FACTORS AFFECTING NEONATAL BEEF CALF METABOLISM AND VIGOR

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By

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FACTORS AFFECTING NEONATAL BEEF CALF METABOLISM AND VIGOR

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Dr. Allison M. Meyer, Thesis Supervisor

ABSTRACT

Two studies were conducted to investigate factors affecting neonatal beef calf metabolism and vigor. In the first study, effects of late gestational nutrient restriction on colostrum yield, neonatal vigor, and blood chemistry and hematology measures were investigated in beef cattle. Colostrum volume and weight from nutrient restricted dams was 40% less compared with control dams. Although gestational nutrition did not affect gestation length or calf birth weight, calves born to control dams had faster times to attempt to stand and to stand. Calves born to nutrient restricted dams had greater serum protein metabolites from 6 to 48 h of age. Serum aspartate aminotransferase and creatine kinase concentrations were greater in nutrient restricted calves until 24 h postnatal. Red blood cells, hemoglobin, and hematocrit were greater in control calves at 6 and 12 h of age. In conclusion, calves born to nutrient restricted dams experienced more trauma at birth, reduced neonatal vigor, and had less colostrum available but greater serum protein concentrations. The objectives of the second study were to determine the effect of calving season on perinatal nutrient availability and neonatal vigor. Fall-born calves tended to have lighter birth weight and faster time to stand than spring-born calves. Spring-born calves had greater circulating 0 h glucose, 0 and 6 h NEFA, and 0, 6, 12, and 48 h triglycerides. Fall-born calves had greater sodium and magnesium during the first 48 h postnatal. Spring-born calves had greater aspartate aminotransferase and creatine kinase concentrations until 48 h of age. Albumin, chloride, calcium, and anion gap were greater in fall-born calves. Bicarbonate and direct bilirubin were greater in spring-born calves. In
conclusion, spring-born calves are heavier at birth but were slower to stand. Additionally, differences in metabolites over time suggest that spring- and fall-born calves adapt to postnatal life differently where thermoregulation plays an important role.
REVIEW OF LITERATURE

INTRODUCTION

In the United States, of beef calves born alive, there is a 5.5% non-predator preweaning death loss (USDA-APHIS, 2017). Of those deaths, two-thirds occur within the first 3 wk postnatal (USDA-APHIS, 2010). Others reported that 57% of calf death loss preweaning occurs during the first 24 h (Patterson et al., 1987). This suggests that the transition to extrauterine life poses one of the greatest challenges to neonatal calves. Survival during this difficult time is dependent on calves displaying proper vigor behaviors to quickly stand and ingest colostrum for necessary nutrients and passive transfer (Besser and Gay, 1994). Several factors or a combination of many, such as altered neonatal metabolic status, fetal growth and development, and environmental conditions, can contribute to impaired neonatal vigor and potentially cause inadequate colostrum consumption or absorption resulting in increased calf morbidity and mortality (Bleul, 2011; Dwyer et al., 2016; Cooke, 2019; Perry et al., 2019).

As beef calves are typically expected to be raised by their dam, the colostrum produced by the dam must meet the nutrient demands of the calf and supply maternal antibodies for passive transfer of immunity. Maternal maturity and metabolic status can directly influence the amount and composition of the colostrum available to their neonate (Swanson et al., 2008; McGee and Earley, 2019). Even with proper vigor behavior, limited colostrum yield or reduced quality can be detrimental to calf survival.
The objective of this review of the literature is to explore current understanding of factors that affect the transition to extrauterine life, colostrogenesis, and the process of passive transfer.

TRANSITION TO EXTRAUTERINE LIFE

Challenges to the Neonate

During the transition to extrauterine life, critical changes and adaptations must take place. Maturation of organs, endocrine and metabolic changes, achieving passive transfer of immunity, and an abrupt switch to a discontinuous oral feeding method all occur during this critical time frame (Danijela, 2015). Endocrine changes, such as fetal surges of glucocorticoids and catecholamines during parturition, play an important part in maturation of organs and metabolic pathways in preparation for oral nutrient consumption (Hammon et al., 2012). When the neonate’s method of nutrient intake changes from parenteral to enteral, likewise the nutrient composition shifts from consisting of mainly glucose, lactate, and amino acids to diet consisting of lactose, lipids various proteins, enzymes, and growth factors (Hammon et al., 2012; McGrath et al., 2016). However, prior to colostrum consumption and when inadequate amount of nutrients are available in the colostrum, the neonate relies on the mobilization of its own glycogen, protein, and body lipids to meet nutrient demands and allow for physical activity and thermogenesis (Danijela, 2015).

The calf experiences a dramatic temperature change at birth as they transition from 38.8°C in utero to ambient temperatures (Danijela, 2015). Additionally, the large surface area-to-body mass ratio and the evaporation of amnionic fluids contribute to
neonatal calf heat loss (Carstens, 1994). Therefore, the calf’s ability to thermoregulate upon parturition is paramount. Normally, calves are able to achieve homeothermy following birth through shivering thermogenesis of the muscle tissue and nonshivering thermogenesis of brown adipose tissue (Carstens, 1994). However, calves that are less vigorous and are slow to stand often become hypothermic, defined as a body temp of about 35.2 to 35.7°C in dairy calves (Okamoto et al., 1986), are slow to ingest colostrum, and have an increased possibility of death (Dwyer and Morgan, 2006; Danijela, 2015). In addition to facing the temperature adjustment, other harsh elements such as cold, wind, and precipitation may affect the calf’s ability to properly thermoregulate (Holmes and McLean, 1975). In one study, an increase in beef calf mortality was associated with increasing amounts of precipitation and decreasing temperatures (Azzam et al., 1993).

Dystocia, a prolonged and difficult birthing process that may require human assistance, can impose more challenges on the calf during this already trying time. The causes of dystocia can fall into two categories: indirect or direct (Zaborski et al., 2009). Fetal abnormal presentation or uterine torsion would be classified as indirect causes (Zaborski et al., 2009). The direct causes of dystocia would include effects such as calf birth weight and sex, dam pelvic area, dam BW and BCS, dam age and parity, and other genetic factors such as dam or sire breed (Zaborski et al., 2009). Even with human intervention, calves that experience dystocia face greater incidences of subclinical trauma (Pearson et al., 2019), inability to properly thermoregulate (Vermorel et al., 1989; Bellows and Lammoglia, 2000), induced metabolic and respiratory acidosis or hypoxia (Homerovsky et al., 2017a). These can all contribute to the increased risk of morbidity and mortality observed in calves born to dystocia (Laster and Gregory, 1973).
**Neonatal Blood Gas**

Hypoxia can occur during the fetal and neonatal time periods. Fetal hypoxia is the result of alterations in umbilical or uterine blood flow and the corresponding oxygen delivery to the fetus (Jensen et al., 1999), whereas improper lung function or cardiac disease impairs the exchange of respiratory gases causing neonatal hypoxia (Bleul et al., 2007). These conditions lead to an acidotic state. In these calves, metabolic acidosis is the result of an accumulation of lactate produced from anaerobic glycolysis, while respiratory acidosis is the result of decreased carbon dioxide exchange and the subsequent increased concentration of carbon dioxide in circulation (Bleul et al., 2007). Although calves from eutocic births normally experience minor metabolic-respiratory acidosis, prolonged acidotic conditions can increase the neonatal calf’s risk for morbidity and mortality (Szenci et al., 1988). Sampling venous blood can be used to determine the pH and acid-base status; however, an arterial blood sample is necessary to accurately determine the arterial pressures of carbon dioxide and oxygen (Kasari, 1999; Bleul et al., 2007).

Dystocia may prematurely cause the rupture or compromised blood flow of the umbilical cord which can cause hypoxia and ultimately lead to an acidotic state (Murray-Kerr et al., 2017). In a eutocic calf, blood pH after birth can range from 7.2 to 7.4; however, dystocic calves will typically have blood pH < 7.2, consistent with an acidotic state (Vannucchi et al., 2015; Homerosky et al., 2017a). One study reported that unassisted calves had average lactate concentrations at 10 min postnatal of 4.1 mmol/L and decreased to 2.3 mmol/L by 24 h of age; conversely, calves that required mechanical assistance had lactate concentrations of 7.3 mmol/L at 10 min after birth and were
considered to be acidotic (Homerovsky et al., 2017a). These studies observed no differences in other blood gas measures, such as carbon dioxide, oxygen, bicarbonate, or anion gap, between calves dystocic and eutocic calves (Vannucchi et al., 2015; Homerovsky et al., 2017a).

Calves forcefully extracted experienced more serious metabolic acidosis and mortality than those from unassisted births (Vermorel et al., 1989; Schuijt and Taverne, 1994). Likewise, Lombard et al. (2007) reported increased odds of calves experiencing respiratory acidosis when human intervention was needed due to dystocia complications. This is evident in a study conducted by Szenci et al. (1988) when applying traction during normal anterior presentation of dairy calves. They measured acid-base balance of calves prior to calving by collecting blood from metacarpalis volaris superficialis vein in the fore limb prior to applying traction (Szenci et al., 1988). Venous blood samples were also collected from the jugular vein following birth and human intervention (Szenci et al., 1988). Approximately 26% of calves had acidosis (blood pH of ≤ 7.2) prior to applying traction; however, following birth over 50% of calves were considered to have acidosis (Szenci et al., 1988). Furthermore, the longest duration of traction led to calves with the most severe acidotic state, all of which did not survive (Szenci et al., 1988).

Additionally, calf sex or breed type can affect neonatal blood gas profile. Dillane et al. (2018) reported male calves having lower blood pH and acid-base status than female calves in their study. Although blood pH was not different between beef and dairy breeds in this study, other blood gas measures, such as pCO₂, HCO₃⁻², and anion gap, were greater in beef calves relative to dairy calves (Dillane et al., 2018). These results may be due to limited number of beef breeds compared with dairy breeds enrolled in this
study; however, others have noted breed differences in other hematology measures. Beef breeds generally have greater concentrations (Roland et al., 2014) and size (Adili et al., 2014) of red blood cells and may be more resistant to changes in their oxidation curve (Gustin et al., 1997).

**Neonatal Vigor**

A scoring system was first developed to assess the condition of the newborn human infant within the first few minutes of life. This is known as the Apgar score which scores the infant based on heart rate, respiratory efforts, reflex response to stimuli, muscle tone, and color (Apgar et al., 1958). An increase in infant mortality and neonatal hypoxia were found to be associated with low Apgar scores (Apgar et al., 1958). However, neonatal hypoxia is not the sole cause of a low Apgar score and not all neonates which are in an acidotic state are given a low Apgar score (Gilstrap III et al., 1989). Therefore, in human medicine a combination of Apgar scores and sampling of the umbilical artery for blood gas concentrations are used to determine neonatal hypoxia shortly following birth (Gilstrap III et al., 1989).

Since then, the Apgar score has been adapted to assess the condition of livestock neonates. In addition to the criteria used in human medicine (heart rate, respiration, reflex response to stimuli, and muscle tone), other common criteria used to evaluate neonatal calf vitality include mucous membrane color, hair coat appearance, rectal temperature, sternal recumbence within 5 min, attempts to stand within 15 min, standing within 1 h, and suckling within 2 h (Mee, 2008). Proper display of the last 3 vigor behaviors, attempting to stand, stand, and suckling, are especially vital for the newborn ruminant
because these directly contribute to the calf obtaining passive transfer. Although hypoxia may be an indicator of overall calf vitality, blood gas analyses are expensive and inconvenient lab procedures that are not practical for most cow-calf producers (Murray-Kerr et al., 2017).

Several studies have observed increased time to stand (Odde, 1988; Barrier et al., 2012; Vannucchi et al., 2015) or reduced vigor in calves from a difficult birth (Colburn et al., 1997; Murray-Kerr et al., 2017). Vannucchi et al. (2015) reported respiratory and metabolic acidosis in dystocic calves with low vigor. This may indicate an acidotic state, which in neonates is often initiated by dystocia and negatively impacts neonatal calf vigor (Vermorel et al., 1989; Homerosky et al., 2017a). Time to reach sternal recumbency by 15 min was used as the neonatal vigor measure in a study conducted by Schuijt and Taverne (1994) to determine calf vitality. They reported calves with more severe acidosis had longer times to reach sternal recumbency (Schuijt and Taverne, 1994). However, Barrier et al. (2012) reported no difference in time to sternal recumbency between assisted and non-assisted calves, stating that this posture takes less muscular coordination than other behaviors. In another study which considered suckle reflex and colostrum consumption by 4 h as calf vigor measures, it was observed that calves with decreased blood pH and increased lactate concentrations failed to consume colostrum by 4 h (Homerosky et al., 2017b). Homerosky et al. (2017b) also reasoned that the blood gas disturbances would have contributed to decreased muscle tone, therefore affecting suckle reflex and other behaviors needed to suckle.

Calves with poor neonatal vigor that do not receive intervention may have delayed intake of adequate amounts of colostrum, which can compromise the calf’s
health, metabolic, and endocrine status. Studies which delayed first colostrum administration to calves observed lower glucose, albumin, total protein, and globulin and elevated concentrations of nonesterified fatty acids (Hadorn et al., 1997; Zanker et al., 2001). These effects on the neonatal calf metabolic status were no longer apparent by 7 d following birth (Hadorn et al., 1997) which gives evidence for the calf’s ability to compensate for the delay in colostrum consumption in regards to its metabolic status (Zanker et al., 2001). When Vermorel et al. (1989) studied the effects of calving difficulty on the metabolism of neonatal calves, they observed dystocic calves to have more severe acidosis and decreased concentrations of glucose, nonesterified fatty acids, triiodothyronine, and thyroxine during the first hours of life than eutocial calves. It was noted that calves from very difficult births were less physically active and had little to no appetite, but no vigor scores were formally assigned (Vermorel et al., 1989).

Maternal Factors on the Transition

Effects of parity. It is thought that primiparous dams have greater incidence of calving difficulty. This is most often due to fetal-maternal size mismatch where the calf is too large for the dam’s pelvic dimensions (Lombard et al., 2007). It has been reported that heifers with small pelvic areas had greater calving difficulty and required more caesarean sections (Vermorel et al., 1989; Colburn et al., 1997). Bellows et al. (1982) and Nix et al. (1998) also reported greater calving difficulty in primiparous dams. Despite these observations, Barrier et al. (2012) observed no effect of parity on calf vigor; however, they did report that calves that required assistance had reduced neonatal vigor.
**Effects of maternal nutritional status.** Insults to maternal nutrition during critical fetal developmental points can cause altered fetal development and have detrimental effects on postnatal calf health and performance (Caton and Hess, 2010; Funston et al., 2010; Perry et al., 2019). During late gestation, Corah et al. (1975) restricted dams to 65% of dietary energy and observed greater preweaning calf death loss and incidence of scours in calves from restricted dams. Corah et al. (1975) hypothesized the greater calf death loss could have been attributed to calves from restricted dams taking longer to suckle after birth. In another study, dairy cows were fed at 3 different energy levels (low, medium, and high) during the last 21 d of gestation (Gao et al., 2012). Despite there being no differences in maternal weight among the 3 energy levels, altered maternal metabolites and reduced calf weight and size were observed in calves born to dams fed low energy versus high energy diets (Gao et al., 2012).

In a study by Kroker and Cummins (1979), primiparous beef dams during late gestation were fed to gain 0.75 kg/d BW, maintain maternal BW, or lose 0.5 kg/d BW. They reported that calves born to dams that gained weight in late gestation had the greatest birth weights, whereas calves born to dams that lost weight in late gestation had the smallest birth weights (Kroker and Cummins, 1979). Additionally, Kroker and Cummins (1979) reported that dams that lost weight during late gestation had longer durations of labor and their calves took longer to stand and suckle compared with calves from dams that gained or maintained body weight in late gestation. This study (Kroker and Cummins, 1979) suggests that maternal nutrient status likely impaired fetal growth and reduced neonatal vigor.
Late gestational protein restriction may impact calf vigor by increasing the time to stand of calves from restricted dams and may also affect thermogenesis of the calf where calves from restricted dams have decreased heat production (Carstens et al., 1987; Odde, 1988). Conversely, Martin et al. (1997) observed no differences in calf vigor score or neonatal thermogenesis between calves born to protein restricted or non-restricted heifers. Although maternal nutrition seems to have varying effects on the neonate and its ability to transition to extrauterine life, confounding effects may exist due to maternal nutritional influences on mammary gland development and colostrum and subsequent milk production (Ferrell et al., 1976; Swanson et al., 2008; Funston et al., 2010; Meyer et al., 2011).

**COLOSTRUM PRODUCTION**

*Colostrogenesis*

Accumulation of colostrum in the mammary gland prior to parturition, or colostrogenesis, occurs when immunoglobulins (Ig) and other macromolecules are transported into mammary secretions directly from maternal circulation during the final weeks of gestation (Barrington et al., 2001; Stelwagen et al., 2009). This event is especially critical to ruminants to provide the means of passive transfer of immunity to their offspring, who are born agammaglobulinemic due to the lack of transplacental exchange of Ig (Larson et al., 1980; Baumrucker and Bruckmaier, 2014). Although most classes of Ig can be found in colostrum, bovine colostrum predominantly contains IgG₁ (Larson et al., 1980; Barrington et al., 2001). The higher concentrations of IgG₁ in bovine
colostrum are thought to be the result of a high selectivity for IgG_1 by the neonatal Fc receptor (FcRn) located in the bovine mammary gland (Mayer et al., 2005).

The FcRn was initially found in the small intestines of neonatal rodents, which facilitated transfer of maternal Ig to the neonate (Cervenak and Kacskovics, 2009; Hine et al., 2019). The lower pH of the small intestinal lumen allows binding of IgG_1 to the FcRn, whereas the internalization and transcytosis to a neutral pH at the basolateral surface causes release of the IgG_1 into circulation (Baumrucker and Bruckmaier, 2014). During colostrogenesis, the transfer of Ig is reversed in the bovine mammary gland but moving from neutral pH on the basal side to the apical side does not promote binding of IgG_1 and FcRn (Cervenak and Kacskovics, 2009; Baumrucker and Bruckmaier, 2014; Hine et al., 2019). Therefore, it is presumed that IgG_1 binding to FcRn is enabled through fluid phase endocytosis followed by endosome acidification (Baumrucker and Bruckmaier, 2014) subsequently leading to the release of IgG_1 on the apical side and accumulation in the colostrum.

A transgenic mouse model with over-expressed bovine FcRn in the mammary gland was used to investigate the receptor’s role in selective IgG_1 transcytosis. The transport of IgG has an inverse relationship with the binding affinity to the FcRn (Lu et al., 2007). Therefore when Lu et al. (2007) observed higher serum to milk ratios of IgG_2 when equal amounts of IgG_1 and IgG_2 were injected into the mice, it was attributed to the higher binding affinity of IgG_2 for the FcRn and the longer half-life of bovine IgG_2. However, this study was carried out while the mice were in mid-lactation which may not have been truly representative of the bovine. Minimal IgG_1 transfer across the bovine mammary cells occurs after parturition and initiation of lactogenesis (Baumrucker and
Bruckmaier, 2014); therefore, an abrupt decrease in concentrations of IgG\textsubscript{1} found in mammary secretions of milk can be noted (Barrington et al., 2001). Mayer et al. (2005) observed that, during lactation the FcRn were strictly localized to the apical side of mammary cells which limit the function of the FcRn to simply recycling the IgG\textsubscript{1}.

Immunoglobulin A is the predominant Ig in most mammalian mammary secretions, with exception of ruminants (Larson et al., 1980; Hunziker and Kraehenbuhl, 1998). It has been widely accepted that the transport of IgA and IgM across the mammary epithelium in humans and mice rely on the polymeric immunoglobulin receptor (pIgR; Hunziker and Kraehenbuhl, 1998; Berry et al., 2013; Hine et al., 2019). Likewise, the role of the pIgR in IgA accumulation in the bovine colostrum was supported by the work of Berry et al. (2013) who found that polymorphisms of the bovine pIgR in the mammary gland accounted for the variation of IgA concentration in the colostrum produced. The specificity for polymeric Ig binding to this receptor is due to the J-chain polypeptide found strictly in polymeric Ig (Hine et al., 2019). The process of Ig transcytosis utilizing the pIgR is similar to the function of the FcRn. When IgA binds to the pIgR on the basal side of the mammary cell it is then transported across the cell (Hunziker and Kraehenbuhl, 1998). Once at the apical surface, proteolytic cleavage of the pIgR results in the release of the extracellular portion of the pIgR, known as secretory component, and the IgA into the lumen (Hunziker and Kraehenbuhl, 1998). Proteolytic cleavage occurs regardless of IgA bound to the pIgR. When IgA is bound to the secretory component at the time of receptor cleavage, it is known as secretory IgA (Hine et al., 2019).

