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Beta-hydroxybutyrate concentration influences timing of pregnancy in young beef cows

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I am submitting herewith a thesis written by Jeremy Daniel Hobbs entitled "Beta-hydroxybutyrate concentration influences timing of pregnancy in young beef cows." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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**Beta-hydroxybutyrate concentration influences timing of pregnancy in young
beef cows**

**A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville**

**Jeremy Daniel Hobbs
December 2016**

DEDICATION

I dedicate my work to my family: Michael J. Hobbs, Tanya L. Hobbs, Christopher P. Hobbs, and
Johnathon M. Hobbs.

ACKNOWLEDGEMENTS

With great sincerity I would like to thank my family for having such a great impact in my life. To my father Michael Hobbs for instilling me with unteachable work ethic and proficiency in decision making. My mother Tanya Hobbs for unconditional love and understanding during the toughest times. Also, to my brothers Chris and Johnathon for which I could not be where I am today without their competitive influence.

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ABSTRACT

Timing of conception can influence productivity within the cow herd and can be negatively influenced by metabolic dysfunctions. Selection for maternal traits like milk production in beef cattle may decrease reproductive efficiency due to metabolic dysfunctions caused by an increased metabolic load of lactation. Therefore, our objective was to analyze the association of milk production, serum metabolites as an indicator of nutrient status, cow BW and BW change, and calf performance with timing of pregnancy in 183 spring-calving beef cows. Cows were classified by timing of pregnancy as cows that were diagnosed pregnant by timed artificial insemination (**TAI**; n=118) or natural breeding (**NAT**; n=65). In addition, cows were grouped by age to represent young (3-to 4-yr-old), mature (5- to 6-yr-old), and old (7- to 9-yr-old) cows. Starting approximately d 30 postpartum, cow BW and BCS were recorded and blood samples were collected weekly through the end of breeding. Weekly serum samples were composited by cow within production periods: (1) pre-breeding; (2) timed- artificial insemination to end of natural breeding. Cow BW and BCS did not influence ($P \geq 0.40$) timing of pregnancy during the entire study. Calf, BW at birth and weaning were not different ($P \geq 0.30$) between timing of pregnancy groups. However, calf BW at weaning and value the subsequent year of the study were greater ($P < 0.001$) for TAI than NAT cows. An age group \times treatment interaction ($P < 0.01$) occurred for serum β -hydroxybutyrate (**BHB**). Serum BHB concentrations for mature and old cows were similar regardless of timing of pregnancy. However, serum BHB concentrations for young NAT cows were greater than young TAI cows. In addition, serum NEFA exhibited ($P = 0.04$) a timing of pregnancy \times sampling period interaction. Pre-breeding serum NEFA concentrations were greater for NAT cows than TAI

cows. Contrarily, serum NEFA concentrations during the natural breeding season were similar regardless of timing of pregnancy. Results from this study indicate that only the young, postpartum beef cows during early lactation were susceptible to measured metabolic dysfunctions of elevated blood BHB concentrations, which may have caused a delay in timing of pregnancy.

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CHAPTER I: LITERATURE REVIEW

Introduction

For cow-calf producers, optimizing production and economic efficiency is key for long-term success. With a growing demand for efficiency within the beef cattle industry, reproduction can be a limiting factor for increasing production efficiency. Such that, reproductive failures account for the greatest economic loss within the cow/calf segment because it represents the single most important factor reducing net calf crop (Bellows and Short, 1978; Dziuk and Bellows, 1983). To illustrate this, Mulliniks et al. (2011) reported an increase in cow-herd profitability due to the sum of an increase in pregnancy rate and a lesser extent a decrease in days to first estrus.

It is imperative that each cow in a herd maintains a yearly 365 calving interval which requires that she is pregnant within 80 days of calving. Therefore, a cow then needs to resume estrus as early as possible, as the earlier a cow conceives in the breeding season, the older and heavier the calf will be in the following year (Wiltbank, 1970). To reach that goal, optimizing nutrition and reproduction are the two greatest controls of economic and production efficiency within a cow/calf herd (Hess et al., 2000). Grazing beef cattle production depends on the ability to graze available forages to meet energy requirements for production. Therefore, beef cow herds are required to be biologically and reproductively efficient in an array of ever changing environmental conditions (Mulliniks et al., 2016). Cows that can easily adapt to these environmental and physiological changes and have the ability to adapt their maintenance requirements to match current environmental conditions may have the capacity for increased reproductive efficiency. In addition, timing of pregnancy has been shown to increase the opportunity for a cow to have an increased longevity and productivity within the cow herd

(Cushman et al., 2013). Therefore, it is imperative to understand the nutritional mediators of reproduction and strive to strategically manage cows to conceive earlier in the breeding season.

Nutrient Requirements of Beef Cows

Maintenance requirements support the animal's body temperature regulation, essential metabolic processes, and physical activity. The expenditure of maintenance energy by livestock is dependent on animal BW, breed or genotype, sex, age, season, temperature, physiological state, and previous nutrition. Ferrell and Jenkins (1987) concluded that metabolizable energy (ME) required for functions supported by the animal's maintenance requirement accounts for approximately 70% of the total ME required by mature, producing beef cows. In comparison, a minimum of 40% and 90% of ME was required by growing cattle and breeding bulls, respectively, to meet these specific animals' maintenance requirements.

The physiological state of beef cattle can directly affect their maintenance energy requirements. Specific nutrient requirements for maintenance and growth are designated for each as well as gestation and lactation for cows (Ferrell and Jenkins, 1985). Heat production increases for cows by 50% during gestation compared to non-gestating cows (Ferrell et al., 1976) and 20% in lactating compared to non-lactating (Ferrell and Jenkins, 1985). Also, lactating beef and dairy cattle have increased maintenance energy requirements to the magnitude of 10 to 49%, with an average of 20% above non-lactating cows (Ritzman and Benedict, 1938; Flatt et al., 1969; Neville and McCullough, 1969; Moe et al., 1970; Neville, 1974; Ferrell and Jenkins, 1987). Therefore, stage of production is an important factor influencing nutritional requirements.

