. It has been established that during late pregnancy mammary ducts develop into lobulo-alveolar tissue with differentiated cells capable of producing milk, and mammary BF, oxygen consumption, and glucose uptake increased in the week prior to parturition (Davis et al. 1979). Additionally, in the first 12 weeks of lactation, total BF to the

mammary gland was highest in German Holstein cattle the day after parturition (Götze et al., 2010).

Distiller's grain with solubles has been utilized by producers as both a protein and energy source. Larson et al. (2009) have attributed the crude protein of DDGS as the component accountable for the postnatal performance measured in calves. Calculated rumen degradable protein balance and urea concentrations of SUP cows were greater than those of CON cows while both treatments had similar forage intakes. Therefore, the response was due to the DDGS supplementation and not simply due to increased forage intake.

Sullivan et al. (2009a) found in the first two trimesters in gestating beef heifers, urea is higher when cows are supplemented with a protein and energy source; moreover, this study also reported no differences in plasma NEFA amongst treatments. Cows were housed in an indoor facility, and calculated NEm balance was positive as gestation advanced. A similar study has observed differences in plasma NEFA concentrations between cows supplemented and unsupplemented, with those unsupplemented having higher serum NEFA levels than protein supplemented (Sletmoen-Olson et al., 2000).

Supplementation with DDGS during late gestation but did not impact BF to the mammary gland. Further research is required to understand the mechanism of enhanced postnatal performance of calves whose dams were supplemented with protein sources. This study serves as a pilot study for future studies that may want to measure mammary gland blood flow, and when to begin monitoring mammary gland flow during late gestation.

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CHAPTER 3. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

The original intent of my discussed research was to investigate the effects of late gestation protein supplementation on the blood flow to the uterus. However, after the animal portion of the experiment was complete, we discovered that the artery that was measured in this thesis was external pudendic artery rather than the uterine artery. While at first disappointed, my study was also important in the establishment of mammary gland blood flow monitoring in the Vonnahme laboratory. Since the time of completed the animal portion of my study, mammary gland blood flow has been monitored in two other studies. This pilot study has established that to determine differences due to maternal diet during the last third of gestation monitoring of mammary gland blood flow during late gestation does not need to take place prior to day 210 of gestation.

Mammary gland function surely is part of the explanation as to why calves from protein supplemented cows outperformed calves from non-supplemented cows since they have similar birth weights (Martin et al., 2007, Stalker et al., 2006). One hypothesis as to how the improved performance of the offspring was achieved was that the increased protein fed to the dam during late gestation allowed for increased mammary gland development which would allow for greater milk production for that calf immediately after birth.

During the time we measured mammary gland blood flow, we did not observe dietary effects. However, we need to further investigate how blood flow is altered as gestation advances. Moreover, perhaps volume is not affected, but nutrients delivered to the gland is. It is currently unknown what nutrients are made available to the developing mammary gland during late gestation in the beef cow. Whether it be increased blood flow or greater nutrient absorption, both scenarios attribute the amount of nutrients delivered to the developing mammary gland.

Likewise, uterine blood flow should be monitored. If blood flow is increased to the uterus, or if increased nutrient absorption occurs, there is the potential for increased fetal development. Since the completion of this study there have been several additional trials that have studied both uterine blood flow as well as mammary blood flow.

Continued research in both uterine and mammary gland blood flow could lead to increased understanding for researchers, nutritionists and producers. Ultimately, understanding the mechanisms that resulted in offspring with greater fertility and improved carcass quality can lead to better ways for the commercial cow/calf operator to feed their herd. With fertility as one of the most important traits for a profitable operation, finding ways to economically improve this would be of value for the beef industry.

Further research to continue exploring the effects of supplementation during late gestation on blood flow and nutrient uptake by the uterus and mammary gland has value. We were unable to collect data on milk production or milk quality. Further research into the effect of nutrition on mammary development, milk production and milk components has merit as we potentially could augment mammary gland development during later gestation, allowing for a better lactation. If there were data that showed improvement in either milk volume or milk quality in supplemented cows, measuring mammary development (alveolar numbers, mammary weight, etc.) could lead to a better understanding of late gestational nutrition.

As the livestock industry tries to improve efficiency, discovering ways to produce females with greater fertility and more feed efficient offspring will become more vital for producer success. Determining the best way to feed the pregnant cow to produce those types of animals while consuming lower quality forages may be one way to accomplish that efficiency.

In conclusion, continued study of fetal programming as well as nutritional impact on mammary development and milk production appears to be a worthy venture to accomplish those goals.