Colostrogenesis is primarily under endocrine control where estrogen and progesterone signal Ig transport into the mammary secretions during the last 4 to 6 wk.
prepartum (Barrington et al., 2001; Baumrucker and Bruckmaier, 2014). Studies have demonstrated that administration of both estrogen and progesterone to nonlactating, nonpregnant Holstein cows induced secretions of the mammary gland that resembled IgG concentrations of colostrum (Smith et al., 1971; Winger et al., 1995). Whereas glucocorticoids and prolactin are the hormones responsible for the cessation of colostrogenesis and initiation of lactogenesis (Barrington et al., 2001). This was supported in work done by Winger et al. (1995), when they observed a decrease in IgG transfer to mammary secretions when glucocorticoids were administered to cows during induced lactation.

**Colostrum Nutrient Composition**

In addition to providing Ig, colostrum contains proteins, lipids, carbohydrates, vitamins, and minerals that are all greatly needed nutrients by the neonate (Blum and Hammon, 2000). The proteins in colostrum, in addition to Ig, provide a quick (alphalactalbumin and betalactoglobuline) and slow (casein) source of amino acids when hydrolyzed that can be used in protein synthesis and gluconeogenesis (Quigley III and Drewry, 1998). Colostral lipids supply the neonate with energy to properly thermoregulate and sustain gluconeogenesis. The protein and energy constituents of colostrum play an important role in the neonate establishing homeostasis (Quigley III and Drewry, 1998). It is believed that the carbohydrates in colostrum, especially oligosaccharides, provide protection to the neonatal intestine because of their competitive inhibitor action against pathogens (Gopal and Gill, 2000). Minimal placental transport of vitamins occurs in the bovine; therefore, the neonate is dependent on colostrum for
providing Vitamins A and E which aid in growth and development and protect against oxidative stress, respectively (Quigley III and Drewry, 1998; Debier et al., 2005). The neonate obtains several minerals through colostrum ingestion, where Ca, P, Mg, Na, Fe, Zn, Cu, and Mn are available in the highest concentration following birth and decrease with time (Kume and Tanabe, 1993).

Colostrum also provides growth factors, cytokines and other macromolecules that can have biological effects in the neonatal calf (Blum and Hammon, 2000). Numerous growth factors, such as insulin-like growth factors, transforming growth factor beta and epidermal growth factor, promote the growth and differentiation of cells (Pakkanen and Aalto, 1997). Cytokines are responsible for B and T cell maturation, stimulating antibody production, and fighting systemic infections (Pandey et al., 2011).

In recent years, the lactocrine hypothesis has been proposed to describe how milk-borne bioactive factors (MbF) in colostrum lead to maternal programming of the postnatal nursing offspring (Bagnell and Bartol, 2019). Studies in pigs show that the tissues of the female reproductive tract are sensitive to MbF in porcine colostrum, such as relaxin (Yan et al., 2006; Bartol et al., 2008). When relaxin-free milk replacer was administered to gilts during the first 2 d postnatal, abnormal gene expression in both uterine and cervical tissues was observed when compared with neonates that were allowed to suckle naturally (Yan et al., 2006; Chen et al., 2011). This supports the idea that lactocrine signaling has an important role in proper development of porcine reproductive tissues. Additionally, relaxin receptors have been found in other female and male mammalian tissues, such as the brain, heart, kidney, and adrenal (Hsu et al., 2000; Yan et al., 2006). This gives reason to believe that lactocrine response may not only
affect the development of vital organs in the pig but may also be evident in other mammalian species.

**Maternal Factors**

*Effects of parity.* Dam age and parity can affect colostrum production. Primiparous heifers entering their first lactation are still growing and developing which can impact both the amount and quality of colostrum produced (Devery-Pocius and Larson, 1983; Cabral et al., 2016). A study conducted on commercial dairy farms found that primiparous and second lactation cows have lower IgG concentration than third or greater lactation cows, but no difference in volume of colostrum produced among lactation groups (Kehoe et al., 2011). Likewise, a more recent study conducted with all multiparous dairy cows reported colostrum from second and third lactation cows had lower IgG concentration than cows that were on their fourth or greater lactation (Silva-del-Río et al., 2017). These results are in contrast to a study done with beef cows in which there was no observed difference in IgG concentration between parities; however, amount of IgG produced and colostrum volume were less in heifers compared with yields from mature cows (McGee et al., 2006). Differences in results may be due to differences between dairy and beef breeds or changes in heifer and dry cow management.

*Effects of maternal nutritional status.* Maternal nutrition can directly influence the colostrum nutrient composition and yield (Swanson et al., 2008). Studies conducted in sheep demonstrate that both maternal restriction and overnutrition prepartum can affect colostrum IgG concentration and quantity produced (Wallace et al., 2005; Swanson et al., 2008). However, studies in cattle, especially beef cattle which often receive low quality
forage during late gestation, focus on the effects of maternal restriction on dam performance. Corah et al. (1975) observed that nutrient restricted dams during the last 100 d prepartum produced less milk than dams whose nutrient requirements had been met. They also reported greater calf mortality and incidence of scours in calves born to nutrient restricted cows than those calves from control dams (Corah et al., 1975). They hypothesized that maternal nutrient restriction affected the colostrum Ig concentrations or Ig absorption by the calf (Corah et al., 1975). Another study nutrient restricted cows during the last 90 d of gestation and reported that nutrient restricted dams had 8.1% more IgG concentrated in their colostrum (Hough et al., 1990). Despite this, calves which received colostrum from nutrient restricted cows had numerically less concentrations of circulating IgG; suggesting maternal nutrient restriction may affect a component of colostrum involved in the absorption of IgG (Hough et al., 1990).

Some studies focused on shorter windows of nutrient restriction at the end of gestation. McGee et al. (2006) fed beef dams either straw, considered to be a nutrient restricted diet, or grass silage ad libitum during the last 15 d prepartum and observed lower IgG mass produced by those dams under restriction compared with controls. In another study where beef dams were restricted to 70% of NEm requirements for 40 d prepartum, the effects of nutrient restriction on colostrum were not measured; however, there were no differences in calf serum IgG concentration at 12 h postnatal (Moriel et al., 2016). This may imply that concentration of IgG was not influenced by maternal nutrient restriction in this study. Based on the above studies, the timing and duration of maternal nutrient restriction can have varied effects on colostrum produced by the dam and ultimately calf health.
Additionally, colostrum composition may be influenced by maternal plane of nutrition. Following a period of either restricted, control, or high nutritional plane, initiated at d 40 of gestation, Meyer et al. (2011) reported reduced colostrum yield and total colostral butterfat, solids-not-fat, lactose, and protein in primiparous ewes. Similar observations in colostrum yield and composition were reported by Swanson et al. (2008) after establishing the same 3 planes of nutrition on d 50 of gestation in ewe lambs. In another study, ewes were fed to meet ME and CP requirements of maintenance of ewes with twins until the last 3 wk of gestation when they were assigned to either control, meeting 100% of ME and CP requirements, restriction of 50% ME and CP requirements following parturition, or restriction of 50% ME and CP requirements during the last 3 wk of gestation and during lactation (Chadio et al., 2014). Lactose concentrations were reduced in 18 h colostrum samples from those ewes experiencing restriction during gestation and lactation compared with control ewes; however, there was no difference in lactose concentration in 1 wk milk samples (Chadio et al., 2014). Additionally, protein and solids non-fat concentrations were greater in ewes that were only restricted after parturition than ewes restricted during gestation and parturition at 18 h colostrum samples (Chadio et al., 2014). This study did not report colostrum yield, unlike Meyer et al. (2011) and Swanson et al. (2008), but attributed lower growth rates in lambs born to undernourished ewes to likely lower milk production of those ewes (Chadio et al., 2014).

**Effects of maternal vaccinations.** Inoculating the dam with specific antigens prior to parturition elicits an immune response resulting in increasing corresponding antibodies in her circulation. Over the past several years, studies have investigated the transfer of maternal antibodies into colostrum following an elicited immune response and
how it may affect the calf. Studies focusing on vaccinating dams for strains of *Escherichia coli* observed greater transfer of specific antibodies (Gay et al., 1964; Wileman et al., 2010) and reduced mortality due to diarrheal disease (Myers, 1976; Cornaglia et al., 1992) in beef calves from those dams that were vaccinated. Likewise, when dairy cows were immunized 3 to 5 times prepartum against *Cryptosporidium parvum*, they had greater antibody titers in serum and colostrum compared with control cows that were not immunized (Jenkins et al., 1999; Askari et al., 2016). Colostrum from immunized dams was considered to be hyperimmunized and when it was administered to calves (Askari et al., 2016) or immunosuppressed adult mice (Jenkins et al., 1999), there was evidence of passive transfer of specific antibodies and partial protection against *C. parvum*. Saif et al. (1984) reported greater antibody titers to bovine rotavirus in colostrum of dams that were inoculated intramuscularly and intramammarily compared with dams immunized only intramuscularly or to noninoculated controls. Because of potential health benefits to the calf, it is common practice for herds to be managed with a prepartum vaccination program that can be tailored to specific threats or needs of that individual farm (Chase, 2012).

**PASSIVE TRANSFER**

Intrauterine transfer of maternal Ig or antibodies to the fetus does not occur in ruminants and calves are born with a naive immune system (Besser and Gay, 1994; Cortese, 2009; Singh et al., 2011). In order to obtain passive immunity or passive transfer, the calf must ingest an adequate amount and quality of colostrum and then also absorb the maternal Ig before intestinal closure occurs (Matte et al., 1982; Besser and
Gay, 1994; Singh et al., 2011). Inadequate absorption of colostral antibodies leads to a condition termed failure of passive transfer which can lead to increased morbidity and mortality in the first weeks of life (Weaver et al., 2000; Furman-Frutchak et al., 2011). Failure of passive transfer can also have long term effects by increasing morbidity and mortality during the preweaning period for both beef and dairy calves and can decrease feed lot performance in beef calves (Wittum and Perino, 1995) and ADG and lactational performance of dairy heifers (Robison et al., 1988; DeNise et al., 1989).

Prenatal absorption of nutrients in the small intestine is limited to active transport of carbohydrates, amino acids, and proteins from ingested amniotic fluid (Pacha, 2000). Shortly following birth important transport of macromolecules obtained through colostrum consumption must occur. Two pathways for macromolecule transport, micropinocytosis and receptor-mediated transcytosis, exist in the small intestine of the neonatal calf (Pacha, 2000). The prominent pathway for Ig transport throughout the small intestine is micropinocytosis during the period before intestinal closure at about 24 h of age, whereas receptor-mediated transcytosis is utilized at a lesser extent and only during the first few hours postnatal (Jochims et al., 1994). The functionality is attributed to presence of an apical tubular system and large vacuoles (Pacha, 2000) due to the ability to peristaltically force Ig from the lumen into circulation (Jochims et al., 1994). A close relationship exists between the maturation state and absorptive function of the small intestine (Pacha, 2000); therefore, preterm calves may experience differed absorptive function of macromolecules when compared with full-term calves (Bittrich et al., 2004).

Neonatal calves have 3 segments of small intestine, duodenum, jejunum, and ileum. Each segment differs in contribution to Ig transport. The location of greatest Ig
absorption is in the caudal portion of the small intestine (James et al., 1979; Staley and Bush, 1985; Jochims et al., 1994). Although some transport of Ig occurs in the duodenum (Jochims et al., 1994), this may be negligible especially when compared with the rate of uptake of the middle and lower segments (James et al., 1979). The initial rate of absorption of Ig following colostrum intake is relatively high but is then followed by rapid cessation of Ig transport, termed intestinal closure (Stott et al., 1979; Weaver et al., 2000). The apparent termination of Ig transport is attributed to further maturation of small intestine cells or enterocytes, disappearance of apical tubular system (Stott et al., 1979), and the emergence of lysosomes in the cells by 24 h after birth (Jochims et al., 1994). Interestingly, some studies suggest that a delay in initial colostrum ingestion may also delay the timing of intestinal closure past 24 h after birth (Stott et al., 1979). However, the total period of absorption of Ig is reduced considerably when compared with administering colostrum following birth (Stott et al., 1979). Additionally, limited Ig absorption occurs after a first colostrum feeding at 24 h postnatal (Matte et al., 1982; Hadorn et al., 1997).

Colostrum-Related Factors

As previously mentioned, the eventual intestinal closure prevents further absorption of Ig by the neonatal calf; therefore, the acquisition of an adequate amount of colostrum is time sensitive. This is especially apparent in work done by Matte et al. (1982) in dairy calves where they reported an Ig absorption of 65.8%, 46.9%, 11.5%, 6.7% and 6.0% when first feeding of colostrum was administered to calves at 6, 12, 24, 36, or 48 h respectively after birth. There is a notable decline in macromolecule
absorption occurring between 12 and 24 h postnatal (Matte et al., 1982; Osaka et al., 2014). Therefore, it has previously been recommended that dairy calves should receive 2 L of colostrum during the first 4 h of life for maximum Ig absorption prior to intestinal closure (Stott et al., 1979). However, after notable inadequate passive transfer following feeding of 2 L (Besser et al., 1991) and reported voluntary consumption of 3 L of colostrum when offered to dairy calves (Hopkins and Quigley III, 1997), the suggested amount increased to 3 L within the first 6 h to prevent failure of passive transfer (Godden et al., 2009; Osaka et al., 2014). Therefore, it has previously been recommended that dairy calves must have at least a serum total protein level of 5.2 g/dL (Weaver et al., 2000) or serum IgG concentrations of 10 mg/mL by 48 h after birth (Yang et al., 2015) for successful passive transfer. However, a more recent study suggest that cut-offs for dairy calves should be increased to 5.7 to 6.0 g/dL of serum total protein for optimal calf health (Todd et al., 2018).

Above recommendations for dairy calves have been modified to apply to beef calves as well. Generally, beef cows do not produce as much colostrum as a dairy cow and may fail to meet the 3 L volume target (McGee and Earley, 2019). Despite this, the concentration of IgG in beef cow colostrum is typically greater than dairy cow colostrum (Guy et al., 1994; McGee et al., 2005; McGee and Earley, 2019), allowing for a lower volume of colostrum, 2 rather than 3 L, to be ingested that would contain roughly an equivalent Ig mass that is adequate for passive transfer (McGee and Earley, 2019). In one study, suggested cut-offs for optimal beef calf health were proposed to be set at 5.6 to 6.1 g/dL of serum total protein (Todd et al., 2018).
Although calves may receive the recommended amount of colostrum, failure of passive transfer may still occur if the colostrum is of poor quality. The concentration of maternal antibodies or Ig present in the colostrum determines the quality (Langel et al., 2015). Subsequent consequences of inadequate quality colostrum are apparent in altered calf health and intestinal development (Langel et al., 2015; Yang et al., 2015).

Studies comparing the feeding of fresh colostrum and diluted colostrum, or milk and milk-based formula have shown striking differences in the intestinal development and immune status of the neonate. Calves that receive fresh colostrum following birth had greater villus length and width, crypt depth, and mucosal thickness in all segments of the small intestine (Yang et al., 2015) along with greater epithelial cell proliferation in the duodenum (Blättler et al., 2001) than those calves that were fed milk or milk-based formula. Differences in intestinal development were largely attributed to the absence of bioactive elements that are otherwise supplied in colostrum (Blättler et al., 2001; Kindlein et al., 2008). Additionally, the absence or reduced concentrations of serum Ig are directly related to Ig content of the meals the calf received after birth (Yang et al., 2015) and were reflected in the increased rates of calf morbidity and mortality in low serum Ig calves (Kindlein et al., 2008; Langel et al., 2015; Yang et al., 2015). Although high quality colostrum can prevent the above complications, Besser et al. (1985) have shown a reduced efficiency of absorption with an increased concentration of colostral Ig. This may suggest a threshold for Ig transport or a saturated transport mechanism exists in the neonatal calf (Besser et al., 1985). Despite this, Besser et al. (1985) do agree that calves fed colostrum with greater Ig concentrations will obtain greater serum Ig concentrations.
Calf-Related Factors

Dystocia can be regarded as a stressful event not only for the dam, but also for the neonate as it transitions to extrauterine life. Metabolic disturbances as a result of dystocia, such as respiratory acidosis and hypoxia, may reduce Ig absorption by the neonate (Besser et al., 1990; Weaver et al., 2000; Quigley et al., 2002; Sangild, 2003). However, Tyler and Ramsey (1991) observed that there was no difference in peak Ig absorption between hypoxic and normoxic calves, only an extension in time prior to intestinal closure in hypoxic calves. During induced hypoxic conditions in fetal sheep, growth retardation in the fetal small intestine was reported (Avila et al., 1989). This was attributed to reduced blood flow to the gut and decreased small intestine growth (Avila et al., 1989). Avila et al. (1989) suggest that neonates with reduced small intestinal development may be more susceptible to infections and diarrhea. Therefore, it is unclear to what extent altered metabolic status has directly or indirectly on both Ig absorption and subsequent health in neonatal calves.

Circulating cortisol concentrations or birth stress may influence the intestinal absorptive capacity of Ig (Sangild, 2003). Hough et al. (1990) demonstrated that elevated cortisol concentrations following birth in lambs is necessary for maximum Ig absorption. Similarly, a study done with neonatal dairy calves showed stimulatory effects of glucocorticoids on intestinal development when administered after birth twice daily at feeding (Sauter et al., 2004). Although elevated concentrations of cortisol at birth are needed for intestinal development and ultimately Ig absorption, potentially prolonged concentrations may induce enterocyte maturation prematurely and limit the window of Ig
absorption (Sangild, 2003). Conversely, elevated cortisol at birth in preterm calves may mitigate Ig absorption (Sangild, 2003) by promoting intestinal development of their rather immature small intestine (Bittrich et al., 2004).

Calves from dystocic births are likely to have increased prevalence of failure of passive transfer because they are weaker and less vigorous after birth (Odde, 1988; Weaver et al., 2000; Furman-Fratczak et al., 2011; Pearson et al., 2019). Reduced vigor coincided with negative influences on serum Ig concentration and ultimately greater incidence of failure of passive transfer (Odde, 1988; Weaver et al., 2000; Furman-Fratczak et al., 2011; Pearson et al., 2019). Logically, calves that lack the ability to stand in a timely fashion have a prolonged time to suckle therefore, reducing the amount of colostrum ingested in the window of Ig absorption.

**Maternal-Related Factors**

Although there is evidence that suggests maternal nutrition during gestation can influence colostrogenesis, indirectly affecting the calf postnatally, reduced fetal nutrient supply during gestation may alter fetal development which can have lasting implications on the offspring throughout its life (Wu et al., 2006; Meyer and Caton, 2016; Osorio, 2020). Inadequate development of the fetal small intestine that results in alterations in the organ’s function, morphology, or physiology at birth may influence the neonate’s ability to absorb necessary macromolecules from colostrum (Wu et al., 2006; Duarte et al., 2013; Meyer and Caton, 2016).

Maternal nutrient restriction during early to mid-gestation had no effect on fetal bovine intestinal mass (Meyer et al., 2010). However, greater cell proliferation and
intestinal vascularity were reported in fetuses from nutrient-restricted and then realimentated dams (Meyer et al., 2010). These responses suggest an increase in efficiency of the fetal intestine and nutrient usage during maternal nutrient restriction, which after realimentation lead to greater intestinal growth and vascularization (Meyer et al., 2010). Others investigated the effects of maternal nutritional plane during mid to late gestation on the development of gastrointestinal tract in bovine fetus (Duarte et al., 2013). Longer small intestines and greater villi lengths were observed in calves from nutrient-restricted dams (Duarte et al., 2013). Duarte et al. (2013) in conjunction with the results from Hammer et al. (2011), which found that lambs from nutrient-restricted ewes had greater circulating IgG concentrations than their contemporaries, suggesting that neonates born to nutrient-restricted dams may have small intestines that are more efficient in macromolecule absorption. Differences in the fetal small intestinal response in these studies may be due to timing of maternal nutrient restriction occurring at different developmental stages of the fetus.