Average required diet nutrient density values for CP and TDN are different throughout the four phases of production (Figure 1).

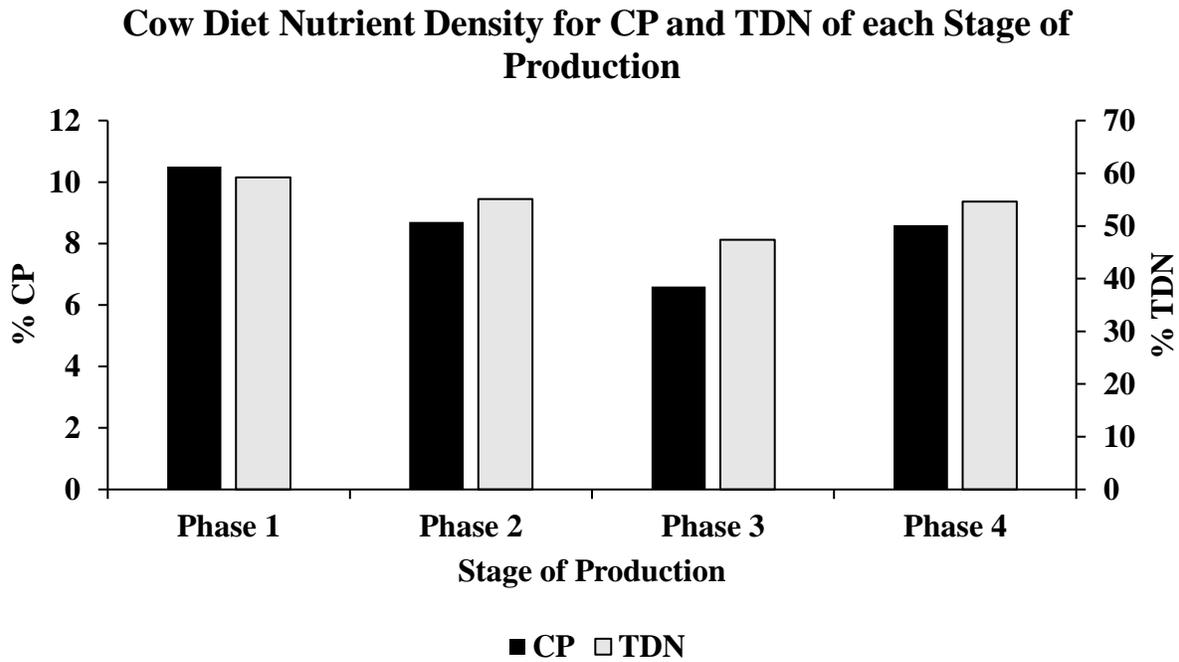


Figure 1: Cow diet nutrient density for CP and TDN of each stage of production; Phase 1: early lactation through breeding, Phase 2: late lactation through weaning, Phase 3: weaning through 60-90 days pre-calving, and Phase 4: 60-90 days pre-calving through calving.

It is important to note that cows within a grazing system may not have the ability to consume the energy to meet maintenance requirements depending on forage quality and availability within its environment. Therefore, maintenance requirements of grazing beef cattle in forage-based pasture systems often have to be met with additional harvested feedstuffs, supplemental feeds, or mobilized tissue stores. However, supplementing to meet this requirement can be costly. It is estimated that over 63% of the expenses associated with a beef cattle

operation is feed costs (Miller et al., 2001). Within forage-based systems, understanding the periods of high and low nutritional demands that could inevitably result in deficiencies and loss in performance is critical to meeting production goals. Therefore, understanding and knowing the necessary protein and energy requirements for cattle is critical to the operation of a successful beef cattle enterprise. When assessing the nutritional environment of livestock coupled with nutrient requirement, it is the goal to determine if any or which nutrients or metabolic intermediates are imbalanced (too little or too much) and if production goals can be met (i.e. calf crop and pregnancy rates) with the existing nutrient equilibrium. If nutrient imbalance(s) exist, then the extent of the imbalance should be appraised and evaluate how to correct the imbalance with nutritional strategies. However, there may be times when the imbalance and its consequences are acceptable, or determine a method to minimize the disproportion of dietary nutrients.

Nutrient Partitioning of Beef Cows

Nutrient partitioning to body tissues includes two types of regulation, homeostasis and homeorhesis. Homeostasis has been well established as the maintenance of physiological equilibrium, in example constant conditions in the internal environment. Homeorhesis is the orchestrated changes for the priorities of a physiological state, in example the coordination of metabolism in various tissues to support a physiological state (Bauman and Currie, 1980). With that in mind, an approximate order of priority of nutrients can be described as 1) basal metabolism, 2) activity, 3) growth, 4) basic energy reserves, 5) maintenance of pregnancy, 6)

lactation, 7) additional energy reserves, 8) estrous cycles and initiation of pregnancy and 9) excessive reserves (Short et al., 1990).