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APPENDIX. NUTRITIONAL IMPACTS ON MAMMARY GLAND HEMODYNAMICS

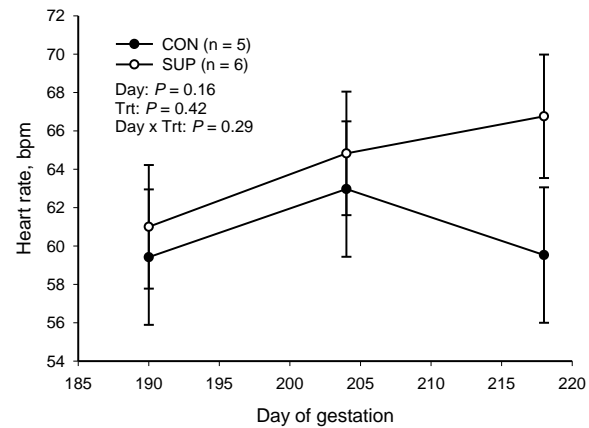


Figure A.1. Heart rate for cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).

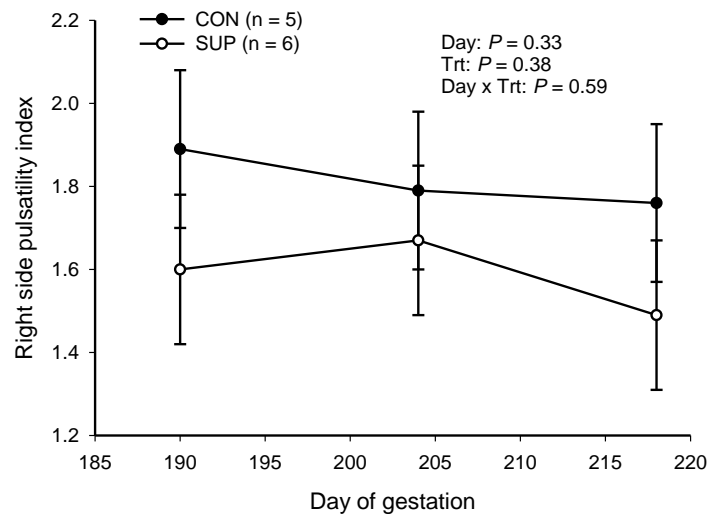


Figure A.2. Pulsatility index of the right mammary artery in cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).

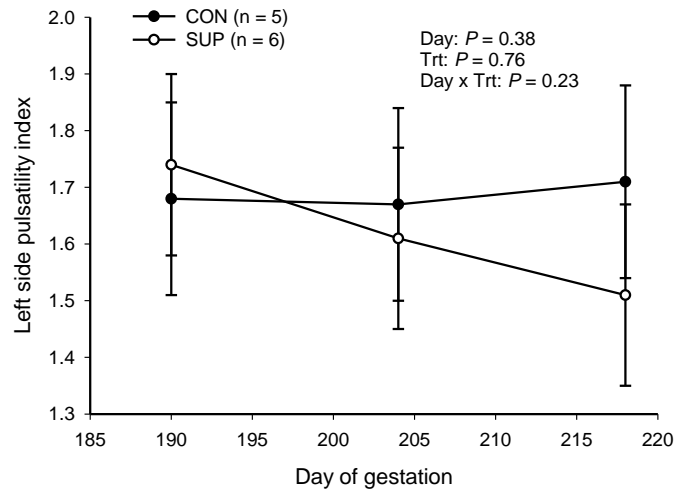


Figure A.3. Pulsatility index of the left mammary artery in cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).

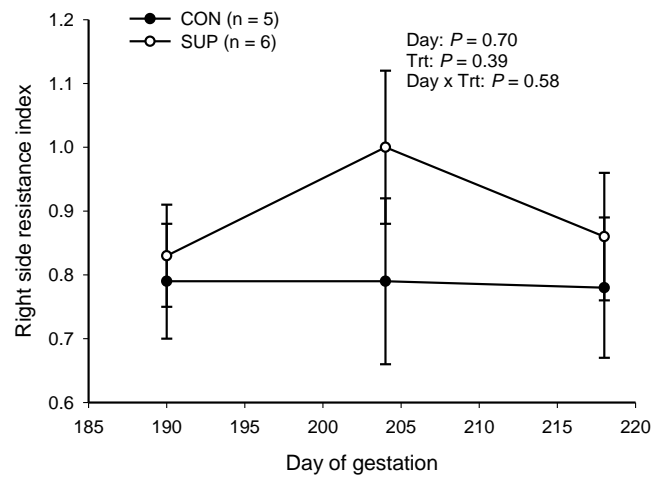


Figure A.4. Resistance index of the right mammary artery of cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).