Studies using sheep as a model to investigate the effects of maternal restriction have also been conducted. Trahair et al. (1997) reduced fetal nutrient supply during the first half of gestation via maternal nutrient restriction and carunclectomy. At 90 d gestation, reduced gastrointestinal mass was observed in restricted fetuses when compared with control fetuses; however, fetal weight was not different (Trahair et al., 1997). Additionally, reduced intestinal circumference and crypt depth in restricted fetuses was reported (Trahair et al., 1997). Trahair et al. (1997) suggested that the diminishment of the intestinal surface area rather than the length of the intestine would have more detrimental effects on intestinal efficiency. However, in another study in which ewes
were subjected to 1 of 3 nutritional planes (restricted, control, or high) beginning at d 40 of gestation, Meyer et al. (2013) observed no differences in small intestinal mass or villus morphology among 20-d old lambs raised independently of the dam following birth. Despite this, maternal plane affected small intestinal cellularity, proliferation, and vascularization where lambs born to high plane of nutrition ewes had greater amounts of intestinal DNA and total intestinal cells, whereas lambs from restricted ewes had increased capillary area (Meyer et al., 2013). This may suggest that small intestines increase capillary area to attempt to compensate postnatally for their dams’ nutritional status during gestation (Meyer et al., 2013). Although there are species differences in fetal developmental stages, maternal nutritional status appears to affect small intestinal growth and development that may be apparent in postnatal life (Meyer and Caton, 2016).

Additionally, maternal nutrition may influence the developing immune system and tissues that may hold repercussions later in the offspring’s life. A short-term energy restriction during the last 40 d of gestation in beef cattle resulted in reduced serum titers following vaccination against bovine respiratory disease in calves born to restricted dams (Moriel et al., 2016). Furthermore, Chadio et al. (2014) observed increased expression of toll-like receptor in the thymus of female lambs that were born to restricted ewes. Toll-like receptors are essential for the proper signaling and control of an innate and adaptive immune response (Chadio et al., 2014). Chadio et al. (2014) suggested that the upregulation of toll-like receptors, particularly in the thymus, serves to compensate for undernutrition during the prenatal period. Both studies suggest maternal nutritional status can influence the development of the fetal immune system and possibly leave offspring more susceptible later in life.


*Environmental Factors*

There is also evidence that suggests heat or cold stress may affect Ig absorption by the neonatal calf. Maternal heat stress during late gestation has been reported to compromise passive transfer in neonatal calves (Donovan et al., 1986; Monteiro et al., 2014; Strong et al., 2015; Almoosavi et al., 2020). In one study, Monterio et al. (2014) observed that dairy calves born to heat stressed dams had lower IgG absorption than calves born to cool treatment dams. It was hypothesized that heat stressed calves had a decreased ability to absorb IgG or that there was reduced intestinal contact surface area with colostrum because of lower birth weights in heat stressed calves (Monteiro et al., 2014). Additionally, colostrum from the heat stressed dairy cows was administered to calves from the cool treatment and a delay in calf humoral immune response was reported, suggesting that other colostral components such as cytokines and growth and metabolic factors could have been altered by heat stress (Monteiro et al., 2014). Almoosavi et al. (2020) observed similar results where dairy calves from heat stressed dams had lower serum IgG and apparent efficiency of absorption than calves from cool treatment dams. Another study reported dairy calves from heat stressed dams had altered innate immune function during the first month of life despite calves receiving the same amount of pooled colostrum (Strong et al., 2015). This indicates that the adverse effects of maternal heat stress extend farther than colostrum quality to the physiological state of the calf.

One study suggests that the Ig concentration of dairy cow colostrum is affected by season where the greatest concentrations are seen in cows that calve during the winter
This may suggest that calves during winter would have greater serum Ig because of potentially greater concentrations of colostral Ig. However, Olson et al. (1980b) observed a decreased rate of Ig absorption and lower serum IgG concentrations up to 18 h after birth in dairy calves that experienced cold stress conditions. They hypothesized that the reason for no difference on the net absorption of colostral Ig was due to the short duration of the induced cold stress, where calves were removed from cold water baths after their body temperature decreased 10°C, about 125 min (Olson et al., 1980a, b). Therefore, calves that are born into prolonged cold stress conditions may have decreased Ig absorption that potentially leads to failure of passive transfer. Olson et al. (1980a) also reported that the cold stressed calves showed muscle weakness and difficulty in muscle coordination for standing and suckling. This suggests that abnormal vigor behaviors from cold stress may contribute to reduced Ig consumption and absorption.

**SUMMARY AND CONCLUSIONS**

In conclusion, the transition from intra- to extrauterine life is one of the most challenging events to a neonate. The normal conversion to self-reliance for thermoregulation, metabolism and endocrine changes, coordination of vigor behaviors, and a naive immune system provides its own difficulties neonatal calves must face. Furthermore, there must be a sufficient amount of colostrum available to meet the neonate’s nutrient demands and provide adequate passive transfer. Maternal, environmental, or neonatal conditions may exacerbate the transition to extrauterine life and effect neonate survivability. However, limited research has been conducted in beef
cattle investigating how perinatal nutrient availability and neonatal vigor may be affected by maternal nutrient status and calving season and how this may impact the transition to postnatal life in beef calves.
CHAPTER 2

MATERNAL NUTRIENT RESTRICTION DURING LATE GESTATION DECREASES ColoSTRUM YIELD, REDUCES CALF VIGOR, AND ALTERS NEONATAL BLOOD CHEMISTRY AND HEMATOLOGY IN BEEF CATTLE

ABSTRACT

We hypothesized that late gestational nutrient restriction (NR) would reduce colostrum yield and impair offspring transition to postnatal life. Primiparous, fall-calving crossbred beef heifers (BW: 451 ± 28 [SD] kg; BCS: 5.4 ± 0.7) were individually-fed either 100% (control; CON; n = 12) or 70% (NR; n = 13) of NASEM metabolizable energy and metabolizable protein requirements for maintenance, pregnancy, and growth from d 160 of gestation to parturition. Calves were reared naturally by their dams and monitored for times from birth to first sternal recumbency, attempt to stand, and stand; vigor scores (1 = very weak, 5 = extremely vigorous) were assigned at 2, 5, 10, and 20 min of age. Total colostrum from one rear quarter was collected pre-suckling. Calf jugular blood was obtained at 0 (pre-suckling), 6, 12, 24, and 48 h postnatally to determine blood chemistry and hematology. Data were analyzed with the fixed effects of treatment (single point) or treatment, hour, and their interaction (over time, using repeated measures). Calving date was a fixed effect; calf sex was included when P < 0.25. Heifers fed CON had greater (P = 0.04) colostrum weight and volume than NR dams. Although gestational nutrition did not affect (P ≥ 0.46) gestation length or calf birth weight, calves born to CON heifers tended to have faster (P = 0.09) times to attempt to stand and had faster (P = 0.02) times to stand. Calves from CON heifers had greater (P
= 0.05) 20 min vigor scores. The treatment × hour interaction ($P \leq 0.10$) affected total protein, globulin, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), creatinine, creatine kinase, red blood cells (RBC), hemoglobin, hematocrit, sodium, anion gap, and potassium. Total protein and globulin at 6 to 48 h were greater ($P \leq 0.02$) in NR calves. Calves from NR heifers had greater ($P < 0.08$) GGT at 6, 12, and 48 h and greater ($P \leq 0.07$) AST at 0 to 24 h. Creatinine at 24 h and creatine kinase at 6 to 24 h were greater ($P < 0.04$) in NR calves. Hematocrit from 6 to 24 h and RBC and hemoglobin at 6 and 12 h were greater ($P \leq 0.09$) in CON calves. Sodium from 0 to 48 h and anion gap at 6 h were greater ($P < 0.09$) in CON calves. Potassium was greater ($P = 0.03$) at 0 h in NR calves. There was a main effect of treatment for chloride, which was greater ($P = 0.08$) in CON calves. These data indicate that heifers nutrient restricted during late gestation have reduced colostrum yield and less vigorous calves that may experience more trauma at calving, which may influence postnatal calf survival.

**INTRODUCTION**

Survival through the neonatal period poses one of the greatest challenges to beef calves and depends on a successful transition to extrauterine life and an adequate postnatal nutrient supply provided by their dam (Danijela, 2015). Following birth, calves must be vigorous to quickly stand and ingest colostrum for necessary nutrients and passive transfer (Weaver et al., 2000). Insults to the maternal nutrient status during gestation can alter nutrient delivery to the fetus which can impair fetal growth and development (Caton and Hess, 2010), neonatal vigor (Dwyer et al., 2003), and preweaning health (Cooke, 2019). Additionally, previous data in sheep suggest that
maternal nutrient restriction can reduce colostrum yield and composition (Swanson et al., 2008; Meyer et al., 2011). Consequently, maternal nutrient restriction may affect perinatal nutrient availability which can exacerbate the challenge of transitioning to extrauterine life and ultimately decrease neonatal survival.

During the last third of gestation, nutrient requirements of beef cows increase rapidly to match the exponential increase in demand for nutrients by the fetus for growth and maturation of organs in preparation for postnatal life (NASEM, 2016). However, beef dams may often not meet their requirements due to poor forage quality and quantity that is available to them. Additionally, primiparous dams may experience more competition for the nutrients that are available when attempting to meet both fetal demands and her own growth requirements. Therefore, we hypothesized that late gestational nutrient restriction in primiparous beef cattle would reduce perinatal calf nutrient supply and impair neonatal vigor. Our objectives were to determine the effects of maternal nutrient restriction during late gestation on colostrum yield, fetal growth, calf vigor, and neonatal calf metabolic and hematologic measures.

MATERIALS AND METHODS

The University of Missouri Animal Care and Use Committee approved animal care and use in this study (Protocol #9877).

Animal Management and Diets

Twenty-six single-sired fall-calving Sim-Angus Herford crossbred beef heifers (initial BW = 451 ± 28 [SD] kg, initial BCS = 5.4 ± 0.7) bred by AI to a single Angus
sire were allocated by BW, BCS, fetal sex, and expected calving date to 1 of 2 late gestation nutritional plane treatments from d 160 of gestation to parturition. Control (CON; n = 12) heifers received 100% of NASEM metabolizable energy and metabolizable protein requirements for maintenance, pregnancy, and growth, whereas nutrient restricted (NR; n = 13) heifers received 70% of NASEM metabolizable energy (ME) and metabolizable protein requirements (MP).

Nutrient requirements were based on NASEM (2016) and Freetly et al. (2005) recommendations, using an expected calf birth weight of 34 kg and maternal ADG of 0.36 kg/d. Metabolizable energy for maintenance was based on data for heifers in confinement (0.138 Mcal ME/kg BW\(^{0.75}\); Freetly and Hales, personal communication). Metabolizable energy for conceptus was based on Freetly et al. (2005). Metabolizable energy for gain was 4.9 Mcal/d based on NASEM (2016). MP requirements for maintenance, conceptus, and gain were based on equations from NASEM (2016). Dam BW were recorded every 21 d (2-day BW every 42 d). Nutrient requirements were adjusted weekly based on previous individual DMI, most recent dam BW, and day of gestation.

From d 160 to d 265 of gestation, diets were based on chopped sorghum sudan hay (1.74 Mcal/kg ME, 6.66% CP, 72.0% NDF, 52.8% ADF; DM basis). From d 266 of gestation to parturition, diets were based on chopped endophyte-infected tall fescue-based hay (1.90 Mcal/kg ME, 7.22% CP, 65.1% NDF, 43.2% ADF). Sorghum sudan and tall fescue hays were offered at 1.3% and 1.7% BW on DM basis, respectively, with an additional 20% estimated waste. Hay refusals were weighed back 2 times per week, on the same days each week, and subsampled for DM analysis. Individual hay DMI was then
calculated by subtracting refusal DM from offered DM and was used to estimate subsequent hay intake. Switching to tall fescue hay at d 266 of gestation allowed for less supplement needed to meet nutritional treatments than the continued use of sorghum sudan. Following parturition, treatments were terminated, and all dams were fed to meet nutrient requirements of lactation, maintenance, and growth using tall fescue-based hay.

Based on expected individual hay intakes, heifers were supplemented with whole corn (2.94 Mcal/kg ME, 7.25% CP, 8.31% NDF), dried distillers’ grains with solubles (DDGS; 2.97 Mcal/kg ME, 29.0% CP, 31.1% NDF, 14.9% ADF), and soyhull pellets (2.67 Mcal/kg ME, 11.2% CP, 59.9% NDF, 42.9% ADF) to meet their assigned nutritional treatment. Supplement for each heifer was formulated individually using corn, DDGS, and soyhull pellets, and then weighed individually. The corn:soyhull and DDGS:soyhull ratios remained similar within treatment.

Core samples of sorghum sudan and tall fescue bales were submitted to Cumberland Valley Analytical Services (CVAS, Inc., Waynesboro, PA) for wet chemistry analysis prior to being ground and fed to heifers. The wet chemistry analysis included dry matter (adapted from Goering et al., 1970 and modified to 105°C for 3 hr per National Forage Testing Association recommendations, 2002), crude protein (AOAC, 990.31), ADF (AOAC, 973.18), NDF (Van Soest et al., 1991), minerals (AOAC, 985.01), and values of TDN and ME were then calculated using the value of ADF. Samples of corn, DDGS, and soyhull pellets were also submitted to CVAS for wet chemistry analysis of DM (AOAC, 930.15) and CP (AOAC, 990.31) between every new batch of feedstuffs. Values of ME from NASEM (2016) were used in diet formulations for the 3 feedstuffs used in supplement.
Heifers were housed in 12 partially-covered 3.7 × 15.8 m pens (n = 2 to 3/pen) with concrete floors bedded with sawdust. Dams were penned together by treatment and randomly allocated to the 12 pens. Electronic feeding gates (Calan Broadbent Feeding System, American Calan, Northwood, NH) allowed for individual feeding of each heifer. Heifers were acclimated to electronic gate feeding system for ≥ 15 d prior to treatment initiation. Supplement was fed every morning at approximately 0700 h in a feed pan to prevent wastage. Equal amounts of hay for each individual heifer were weighed and fed every morning (0730) and evening (1900). Dams had ad libitum access to water and a mineral lick block (Big 6 Mineral Salt, Compass Minerals America Inc., Overland Park, KS).

**Calving Monitoring and Data Collection**

Heifers remained in partially-covered pens during the peripartum period. Overhead lighting in the barn and LED string lights allowed for continuous monitoring of heifers and calves throughout the night. Beginning on d 274 of gestation, heifers were closely monitored 24 h a day by trained personal to detect when heifers were in stage II of parturition by walking down aisles located in the front and back of the pens at least once every 15 to 30 min. Once stage II was detected the heifer was continuously monitored to allow for all data collection. Delivery duration was determined by subtracting the time of first expulsion of fetal feet and time of birth (expulsion of entire calf, including all 4 legs). Calving assistance was provided (CON n = 2; NR n = 4) in the pen or the dam was moved to the chute for delivery assistance if the calf was presenting abnormally, there was a prolonged duration since first appearance of fetal membranes, or
if progress slowed during contractions. A calving difficulty score ranging from 1 to 5 was assigned with 1 indicating no assistance, 2 an easy pull, 3 a mechanically assisted pull, 4 an abnormal presentation, and 5 indicating a caesarian-section.

Calf vigor behaviors were recorded, which included time of sternal recumbency (time when calf first achieves sternal recumbency), time of attempt to stand (time when calf first supports weight on hind legs while balanced on the front knees for at least 5 seconds) and time of successful stand (time when calf is standing on all 4 legs for at least 5 seconds). The time of birth was then subtracted from the time of each vigor behavior to determine the time to display each behavior in minutes. The number of failed attempts to stand (calf attains the posture of attempt to stand but falls) were also recorded. A vigor score was also assigned to calves at 2, 5, 10, and 20 min of age on a scale of 1 to 5 (adapted from Matheson et al., 2012) as shown in Table 2.1.

Following the successful standing of the calf but prior to suckling, calves were removed from the dam, processed, and measured (0.9 ± 0.3 h [SD] of age). Calf sex was recorded. Calf birth weight was measured using an electronic hanging scale with a calf sling placed under the abdomen of the calf and was lifted until all 4 feet were off the ground. Using a flexible measuring tape, calf body size was recorded. Heart girth was measured as the body circumference immediately posterior to the shoulders and front legs, perpendicular to the spine. Abdominal girth was measured by placing the tape measure around the abdomen over the umbilicus, perpendicular to the spine. Flank girth was measured as the body circumference immediately anterior to the hooks, perpendicular to the spine. Shoulder to rump length was measured along the spine from front of the shoulder blades to end of the tailhead. Cannon bone circumference was
measured at the smallest circumference of a rear cannon bone. Cannon bone length was measured from center of the knee to between the declaws along the back of a calf’s front cannon bone. Coronet circumference was measured at the hairline (line between hoof and hair) of a front leg. Height at the shoulder was recorded as height at the dorsal edge of the shoulder, with the head up and feet set square, using an aluminum height measuring stick. As an indicator of calf shape, calf ponderal index was calculated using the equation

\[
\text{ponderal index} = \frac{\text{calf birth weight (kg)}}{\text{shoulder to rump length (m)}}
\]

Calf heart girth:length ratio was calculated as a second indicator of shape, using the equation

\[
\text{heart girth:length} = \frac{\text{heart girth (cm)}}{\text{shoulder to rump length (cm)}}
\]

Each calf was given an ear tag for visual identification, had their umbilicus sprayed with chlorhexidine solution, and was administered a Bo-Se injection (5.5 cc/kg BW) subcutaneously.

**Colostrum Collection**

After the successful standing of the calf but prior to suckling, dams were moved into a chute for colostrum collection (50.5 ± 17 min [SD] postpartum). Through visual assessment and palpation of the 2 rear quarters, the most full rear quarter was determined by at least 2 trained personnel. Without administration of oxytocin, colostrum from the most full rear quarter was completely hand-milked to determine total yield from that quarter. This method has been previously used in our lab to allow for natural suckling of the calf without failure of passive transfer of the calf and still allowed for differences to be observed in a previous parity study (Meyer et al., unpublished data). Colostrum volume and weight were recorded. Following colostrum collection and processing of the
calf, the cow-calf pair was returned to their pen to allow for natural rearing of the calf by its dam.

**Neonatal Blood Collection**

Calf jugular blood samples and rectal temperature were collected at 0 (prior to suckling but after standing), 6, 12, 24, and 48 h postnatally (0.62 ± 0.3 h, 6.03 ± 0.2 h, 12.08 ± 0.2 h, 24.20 ± 0.2 h, 48.09 ± 0.3 h, respectively). Blood samples obtained at 0 and 48 h were collected into 4 collection tubes (2 Vacutainer serum collection tubes containing no additives [10 mL draw; Becton Dickinson, Franklin Lakes, NJ] and 1 Monoject plasma collection tube containing 0.10 mL of 15% K₃EDTA [10 mL draw; Covidien, Mansfield, MA]). For samples collected at 6, 12, and 24 h, only 2 collection tubes were used (1 Vacutainer serum collection tubes containing no additives [10 mL draw; Becton Dickinson, Franklin Lakes, NJ] and 1 Monoject plasma collection tube containing 0.10 mL of 15% K₃EDTA [10 mL draw; Covidien, Mansfield, MA]). Following collection, all tubes were inverted several times. Plasma tubes were immediately placed on ice while serum tubes were allowed to clot prior to being placed on ice. Between 1 and 8 h after collection, all samples were centrifuged at 1,500 × g at 4°C for 30 min. Plasma and serum were transferred into multiple 2 mL microcentrifuge tubes and stored at -20°C until analysis.

**Serum Chemistry and Hematology Analysis**

One aliquot of serum and plasma from each sampling time was refrigerated and then transported on ice to the University of Missouri Veterinary Medical Diagnostic
Clinical Pathology Laboratory for a complete chemistry profile analysis and blood cell count. Serum glucose, urea nitrogen, creatinine, globulin, albumin, total protein, sodium, potassium, chloride, phosphorus, calcium, magnesium, bicarbonate, anion gap, direct bilirubin, total bilirubin, aspartate aminotransferase, gamma-glutamyl transpeptidase, and creatine kinase concentrations were determined using a Beckman Coulter AU480 Chemistry Analyzer (Beckman Coulter, Inc., Brea, CA). Internal quality control and verification of performance within specific CV’s are conducted daily. Upon delivery, samples were analyzed at the same time through the instrument’s completely automated process. White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count were determined from neonatal plasma using a Sysmex XT-2000iV (Sysmex Nordic Aps Filial Sverige, Landskrona, Sweden). Fluorescence flow cytometry used to determine WBC, MCV, MCH, and MCHC. DC sheath flow to determine RBC, hematocrit, and platelet count. Cyanide-free SLS-hemoglobin methods to determine hemoglobin. Samples were analyzed within 24 h of collection.