During early lactation cows may enter a state of negative energy balance due to the inept capacity to meet nutrient requirements. In this case, milk synthesis requires increased use of nutrients by the mammary gland. Glucose is the only precursor for lactose synthesized in milk. Approximately 1.5 units of glucose are needed to synthesize 1 unit of milk lactose, and the net daily requirement may be as much as 2.8-3 kg of glucose in the case of high milk production (Van Soest, 1994). Circulating glucose is transported into mammary gland tissue via the insulin independent GLUT 1 transporter and subsequently transported into the trans-Golgi, the organelle of lactose production via GLUT 1 (Rudolph et al., 2007). Most important to note is that nutrient interactions within the mammary gland are regulated independent of insulin, henceforth it may be used as a glucose sink under insulin resistance circumstances. In response to meet the glucose demand of lactation extra-hepatic insulin resistance, which is developed during late gestation increases postpartum. Growth hormone can block the phosphorylation of glucose inside the cell after cellular uptake (Ojeda and McCann, 2000). Glucose can then freely diffuse back out of the cell and limit insulin response. During times of hypoglycemia after calving, cows will also display an increased sensitivity to adrenergic agents. The lipid metabolism within adipose tissue will undergo increased lipolysis and decreased lipogenesis. Within the liver glucose metabolism is altered by increased gluconeogenesis and increased glycogenolysis. As well as an increased utilization of lipid use as energy source and decreased glucose. Muscle and other body tissues mobilize protein reserves, and kidney, liver, gut and bone will increase absorption and mobilization of calcium (Bauman and Currie, 1980). Because there is variation in nutrients available and nutrients required, cows may mobilize body tissue to support lactation.

With mechanisms by which satisfactory supply of metabolites are available for milk synthesis are established, it is important to note that insulin concentrations relative to the hormones which inhibit tissue deposition (GH, glucagon, and glucocorticoids) can influence how nutrients are partitioned between the mammary gland and peripheral tissues (Hart, 1983). Kronfield et al. (1963) reported lactating cows treated with insulin displayed an immediately decrease in milk yield. When comparing high- and low- milk yielding dairy cattle Hart et al. (1979) reported insulin concentrations were greater in the plasma of low-yielding cows. The authors also reported that changes in insulin were positively correlated with changes in BW. Thus, high milking ability of beef cows may be influenced by peripheral insulin resistance increasing glucose uptake by the mammary gland and increasing milk production.

Nutritional Assessments of Reproductive Status

Monitoring the nutritional status of beef cattle is critical to enhance cow reproductive efficiency and optimize overall cow performance. The simplest and most economical way to assess the nutritional status of cows is BCS. The increased demands of nutrients during early lactation results in a loss in body condition subsequently influencing a change in the metabolic profile of the cow. Thus, BCS and body weight change at and prior to parturition have been used as an indicator for reproductive performance (Selk et al., 1988; DeRouen et al., 1994). However, the majority of the studies that have assessed prepartum and postpartum BCS have manipulated cow BW prior to calving to achieve a certain BCS, which may have confounded BCS results with BW change. Furthermore, Mulliniks et al. (2012) reported that cows can be managed at calving with a BCS 4 to 4.5 with the same final pregnancy rate as cows in BCS 5 or greater.

Due to the subjective nature of BCS and the lack of sensitivity, BCS may not be the most accurate tool for producers to use for assessing nutritional status. Concentrations of glucose, insulin, NEFA, and β -hydroxybutyrate (BHB) display the effects of an animal's adaptive response to negative energy balance (NEB) and may also be used as an indicator of reproductive competence (Bell, 1995; Pushpakumara et al., 2003; Mulliniks et al., 2013). Therefore, utilizing metabolic measurement of nutrient status may provide livestock producers the opportunity to select cows that conceive earlier in the breeding season.

Glucose

Ruminants depend on gluconeogenesis in the liver for glucose to be synthesized from VFA. It is suggested that less than 10% of glucose requirement will be met by absorbed glucose regardless of diet (Annison et al., 1957, and Armstrong, 1965). Therefore, propionate is the primary precursor for gluconeogenesis. In addition, propionate supply needs to be in adequate quantities to meet glucose energy requirements for metabolism (Leng et al., 1967). Glucose is an important regulator for milk synthesis (Bell and Bauman, 1997). Such that it takes approximately seventy-two g of glucose for each kg of milk produced (Bell, 1995). Also, glucose is an important mediator of the hypothalamic-pituitary-gonadal axis (Funston et al., 1995). During critical physiological and productive periods such as lactation and breeding season glucose demand and utilization rate is high and may limit cow productivity (Mulliniks et al., 2012). Garverick et al. (2013) reported early postpartum dairy cows that became pregnant after first artificial insemination (AI) had greater blood glucose concentrations at calving than those that did not become pregnant during first AI. Whereas, Mulliniks et al. (2013) reported elevated

serum glucose concentrations with late conception cows compared to early conception beef cows. However, Mulliniks et al. (2013) also reported elevated serum insulin concentrations in early conception cows when compared to late conception cows. The variation in glucose reports across these studies may be influenced by diet quality and insulin sensitivity.

Insulin

Insulin is a polar peptide hormone released from the beta cells of the islets of Langerhans of the pancreas in response to high blood glucose. Moreover, insulin acts on liver, muscle, and adipose cells to decrease blood glucose by increasing glycolysis (Ozand and Narahara, 1964), glycogen synthesis (Dent et al., 1990), fatty acid synthesis (Stransbie et al., 1976), and translocation of glucose transport protein (Glut4) to the plasma membrane (Fischer, et al., 1997). With insulin being a regulator of glucose metabolism, it may be an important aspect to analyze when considering nutritional mediators of reproduction (Hess et al., 2005). In the liver, elevated insulin concentration results in reduced activity of Carnitine palmitoyltransferase I, reduced NEFA transport into mitochondria and suppressed ketogenesis (Herdt, 2000). In dairy cows during early lactation lowered responsiveness of extrahepatic tissues to insulin plays a role in development of hepatic lipidosis and ketosis (Hayirli, 2006). Both hepatic lipidosis and ketosis impair immune function and fertility (Hayirli, 2006). With that, insulin combined with glucose can stimulate the release of GnRH from the hypothalamus (Burcelin et al., 2003). Whitley et al. (1998a) reported sows treated with exogenous insulin had increased follicular steroidogenesis and possibly follicular development. However, insulin has been reported to be involved in follicular growth and ovulation without changes in gonadotropins (Gong et al., 2002). In

addition, Whitley et al. (1998b) indicated that insulin treatment dramatically decreased estradiol production in large follicles and did not affect estradiol in small- and medium sized follicles. Ramirez et al. (1997) reported sows that were treated 4 d with exogenous insulin had increased litter sizes when compared to sows treated with 2 d exogenous insulin or saline. Thus, the combination of insulin and glucose may be used as an indicator of reproductive competence.