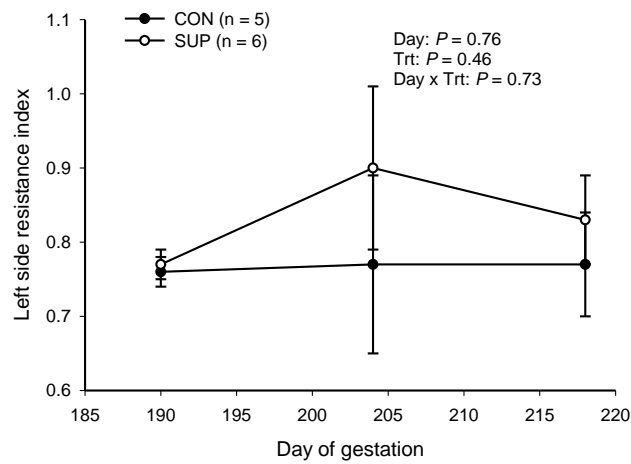


Figure A.5. Resistance index of the left mammary artery of cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).

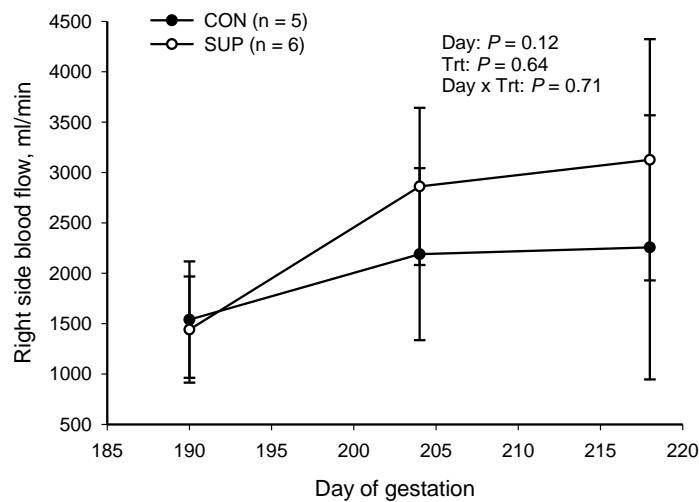


Figure A.6. Blood flow through the right mammary gland artery of cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).

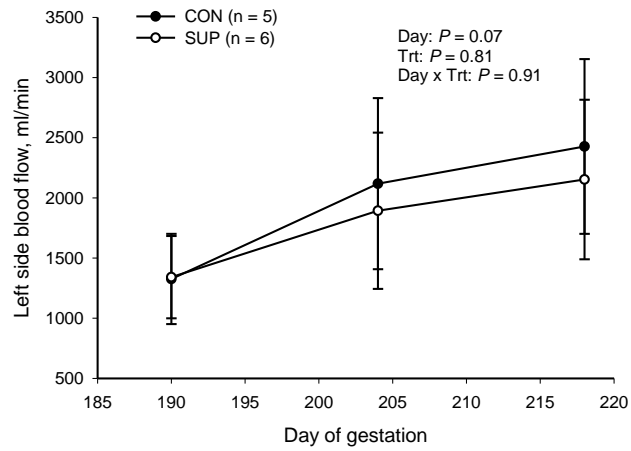


Figure A.7. Blood flow through the left mammary gland artery of cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).

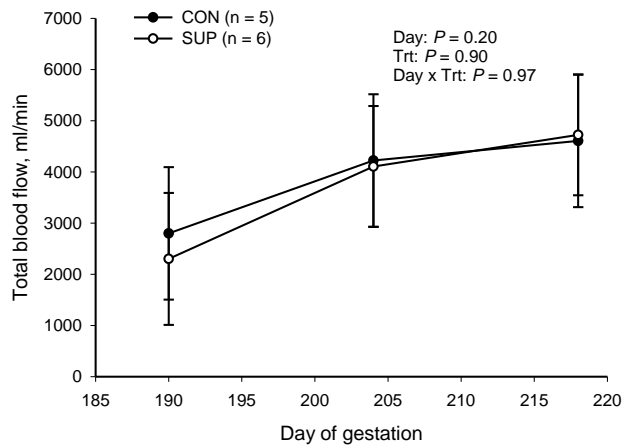


Figure A.8. Total mammary blood flow for cows fed low quality forage at 2% body weight (CON) and CON plus corn dried distiller's grain with solubles (DDGS) at 1.7 g/kg of body weight (SUP).