**Circulating Metabolite Analysis**

Concentrations of calf serum NEFA were analyzed using a modified procedure of the NEFA C kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan), based on the acyl-CoA synthase-acyl-CoA oxidase method. Calf plasma triglycerides were analyzed using a commercially available Infinity Triglycerides kit (Thermo Scientific) based on the glycerol-3-phosphate oxidase methods. For each assay, samples were read in duplicate in
96-well plates on a microplate reader (Biotek Synergy HT, Biotek Instruments Inc., Winooski, VT). Plates for determination of NEFA were read at 550 nm whereas, plates for plasma triglycerides were read at 500 and 660 nm and final results were calculated by subtracting the 660 nm reading from the 500 nm reading. The intraassay and interassay CV for serum NEFA were 3.83% and 4.46%, respectively. The intraassay and interassay CV for plasma triglycerides were 3.30% and 1.05%, respectively.

**Statistical Analyses**

One heifer was removed from the study due to late gestational abortion resulting in 12 CON and 13 NR heifers. Colostrum yield from one CON cow was not included in analysis due to being nursed prior to parturition by another calf. Vigor score at 2 min was not included for 1 NR calf because it was born through the fence into an adjacent pen and required human intervention to be put back into its respective pen. This calf’s time to sternal recumbency was excluded from analysis along with 2 others (CON n = 1; NR n = 1) that were sternal before final expulsion of hind feet and technical time of birth. The only vigor measures recorded from calves that had a calving difficulty score of 4 (NR n = 3) included time to stand and 20 min vigor score because calves were pulled in the chute and likely disrupted early vigor behaviors. Vigor data from calves with a calving difficulty of 2 or 3 (CON n = 2; NR n = 1) were included in analysis because assistance was provided in the pen and no further intervention was provided following the birth of the calf. In order for blood chemistry, hematology, and rectal temperature data to be included in over time analyses, data from 4 of the 5 time points were required (3 animals
missing one time point for rectal temperature and 1 missed time point for hematology).

Table 2.2 depicts the final number of animals and samples used in analysis.

Colostrum yield, calf size, and calf vigor measures were analyzed using the MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC) with late gestational nutritional plane as a fixed effect and animal as the experimental unit. For blood chemistry, calf hematology, and rectal temperature over time, late gestational nutritional plane, hour, and their interaction were considered fixed effects. These were considered repeated measures using the majority best-fit covariance structure (based on AIC, BIC, and BICC) specific for each variable (chosen from unstructured, compound symmetry, heterogeneous compound symmetry, autoregressive, and heterogeneous autoregressive). Vigor score data and calving difficulty score were analyzed using a cumulative logistic regression model with the GLIMMIX procedure in SAS 9.4 with late gestational nutritional plane as a fixed effect. For all measures, either date of study initiation (colostrum analyses only) or date of calving (all calf measures) was included as a fixed effect; calf sex was also included as a fixed effect when $P \leq 0.25$. Main effects and interactions were reported when $P \leq 0.05$ with tendencies considered when $0.05 < P \leq 0.10$. In the absence of interactions, main effects of season are reported. Means were separated using least significant difference and considered different when $P \leq 0.05$.

**RESULTS AND DISCUSSION**

*Colostrum Yield*

Late gestational nutritional plane affected ($P = 0.04$) colostrum yield, where colostrum volume and weight from nutrient restricted dams was 40% less compared with
CON dams. Colostrum volume and weight were greater from CON heifers (Table 2.3).
To our knowledge, this is the first report on colostrum production in primiparous beef cattle following late gestational nutrient restriction.

Although the energy demands for mammary gland development in heifers appears to be minimal (Ferrell et al., 1976), gestational nutrient status can influence the gland’s development and subsequent colostrum or milk production. Studies investigating maternal nutritional plane in sheep agree with results from the present study where reduced colostrum weight and volume at 3 h postpartum was observed in primiparous ewes that were nutrient restricted (Swanson et al., 2008; Meyer et al., 2011). Additionally, Kennedy et al. (2019) reported a tendency for greater colostrum weight produced by multiparous beef cows supplemented with DDGS while consuming low quality forage during the final 10 wk of gestation than non-supplemented dams. Results from the current study indicate that maternal nutrient intake can impact colostrum yield. Differences between this study and the data reported by Kennedy et al. (2019) may be related to use of primiparous dams and longer duration of nutritional treatment in the current study.

Reasons for reduced colostrum yield in nutrient restricted dams may be due to altered mammary development, endocrine status, or mammary blood flow. Swanson et al. (2008) reported that restricted ewes when compared to controls had reduced mammary gland weight but greater alveolar area. However, Neville et al. (2013) observed no difference in mammary gland weight or alveolar area at parturition between nutrient restricted and control ewes, but by d 20 of lactation, nutrient restricted ewes had less alveolar numbers than control ewes and maintained lower lactational performance.
(Meyer et al., 2011). In both studies, they hypothesized that maternal nutrition also may have affected the endocrine status of the dam which influenced mammary development and production (Swanson et al., 2008; Neville et al., 2013). Previously it has been reported that increased circulating progesterone concentrations (Dwyer et al., 2003) and a delay in decreasing concentrations of progesterone prior to parturition (Mellor et al., 1987) in nutrient restricted ewes contributed to lower colostrum production.

Additionally, Kennedy et al. (2019) reported greater ipsilateral mammary blood flow at d 245 of gestation in supplemented cows which may have attributed to greater colostrum production by greater nutrient delivery to the mammary gland. Because primiparous dams are still growing, nutrient restricted animals may partition less nutrients to mammary development in attempts to support fetal and their own nutrient demands for growth. These data suggest that maternal nutrition during late gestation can influence mammary gland development and ultimately impair colostrum production in nutrient restricted dams.

Furthermore, previous studies in ewes have reported that maternal nutrient restriction can reduce total colostrum nutrient content in addition to reduced colostrum yield (Swanson et al., 2008; Meyer et al., 2011). It was suggested that the nutrient restricted ewes likely did not have adequate nutrients to support colostrogenesis (Meyer et al., 2011) and was hypothesized that the 30 to 40% reduced amount of total colostral butterfat, protein, lactose, solids not fat, and milk urea nitrogen during the first few days of life would not have met neonatal lamb requirements, potentially leading to poor health and performance (Swanson et al., 2008). Lower colostrum yield and nutrient content may not meet the requirements of the neonate causing alterations in their metabolic status
Gestation Length, Calving Characteristics, and Calf Birth Weight and Size

Late gestational nutritional plane did not affect \( P \geq 0.33 \) gestation length, calving difficulty score, or delivery duration (Table 2.4). Contrary to previous studies in beef cattle that observed late gestational nutrient restriction to reduce gestation length in primiparous (Kroker and Cummins, 1979) and multiparous beef dams (Corah et al., 1975), there was no difference in gestation length between late gestational nutritional planes in the current study. Previous studies observed greater calving difficulty in primiparous heifers than multiparous dams (Vermorel et al., 1989; Lombard et al., 2007). As primiparous dams are still growing themselves during gestation, nutrient restriction may increase calving difficulty by impairing maternal growth and causing fetal-pelvic size disproportion (Kroker and Cummins, 1979; Lombard et al., 2007). However, there is no evidence from the current study to support that nutrient restriction induced fetal-pelvic disproportion in primiparous dams, as there was no difference in the duration of labor or calving difficulty between CON and NR heifers.

Calf birth weight, which is an indicator of final fetal growth, was not affected \( P = 0.72 \) by late gestational nutritional plane in the current study (Table 2.4). This contradicts previous late gestational studies in primiparous beef heifers that received 3 different planes of nutrition (Kroker and Cummins, 1979), were restricted digestible energy (Corah et al., 1975), were managed to achieve differing BCS (used as an indicator for maternal nutrient status; Spitzer et al., 1995), or experienced energy restriction from
d 94 of gestation to parturition (Freetly et al., 2005) in which calf birth weight was impaired by low maternal nutrition or BCS and energy restriction. In another study, Winterholler et al. (2012) observed greater birth weights of calves that were born to cows that were supplemented during late gestation with DDGS when compared to dams that did not receive DDGS supplementation. On the contrary, calf birth weight was not affected when cows were fed 57% of nutrient requirements during the last 90 days of gestation (Hough et al., 1990). In another study, Long et al. (2021) reported no differences in d 265 fetal weights from heifers that were restricted to 70% of energy requirements during late gestation. Additionally, other studies reported no influence of maternal treatment on calf birth weight when multiparous dams were supplemented protein during the last third of gestation (Stalker et al., 2006; Martin et al., 2007; Larson et al., 2009) or when primiparous dams were protein restricted for the last 75 d of gestation (Anthony et al., 1986).

Other calf size measures and indicators of calf shape were also not affected ($P \geq 0.27$) by late gestational nutritional plane in the current study (Table 2.4). The lack of differences in other calf size measures and shape measures may indicate that there was no difference in muscle or organ mass of CON and NR calves. Similar to reports on calf birth weight, there are varying observations of the effects of maternal nutritional treatments on calf size measures. Kennedy et al. (2019) reported no difference in calf heart girth or crown-rump length when dams received supplemental DDGS during late gestation even though calves from supplemented dams had greater birth weight. Additionally, Anthony et al. (1986) observed no effect of prepartum protein restriction on calf body length or heart girth. Another study reported that calf body length and hip width
was impaired when dams were managed on a low plane of nutrition (Kroker and Cummins, 1979).

Discrepancies among studies investigating the effects of maternal nutrient status on fetal growth could be attributed to many factors such as differences in dam age, timing and extent of nutritional restriction, or other environmental factors. The current study was conducted with fall-calving females which likely experienced heat stress during late gestation, which has been reported to reduce calf birth weight in dairy cattle (Dahl et al., 2016). Maternal heat stress could have influenced fetal growth in the current study and contributed to the lack of differences between CON and NR fetal growth. Additionally, previous studies suggest that nutrient delivery to the fetus, through uterine artery blood flow and placental transporters, may be influenced by maternal nutrient status which may cause altered fetal growth (Mordhorst et al., 2017; Kennedy et al., 2019). During late gestation, nutrient supply to the fetus is often prioritized to allow for optimal fetal growth for survival at the expense of maternal body reserves (Bell and Ehrhardt, 2000). In the current study, NR dams weighed 64 kg less and were 2.0 BCS lower than CON dams at calving (Redifer et al., 2021). These data show that NR dams sacrificed their own body reserves to support fetal growth (Redifer et al., 2021). Despite a lack of differences in calf birth weight and size, maternal nutrient restriction may have long lasting implications that become more apparent later in calf performance and preweaning growth due to reduced dam lactational performance or reduced calf feed efficiency (Greenwood and Cafe, 2007; Funston et al., 2010; Robinson et al., 2013).
**Calf Vigor**

Calf vigor measures are presented in Table 2.4 and Figure 2.1. There was no effect \( P = 0.36 \) of late gestational nutritional plane on calf time to sternal recumbency. Calves born to CON heifers tended to have faster \( P = 0.09 \) time to attempt to stand; however, there was no difference \( P = 0.58 \) in the number of failed attempts to stand between calves born to CON or NR heifers. Calves born to CON heifers stood 14 min faster \( P = 0.02 \) than calves born to NR dams. Calves born to CON heifers were more likely to have a greater (more vigorous; \( P = 0.05 \)) vigor score at 20 min of age compared with NR calves (Figure 2.1). There was no difference \( P \geq 0.55 \) in vigor scores assigned at 2, 5, and 10 min of age (Figure 2.1).

Although previous studies have observed reduced neonatal calf vigor related to dystocia at birth (Barrier et al., 2012; Vannucchi et al., 2015), there was no difference in calving difficulty in the present study. However, 23.1% of NR dams had fetal malpresentations where 2 calves had a front leg folded back and 1 calf was backwards, while fetal presentation in all CON births was normal in the current study (Redifer et al., 2021). Previous studies suggest that maternal nutrient restriction during gestation may reduce fetal movement in preparation for parturition and increase the incidence of fetal malpresentations (Kroker and Cummins, 1979; Dwyer et al., 2003) and nutrient restricted ewes required more delivery assistance during lambing (Dwyer et al., 2003). These studies also reported greater duration of labor (Kroker and Cummins, 1979) or interval between twins (Dwyer et al., 2003) in nutrient restricted dams which may be attributed to muscle weakness of nutrient restricted dams. Despite these observations, delivery duration in the current study was not different between CON and NR dams. This may
suggest that preparation of the fetus for normal presentation may be affected by late
gestational nutrient plane and contribute to reduced vigor in NR calves.

Additionally, the differences observed in time to attempt to stand, time to stand,
and 20 min vigor score are likely due to impaired fetal development in calves born to NR
heifers. Kroker and Cummins (1979) reported similar findings in neonatal vigor
compared with the current study among heifers fed to gain 0.75 kg/d BW, to maintain
BW, or to lose 0.5 kg/d BW during late gestation in primiparous beef dams. Calves from
heifers that lost BW took longer to stand and to suckle compared with calves born to
heifers that gain or maintain BW (Kroker and Cummins, 1979). However, Kroker and
Cummins (1979) also reported that maternal treatment affected calf birth weights which
may have contributed to differences in the vigor behaviors. In a study conducted by
Dwyer et al. (2003), they reported smaller birth weight lambs were born to ewes that
were undernourished but no direct effect of maternal nutrient status on neonatal lamb
vigor behaviors. However, Dwyer et al. (2003) did report that smaller lambs took longer
to display normal vigor behaviors than heavier lambs.

Because there was no difference in calf birth weight or size in the current study,
the reduced vigor in NR calves is not likely related to calf size, but potentially reduced
fetal neurodevelopment leading to impairment of vigor and complex movement
coordination as previously suggested in Dwyer et al. (2003). In other species, such as
rats, guinea-pigs, and rabbits, maternal undernutrition during gestation has been reported
to lead to brain network rearrangement (Illa et al., 2017), impairment of coordination for
neurobehaviors (Gramsbergen and Westerga, 1992), and even possible underdevelopment
of brain sections and neurons (Mallard et al., 2000). Similar conditions may have
potentially occurred in the NR calves in the current study which contributed to their reduced neonatal vigor, particularly increasing the time to stand after birth. In one study, it was reported that beef calves that failed to consume colostrum by 4 h postnatal had decreased serum IgG by 24 h of age and were considered to have lower than optimal passive immunity and greater incidence of morbidity than calves that had successfully consumed colostrum prior to 4 h postnatal (Homerosky et al., 2017b). Additionally, Homerosky et al. (2017b) suggested that the calves with reduced neonatal vigor not only may have a delay in colostrum consumption but also consume smaller volumes of colostrum that contributed to greater medical treatment preweaning.

**Calf Rectal Temperature**

There was an interaction ($P = 0.02$) of late gestational nutritional plane × hour for neonatal calf rectal temperature (Figure 2.2). Calves born to CON heifers had greater ($P = 0.02$) rectal temperature at 0 h of age; however, at 24 h postnatal calves from NR heifers had greater ($P = 0.04$) rectal temperature. Within CON calves, rectal temperature at 0 h was greater ($P = 0.05$) than at 12 h postnatal and then increased ($P = 0.004$) from 24 to 48 h of age. Rectal temperature of NR calves increased ($P = 0.01$) from 0 to 6 h of age.

Following birth, thermogenesis is vital in preventing hypothermia as the neonate transitions to extrauterine environment (Carstens, 1994; Danijela, 2015). In a late gestational protein restriction study by Carstens et al. (1987), there was no difference in calf rectal temperature at birth or at approximately 13 h of age. However, calves from restricted dams had 11.4% less heat production between 5 and 13 h postnatal than calves
born to control dams (Carstens et al., 1987). In combination with observations in calf rectal temperature in the current study, this suggests that maternal nutrient restriction can influence thermoregulatory mechanisms in neonatal calves. However, Martin et al. (1997) reported no differences in thermoneutral metabolic rate between calves born to control or protein restricted dams during late gestation. Lack of differences in the study by Martin and others may be due to an increase in metabolizable energy included in the diet for restricted dams beginning on d 238 of gestation which may have allowed for rescue of fetal development as no differences were also observed in calf birth weight or organ weights (Martin et al., 1997). In the current study, fall-calving dams were utilized. Therefore, calves were born into more thermoneutral conditions compared with the experimentally induced cold stress conditions of Carstens et al. (1987), easing their thermoregulatory transition to postnatal life.

**Neonatal Calf Metabolites and Blood Chemistry**

*Energy-related metabolites.* Energy-related metabolites are presented in Figure 2.3 and Table 2.5. Late gestational nutritional plane did not affect \((P \geq 0.18)\) serum glucose, NEFA, or plasma triglycerides in the current study (Table 2.5) Additionally, there was no effect \((P \geq 0.36)\) of nutritional plane on the area under the curve for glucose, NEFA, or triglycerides (data not shown). However, there was a main effect of hour \((P < 0.001)\) for glucose, NEFA, and triglycerides (Figure 2.3). Serum glucose increased \((P < 0.001)\) from 6 to 24 h of age. Whereas serum NEFA decreased \((P \leq 0.03)\) between 12 and 48 h of age. Plasma triglycerides also increased \((P < 0.001)\) but from 0 to 6 h and again from 24 to 48 h postnatal. Previous studies reported that maternal nutrient restriction in
beef (Long et al., 2021) and dairy beef crossbred cattle (Prior and Scott, 1977) had no effect on fetal plasma concentrations of glucose or fetal liver gluconeogenic activity, which suggests glucose supply to meet fetal demands is prioritized over maternal demands (Hammon et al., 2012). Results from the current study are in agreement with aforementioned studies and support this notion of prioritization of fetal demands as there was no effect of maternal treatment on neonatal energy metabolites. Additionally, this may suggest that colostrum lactose absorption was not different between CON and NR dams despite the reduced yield in NR dams.

Postnatal glucose and triglyceride concentrations increase following colostrum intake due to lactose and fat provided in colostrum (Blum et al., 1997; Rauprich et al., 2000). However, prior to colostrum consumption, the neonate must rely on mobilization of its fat stores for energy (Hammon et al., 2012). This can be estimated through NEFA concentrations, which are usually high at birth but rapidly decline if colostrum consumption meets the neonate’s energy demands (Egli and Blum, 1998; Rauprich et al., 2000). Despite NR calves having less colostrum available, there may have been no difference in lipid concentration between CON and NR colostrum. However, as the current study was conducted with fall-calving heifers, future studies are warranted to investigate how maternal nutrient restriction during late gestation in spring-calving heifers influences a neonate’s transition to postnatal life, because of greater energy demands during cold stress conditions (Okamoto et al., 1986; Godfrey et al., 1991).

**Protein-related metabolites.** Protein-related metabolites are presented in Figure 2.4 and Table 2.5. There was an interaction ($P = 0.04$) of late gestational nutritional plane $\times$ hour for urea nitrogen. Although concentrations of urea nitrogen between treatments
did not differ \((P \geq 0.17)\) at any of the 5 sample timepoints, within calves born to CON heifers, serum urea nitrogen increased from 0 to 6 h of age. Serum urea nitrogen increased \((P < 0.001)\) from 0 to 12 h, but then decreased \((P < 0.001)\) from 24 to 48 h postnatal in calves born to NR dams. Neonatal blood urea nitrogen at birth has been shown to be highly correlated with maternal concentrations immediately postpartum (Bertoni et al., 2009; Meyer et al., 2018b). Contrary to observations by Bull et al. (1991), where beef calves born to dams that were protein restricted during the last half of gestation had lower serum urea nitrogen concentrations compared with calves born to dams receiving adequate protein, there was no difference in calf serum urea nitrogen concentrations at birth in the current study. In the present study, NR calves had increasing urea nitrogen from 0 to 12 h of age, 6 h longer than CON calves. This may indicate that NR calves had greater protein catabolism and amino acid deamination for energy as suggested by others (Rauprich et al., 2000; Alharthi et al., 2021).

There was also an interaction \((P = 0.03)\) of late gestational nutritional plane \(\times\) hour for serum creatinine, where NR calves had greater \((P = 0.03)\) concentrations of creatinine at 24 h of age when compared with CON calves. Serum creatinine decreased \((P \leq 0.004)\) from 0 to 48 h of age in CON and NR calves (Figure 2.4). Serum creatinine concentrations are reflective of normal muscle metabolism and renal function (Russell and Roussel, 2007) and are not influenced by neonatal colostrum consumption (Kurz and Willett, 1991; Hadorn et al., 1997) or maternal BCS during the last weeks of gestation in dairy cattle (Kurz and Willett, 1991; Alharthi et al., 2021). Both CON and NR creatinine concentrations were within values reported for neonatal calves at similar time points (Egli and Blum, 1998; Knowles et al., 2000); however, NR calves potentially had slight muscle
damage from more traumatic births, and the greater creatinine at 24 h of age is likely due to reduced variation, as observed in decreased standard errors, as creatinine concentrations begin to decline and reach adult reference ranges (Knowles et al., 2000). Creatinine concentrations can also be influenced by muscle mass (Russell and Roussel, 2007), but as there was no differences in calf size or shape measures at birth, this is unlikely the cause of the difference seen at 24 h of age in the present study.