Nonesterified Fatty Acids and Beta-Hydroxybutyrate

During early lactation there is a rapid shift in metabolic demands that will increase the mobilization of adipose tissues in the form of NEFA. However, this shift may initiate during late gestation due to a decrease in DMI (Hayirli et al., 2002), resulting in a negative energy balance (Grummer, 1995). Nonesterified fatty acids provide peripheral tissues with energy, however elevated NEFA concentrations can display consequences. Wathes et al. (2007) reported NEFA concentrations to be negatively correlated with days from calving to conception in multiparous cows one week prior to calving. In addition, Garverick et al. (2013) reported elevated circulating NEFA concentrations in cows that did not conceive at first insemination when compared to cows that did conceive by first insemination. The liver has the ability to metabolize NEFA into triacylglycerides (TAG). Triacylglycerides then have two fates within the liver which are to be oxidized to acetyl CoA, or exported as very low density lipoproteins (VLDL). When acetyl CoA resulting from oxidation of free fatty acids is not utilized within the tricarboxylic acid cycle (TCA) it is converted to ketone bodies. As well as large accumulation of TAG in the liver can result in fatty liver syndrome, which may lead to insulin sensitivity issues and increased ketogenesis (Grummer, 1993). Energy homeostasis is attained by the incorporation of lipid

energy metabolism with carbohydrate and protein metabolism. Ketone bodies such as BHB and acetoacetate can be oxidative fuels, as lipogenic precursors, and regulators of metabolism. Such that, they may be seen as signals of carbohydrate deficiency as they are convoluted within whole-body metabolism. However, ruminal fermentation of forages often produces inadequate amounts of propionate the primary glucogenic precursor for ruminants (McCollum and Gaylean, 1985). Due to the limited availability of glucose relative to acetate, acetate utilization may be less efficient (Preston and Leng, 1987). With an increase in acetate and free fatty acids an adequate supply of glucose is needed for oxidative energy metabolism. Additionally, if glucose is unavailable then acetate and free fatty acids will be converted into ketones. With that, Tardiff et al. (2001) reported that chronic exposure to the ketone body β -hydroxybutyrate in-vitro impairs insulin action in primary cultures of adult cardiomyocytes specifically the GLUT 4 transporter. The insulin dependent GLUT 4 transporter has been detected in all insulin-responsive tissue including heart, skeletal muscle, and adipose tissue (Hocquette et al., 1995). Correspondingly, Kerestes et al. (2009) reported that pancreatic β -cell function and biological potency of insulin is impaired in dairy cows with long-term elevated levels of circulating BHB. However, these authors indicated that short-term elevations in plasma NEFA and BHB may not induce insulin resistance during early lactation. With that, blood ketone concentrations in dairy cows increase in late conceiving and non-pregnant cows when compared to early conception cows (Pushpakumara et al., 2003). In addition Mulliniks et al. (2013) reported elevated BHB concentrations in late conception cows when compared to late conception cows. Cope et al. (2016) reported wethers injected in the lateral ventricle with β -hydroxybutyrate suppresses LH amplitudes. In agreement, BHB suppressed LH pulses when injected into the hypothalamic paraventricular nucleus in female rats (Iwata, 2011). These studies support that elevated

circulating BHB may negatively influence LH secretion from the anterior pituitary and increase days to conception post-partum.

With reports of variation in BCS in relation to reproductive competence other nutritional assessments may be more efficient in diagnosing metabolic dysfunction in postpartum beef cows. Recent reports consider circulating concentrations of glucose, insulin, NEFA, and BHB as indicators of nutritional status and reproductive capability. Thus, further validation of these methods for diagnosing metabolic dysfunction and predicting reproductive performance is desirable.

Conclusion

While longevity within the cow herd is easily defined, there are no tools available to select for longevity. However, timing of conception within the breeding season has been reported to influence productivity and longevity within the cow herd. During early lactation cows nutrient requirements increase as well as the cow will partition nutrients to lactation. Mobilization of body storage to meet energy demands during this critical period results in altered metabolic profile which can influence reproductive performance of the cow and delay timing of conception. Overall, the specific mechanisms linking nutrition and reproduction are still misunderstood. However, it is known that reproduction is extremely complex and cannot be controlled by one single aspect being nutrients, metabolites, or hormones. Each of these facets are coordinated within a complex integrated system that is imperative for overall beef cow productivity and efficiency.

**CHAPTER II: BETA-HYDROXYBUTYRATE CONCENTRATION
INFLUENCES TIMING OF CONCEPTION IN YOUNG BEEF COWS IN
TENNESSEE**

Introduction

Longevity of beef cows is imperative for sustainability and profitability within any cow-calf operation. However, there are no selection tools available for longevity. Timing of pregnancy and subsequent calving date has been shown to be a major influence on longevity and life-time productivity (Cushman et al., 2013). Traditionally, BCS has been used as a tool for predicting reproductive competence (Selk et al., 1988). However, Mulliniks et al. (2012) suggests BCS has limitations in predicting reproductive competence. Mobilization of body storage to meet energy demands during early lactation results in altered metabolic profiles which may influence reproductive performance. With this in mind, elevated BHB concentrations prior to the breeding season have been linked to delayed timing of pregnancy in both beef (Mulliniks et al., 2013) and dairy (Pushpakumara et al., 2003; Walsh et al., 2007) cows. Elevated concentrations BHB in the brain may act as a negative signal to suppress pulsatile LH secretion (Cope et al., 2016). While each facet of reproductive control is coordinated within a complex system, evidence from these studies suggests that circulating BHB concentrations may be a nutritional indicator of reproductive status. Thus, the hypothesis of our research was that an increase in metabolic load of lactation will cause a metabolic dysfunction resulting in elevated BHB concentrations and a delay in timing of pregnancy. Therefore, our objective was to analyze the association of milk production, serum metabolites, cow BW change, and calf performance with timing of pregnancy in spring-calving beef cows in Tennessee.