Globulin is calculated by subtracting measured albumin from measured total protein. There was an interaction \( (P \leq 0.006) \) of late gestational nutritional plane × hour for serum globulin and total protein (Figure 2.4). Calves born to NR heifers had greater \( (P \leq 0.02) \) serum globulin and total protein at 6, 12, 24, and 48 h of age when compared with CON calves. Serum globulin increased \( (P < 0.001) \) between 6 and 24 h and then decreased \( (P \leq 0.01) \) from 24 to 48 h in both CON and NR calves. In calves born to NR heifers, an increase \( (P < 0.001) \) from 0 to 6 h also occurred. Serum total protein had a similar pattern to globulin with increasing \( (P < 0.001) \) concentrations from 6 to 24 h of age for calves from both CON and NR heifers. Calves born to NR also had an increase \( (P < 0.001) \) from 0 to 6 h and a decrease \( (P < 0.001) \) from 24 to 48 h of age in serum total protein. There was no effect of late gestational nutritional plane \( (P \geq 0.39) \) on serum albumin in the current study (Table 2.5).

Increases in total protein and globulin are expected following the ingestion of colostrum (Hammon and Blum, 1999). Additionally, total protein is often used as a clinical indicator of passive transfer because globulin is mostly comprised of Ig (Bush and Staley, 1980; Weaver et al., 2000). Despite less colostrum produced by NR cows, their calves had greater total protein and globulin from 6 to 48 h postnatal which suggests
that NR calves had improved passive transfer compared with CON calves. It has previously been reported that lambs born to nutrient restricted primiparous ewes and reared independently had greater serum Ig concentrations at 24 h after birth (Hammer et al., 2011). These results were independent of dam colostrum production, therefore suggests that maternal nutrient restriction alters the mechanism of Ig absorption in the neonate through altered rate of absorption or small intestinal maturation (Hammer et al., 2011). Other studies observed altered development of the small intestine in neonatal lambs (Meyer et al., 2013) and fetal Nellore calves (Duarte et al., 2013) which likely affects macronutrient absorption in neonates. In this study, potentially total mass of globulin proteins in colostrum differed between CON and NR dams contributed to differences observed in neonatal circulation concentrations.

Concentrations of albumin are reflective of hydration status, and greater concentrations suggest a state of dehydration (Russell and Roussel, 2007). There was no effect of maternal nutritional plane on serum albumin concentrations in the current study. This likely suggests that hydration status of calves did not differ.

**Electrolytes.** Neonatal calf electrolytes are presented in Figure 2.5 and Table 2.5. There was an interaction ($P = 0.03$) of late gestational nutritional plane × hour for serum potassium and a tendency of a maternal treatment and hour interaction ($P = 0.08$) for serum sodium. Calves born to CON heifers tended to have greater ($P \leq 0.09$) serum sodium at 0 h and had greater ($P \leq 0.004$) sodium from 6 to 48 h postnatal. Within calves born to CON heifers, sodium concentration decreased ($P < 0.001$) between 6 and 24 h of age. Serum sodium of NR calves decreased ($P \leq 0.003$) from 0 to 24 h and then increased ($P = 0.01$) from 24 to 48 h of age. Serum potassium was greater ($P = 0.03$) in NR calves
at 0 h postnatal when compared with CON calves. Potassium concentrations in CON did not change \((P \geq 0.06)\) across the 5 timepoints. Within NR calves, there was a decrease \((P = 0.01)\) in serum potassium from 0 to 6 h of age.

Transplacental exchange of sodium and potassium occurs in cattle; therefore, the differences at 0 h of age in these two electrolytes is likely due to differences in maternal circulation (Bertoni et al., 2009). Additionally, because blood samples were collected after the first standing of the calf, NR calves tended \((P = 0.07)\) to be older than CON calves at the first sample point \((1.08 \pm 0.09 \text{ h and } 0.84 \pm 0.09 \text{ h for NR and CON calves, respectively})\). This may have also contributed to the observed differences in sodium and potassium. Furthermore, sodium concentrations are greatest in colostrum and decrease with production of milk (McGrath et al., 2016). As NR heifers had reduced colostrum yield, sodium content may have also been reduced in NR dam colostrum leading to lower concentrations in NR calves. However, sodium concentrations in CON and NR calves were within the reported range for healthy dairy calves at 24 to 48 h of age (Mohri et al., 2007) and adult reference ranges (Knowles et al., 2000).

There tended to be a main effect of late gestational nutritional plane \((P = 0.08)\) for serum chloride where calves born to CON heifers had greater concentrations compared with NR calves (Table 2.5). There was no effect of maternal treatment \((P \geq 0.12)\) on serum phosphorus, calcium, or magnesium in the current study (Table 2.5). Concentrations of chloride often follow the patterns of sodium, because renal reabsorption of both occurs simultaneously (Russell and Roussel, 2007). As previously discussed, CON calves had greater concentrations of sodium; therefore, it would be expected that CON calves would also have greater serum chloride. Concentrations of
chloride, phosphorus, and magnesium in the current study are similar to suggested reference range for neonatal calves (Dillane et al., 2018). However, serum calcium concentrations of calves in the present study were above neonatal calf reference ranges (Dillane et al., 2018) and above reported values for dairy calves at a similar age (Mohri et al., 2007). A reason for this is likely due to breed differences, as suggested by Dillane et al. (2018) when utilizing calves of dairy and beef breeds in their study.

**Acid-base status.** There tended to be an interaction \( P = 0.10 \) of late gestational nutritional plane \( \times \) hour for serum anion gap (Figure 2.5 and Table 2.5). Calves born to CON heifers had greater \( P = 0.02 \) anion gap at 6 h of age compared with NR calves. Anion gap decreased \( P \leq 0.008 \) between 6 and 24 h in CON calves. Within NR calves, a decrease \( P < 0.001 \) in anion gap occurred between 12 and 24 h. There was no effect \( P \geq 0.46 \) of maternal treatment on neonatal calf serum bicarbonate concentrations in the current study (Table 2.5).

Anion gap is calculated by adding cations, sodium and potassium, and subtracting the anions, chloride and bicarbonate, and is typically used to evaluate metabolic acidosis (Russell and Roussel, 2007). Metabolic acidosis occurs with an imbalance of electrolytes, altered buffer capacity, or moderate hypoxia (Bleul and Götz, 2013). Typically, an increased state of metabolic acidosis will also depress vigor and muscle coordination and lead to increased mortality (Vannucchi et al., 2015; Homerosky et al., 2017b). However, the anion gap difference between CON and NR calves at 6 h is likely not due to CON calves experiencing metabolic acidosis, as CON calves were more vigorous than NR calves, but rather caused by persistently elevated sodium concentrations at that sample time point in CON calves.
**Bilirubin.** There was no effect \((P \geq 0.24)\) of late gestational nutritional plane on neonatal calf serum direct bilirubin or total bilirubin in the current study (Table 2.5). Bilirubin is produced after heme catabolism and is also related to liver function (Russell and Roussel, 2007). In a study conducted with dairy cows, Alharthi et al. (2021) reported that maternal BCS during the final weeks of gestation tended to influence neonatal calf bilirubin. They hypothesized that calves with higher bilirubin had a more mature metabolism at birth (Alharthi et al., 2021). From this, it could be expected that calves born to nutrient restricted dams would have a more immature metabolism at birth; however, there is no evidence of this in the current study based on calf bilirubin concentrations.

**Metabolic enzymes.** There was an interaction \((P \leq 0.03)\) of late gestational nutritional plane \(\times\) hour for serum aspartate aminotransferase and gamma-glutamyl transferase concentrations and a tendency for an interaction \((P = 0.10)\) of late gestational nutritional plane \(\times\) hour for serum creatine kinase (Figure 2.6). Calves born to NR heifers had greater \((P \leq 0.01)\) aspartate aminotransferase at 0 to 12 h and tended to have greater \((P = 0.07)\) aspartate aminotransferase at 24 h postnatal compared with CON heifers. In calves born to CON heifers, serum aspartate aminotransferase increased \((P \leq 0.01)\) from 0 to 24 h, followed by a decrease \((P < 0.001)\) from 24 to 48 h of age. Serum aspartate aminotransferase in NR calves also increased \((P < 0.001)\) between 0 and 12 h and then decreased \((P < 0.001)\) from 24 to 48 h of age. Serum gamma-glutamyl transferase was greater \((P = 0.006)\) at 6 h and tended to be greater \((P \leq 0.08)\) at 12 and 48 h of age in calves born to NR heifers. In calves born to CON heifers, serum gamma-glutamyl transferase increased \((P < 0.001)\) from 6 to 12 h and then decreased \((P < 0.001)\) from 24
to 48 h postnatal. In NR calves, serum gamma-glutamyl transferase increased ($P < 0.001$) from 0 to 12 h and then decreased ($P < 0.001$) from 24 to 48 h postnatal. Serum creatine kinase was greater ($P \leq 0.04$) at 6, 12, and 24 h in calves born to NR heifers.

Concentrations of serum creatine kinase in CON calves increased ($P = 0.02$) from 0 to 6 h postnatal. In calves born to NR heifers, creatine kinase increased ($P < 0.001$) from 0 to 6 h but then decreased ($P \leq 0.006$) between 12 and 48 h of age.

Aspartate aminotransferase and creatine kinase are important enzymes for amino acid and energy metabolism, respectively, that are commonly found in liver and muscle cells; however, when muscle damage or inflammation occurs these enzymes are released into circulation causing increased concentrations (Russell and Roussel, 2007; Pearson et al., 2019). Pearson et al. (2019) observed greater aspartate aminotransferase and creatine kinase concentrations in calves that had experienced dystocia compared with calves that were born unassisted and reported reduced vigor in calves with higher aspartate aminotransferase and creatine kinase concentrations. This may suggest that in the current study maternal growth of the pelvis was slightly impaired during nutrient restriction and caused more trauma to NR calves. This could have also contributed to the reduced neonatal vigor that was displayed by the NR calves.

Colostrum contains high concentrations of gamma-glutamyl transferase; therefore, neonatal serum gamma-glutamyl transferase concentrations will sharply increase following colostrum intake (Knowles et al., 2000; Maden et al., 2003). Some studies suggest that gamma-glutamyl transferase can be used to indicate successful passive transfer in both lambs (Maden et al., 2003) and calves (Perino et al., 1993) because neonates that ingested colostrum had greater serum gamma-glutamyl transferase
concentrations compared with those that had not (Weaver et al., 2000; Russell and Roussel, 2007). Therefore, because NR dams had reduced colostrum yield, this potentially caused more concentrated gamma-glutamyl transferase in their colostrum or differing intestinal macromolecule absorption between CON and NR calves that lead to NR having greater concentrations than CON calves. Additionally, when the cow-calf pair was returned to their pen descriptive notes were taken in regard to when calves first appeared to suckle. Based on these notes, more CON calves had first suckle events closer to 6 h postnatal than calves born to NR dams, despite the faster times to stand of CON calves. Lower concentrations of gamma-glutamyl at 6 h of age in CON calves support this observation.

**Neonatal Calf Hematology**

Neonatal calf hematology measures are presented in Figure 2.7 and Table 2.6. There was an interaction \( P \leq 0.04 \) of late gestational nutritional plane × hour for RBC, hemoglobin, and hematocrit (Figure 2.7). Calves born to CON heifers tended to have greater \( P \leq 0.09 \) RBC and hemoglobin at 6 and 12 h postnatal compared with NR calves. In CON calves, RBC decreased \( P \leq 0.02 \) between 0 and 48 h postnatal. In calves born to NR heifers, RBC decreased \( P \leq 0.04 \) from 0 to 12 h and then from 24 to 48 h of age. Hemoglobin followed a similar pattern where in CON calves hemoglobin decreased \( P \leq 0.007 \) from 0 to 48 h of age and in NR calves hemoglobin decreased \( P < 0.001 \) from 0 to 12 h postnatal. Hematocrit concentrations were greater \( P \leq 0.04 \) at 6 and 12 h and tended to be greater \( P \leq 0.10 \) at 24 h of age in calves born to CON heifers.
compared with NR calves. In both CON and NR calves, hematocrit decreased \( (P \leq 0.001) \) between 0 and 48 h postnatal.

Concentrations of RBC, hemoglobin and hematocrit for both CON and NR calves in the current study fall within previous reported reference ranges for neonatal dairy calves (Kurz and Willett, 1991; Knowles et al., 2000). Considering that all 3 parameters were increased in CON calves at 6 and 12 h of age, this may indicate relative polycythemia to some degree in these calves (Roland et al., 2014). Relative polycythemia may be caused by a decrease in plasma volume or following a splenic contraction (Roland et al., 2014). An accompanying increase in total protein or albumin under dehydration conditions was not observed in the CON calves, which excludes a decrease in plasma volume as the cause of polycythemia in these calves. When goats received an injection of epinephrine, an increase in packed cell volume was observed within 5 min, suggesting that splenic contractions released stored red blood cells (Abdelatif and Abdalla, 2012). This may suggest that the relative polycythemia observed in CON calves in the present study may be related to handling stress at the early sampling times and that maternal nutrient restriction may have altered calves’ response to handling stress related to blood draw restraint. Additionally, Egli and Blum (1998) reported lower RBC, hemoglobin, and hematocrit in blood samples collected at approximately 2 h postnatal from beef calves requiring delivery assistance at birth. This may suggest that the lower concentrations of RBC, hemoglobin, and hematocrit in NR calves were a result of more trauma experienced during birth.

There was no effect of late gestational nutritional plane \( (P \geq 0.29) \) on neonatal calf WBC in this study (Table 2.6). Increased WBC typically signifies disease or
inflammation (Roland et al., 2014), but in calves it may reflect immune system
development in early neonatal period (Ježek et al., 2011). However, values of WBC in
the CON and NR calves were within the recommended ranges used in dairy calves
(Knowles et al., 2000; Mohri et al., 2007) and are expected to decrease during the first
month of life (Roland et al., 2014).

There was no effect of late gestational nutritional plane \( (P \geq 0.12) \) on neonatal
calf MCV, MCH, or MCHC in the current study (Table 2.6). These values are termed red
cell indices because MCV defines red blood cell size, MCH quantifies the amount of
hemoglobin per red blood cell, and MCHC indicates amount of hemoglobin per unit
volume (hematocrit; Sarma, 1990). Values from the present study are slightly higher
compared with values reported by Mohri et al. (2007) for MCV, MCH, and MCHC at 24
to 48 h postnatal in dairy calves. Rather, results from CON and NR calves in the present
study resided in the recommended range for 1 to 4 wk old calves (Ježek et al., 2011) and
adult reference ranges of adult cattle (Knowles et al., 2000).

There was no effect of late gestational nutritional plane \( (P \geq 0.70) \) on neonatal
calf platelet count in the current study (Table 2.6). Platelet count in CON and NR calves
were within adult reference ranges (Knowles et al., 2000; Roland et al., 2014). Again,
results from the current study were slightly higher than those observed in dairy calves
(Mohri et al., 2007).

**CONCLUSION**

Although no differences were observed in fetal growth in the current study, fetal
development and perinatal nutrient availability was affected by late gestational nutritional
plane. Despite reduced colostrum yield from nutrient restricted dams, their calves had
greater total protein and globulin concentrations. This may suggest that colostrum
composition or neonatal intestinal development was altered by maternal nutrient
restriction. Calves that were born to nutrient restricted dams were less vigorous at birth
which could have resulted from impaired fetal neurodevelopment. There was also
indication that calves born to restricted dams may have experienced more trauma at
calving; this in combination with reduced vigor could hinder the calves in their transition
to postnatal life. Further investigation is needed to ascertain if the greater total protein
and globulin holds any advantage for neonatal calf health and whether nutrient restriction
also impairs subsequent lactational performance of dams that would further influence
preweaning calf performance.
Table 2.1. Vigor score used to assess neonatal beef calf vigor at 2, 5, 10, and 20 min of age

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very weak, laying flat on side, unable to lift head, minimal movement</td>
</tr>
<tr>
<td>2</td>
<td>Weak, lying flat on side, but holding head up</td>
</tr>
<tr>
<td>3</td>
<td>Active and vigorous, on chest and holding head up</td>
</tr>
<tr>
<td>4</td>
<td>Very active and vigorous, standing on back legs and front knees</td>
</tr>
<tr>
<td>5</td>
<td>Extremely active and vigorous, standing on all 4 feet</td>
</tr>
</tbody>
</table>

Scores were assigned respectively to vigor behaviors that had been displayed leading up to exact times of scoring, even if calf was no longer displaying the behavior at the actual time of scoring.
Table 2.2. Number of animals and samples included in analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>CON</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-suckling colostrum</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Calf size measures</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Delivery duration</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Calf vigor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Time to sternal recumbency</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>2. Time to attempt to stand</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>3. Failed attempts to stand</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>4. Calf time to stand</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>5. Vigor score at 2 min</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>6. Vigor score at 5 min</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>7. Vigor score at 10 min</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>8. Vigor score at 20 min</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>9. Calf rectal temperature</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>10. Calf blood chemistry and metabolites</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>11. Calf hematology</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

1 Primiparous dams were individually fed either 100% (CON) or 70% (NR) of metabolizable energy and metabolizable protein requirements for maintenance, pregnancy, and growth from d 160 of gestation to calving.
2 Calf size measures include birth weight, shoulder to rump length, heart girth, abdominal girth, flank girth, cannon circumference, cannon length, coronet circumference, and height at shoulder.
3 Time to stand and 20 min vigor scores were the only vigor measures recorded for calves with calving difficulty of 4 (NR: n = 3).
4 Collected at 0 (after standing and prior to suckling), 6, 12, 24, and 48 h postnatal. Includes calves with data point for at least 4 of the 5 time points.
Table 2.3. Effects of late gestational nutritional plane on primiparous dam single rear quarter colostrum yield

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>CON</th>
<th>NR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum volume, mL</td>
<td></td>
<td>471 ± 63</td>
<td>282 ± 58</td>
<td>0.04</td>
</tr>
<tr>
<td>Colostrum weight, g</td>
<td></td>
<td>509 ± 68</td>
<td>304 ± 62</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 Mean ± SEM presented for measures.
2 Primiparous dams were individually fed either 100% (CON) or 70% (NR) of metabolizable energy and metabolizable protein requirements for maintenance, pregnancy, and growth from d 160 of gestation to calving.
Table 2.4. Effects of late gestational nutritional plane on neonatal beef calf size and vigor

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>NR</td>
</tr>
<tr>
<td>Gestation length, d</td>
<td>278 ± 1</td>
<td>279 ± 1</td>
</tr>
<tr>
<td>Calf sex, % male</td>
<td>66.7</td>
<td>69.2</td>
</tr>
<tr>
<td>Calving difficulty score&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.25 ± 0.30</td>
<td>1.77 ± 0.29</td>
</tr>
<tr>
<td>Delivery duration&lt;sup&gt;4&lt;/sup&gt;, min</td>
<td>41.9 ± 6.6</td>
<td>40.9 ± 7.4</td>
</tr>
<tr>
<td>Calf size&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf birth weight, kg</td>
<td>30.7 ± 1.5</td>
<td>29.9 ± 1.4</td>
</tr>
<tr>
<td>Heart girth, cm</td>
<td>70.2 ± 1.1</td>
<td>69.1 ± 1.1</td>
</tr>
<tr>
<td>Abdominal girth, cm</td>
<td>63.2 ± 1.2</td>
<td>62.5 ± 1.2</td>
</tr>
<tr>
<td>Flank girth, cm</td>
<td>58.2 ± 1.3</td>
<td>56.9 ± 1.3</td>
</tr>
<tr>
<td>Shoulder to rump length, cm</td>
<td>55.4 ± 0.9</td>
<td>55.0 ± 0.9</td>
</tr>
<tr>
<td>Cannon circumference, cm</td>
<td>11.5 ± 0.2</td>
<td>11.3 ± 0.2</td>
</tr>
<tr>
<td>Cannon length, cm</td>
<td>17.2 ± 0.3</td>
<td>17.7 ± 0.3</td>
</tr>
<tr>
<td>Coronet circumference, cm</td>
<td>17.7 ± 0.3</td>
<td>17.3 ± 0.3</td>
</tr>
<tr>
<td>Height at shoulder, cm</td>
<td>67.0 ± 0.7</td>
<td>66.6 ± 0.7</td>
</tr>
<tr>
<td>Calf ponderal index&lt;sup&gt;6&lt;/sup&gt;, kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>180 ± 6</td>
<td>180 ± 5</td>
</tr>
<tr>
<td>Heart girth:length&lt;sup&gt;7&lt;/sup&gt;</td>
<td>1.27 ± 0.01</td>
<td>1.26 ± 0.01</td>
</tr>
<tr>
<td>Calf vigor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to sternal recumbency&lt;sup&gt;8&lt;/sup&gt;, min</td>
<td>3.64 ± 0.44</td>
<td>3.00 ± 0.51</td>
</tr>
<tr>
<td>Time to attempt to stand&lt;sup&gt;9&lt;/sup&gt;, min</td>
<td>11.7 ± 2.7</td>
<td>18.2 ± 2.7</td>
</tr>
<tr>
<td>Time to stand&lt;sup&gt;10&lt;/sup&gt;, min</td>
<td>20.8 ± 4.1</td>
<td>34.8 ± 4.0</td>
</tr>
<tr>
<td>Number of failed attempts to stand&lt;sup&gt;11&lt;/sup&gt;</td>
<td>5.33 ± 1.14</td>
<td>4.41 ± 1.25</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean ± SEM presented for measures.
<sup>2</sup> Primiparous dams were individually fed either 100% (CON) or 70% (NR) of metabolizable energy and metabolizable protein requirements for maintenance, pregnancy, and growth from d 160 of gestation to calving.
<sup>3</sup> Assigned on a scale of 1 to 5 (1 = unassisted, 2 = easy pull, 3 = mechanically assisted pull, 4 = abnormal presentation, 5 = caesarian-section).
<sup>4</sup> Determined as time from first expulsion of feet to birth of the calf.
<sup>5</sup> Calves were weighed and measured at 0.9 ± 0.3 h [SD] of age.
<sup>6</sup> Ponderal index = calf birth weight (kg)/shoulder to rump length (m)<sup>3</sup>.
<sup>7</sup> Ratio of heart girth (cm):shoulder to rump length (cm).
<sup>8</sup> Determined as time from birth to when calf first achieves sternal recumbency.
<sup>9</sup> Determined as time from birth to when calf first supports weight on hind legs while balanced on the front knees for ≥ 5 sec.
<sup>10</sup> Calf attains the posture of attempting to stand but falls.
<sup>11</sup> Determined as time from birth to when calf is first standing on all 4 legs for ≥ 5 sec.
Table 2.5. Effects of late gestational nutritional plane on neonatal beef calf blood chemistry during the first 48 h postnatal

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>P-values</th>
<th></th>
<th>Treatment</th>
<th>P-values</th>
<th>Treatment</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>NR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>79.8 ± 3.1</td>
<td>75.5 ± 3.0</td>
<td>0.33</td>
<td>&lt; 0.001</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-esterified fatty acids, μEq/L</td>
<td>403 ± 35</td>
<td>387 ± 33</td>
<td>0.73</td>
<td>&lt; 0.001</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>301 ± 28</td>
<td>297 ± 27</td>
<td>0.93</td>
<td>&lt; 0.001</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea nitrogen, mg/dL</td>
<td>5.21 ± 0.53</td>
<td>5.62 ± 0.51</td>
<td>0.59</td>
<td>&lt; 0.001</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.78 ± 0.14</td>
<td>2.03 ± 0.13</td>
<td>0.20</td>
<td>&lt; 0.001</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Globulin, g/dL</td>
<td>2.35 ± 0.15</td>
<td>3.13 ± 0.15</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>2.28 ± 0.06</td>
<td>2.21 ± 0.05</td>
<td>0.39</td>
<td>&lt; 0.001</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td>4.63 ± 0.17</td>
<td>5.34 ± 0.17</td>
<td>0.007</td>
<td>&lt; 0.001</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium, mEq/L</td>
<td>143 ± 0.3</td>
<td>141 ± 0.3</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>5.51 ± 0.08</td>
<td>5.60 ± 0.08</td>
<td>0.44</td>
<td>0.20</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride, mEq/L</td>
<td>100.6 ± 0.5</td>
<td>99.3 ± 0.5</td>
<td>0.08</td>
<td>&lt; 0.001</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus, mg/dL</td>
<td>6.91 ± 0.16</td>
<td>7.21 ± 0.16</td>
<td>0.20</td>
<td>&lt; 0.001</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>11.5 ± 0.1</td>
<td>11.3 ± 0.1</td>
<td>0.27</td>
<td>&lt; 0.001</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium, mg/dL</td>
<td>2.35 ± 0.08</td>
<td>2.49 ± 0.08</td>
<td>0.23</td>
<td>&lt; 0.001</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicarbonate, mEq/L</td>
<td>26.6 ± 0.5</td>
<td>26.1 ± 0.5</td>
<td>0.46</td>
<td>0.05</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anion gap, mEq/L</td>
<td>21.8 ± 0.4</td>
<td>21.3 ± 0.4</td>
<td>0.43</td>
<td>&lt; 0.001</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct bilirubin, mg/dL</td>
<td>0.36 ± 0.02</td>
<td>0.34 ± 0.02</td>
<td>0.47</td>
<td>&lt; 0.001</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>1.03 ± 0.08</td>
<td>0.91 ± 0.08</td>
<td>0.29</td>
<td>&lt; 0.001</td>
<td>0.70</td>
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<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>47.7 ± 3.1</td>
<td>58.7 ± 3.0</td>
<td>0.02</td>
<td>&lt; 0.001</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma-glutamyl transferase, U/L</td>
<td>499 ± 76</td>
<td>782 ± 73</td>
<td>0.01</td>
<td>&lt; 0.001</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine kinase, U/L</td>
<td>180 ± 75</td>
<td>414 ± 72</td>
<td>0.03</td>
<td>&lt; 0.001</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Mean ± SEM presented for measures.
2 Primiparous dams were individually fed either 100% (CON) or 70% (NR) of metabolizable energy and metabolizable protein requirements for maintenance, pregnancy, and growth from d 160 of gestation to calving.
3 Jugular blood samples collected at 0 (after standing and prior to suckling), 6, 12, 24, and 48 h postnatal.
Table 2.6. Effects of late gestational nutritional plane on neonatal beef calf hematology

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>P-values</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>NR</td>
<td>Treatment</td>
<td>Hour</td>
</tr>
<tr>
<td>White blood cell count, x10⁶/uL</td>
<td>11.9 ± 0.8</td>
<td>10.9 ± 0.8</td>
<td>0.38</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Red blood cell count, x10⁶/uL</td>
<td>7.98 ± 0.32</td>
<td>7.38 ± 0.31</td>
<td>0.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>9.77 ± 0.41</td>
<td>8.94 ± 0.40</td>
<td>0.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>32.7 ± 1.3</td>
<td>29.5 ± 1.3</td>
<td>0.09</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean corpuscular volume, fL</td>
<td>40.9 ± 0.5</td>
<td>39.8 ± 0.4</td>
<td>0.12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin, pg</td>
<td>12.2 ± 0.1</td>
<td>12.1 ± 0.1</td>
<td>0.42</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration, g/dL</td>
<td>30.0 ± 0.2</td>
<td>30.4 ± 0.2</td>
<td>0.14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Platelet count, x10⁹/uL</td>
<td>517 ± 32</td>
<td>503 ± 31</td>
<td>0.75</td>
<td>0.002</td>
</tr>
</tbody>
</table>

1 Mean ± SEM presented for measures.
2 Primiparous dams were individually fed either 100% (CON) or 70% (NR) of metabolizable energy and metabolizable protein requirements for maintenance, pregnancy, and growth from d 160 of gestation to calving.
3 Jugular blood samples collected at 0 (after standing and prior to suckling), 6, 12, 24, and 48 h postnatal.
Figure 2.1. Frequency of neonatal beef calf vigor score on scale of 1 to 5 (1 = very weak; 5 = extremely active and vigorous) at 2 min (Panel A), 5 min (Panel B), 10 min (Panel C), and 20 min (Panel D) of age. Solid bars (■) represent calves born to heifers fed 100% (CON) and open bars (□) represent calves born to heifers fed 70% (NR) of metabolizable energy and metabolizable protein requirements beginning on d 160 of gestation. There was an effect of treatment ($P = 0.05$) on vigor score at 20 min of age.
Figure 2.2. Effects of late gestational nutritional plane on rectal temperature of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Solid circles (●) represent calves born to heifers fed 100% (CON) and open circles (○) represent calves born to heifers fed 70% (NR) of metabolizable energy and metabolizable protein requirements beginning on d 160 of gestation. There was a treatment × hour interaction ($P = 0.02$). *Treatment means within hour differ ($P \leq 0.05$). $^{ab}$ Means differ ($P \leq 0.05$) for control calves across hours. $^{yz}$ Means differ ($P \leq 0.05$) for nutrient restricted calves across hours.
Figure 2.3. Effects of late gestational nutritional plane on serum energy metabolites glucose (Panel A), non-esterified fatty acids (NEFA; Panel B), and triglycerides (Panel C) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Solid circles (●) represent calves born to heifers fed 100% (CON) and open circles (○) represent calves born to heifers fed 70% (NR) of metabolizable energy and metabolizable protein requirements beginning on d 160 of gestation. There was no interaction of season × hour (P ≥ 0.18) for glucose, NEFA, and triglycerides. There was a main effect of hour (P < 0.001) for glucose, NEFA, and triglycerides. ABC Means differ (P ≤ 0.05) across hours.
Figure 2.4. Effects of late gestational nutritional plane on serum protein metabolites including urea nitrogen (urea N; Panel A), creatinine (Panel B), globulin (Panel C), and total protein (Panel D) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Solid circles (●) represent calves born to heifers fed 100% (CON) and open circles (○) represent calves born to heifers fed 70% (NR) of metabolizable energy and metabolizable protein requirements beginning on d 160 of gestation. There was an interaction of treatment × hour for urea N ($P = 0.04$), creatinine ($P = 0.03$), globulin ($P = 0.006$), and total protein ($P = 0.004$). *Treatment means within hour differ ($P \leq 0.05$). $^{abcde}$Means differ ($P \leq 0.05$) for control calves across hours. $^{vwxyz}$Means differ ($P \leq 0.05$) for nutrient restricted calves across hours.
Figure 2.5. Effects of late gestational nutritional plane on serum sodium (Panel A), phosphorus (Panel B), and anion gap (Panel C) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Solid circles (●) represent calves born to heifers fed 100% (CON) and open circles (○) represent calves born to heifers fed 70% (NR) of metabolizable energy and metabolizable protein requirements beginning on d 160 of gestation. There was an interaction of treatment × hour (P = 0.03) for potassium and a tendency (P ≤ 0.10) for sodium and anion gap. *Treatment means within hour differ (P ≤ 0.05), + tend to differ (P < 0.10). abc Means differ (P ≤ 0.05) for control calves across hours. wxyz Means differ (P ≤ 0.05) for nutrient restricted calves across hours.
Figure 2.6. Effects of late gestational nutritional plane on serum metabolic enzymes including aspartate aminotransferase (AST; Panel A), gamma-glutamyl transferase (GGT; Panel B), and creatine kinase (CK; Panel C) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Solid circles (●) represent calves born to heifers fed 100% (CON) and open circles (○) represent calves born to heifers fed 70% (NR) of metabolizable energy and metabolizable protein requirements beginning on d 160 of gestation. There was a treatment × hour interaction ($P \leq 0.03$) for AST and GGT and a tendency ($P = 0.10$) for CK. *Treatment means within hour differ ($P \leq 0.05$). †tend to differ ($P < 0.10$). $^{abcd}$Means differ ($P \leq 0.05$) for control calves across hours. $^{wxyz}$Means differ ($P \leq 0.05$) for nutrient restricted calves across hours.
Figure 2.7. Effects of late gestational nutritional plane on red blood cell count (RBC; Panel A), hemoglobin (Panel B), and hematocrit (Panel C) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Solid circles (●) represent calves born to heifers fed 100% (CON) and open circles (○) represent calves born to heifers fed 70% (NR) of metabolizable energy and metabolizable protein requirements beginning on d 160 of gestation. There was a treatment × hour interaction for RBC (P = 0.01), Hgb (P = 0.04), and Hct (P = 0.01). *Treatment means within hour differ (P ≤ 0.05), + tend to differ (P < 0.10). abcd Means differ (P ≤ 0.05) for control calves across hours. vwxyz Means differ (P ≤ 0.05) for nutrient restricted calves across hours.
CHAPTER 3
EFFECTS OF SPRING VERSUS FALL CALVING ON PERINATAL NUTRIENT AVAILABILITY AND NEONATAL VIGOR IN BEEF CATTLE

ABSTRACT

To determine the effect of calving season on perinatal nutrient availability and neonatal calf vigor, multiparous and primiparous dams (BW: 661 ± 84 kg; BCS: 5.4 ± 0.6; age: 4.7 ± 2.4 yr) from 4 spring (average calving date: February 4; n = 205) and 4 fall (average calving date: September 19; n = 181) calving experiments were observed during parturition. Time to stand (spring: n = 67; fall: n = 104) was determined as minutes from birth to standing for 5 sec. After birth, calf BW (spring: n = 202; fall: n = 177) and size (spring: n = 99; fall: n = 169; length, heart girth, abdominal girth, and cannon circumference) were recorded. Jugular blood samples (spring: n = 63; fall: n = 89) were obtained at 0 (pre-suckling), 6, 12, 24, and 48 h postnatally. Data were analyzed with the fixed effects of season (single point) or season, hour, and their interaction (over time, using repeated measures). Experiment was a random effect; calf sex was included when P < 0.25. Within calving season, correlations were determined between calf size, vigor, and 48-h serum total protein. Fall-born calves tended to have lighter (P = 0.09) birth weight and faster (P = 0.05) time to stand than spring-born calves. Season did not affect (P ≥ 0.18) gestation length, other calf size measures, or 48-h serum total protein. Fall-born calves had greater (P ≤ 0.003) rectal temperature at 0, 24, and 48 h postnatal when compared with calves born in the spring. Spring-born calves had greater (P ≤ 0.009) circulating 0 h glucose, 0 and 6 h NEFA, and 0, 6, 12, and 48 h triglycerides. Fall-born calves had greater (P = 0.03) serum total protein at 24 h and tended to have greater (P ≤ 0.10) total protein and urea nitrogen at 48 h and globulin at 24 h. Fall-born calves had
greater \((P \leq 0.03)\) sodium from 6 to 48 h and magnesium from 0 to 24 h of age. Phosphorus was greater \((P \leq 0.02)\) at 6 and 12 h of age in spring-born calves. Spring-born calves had greater \((P \leq 0.04)\) aspartate aminotransferase at 12 and 24 h and creatine kinase at 0 and 12 h of age. There was a main effect of calving season for albumin, chloride, and calcium and tendency for anion gap, which were greater \((P \leq 0.10)\) in fall-born calves. Bicarbonate and direct bilirubin were greater \((P \leq 0.03)\) in spring-born calves. Within fall-born calves, calf birth weight had a weak positive relationship with 48-h serum total protein and time to stand. These data suggest that calving season influences perinatal nutrient availability, which may impact the transition to postnatal life.

INTRODUCTION

Calf survival to weaning is important to cow-calf producers. Of beef calves born alive, 5.5% die prior to weaning with two-thirds of those losses occur during the first 3 wk of life (USDA-APHIS, 2010, 2017). This is because the transition to postnatal life, where timely ingestion of colostrum is imperative for obtaining necessary nutrients and passive transfer, poses one of the greatest challenges to neonatal calves.

Beef calves are often born into seasonal extremes that can impact their transition. In the lower Midwest, 2 of the most common calving seasons are often spring or fall. Calves born in fall often experience late gestational heat stress in utero, which has been shown in dairy calves to compromise fetal growth and alter neonatal metabolism and immune function (Dahl et al., 2019). Conversely, calves born in the spring may experience cold stress conditions following birth which may suppress neonatal vigor.
(Olson et al., 1980b) and increase mobilization of their energy stores to maintain body temperature, impacting their metabolic status (Vermorel et al., 1983).

Additionally, studies have shown that maternal nutrition during gestation influences fetal growth and development in cattle (Caton and Hess, 2010; Funston et al., 2010; Perry et al., 2019). Despite this, limited research has been conducted on how seasonal differences in the nutritional management of pregnant beef females driven by forage availability, along with differing energy demands and nutritional intakes of late gestation dams, may affect perinatal nutrient availability of calves. Therefore, we hypothesized that fall-born calves have decreased fetal growth but faster times to stand and improved passive transfer compared with spring-born calves, and that neonatal circulating metabolites differ between spring- and fall-born calves. Our objectives were to determine the effect of calving season on perinatal nutrient availability and neonatal vigor and to determine the relationships of calf vigor and size with passive transfer in beef calves within calving season.

MATERIALS AND METHODS

The University of Missouri Animal Care and Use Committee approved animal care and use in this study (Protocol #7936, #8952, #8956, #9045, #9815, and #9877).

Gestating Animal Management

All Experiments. Four spring and 4 fall calving experiments with an average calving date of February 13 and September 19, respectively, were conducted at the University of Missouri Beef Research and Teaching Farm (Table 3.1). Multiparous and
primiparous beef cows were utilized in 1 spring and 2 fall experiments, whereas the other 3 spring and 2 fall experiments used strictly multiparous beef dams. Females were either bred by AI or natural service and were housed in well-drained 18 × 61 m drylots and observed during the peripartum period (Figure 3.1). In all 8 experiments, animals were part of larger groups of females observed for peripartum data collection and only animals included in this analysis are reported for each experiment in Table 3.1.

Summer-baled, endophyte-infected tall fescue-based grass hay or harvested haylage was fed ad libitum in round bale feeders with cone chains placed on 9.1 × 9.1 m concrete pad to minimize waste and excessive mud accumulation around feeders. Cows had free access to automatic watering systems and a mineral and vitamin supplement (Exp. 1, 2, and 4: Gold Star MFA Breeder 12 Mineral: MFA, Columbia, MO; Exp. 3, 6, 7, and 8: MLS #12 MINERA-LIX, Midcontinent Livestock Supplements, Inc., Moberly, MO). Sheds were kept closed unless during inclement weather during spring-calving (Exp. 1, 2, 4, and 5) when a single pair was then moved under cover. Gates were hung from the front of each shed to provide a clean, shaded area for calves during fall-calving experiments (Exp. 3, 6, 7, and 8). Approximately 20% of pen area at the opposite end of waterers was bedded with fescue straw to mitigate calf cold stress during experiments 1, 2, 4, and 5. Experiments 3, 6, 7, and 8 occurred during the fall; therefore, no bedding was used.

**Experiments 1, 2, and 4.** A 2-yr study (Exp. 1 and 2) and Exp. 4 were conducted during late gestation with multiparous, spring-calving, crossbred beef cows. The number of females used in this analysis (Exp. 1: n = 46; Exp. 2: n = 53; Exp. 4: n = 50) was part of a larger group of animals in each experiment. Experiments 1 and 2 have been
previously described in Niederecker et al. (2018) and Exp. 4 followed a similar experimental design. Briefly, cows were assigned to either strip-graze endophyte-infected stockpiled tall fescue or receive endophyte-infected tall fescue hay beginning on d 188 ± 2 (SD throughout) of gestation (Exp. 1 and 2) or 75 ± 12 d prior to calving in experiment 4. Cows receiving hay were housed in the same drylots as those used during calving. Prior to parturition (Exp. 1: -19.2 ± 14.2 d, Exp. 2: -13.8 ± 11.8 d, Exp. 4: -14.7 ± 11.8 d), cows grazing stockpiled tall fescue were moved to drylots adjacent to pens receiving hay and were fed rye haylage. Cows were kept within the same forage system treatment groups and penned together in groups of 8 to 10 dams during calving.

**Experiment 3.** Fall-calving crossbred or purebred Hereford beef cows (n = 37) and heifers (n = 13) grazed tall fescue-based pasture during late gestation. Number of animals reported were used in this analysis and were part of a larger group of females used in this experiment. Females were then confined to 6 drylots for observation beginning at 4.1 ± 2.1 d prepartum. Endophyte-infected tall fescue haylage was available ad libitum while in drylots.