Materials and Methods

All animal handling and experimental procedures were conducted according to the guidelines of the Institutional Animal Care and Use committee of the University of Tennessee.

Animals

In a 2-yr study, 183 Angus-sired, spring calving beef cows (3- to 9-yr-old) were utilized to determine the association of milk production, serum metabolites, cow BW and BW change, and calf BW with time of pregnancy. This study was conducted at three locations: (1) Highland Rim Research and Education Center (HRREC) in Springfield, TN; (2) Plateau Research and Education Center (PREC) in Crossville, TN; and (3) Middle Tennessee Research and Education Center (MTREC) in Spring Hill, TN. Average rainfall across the three locations is 1354 mm annually with Tall Fescue (*Schedonorus arundinaceus*) as the predominate grass specie. From December to May in each year, cows at each location were fed either ad libitum corn silage (PREC; 9 % CP and 47 % NDF), orchard grass hay (MTREC; 17 % CP and 48 % NDF), or rye haylage (8 % CP and 61 % NDF) with 5 % dried distiller's grain (HRREC; 30 % CP). Forage samples were ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ) before analysis was performed. Crude protein analysis was determined by combustion (Leco-NS2000, Leco Corp., St. Joseph, MI). Neutral detergent fiber concentrations were determined using by a fiber analyzer vessel using methods described by ANKOM Technology (ANKOM A200, ANcom Technology, Macdon, NY).

In April every year, all cows were synchronized using a controlled internal drug-releasing (CIDR) device (Eazi-Breed CIDR, Zoetis Inc., Kalamazoo, MI) with a 7 d CO-Synch protocol. Cows were administered a single 2-mL intramuscular (i.m.) injection of GnRH (Cystorelin, Merial) and CIDR on -9 d. On -2 d, CIDR was removed and cows were injected with 5-mL i.m. injection of PGF (Lutelyse, Zoetis INC., Kalamazoo, MI). Approximately 66 h after CIDR removal, all cows were given an i.m. injection of 2 mL GnRH (Cystorelin, Merial) and time artificially inseminated (**TAI**). Fourteen days after TAI occurred cleanup bulls were utilized for natural service with a cow-to-bull ratio of 1:20 at PREC, and 1:30 at MTREC and HRREC. Bulls were removed from pastures after a 60 d natural breeding season. In both years, TAI pregnancy diagnosis occurred 30 d after TAI, and overall pregnancy diagnosis occurred in September. Pregnancy diagnosis was determined at PREC by circulating concentrations of pregnancy-specific protein B greater than 0.15 ng/ml in serum (BioPRYN, Golden Standard Labs, Bowling Green, KY) and by transrectal ultrasonography by scanning the uteri of cows at HRREC and MTREC.

Experimental Groups

To determine the association of timing of pregnancy with predictive measurements, cows were retrospectively classified as diagnosed pregnant by timed-artificial insemination (**TAI**; n = 118) or during natural service period (**NAT**; n = 65). Timing of pregnancy was verified by back-calculating from the calving date of the subsequent year minus 285 d for gestation. Cows were also classified by age groups as Young (3- to 4yr-old; n = 73), Mature (5- to 7-yr old; n = 65), and Old (8- to 9-yr old; n = 45).

Sampling and Analyses

Blood samples were collected weekly via coccygeal venipuncture (Corvac, Sherwood Medical, St. Louis, MO) starting approximately 30 d post-partum until the end of the breeding season. Blood samples were centrifuged at 2,000 x g at 4°C for 20 min. Serum was separated and stored in plastic vials at -20°C until further analysis. Weekly serum samples were composited by cow within two production periods: (1) pre-breeding; (2) timed- artificial insemination to end of natural breeding. Composited serum samples were analyzed for insulin, glucose, NEFA, urea N (SUN), and β -hydroxybutyrate (BHB). Serum composites were analyzed using a 96-well microplate reader spectrophotometer with commercial kits for NEFA (Wako Chemicals, Richmond, VA), glucose (Thermo Electron Corp., Waltham, MA), SUN (Thermo Electron Corp., Waltham, MA). Serum BHB concentrations were determined with the use of DL- β -Hydroxybutyric acid sodium salt, β -Nicotinamide adenine dinucleotide hydrate, and 3-Hydroxybutyrate dehydrogenase (Sigma-Aldrich, St. Louis, MO) as described by McCarthy et al. (2015). Serum insulin concentrations were determined by RIA (EMD Millipore's Porcine Insulin RIA) using Wizard² Gamma Counter (Perkin Elmer, Waltham, MA). Inter- and intra-assay CV were <15% for all serum metabolites.

After calving, cows were weighed weekly until the termination of the breeding season. The number of days to BW nadir was calculated from the least BW after calving. Body condition scores (1 = emaciated, 9 = obese; Wagner et al., 1988) were assigned to each cow by visual observation and palpation weekly by 2 trained technicians. Calves were weighed at birth, approximately d 58 postpartum, and weaning each year. Calf weights at weaning were adjusted to a 205-d age constant BW. From 2000 to 2015 national average real prices (\$/kg) were

collected for steers and heifers in the following weight divisions: 227 to 272 kg, and 273 to 319 kg. Calf value was then calculated for every calf weaned in the subsequent year of the study.

On approximately d 58 postpartum, all cows were milked utilizing a modified version of weigh-suckle-weigh by a portable machine (Porta-Milker, Coburn Company Inc., Whitewater, WI) as described by Mulliniks et al., (2011). On the day of the milking, cows were gathered from their pasture and calves were removed. Ten minutes before milking, cows were administered an injection of oxytocin (20 IU; Vedo Inc., St. Joseph, MO) to facilitate milk ejection. Cows were milked until machine pressure could not extract any additional fluid, and milk collected was subsequently discarded. After first milking, cows were kept separate from calves for approximately 3 h and then milked a second time following the same procedures as in the first milking. Milk weights were recorded and extrapolated to calculate 24-h milk production.

Statistical Analysis

Cow and calf performance data were analyzed as a completely randomized design with cow as the experimental unit using the Kenward-Roger degrees of freedom method. The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to test a model that included fixed effects of conception time, location, year, cow age group, calf sex, and their interaction. Serum metabolites were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) with sampling period as the repeated factor, cow as subject with Unstructured as the Covariance structure. The model included conception time, location, year, period, age group, calf sex, and their interaction. Separation of least squares means was performed by the PDIFF option of SAS when a significant ($P \leq 0.05$) effect of conception date classification was detected. Data was pooled when no

significance of period was detected. The usefulness of each potential predictor variables for pregnancy at TAI (BHB, NEFA, cow BCS at TAI, Cow BW at TAI, days to BW nadir, calf WW, and 24-hr milk yield) when used alone to predict pregnancy were compared using area under the curve (AUC) of the receiver operating characteristic (ROC) curve for binary outcomes (MedCalc, Ostend, Belgium).

Results and Discussion

Management of multiparous beef cows from calving through breeding is a major factor of total operation productivity and efficiency (Short, 1990). The greatest opportunity for profit is getting the maximum percentage of cows rebred as early as possible during the breeding season (Spitzer et al., 1975). Calving date has been shown to positively influence calf BW at weaning and cow longevity (Marshall et al., 1990; Cushman et al., 2013). In addition steers born earlier in the calving season have greater HCW and carcass values (Funston et al., 2011). Thus, minimizing the time from calving to pregnancy in beef cows potentially has tremendous impact on cow-calf productivity.

Calving date of the initial years of the study was not different ($P = 0.15$: Table 1) between TAI and NAT cows, which was expected due to selecting cows TAI bred cows from the previous year. Osoro and Wright (1992) reported spring-calving beef cows that calve earlier in the calving season are more likely to conceive earlier in the breeding season than those calving later. In the current study, BCS did not differ ($P \geq 0.40$: Table 1) between TAI and NAT cows at the initial, breeding, and end of breeding time points. Also, no differences ($P \geq 0.47$) were detected for cow BW between TAI and NAT cows at initial, beginning of breeding, end of

breeding, or at nadir. Likewise, cow BW change between TAI and NAT cows was not different ($P \geq 0.40$) during the entire course of this study. Body condition score, BW, and BW change are often used as an indicator of reproductive competence (Derouen et al., 1994; Randel, 1990). However, BCS at calving in some management schemes and production systems may not also be a good indicator of reproductive efficiency as indicated by Mulliniks et al. (2012). In addition Lake et al. (2005) reported no difference in first-service conception rates in BCS 6 compared to BCS 4 cows, but BCS 6 cows did have an increased overall pregnancy rate. In addition, Cicciooli et al. (2003) showed no effect of BCS at calving on estrus, ovarian function, or reproductive performance. Thus, within the given environment of the current study, BCS and BW were not good indicators of timing of pregnancy.

Twenty-four hour milk production at d 58 postpartum did not differ ($P = 0.75$: Table 2) between TAI and NAT cows. Similarly, Pushpakumara et al. (2003) noted no differences in 24-h milk yield between dairy cows that were diagnosed as pregnant by early or late service. However, Edwards et al. (2016) reported a decreased timed-artificial insemination rate in high milk producing multiparous beef cows compared to low and moderate milking multiparous beef cows.

Serum glucose concentrations were not different ($P = 0.23$: Table 2) between TAI and NAT cows. Garverick et al. (2013) reported an increase in circulating glucose concentrations in post-partum dairy cows that were pregnant at first AI compared to cows that failed to conceive at first AI. Similarly, Green et al. (2012) reported decreased plasma glucose concentration during the first 30 d postpartum in dairy cows that did not conceive at first AI when compared to pregnant dairy cows. Mulliniks et al. (2013) reported elevated serum glucose concentrations with late conception cows compared to early conception cows. Indeed, Mulliniks et al. (2013)

reported elevated serum insulin concentrations in early conception cows compared to late conception cows. Within the current study, serum insulin concentrations were not different ($P = 0.31$) between TAI and NAT cows. Chagas (2003) reported increased serum insulin concentrations to display a positive effect in the restoration of LH pulse frequency. However, Vizcarra et al. (1998) reported no difference in insulin concentrations between cows with or without luteal activity in primiparous beef cows. Serum urea N did not differ ($P = 0.99$) between TAI and NAT cows. In contrast, Butler et al. (1996) reported pregnancy rate after AI was reduced in lactating dairy cows that had an elevated plasma urea N concentration.

Whole-blood BHB concentrations can accumulate in blood when the rate of acetate oxidation is inhibited by an inadequate supply of cellular oxaloacetate derived from serum glucose (Kaneko, 1997). Circulating BHB concentrations can increase when the rate of acetate supply is greater than the rate of acetate oxidation (Yamashita et al., 2001). In this study, an age group \times conception date interaction ($P < 0.01$; Table 3) occurred for serum BHB concentration. In mature and old cows, BHB concentrations did not differ by conception date classification. However, young NAT cows had an increased ($P < 0.01$) in serum BHB concentrations when compared to young TAI cows. Similarly, Mulliniks et al. (2013) reported that 2- and 3-yr-old cows classified as becoming pregnant late in the breeding season displayed an elevated whole-blood BHB concentration prior to breeding. The similarity among old and mature NAT and old and mature TAI cows in the current study suggests that young post-partum cows may be more susceptible to the negative impact of elevated BHB concentrations as a result of metabolic imbalances. In addition, the lack of differences in mature and old cows may suggest that cows that couldn't tolerate the metabolic load earlier in life had been culled from the herd at a younger age due to reproductive failure. Even though some inconsistencies between ages of cows existed

when comparing circulating BHB concentration and timing of conception, the results of the current study and from Mulliniks et al. (2013) may indicate that circulating BHB concentration during early lactation may be indicative of reproductive efficiency in young beef cows. Therefore, circulating BHB concentrations may be a more sensitive measurement for reproductive efficiency than pre-breeding BCS in young cows.