**Experiment 5.** Spring-calving primiparous (n = 18) and multiparous (n = 38) crossbred beef cows were managed in 2 groups (parities 1 and 2 vs. parity ≥ 3) grazed tall fescue-based pasture and then fed harvested forage until 6.7 ± 3.0 d prior to calving when females were moved to 6 drylots for observation. More females were observed during Exp. 5 than are reported above for this analysis. Animals were allowed ad libitum access to endophyte-infected tall fescue hay and supplemented with dried distillers’ grains with solubles (DDGS) at a rate of 1.0 kg DM · animal⁻¹ · d⁻¹ at approximately 1700 h daily.
**Experiment 6.** Multiparous fall-calving crossbred beef cows (n = 42; subset of females used in this analysis) were moved at 17.3 ± 7.0 d pre-partum to 6 drylots following an individual feeding study using a Calan gate system to investigate the effects of copper, zinc, and manganese source and inclusion during late gestation (Stephenson, 2019). While in drylots, cows had ad libitum access to endophyte-infected tall fescue hay and received soyhulls and DDGS-based supplement fed to be 11.5% of total pen DMI daily at 1800 h.

**Experiment 7.** Primiparous (n = 18) and multiparous (n = 14) fall-calving crossbred beef cows were nutritionally managed together on tall fescue-based pasture and supplemented 4 to 7 d/wk with DDGS and corn at a rate of 0.91 kg DM · animal⁻¹ · d⁻¹ until being moved to drylots 30 ± 9.9 d prior to calving. The number of animals reported above were used in this analysis and were part of a larger group of females used in this experiment. While in the drylots, dams had ad libitum access to endophyte-infected tall fescue and were supplemented with DDGS and soyhulls at a rate of 0.91 kg DM · animal⁻¹ · d⁻¹ at 1700 h daily.

**Experiment 8.** Multiparous fall-calving crossbred beef cows (n = 57; part of larger group of females used for this analysis) were nutritionally managed together on tall fescue-based pasture until 95.1 ± 14.1 d prior to parturition when a subset (n = 20) of cows were managed separately on a tall fescue-based paddock and supplemented 4 to 5 d/wk with DDGS and corn at a rate of 1.19 kg DM · animal⁻¹ · d⁻¹. The remaining dams remained rotationally grazing tall-fescue based pasture. Dams were then moved to 6 drylots for observation 11.6 ± 6.9 d prior to calving and were nutritionally managed
together. Dams had ad libitum access to endophyte-infected tall fescue hay and were supplemented with DDGS and corn at a rate of 1.19 kg DM · animal⁻¹ · d⁻¹ at 1700 h.

**Pre-calving Blood Collection.**

Jugular blood samples were collected from prepartum dams prior to being moved into the drylot calving pens. Blood samples were collected into 4 tubes (2 Vacutainer serum collection tubes containing no additives [10 mL draw; Becton Dickinson, Franklin Lakes, NJ], 1 Monoject plasma collection tube containing 0.10 mL of 15% K₃EDTA [10 mL draw, Covidien, Mansfield, MA], 1 Vacutainer plasma collection tube containing 15 mg of sodium fluoride and 12 mg of potassium oxalate [6 mL draw; Becton Dickinson, Franklin Lakes, NJ] for glucose determination). All tubes were inverted, and plasma tubes immediately placed on ice whereas serum tubes were allowed to clot prior to being placed on ice. Within 8 h of collection, samples were centrifuged at 1,500 × g at 4°C for 30 min. Serum and plasma were then aliquoted into 2 mL microcentrifuge tubes and stored at -20°C until analysis.

**Calving Monitoring and Data Collection**

Trained personnel closely monitored cows and heifers in calving pens for physical signs of labor from at least 0600 to 2200 h, with additional checks occurring overnight during periods of heavy calving and as blood sampling times required. Stadium lights were located in the back of every other pen to illuminate the calving pens during the night to allow for monitoring of cows and calves. Continuous monitoring of dams began when evidence of stage II parturition (appearance of amniotic membranes or calf feet) in order
to record actual time of birth (expulsion of entire calf, including all 4 legs). Calving assistance was provided if malpresentation was suspected or after a prolonged duration since first appearance of fetal membranes or if progress slowed during contractions. Calving difficulty score ranging 1 to 5 was assigned with 1 indicating no assistance, 2 an easy pull, 3 was mechanically assisted pull, 4 an abnormal presentation, and 5 indicating a caesarian-section. Beginning in Exp. 2, calves were then closely monitored to record time of first standing (defined as the calf standing on all 4 legs for ≥ 5 consecutive seconds) to quantify calf vigor. The time of birth was then subtracted from the time of first standing to obtain a calf’s time to stand in minutes.

Calves were measured and processed at 8.9 ± 10.8 h of age. Calf sex was recorded and birth BW was measured using a hanging scale with a calf sling placed under the abdomen of the calf and lifted until all 4 feet were off the ground. Using a flexible measuring tape, calf body size was recorded. Shoulder to rump length was measured along the spine from front of the shoulder blades to the rump. Heart girth was measured as the body circumference immediately posterior to the shoulders and front legs, perpendicular to the spine. Abdominal girth was measured by placing the tape measure around the abdomen over the umbilicus, perpendicular to the spine. Cannon bone circumference was measured at the smallest circumference of a rear cannon bone. As an indicator of calf shape, calf ponderal index was calculated using the equation ponderal index = calf birth weight (kg)/ shoulder to rump length (m³). Calf heart girth:length ratio was calculated as a second indicator of shape, using the equation heart girth:length = heart girth (cm)/ shoulder to rump length (cm).
Jugular blood samples were collected from all possible calves at 48 h of age in all 8 experiments. Beginning in Exp. 3, calf jugular blood samples were obtained from a subset of calves in each experiment at 0 (prior to suckling but after standing), 6, 12, 24, and 48 h postnatally (0.63 ± 0.4 h; 6.2 ± 0.4 h; 12.2 ± 0.4 h; 24.2 ± 0.4 h; 48.1 ± 0.4 h, respectively). Calf rectal temperatures were recorded using a digital thermometer prior to blood sample collection at all 5 time points. Blood was collected into at most 4 tubes at each time point (at least 1 Vacutainer serum collection tube containing no additives [10 mL draw; Becton Dickinson, Franklin Lakes, NJ], 1 Monoject plasma collection tube containing 0.10 mL of 15% K$_3$EDTA [10 mL draw, Covidien, Mansfield, MA], 1 Vacutainer plasma collection tube containing 15 mg of sodium fluoride and 12 mg of potassium oxalate [6 mL draw; Becton Dickinson, Franklin Lakes, NJ] for glucose determination). All tubes were inverted, and plasma tubes immediately placed on ice, whereas serum tubes were allowed to clot prior to being placed on ice. Within 8 h of collection, samples were centrifuged at 1500 × g at 4°C for 30 min. Serum and plasma were aliquoted into 2 mL microcentrifuge tubes and either submitted for analysis or stored at -20°C until analysis.

**Blood Chemistry and Metabolite Analyses**

One aliquot of calf serum (48 h samples in Exp. 2 and 0 through 48 h samples in Exp. 3 to 6) was refrigerated and transported to the University of Missouri Veterinary Medical Diagnostic Clinical Pathology Laboratory (VMDL) on the day of collection or within 48 h when collected in the evening or on weekends for a complete chemistry analysis. Serum glucose, urea nitrogen, creatinine, globulin, albumin, total protein,
sodium, potassium, chloride, bicarbonate, anion gap, phosphorus, calcium, magnesium, direct bilirubin, total bilirubin, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and creatine kinase (CK) were determined using a Beckman Coulter AU 400e Chemistry System (Beckman Coulter Inc., Brea, CA). Frozen 48-h serum samples from Exp. 7, 8, and a subset of samples (n = 17; not previous analyzed by VMDL) from Exp. 3 were delivered to VMDL for determination of total protein concentration using a Beckman Coulter AU480 Chemistry Analyzer (Beckman Coulter Inc., Brea, CA). The 2 analyzers used the same methodology in determination of serum total protein. Additionally, for both instruments used internal quality control and verification of performance within specific CV’s are conducted daily. Upon delivery, samples were analyzed at the same time through the instrument’s completely automated process.

Calf serum samples in Exp. 7 and 8 and prepartum cow serum were analyzed for urea nitrogen using a commercially available urea nitrogen kit (Urea Nitrogen Procedure Number 0580; Stanbio Laboratory, Boerne, TX) based on the diacetylmonoxime method. Plasma samples (collected in treated tubes described above) for calves in Exp. 7 and 8 and prepartum cow plasma were analyzed for glucose concentration using the Infinity glucose hexokinase commercially available kit (Fisher Diagnostics, Middletown, VA) based on the glucose-6-phosphate dehydrogenase method. Serum concentrations of NEFA for all experiments of calf and prepartum cow samples were determined using a modified procedure of the NEFA C kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan), using the acyl-CoA synthase-acyl-CoA oxidase method. Calf plasma triglycerides
were analyzed using a commercially available Infinity Triglycerides kit (Thermo Scientific) based on the glycerol-3-phosphate oxidate methods.

For each assay, samples were read in duplicate in 96-well plates on a microplate reader (Biotek Synergy HT, Biotek Instruments Inc., Winooski, VT) at 520, 340, and 550 nm for urea nitrogen, glucose, and NEFA, respectively. Plates were read at 500 and 660 nm and final results were calculated by subtracting the 660 nm reading from the 500 nm reading for plasma triglycerides. The intraassay and interassay CV were < 10% for all assays across all experiments. Meyer et al. (2017) have previously reported that serum urea nitrogen values determined with commercial kit and reported by VMDL were highly correlated. Additionally, plasma glucose (commercial kit) and serum glucose (VMDL) were also highly correlated. Therefore, results from VMDL in Exp. 3, 4, 5, and 6 for neonatal calf circulating urea nitrogen and glucose were used.

Statistical Analysis

Data collected from the 8 calving experiments were entered into and managed in a custom-designed Microsoft Access database (Microsoft Cooperation, Redmond, WA). The select query function was then used to retrieve desired data from multiple tables within Access and was consolidated into one Microsoft Excel datasheet.

Data were checked for outliers prior to analysis and data from any twins (n = 3) or stillborn (n = 6) calves were excluded. Data from calves with a calving difficulty score > 1 (n = 19) were excluded from all analyses except calf size. If exact time of birth or first standing was unknown, time to stand was not calculated. Data from calves that received colostrum via an esophageal tube (n = 6) within the first 48 h postnatal were excluded.
from analyses of metabolites and serum chemistry. In order for data to be included in over time analyses of circulating metabolites, serum chemistry, and rectal temperature data from at least 4 of the 5 time points were required. Table 3.2 depicts the final number of calves split by season used in analyses.

Data were analyzed using the MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC) with calving season as a fixed effect and animal as the experimental unit. For circulating metabolites, serum chemistry, and rectal temperature over time, season, hour, and their interaction were considered fixed effects. These were considered repeated measures using the majority best-fit covariance structure (based on AIC, BIC, and BICC) specific for each variable (chosen from unstructured, compound symmetry, heterogeneous compound symmetry, autoregressive, and heterogeneous autoregressive). For all measures, experiment was included as a random effect to account for variation among experiments. Additionally, calf sex was included as a fixed effect in all models when \( P \leq 0.25 \). Means were separated using least significant difference and considered different when \( P \leq 0.05 \). Main effects and interactions were reported when \( P \leq 0.05 \) with tendencies considered when \( 0.05 < P \leq 0.10 \). In the absence of interactions, main effects of season are reported. Pearson correlation coefficients were determined between calf size, vigor, and 48 h serum total protein concentration within calving season.

**RESULTS AND DISCUSSION**

**Dam BW, BCS, Age, and Prepartum Metabolites**

Dam BW, BCS, age, and prepartum metabolites are described in Table 3.2. Fall-calving dams were younger \( (P = 0.05) \) than those dams calving in the spring. Spring-
calving dams had greater \((P < 0.001)\) prepartum serum NEFA. Prepartum plasma triglycerides were greater \((P < 0.001)\) in fall-calving dams. However, there was no effect of calving season on dam prepartum BW \((P = 0.59)\), peripartum BCS \((P = 0.81)\), prepartum plasma glucose \((P = 0.98)\), or prepartum urea nitrogen \((P = 0.14)\).

The age difference between seasons in this study is due to 2 experiments in the fall utilizing primiparous dams whereas only 1 experiment in the spring used primiparous dams. Additionally, dams used in the spring experiments had a wider age range than dams used in 2 of the fall experiments. The differences in prepartum NEFA are likely due to spring-calving dams mobilizing greater amounts of body reserves during colder conditions (Slee and Halliday, 1968). Fall-calving dams had greater prepartum triglycerides which may indicate greater lipid intake or altered lipid metabolism. Less than 10\% of glucose is absorbed in the small intestine of grazing ruminants, therefore these animals rely on mainly on gluconeogenesis (Young, 1977). Similar maternal glucose concentrations between the 2 seasons suggest no difference in gluconeogenesis between spring- and fall-calving dams. There was no difference in maternal serum urea nitrogen concentrations, suggesting no difference in absorption of ammonia N or deamination of amino acids.

**Gestation Length and Calf Size**

There was no effect of calving season \((P = 0.52)\) on gestation length (Table 3.4). Gestation length has previously been reported to be shorter in dairy cows that experienced heat stress during late gestation (Tao et al., 2011; Dahl et al., 2016; Almoosavi et al., 2020). Therefore, in the current study it was expect that fall calving
beef dams would have shorter gestation lengths because they are more likely to experience heat stress during late gestation compared to spring calving beef dams. However, fall-calving dams may not have been heat stressed to the same extent of dams in the previously mentioned studies to cause shorter gestation lengths.

Calves born in the fall tended to have lighter \((P = 0.09)\) birth weight than calves born in the spring (Table 3.4). Other calf size measures, shoulder to rump length, heart girth, abdominal girth, and cannon circumference, were not affected by season \((P \geq 0.18)\) in the current study. There was no effect of calving season \((P \geq 0.40)\) on the indicators of calf shape, calf ponderal index and heart girth:length ratio.

Tao et al. (2012) suggested that 33% of the decreased birth weights in calves born to heat stressed dams could be attributed to the shorter gestation lengths of heat stressed dams. However, because of the lack of an effect of calving season on gestation length in the current study, the difference in calf birth weight can be attributed to difference in fetal growth rather than fetal age. One study observed changes in spring-born calf birth weights to have a 1:1 ratio with the change in ambient air temperatures where birth weights were heavier for calves from dams that experienced colder temperatures during late gestation (Colburn et al., 1997). Ambient air temperatures for spring calving experiments used in this study ranged from -6.2 to 3.8 °C (Table 3.1); whereas air temperatures ranged from -7.2 to 1.1 °C during late gestation in the study by Colburn et al. (1997). Temperatures in the current study were slightly warmer and had a greater range than those reported in Colburn et al. (1997) possibly contributing to only a tendency of greater birth weights of spring-born calves in this analysis.
Colburn et al. (1997) suggested that the colder conditions during late gestation increased blood flow to the uterus and decreased blood flow to the extremities to maintain maternal body temperature. Uterine blood flow is related to fetal growth because it is one of the determinants for fetal nutrient supply (Ferrell, 1991); therefore, greater fetal nutrient availability may potentially support more fetal growth (Colburn et al., 1997). Additionally, spring-calving dams are likely mobilizing more body reserves as seen by their greater NEFA concentration that potentially contributed to greater energy supply to support more fetal growth (Guedon et al., 1999; Abeni et al., 2004).

Maternal heat stress during gestation often depresses maternal nutrient intake and uterine blood flow, leading to reduced fetal growth (Reynolds et al., 1985). Studies in dairy cattle have reported that maternal heat stress during late gestation reduced calf birth weights (Tao et al., 2012), even when independent of reduced maternal nutrient intake (Almoosavi et al., 2020). Both Tao et al. (2012) and Almoosavi et al. (2020) suggest that the reduced fetal growth observed in calves from heat stressed dams may have resulted from altered uterine environment, placental insufficiency, and fetal hyperthermia.

Conversely, Bagley (1987) reported no difference in calf birth weights between those born in the fall or spring calving seasons. The lack of differences between calving seasons on other calf size measures and the indicators of calf shape despite the tendency for greater birth weights for spring-born calves may suggest that fall-born calves had slightly reduced skeletal, muscle, and organ growth that contributed to reduced total birth weight but was not impaired enough independently for statistical differences.
Calves born in the fall had faster \((P = 0.05)\) time to stand immediately after birth than calves born in the spring (Table 3.4). Table 3.1 includes average air temperatures for each calving experiment. These data show that spring-born beef calves were more likely to encounter cold stress postnatally, which may have caused greater heat loss and depressed vigor when compared with fall-born calves who were more likely to be born into thermoneutral or warmer conditions. Induced cold stress in dairy calves has been reported to reduce neonatal vigor where hypothermic calves were reluctant to stand and nurse and showed signs of muscle weakness (Olson et al., 1980b). Additionally, Dwyer and Morgan (2006) observed that lambs with lower rectal temperatures were slower to stand and suckle after birth. Those lambs that took longer to stand and suckle also struggled to maintain their body temperature during the first 72 h postnatal (Dwyer and Morgan, 2006). When neonates display the proper vigor behaviors and rapidly stand and suckle, particularly in cold stress conditions, heat loss to the ground is reduced and neonates are able to better maintain body temperature (Dwyer and Morgan, 2006).

There was no effect of calving season \((P = 0.91)\) on concentrations of 48-h serum total protein (Table 3.4), which is often used as a clinical indicator of passive transfer (Besser and Gay, 1994). Lamb mortality has been reported to be related with longer times to stand and suckle (Dwyer et al., 2001). Additionally, time to stand and time to suckle has been reported to be positively correlated (Wichman et al., 2019). Therefore, it may be inferred that because spring-born calves had longer times to stand, they would also have longer times to reach the udder and suckle. A delay in suckling may limit the colostrum ingested during the period of absorption for successful passive transfer (Stott et al.,
1979). Furthermore, in previous dairy calf studies, cold stress or hypothermic conditions
decreased concentrations of serum IgG up to 18 h after the first ingestion of colostrum
(Olson et al., 1980b) and maternal heat stress during late gestation decreased dairy calf
plasma total protein and serum IgG (Tao et al., 2012; Monteiro et al., 2014).

Despite the differences in the time to stand, in this study there was no difference
between fall and spring calves and their 48-h serum total protein concentrations. Along
with previous data from Guy et al. (1994), that reported that beef cows had greater
colostral concentrations of IgG than dairy cows, this may suggest that beef calves are
able to ingest and absorb adequate amounts of IgG despite cold or heat stress challenges.
Although previous data in dairy calves have recommended serum total protein
concentrations of 5.2 g/dL for passive transfer of immunity (Weaver et al., 2000), this
value is not necessarily applicable to beef calves (McGee and Earley, 2019).
Additionally, a more recent study has suggested serum total protein concentrations of 5.7
to 6.0 g/dL and 5.6 to 6.1 g/dL as new cut-offs for optimal dairy and beef calf health
outcomes, respectively (Todd et al., 2018).

**Calf Rectal Temperature**

There was an interaction ($P = 0.009$) of calving season × hour for neonatal calf
rectal temperature (Figure 3.2). Fall-born calves had greater ($P \leq 0.003$) rectal
temperature at 0, 24, and 48 h postnatal when compared with calves born in the spring. In
calves born in the fall, rectal temperature decreased ($P \leq 0.04$) from 6 to 12 h, followed
by an increase ($P \leq 0.007$) from 12 to 48 h of age. Rectal temperature in spring-born
calves increased ($P \leq 0.005$) from 0 to 6 h and 24 to 48 h.
Following parturition, the neonate must generate adequate quantities of heat to offset the heat loss caused by a large surface area-to-body mass ratio, evaporation of amniotic fluids, and weather conditions such as wind, precipitation, and air temperature (Vermorel et al., 1983; Azzam et al., 1993; Carstens, 1994). Failure to thermoregulate leads to hypothermia and can increase incidence of neonatal mortality; this has especially been shown in calves born in the spring calving season that often experience cold stress due to low ambient temperatures and greater incidence of precipitation at birth (Bagley, 1987; Azzam et al., 1993). The difference in rectal temperature at 0 h postnatal, which is following standing but prior to suckling, may be indicative of the cold stress spring calves are experiencing shortly after birth. Because there was no difference ($P = 0.11$; data not shown) between seasons for the time the rectal temperatures were recorded, differences are likely due to colder conditions during the spring experiments as described in Table 3.1. In attempts to cope with these conditions and to increase their body temperature, spring-born calves are likely relying on mobilization of their body stores and brown adipose tissue during the first 6 h postnatal more than the calves born in the fall (Vermorel et al., 1983; Hammon et al., 2012).