Circulating concentrations of NEFA have been linked to reproductive incompetence due to its association with negative energy balance (Bell, 1995). In the current study a sampling period \times conception date interaction ($P = 0.04$; Table 4) occurred for serum NEFA concentrations. During the pre-breeding period serum NEFA concentrations for NAT cows were greater ($P < 0.01$) than TAI cows. Conversely, during the breeding period, serum NEFA concentrations were similar regardless of timing of conception. In support, Wathes et al. (2007) reported NEFA concentrations to be negatively correlated with days from calving to conception in multiparous cows one week prior to calving. In addition, Ospina et al. (2010) reported that NEFA concentrations had a greater association with reproductive performance than BHB in transition dairy cattle. However, Reist et al. (2000) reported no differences in serum NEFA concentrations in postpartum dairy cows that displayed first ovulation within the first 30 d postpartum compared to cows that ovulated between d 31 and 87 postpartum, whereas elevated BHB concentrations were reported in cows that ovulated after d 30 postpartum compared to those that ovulated during the first 30 d postpartum. Results from the current study indicate that NEFA concentrations could be an indicator of energy status, however the variation from other studies imply that the consistency of NEFA impact on reproduction may depend on fatty acid oxidation ability of the animal.

Within the current study calf BW at birth, 58d, weaning, and 205 adj. BW did not differ ($P \geq 0.30$; Table 5) between TAI and NAT cows. These results were expected due to no differences in dam milk production or calving date the year of the study. However, TAI cows had greater ($P < 0.001$) calf weaning weights in the subsequent year than NAT cows, which was expected due to becoming pregnant and calving earlier than NAT cows. Calf 205 adj. BW of the subsequent year of the study was not different ($P = 0.32$) between TAI and NAT cows. Thus, increased calf BW at weaning of the subsequent year was greater influenced by timing of pregnancy than increased genetic potential from using AI sires. In addition, calf value at weaning of the subsequent year was greater ($P < 0.001$) for TAI than NAT cows. French et al. (2013) reported yearling heifers that were pregnant by AI had greater lifetime revenue than those that conceived during natural breeding. Dunn and Kaltenbach (1980) concluded that allowing a greater number of females to be inseminated at one time results in earlier conception leading to older and heavier calves at weaning. Similarly, Garcia Paloma et al. (1992) concluded that an earlier date of calving contributed to greater concentrations of early calving and production efficiency in subsequent calving seasons. In addition, Cushman et al. (2013) reported that calving date as a heifer influences calf weaning weights through multiple parturitions suggesting there is a long-term effect of timing of conception in young cows.

Receiver-operating characteristic (ROC) analysis can be a useful tool not only to predict pregnancy outcome, but also a selection tool for fertility (Holm et al., 2009). For all ages of cows, the AUC of the ROC curve for circulating BHB concentrations (0.56) and young cows circulating BHB concentrations (0.66) both were acceptable predictors for pregnancy by TAI ($P \leq 0.04$; Table 6). However, prebreeding circulating NEFA concentration, cow BCS at TAI, cow BW at TAI, days to BW nadir, calf WW, and 24-hr milk production were not acceptable

predictors ($P \geq 0.22$). It is important to note when the circulating BHB concentrations are not pooled across all age groups only young cows are an acceptable predictor for pregnancy after TAI. Young cows BHB was not representative of a perfect test (AUC=1). However, AUC for young cows was significantly different from 0.5, which would represent a test with no discriminative capability. Young cows with circulating BHB concentrations less than or equal to 138.8 $\mu\text{mol/L}$ were 95% likely to become pregnant by TAI. Whereas, young cows with circulating BHB concentrations greater than or equal to 301 $\mu\text{mol/L}$ were 96% likely to not become pregnant by TAI.

Conclusion

The results from this study indicate that only young, postpartum beef cows during early lactation were susceptible to metabolic dysfunction, resulting in elevated BHB concentrations and a causing delayed time to conception. Hence, by monitoring BHB concentrations postpartum may provide the opportunity to identify young cows that would have a delayed conception and manage them differently to decrease days from calving to conception and potentially increase their longevity and productivity.

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APPENDIX

Table 1. Cow BW, BW change, and BCS for cows classified as becoming pregnant by timed-artificial insemination or natural service

Measurement	Conception ¹		SEM	P-value
	AI	NAT		
<i>n</i>	118	68		
Calving date, ² Julian Date	28.07	30.87	1.96	0.15
BCS ³				
Initial	5.24	5.31	0.16	0.71
Breeding	5.15	5.06	0.12	0.44
End of breeding	5.43	5.33	0.11	0.40
Cow BW, kg				
Initial	616.9	624.48	11.26	0.47
Nadir	571.82	577.73	11.42	0.61
Begin of breeding	599.68	608.79	12.89	0.48
End of breeding	619.5	625.5	11.03	0.58
Cow BW change, kg				
Initial to nadir	-47.65	-46.93	3.95	0.86
Initial to breeding	-17.12	-16.08	6.64	0.88
Initial to end of breeding	3.79	1.14	4.53	0.56
Days to nadir	73.35	75.44	2.48	0.40

¹Conception time was determined by ultrasound and calving date in the subsequent year: TAI= conceived at timed artificial insemination: NAT= conceived during the natural breeding season.

²Calving date of the study year.

³For BCS, 1 = emaciated, 9 = obese; Wagner et al. (1988).

Table 2. Milk production at 58 d and serum metabolites for cows classified as becoming pregnant by at timed-artificial insemination or natural service

Measurement	Conception ¹		SEM	<i>P</i> -value
	AI	NAT		
24-h milk production, kg/d	9.85	9.70	0.49	0.75
Serum metabolites				
Glucose, mg/dL	60.16	62.34	1.82	0.23
Insulin, ng/ml	0.37	0.40	0.03	0.31
Serum urea N, mg/dL	13.42	13.42	0.38	0.99

¹Conception time was determined by ultrasound and calving date in the subsequent year: TAI= conceived at timed artificial insemination: NAT= conceived during the natural breeding season.

Table 3. Concentrations of serum β -hydroxybutyrate (BHB) in young, mature, and old cows classified as becoming pregnant by timed-artificial insemination or natural service

Measurement	Conception ¹		SEM
	AI	NAT	
Serum BHB, $\mu\text{mol/L}$			
Young ²	221.83 ^{ax}	250.68 ^{by}	9.94
Mature ²	243.70 ^{ay}	230.21 ^{axy}	10.17
Old ²	226.40 ^{ax}	221.87 ^{ax}	11.74

^{a,b}For each interaction within timing of sample, means in rows with different superscripts differ ($P < 0.05$).

^{x,y}For each interaction within timing of sample, means in columns with different superscripts differ ($P < 0.05$).

¹Conception time was determined by ultrasound and calving date in the subsequent year: TAI= conceived at timed artificial insemination: NAT= conceived during the natural breeding season.

²Cows were classified by age group: Young= 3-4 yr old: Mature=5-7 yr old: Old=8-9 yr old.

Table 4. Concentrations of serum non-esterified fatty acids during the prebreeding and breeding period in cows classified as becoming pregnant by timed-artificial insemination or natural service

Measurement	Conception ¹		SEM
	AI	NAT	
Prebreeding ² NEFA, mmol/L	671.89 ^{ay}	750.76 ^{by}	28.96
Breeding ² NEFA, mmol/L	417.83 ^{ax}	427.73 ^{ax}	28.91

^{a,b}For each interaction within timing of sample, means in rows with different superscripts differ ($P < 0.05$).

^{x,y}For each interaction within timing of sample, means in columns with different superscripts differ ($P < 0.05$).

¹Conception time was determined by ultrasound and calving date in the subsequent year: TAI= conceived at timed artificial insemination: NAT= conceived during the natural breeding season.

²Weekly serum samples were aliquoted into two separate composites: Prebreeding= Approximately 30 days post calving to the week prior to timed-artificial insemination: Breeding= Timed artificial insemination to the end of the natural breeding season.

Table 5. Suckling calf body weight and subsequent calf value of cows classified as becoming pregnant by artificial insemination or natural service

Measurement	Conception ¹		SEM	P-value
	AI	NAT		
Calf BW, kg				
Birth	35.48	35.47	0.61	0.99
58 d	66.53	64.34	1.73	0.21
Weaning	290.75	286.42	4.18	0.30
205 Adj.	269.46	267.28	2.19	0.60
Subsequent Weaning ²	298.98	269.73	5.24	<0.001
Subsequent 205 Adj.	275.44	269.05	6.34	0.32
Subsequent Calf Value, \$	824.21	755.53	13.26	<0.001

¹Conception time was determined by ultrasound and calving date in the subsequent year: TAI= conceived at timed artificial insemination: NAT= conceived during the natural breeding season.

²Weaning weight the following year of the study.

Table 6. Univariable predictive ability of 7 variables for pregnancy after timed-artificial insemination (TAI) of beef cows.

Predictor variable	Pregnancy after timed-artificial insemination ¹	P-Value
Serum BHB ²	0.56	0.04
Young ³	0.66	<0.001
Mature ³	0.51	0.79
Old ³	0.54	0.49
Prebreeding NEFA	0.54	0.38
BCS at TAI	0.52	0.58
Cow BW at TAI	0.53	0.45
Days to BW Nadir	0.53	0.59
Calf WW	0.53	0.55
24-hr Milk Yield	0.56	0.22

¹Area under the curve for receiver operating characteristic analysis.

²Circulating concentrations of beta-hydroxybutyrate pooled across all age groups

³Cows were classified by age group: Young=3-4 yr old; Mature=5-7 yr old; Old= 8-9 yr old.

VITA

Jeremy Daniel Hobbs was born January 15, 1993 in Amarillo, TX to Michael and Tanya Hobbs. Throughout his childhood and into high-school Jeremy spent his time raising show pigs and commercial cattle, playing sports, hunting, and fishing with his family. This is when he developed a passion for progressive agriculture and service for rural America. In May, 2011 he graduated from Shamrock High School in Shamrock, TX. After high school Jeremy followed his passion in agriculture and completed a Bachelor's of science degree in Animal Science at Oklahoma State University in Stillwater, OK where he graduated December, 2014. During his time at Oklahoma State, Jeremy developed a great interest in feeding beef cattle. He sought out numerous opportunities to expand his knowledge of ruminant nutrition. Jeremy was recruited by the University of Tennessee to pursue a MS degree in animal science. Jeremy accepted a graduate research assistantship at the University of Tennessee where he conducted research focused on metabolic efficiency in grazing beef cows. He spent time specifically elucidating the adaptive mechanisms that promote energy efficiency and metabolic indicators for increased reproductive efficiency. Jeremy graduated with his MS in Ruminant Nutritional Physiology in December, 2016. Following completion of his MS degree Jeremy pursued a career in the beef cattle industry.