Within calves born in the fall, which are thought to be born into more thermoneutral conditions, their rectal temperature during the first 48 h postnatal were similar to previous reports of calves considered high vitality (Vermorel et al., 1983) or calves in a 31°C environment (Godfrey et al., 1991). Despite this, fall-born calves had greater rectal temperature at 24 and 48 h of age, which may suggest some fall-born calves experiencing heat stress due to the warmer conditions during fall-calving experiments (Table 3.1). Although calves born in the spring had slightly lower rectal temperature at 0
h than a previous report in spring born beef calves (Egli and Blum, 1998), calves in the current study were not hypothermic, which has been considered to occur at a body temperature of about 35.4°C in dairy calves (Okamoto et al., 1986). However, by 24 h spring-born calves’ rectal temperatures were similar to other studies in beef (Egli and Blum, 1998) and dairy calves (Hadorn et al., 1997). This may also suggest that the cold-stress mitigation efforts in spring experiments in the current study may have aided in preventing calf heat loss to the ground.

**Neonatal Calf Metabolites and Blood Chemistry**

*Energy-related metabolites.* Energy-related metabolites for neonatal beef calves are presented in Figure 3.3. There was an interaction ($P < 0.001$) of season × hour for circulating glucose, NEFA, and triglycerides. Calves born in the spring had greater ($P = 0.007$) glucose at 0 h of age when compared with calves born in the fall. Within fall-born calves, circulating glucose increased ($P < 0.001$) from 0 to 48 h postnatal. In calves born in the spring, circulating glucose increased ($P < 0.001$) from 6 to 24 h of age.

The increasing concentrations of glucose over time in the fall-born calves follows a similar pattern to previous reports and is attributed to the ingestion of lactose and the progressive production of endogenous glucose through glycogenolysis and gluconeogenesis (Hadorn et al., 1997; Hammon et al., 2012). Additionally, mobilization of hepatic glycogen serves as the initial energy source for neonatal calves (Hammon et al., 2012). Studies that exposed dairy (Okamoto et al., 1986) or brahman influenced (Godfrey et al., 1991) calves to cold stress conditions within the first 24 h postnatal reported increased circulating glucose concentrations following the cold exposure which
were attributed to calves having to rely on mobilization of their glycogen stores during hypothermia. The higher circulating glucose at 0 h in spring-born calves than calves born in the fall suggests that spring-born calves are mobilizing more glycogen immediately after birth to support thermogenesis.

Serum NEFA concentrations were greater ($P < 0.001$) at 0 and 6 h of age in calves born in the spring than those born in the fall (Figure 3.3). Serum NEFA in fall-born calves increased ($P < 0.001$) from 0 to 6 h and then decreased ($P < 0.001$) from 12 to 48 h postnatal. In calves born in the spring, NEFA concentrations decreased ($P < 0.001$) from 6 to 24 h of age. In cold stressed calves, lipid mobilization may be a greater energy source than glucose as NEFA are used as a substrate in brown adipose tissue (Vermorel et al., 1983; Okamoto et al., 1986; Carstens, 1994). Spring-born calves in the present study had greater serum NEFA during the first 6 h postnatal and likely relied on mobilization of their body lipid reserves for energy compared to calves born in the fall. Nonetheless, the peak in NEFA in fall-born calves at 6 to 12 h is likely due to mobilization of lipid reserves as an energy source prior to adequate lipid supply received through colostrum.

Circulating plasma triglycerides were greater ($P \leq 0.009$) in calves born in the spring at 0, 6, 12, and 48 h of age compared with calves born in the fall (Figure 3.3). Plasma triglycerides increased ($P < 0.001$) from 0 to 6 and 24 to 48 h of age in both spring and fall-born calves. In calves born in the spring, a decrease ($P = 0.03$) between 6 and 12 h also occurred. The difference at 0 h for plasma triglycerides is not due to ingestion of colostrum because the 0-h sampling was pre-suckling in all experiments. Lower triglycerides were reported in ovine fetuses that were considered small for
gestational age in nutrient restricted ewes (Steinhauser et al., 2021). Similar conditions may be imposed on calves that are born in the fall due to potential reduced prenatal nutrient supply and reduced fetal growth. However, increasing plasma triglycerides during the first 48 h postnatal has been reported to correspond with consumption of colostrum (Blum et al., 1997; Rauprich et al., 2000). Therefore, the difference at 6, 12, and 48 h in plasma triglyceride concentrations may be due to spring dams having greater colostral lipid content.

**Protein-related metabolites.** Protein-related metabolites are presented in Figure 3.4 and Table 3.5. There was an interaction ($P = 0.02$) of calving season $\times$ hour for serum urea nitrogen, where fall-born calves tended to have greater ($P < 0.10$) urea nitrogen at 48 h than calves born in the spring. Concentrations of serum urea nitrogen increased ($P < 0.001$) from 0 to 12 h of age in calves born in the fall. Similarly, urea nitrogen in spring-born calves also increased ($P < 0.001$) from 0 to 12 h, but then decreased ($P = 0.003$) from 24 to 48 h postnatal. As protein is being catabolized, ammonia is produced and then is converted to urea in the liver (Russell and Roussel, 2007). Heat stress may alter the glucose utilization hierarchy causing an increase in protein catabolism for gluconeogenic precursors (Baumgard and Rhoads Jr, 2013) and therefore potentially lead to higher blood urea concentrations (Wang et al., 2020). Additionally, concentrations of maternal serum urea nitrogen has been reported to be positively correlated with neonatal calf serum urea nitrogen concentrations at 1 h post-calving (Meyer et al., 2018a). Maternal concentrations of urea nitrogen were numerically greater in fall-calving dams which may have contributed to greater concentrations in the fall-born calves. Furthermore, in incidences of dehydration, the inability to concentrate urine may lead to increased urea in
the blood (Russell and Roussel, 2007). Calves born in the fall are more likely to experience heat stress which could lead to dehydration and result in greater urea nitrogen as seen at 48 h in the current study.

Globulin is calculated by subtracting measured albumin from measured total protein. There was a tendency ($P = 0.06$) of calving season × hour interaction for serum globulin and total protein (Figure 3.4). Serum globulin tended ($P = 0.09$) to be greater in fall-born calves at 24 h of age compared with spring-born calves. Globulin increased ($P < 0.001$) between 0 and 24 h and then decreased ($P < 0.001$) from 24 to 48 h in both fall and spring-born calves. Serum total protein was greater ($P = 0.03$) at 24 and tended to be greater ($P = 0.09$) at 48 h for fall-born calves. Following a similar pattern to globulin, total protein for both fall- and spring-born calves increased ($P < 0.001$) from 0 to 24 h and decreased ($P < 0.001$) from 24 to 48 h of age. Serum albumin was affected by the main effect of calving season ($P = 0.003$), where albumin was greater in calves that were born in the fall (Table 3.5).

Serum total protein is often used as an indicator of passive transfer, due to the main constituent of globulin being IgG (Weaver et al., 2000). The differences in circulating total protein at 24 h is likely due to the greater serum globulin at 24 h in fall-born calves which may be suggest greater absorption of IgG. However, by 48 h there was no difference in globulin between calves born in the fall and spring meaning that the difference in total protein at 48 h in fall-born calves may be due to greater albumin concentrations. Furthermore, as previously discussed, 48-h serum total protein between spring and fall-born calves was likely not indicative of improved passive transfer in fall-born calves. Rather, the greater concentrations of total protein, globulin, and albumin
observed in fall-born calves may be indicative of slight dehydration status (Thornton et al., 1972; Russell and Roussel, 2007).

There was no effect of calving season \((P = 0.31)\) on serum creatinine in this study (Table 3.5). Despite this, there was a main effect of hour \((P < 0.001)\) for serum creatinine concentrations. This may suggest that calving season does not influence muscle metabolism or renal function in eutocic calves. Rather, the decrease in serum creatinine over time during the neonatal period may be of more importance as previously reported by (Hadorn et al., 1997) and (Rauprich et al., 2000).

**Electrolytes.** Neonatal calf electrolytes are presented in Figure 3.5 and Table 3.5. There was an interaction \((P \leq 0.01)\) of calving season \(\times\) hour for neonatal serum sodium, phosphorus, and magnesium (Figure 3.5). Calves born in the fall had greater \((P \leq 0.01)\) serum sodium concentrations from 6 to 48 h of age compared with spring-born calves. In fall calves, there was a decrease \((P < 0.002)\) from 0 to 12 h postnatal in the sodium concentrations. In calves born in the spring, sodium decreased \((P < 0.001)\) from 0 to 24 h of age. Phosphorus concentrations were greater \((P \leq 0.02)\) in spring-born calves at 6 and 12 h of age. Phosphorus decreased \((P < 0.001)\) from 0 to 6 h of age in both fall- and spring-born calves followed by an increase \((P \leq 0.02)\) from 12 to 24 h and 12 to 48 h for spring- and fall-born calves, respectively. Serum magnesium was greater \((P \leq 0.03)\) in calves born in the fall between 0 and 24 h of age. Magnesium concentrations in calves born in both the fall and spring increased \((P < 0.001)\) from 0 to 12 h of age, followed by a subsequent decrease \((P < 0.001)\) from 12 to 48 h postnatal.

There was a main effect of calving season \((P \leq 0.03)\) for serum chloride and calcium (Table 3.5). Calves born in the fall had greater serum chloride \((P = 0.006)\) and
calcium ($P = 0.03$) compared to spring-born calves. Season did not affect ($P = 0.16$) serum potassium in this study (Table 3.5).

The differences observed in serum electrolytes where concentrations of sodium, chloride, magnesium, and calcium were greater in fall-born calves may suggest that they were moderately dehydrated (Russell and Roussel, 2007). This is likely due to the higher ambient temperatures fall-born calves are likely to experience following birth when compared with calves born in the spring. Additionally, electrolyte status can be influenced by metabolic-respiratory acidosis (Russell and Roussel, 2007), which calves born in the fall may have experienced as described in the following section. Furthermore, the greatest concentrations of sodium, magnesium and calcium are found in colostrum when compared to milk (Klimeš et al., 1986; McGrath et al., 2016). Klimeš et al. (1986) observed seasonal differences in dairy cow colostral phosphorus and sodium content by d 3 and 5 postpartum, respectively, where concentrations where greater in dams calving in February than in June. This may suggest seasonal differences of colostral macro elements also occur in beef cattle. However, in the current study fall-born calves had greater sodium, magnesium, and calcium suggesting further research is warranted to evaluate colostrum composition of beef between spring- and fall-calving seasons.

The patterns of phosphorous and magnesium in both fall and spring-born calves followed a similar pattern to those previously reported by Egli and Blum (1998). Sodium concentrations in the current study were within adult cattle reference ranges expect for the 0 h time point which was slightly above (Mohri et al., 2007). Serum potassium, chloride, and calcium also were within ranges that were suggested by Dillane et al. (2018) for healthy neonatal dairy calves.
**Acid-base status.** There was a main effect of calving season ($P = 0.03$) for bicarbonate and a tendency ($P = 0.10$) for serum anion gap (Table 3.5). Serum bicarbonate was greater ($P = 0.03$) in spring-born calves. Anion gap is calculated by adding cations, sodium and potassium, and then subtracting the anions, chloride and bicarbonate, which is used to indicate whether a calf is in an acidotic state (Russell and Roussel, 2007). Anion gap tended to be greater ($P = 0.10$) in calves born in the fall.

A study investigating seasonal differences in acid-base status in dairy calves reported calves born in the summer, comparable to fall-born calves in the current study, had greater anion gap and lower bicarbonate concentrations during the first 24 h postnatal (Kovács et al., 2017). This reflects a greater degree of acidosis in calves born in the summer (Kovács et al., 2017). Similar results were observed in the current study, where fall-born calves also had greater anion gap and lower concentrations of bicarbonate compared to calves born in the spring. This may suggest that fall-born beef calves may experience greater acidotic conditions during the first 48 h postnatal. A potential reason for these differences is fall-calves experience warmer ambient temperatures that could cause greater respiratory rates that rapidly expel CO$_2$ that leads to respiratory alkalosis (West, 2003). In attempts to compensate for the induced respiratory alkalosis, increased amounts of bicarbonate are excreted in the urine leading to decreased blood bicarbonate leading to metabolic acidosis (West, 2003).

**Bilirubin.** There was a main effect of calving season ($P = 0.007$) for direct bilirubin; however, there was no effect of calving season ($P = 0.15$) for total bilirubin (Table 3.5). Calves born in the spring had greater ($P = 0.007$) direct bilirubin compared with calves born in the fall. Indirect bilirubin is a byproduct of heme catabolism and
usually binds to albumin to be transported to the liver to be conjugated into direct bilirubin and then excreted into bile (Russell and Roussel, 2007). Total bilirubin measures both indirect and direct bilirubin.

Olson et al. (1980a) reported greater incidence of subcutaneous hemorrhage in extremities of dairy calves that were subjected to cold stress by lowering their core body temperature 10°C. The greater incidence of hemorrhage likely leads to greater hemoglobin breakdown and increased bilirubin concentrations in cold stressed calves (Bull et al., 1991). This may have occurred in spring-born calves, which could have contributed to their greater direct bilirubin concentrations. However, Bull et al. (1991) reported greater total bilirubin concentrations in neonatal beef calves experiencing ambient temperature of 0°C during the first 72 h postnatal, in the current study there was no difference between season for total bilirubin.

**Metabolic enzymes.** There was a tendency ($P = 0.06$) of calving season × hour interaction for aspartate aminotransferase and an interaction ($P = 0.004$) of calving season × hour for creatine kinase (Figure 3.6). Spring-born calves had greater ($P = 0.03$) serum aspartate aminotransferase at 12 and 24 h of age compared with calves born in the fall. In both fall- and spring-born calves, serum aspartate aminotransferase increased ($P < 0.001$) from 0 to 12 h, and then decreased ($P < 0.001$) from 24 to 48 h of age. Serum creatine kinase was greater ($P \leq 0.04$) at 0 and 12 h and tended to be greater ($P = 0.07$) at 6 h of age for calves born in the spring. In fall calves, serum creatine kinase increased ($P < 0.001$) from 0 to 6 h of age, then decreased ($P \leq 0.06$) between 24 and 48 h. In calves born in the spring, serum creatine kinase increased ($P < 0.001$) from 0 to 12 h postnatal, followed by a decrease ($P < 0.001$) from 12 to 48 h. There was no effect of calving
season \((P = 0.14)\) on neonatal circulating gamma-glutamyl transferase in this study (Table 3.5).

Aspartate aminotransferase is an enzyme involved in amino acid metabolism and is commonly found in muscle and liver tissue in cattle (Sattler and Fürll, 2004). Creatine kinase is an enzyme found in muscle and other tissues with high energy demands because it plays an important role in energy metabolism (Sattler and Fürll, 2004). When muscle or organ damage occurs, concentrations of aspartate aminotransferase and creatine kinase will increase and therefore be used as indicators of muscle or organ trauma (Russell and Roussel, 2007; Pearson et al., 2019). This may suggest that calves born in the spring experience more trauma during parturition. Additionally, Pearson et al. (2019) observed that calves with greater aspartate aminotransferase and creatine kinase concentrations were also less vigorous. This demonstrates that calves that experience trauma birth can also have reduced neonatal vigor, which was observed in NR calves in the current study.

Concentration of gamma-glutamyl transferase can also be an indicator of passive transfer; the lack of difference in serum gamma-glutamyl transferase in the present study further supports that calving season did not affect the ability of calves to achieve passive transfer (Perino et al., 1993).

**Relationship of 48-h Serum Total Protein with Calf Size and Vigor**

Correlation coefficients split by calving season between 48-h total protein, time to stand, and calf size measures are presented in Table 3.6. Time to stand was not correlated \((P = 0.27)\) with total protein at 48 h in fall- or spring-born calves. However, in fall-born calves, calf birth weight had a weak positive correlation \((P = 0.03)\) with time to stand. In
calves born in the fall, 48-h serum total protein had a weak positive correlation \((P \leq 0.02)\) with calf birth weight and calf heart girth. Abdominal girth also tended to have a very weak positive correlation \((P = 0.09)\) with 48-h serum total protein. Other calf size measures were not correlated \((P \geq 0.17)\) with 48-h serum total protein in fall-born calves. In calves born in the spring, calf size was not correlated \((P \geq 0.31)\) with 48-h serum total protein or time to stand.

The timely ingestion of colostrum is paramount for neonatal calf survival, not only for successful passive transfer but also the consumption of vital nutrients (Blum, 2006; Singh et al., 2011). Dairy calves that are unwilling to suckle and are generally less vigorous at birth often also have low consumption of colostrum (Furman-Fratczak et al., 2011). The lack of a relationship between time to stand and 48-h serum total protein for fall or spring-born calves in the current study may be due to type of calves being included in this analysis. Calves that experienced any calving difficulty were excluded from the correlation analysis and calves that did not stand within 2 h postnatal were removed from calving pens, processed, and assisted in suckling; therefore, no time to stand was calculated and metabolite data were excluded. Additionally, maintaining strong vigor behaviors that contribute to subsequent standing and suckling events occurring during the neonatal period may be of more importance to beef calf survival and prevention of starvation.

In fall-born calves, which experienced thermoneutral or warmer conditions after birth (Table 3.1), calf size was related to time to stand where lighter calves stand faster. This may be due to lighter calves having less body mass to coordinate to achieve standing compared to heavier calves or that heavier calves may experience more calving difficulty,
as suggested for dairy calves by Johanson and Berger (2003). Despite this, the weak relationship between calf size and 48-h serum total protein in fall-born calves may suggest that bigger calves are able to consume more colostrum during the first 48-h than smaller calves. Although not present in the current study, a negative relationship of calf size and time to stand along with a positive relationship between calf size and 48-h total protein for calves born in the spring could be hypothesized based on previous reports of reduced vigor in calves experiencing cold stress and that smaller calves are likely more susceptible to cold stress (Olson et al., 1980b; Carstens, 1994).

**CONCLUSION**

The results from this study indicate that calving season influences perinatal nutrient availability, which may impact the transition to postnatal life for beef calves. Although fall-born calves were lighter at birth, they were faster to stand immediately after birth which is important for timely ingestion of colostrum. However, in the present study there was no effect of calving season on passive transfer, indicated by 48-h passive transfer. Differences between seasons in other metabolites over time suggest neonates adapt to postnatal life differently across different calving seasons where thermoregulation plays an important role. Future studies are needed to compare colostrum production and composition between calving seasons in beef cattle as colostrum is likely contributing to the changes in circulating metabolites. Additionally, these data will help us to appropriately manage and interpret data from neonatal beef calves from spring and fall calving seasons.
Table 3.1 Description of calving experiments for determination of season effects (mean ± SD)\(^1\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 4</th>
<th>Exp. 5</th>
<th>Exp. 3</th>
<th>Exp. 6</th>
<th>Exp. 7</th>
<th>Exp. 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>First calving date</td>
<td>2/2/14</td>
<td>1/27/15</td>
<td>2/2/16</td>
<td>1/28/17</td>
<td>9/5/15</td>
<td>9/7/17</td>
<td>9/9/18</td>
<td>9/1/19</td>
</tr>
<tr>
<td>Length of calving season, d</td>
<td>46</td>
<td>83</td>
<td>44</td>
<td>15</td>
<td>11</td>
<td>36</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Average calving date</td>
<td>2/17/14</td>
<td>2/18/15</td>
<td>2/17/16</td>
<td>2/4/17</td>
<td>9/10/15</td>
<td>9/19/17</td>
<td>9/28/18</td>
<td>9/25/19</td>
</tr>
<tr>
<td>Average air temperature, °C</td>
<td>-6.2 ± 7.4</td>
<td>1.7 ± 6.8</td>
<td>2.0 ± 6.9</td>
<td>3.8 ± 5.5</td>
<td>22.0 ± 4.1</td>
<td>21.5 ± 3.1</td>
<td>18.8 ± 5.9</td>
<td>20.9 ± 6.2</td>
</tr>
<tr>
<td>Minimum air temperature, °C</td>
<td>-12.7 ± 7.0</td>
<td>-4.4 ± 6.6</td>
<td>-2.8 ± 5.7</td>
<td>-2.3 ± 4.4</td>
<td>15.9 ± 5.3</td>
<td>15.5 ± 3.9</td>
<td>13.0 ± 5.6</td>
<td>15.5 ± 6.7</td>
</tr>
<tr>
<td>Maximum air temperature, °C</td>
<td>0.13 ± 8.7</td>
<td>7.7 ± 7.9</td>
<td>7.6 ± 8.6</td>
<td>9.9 ± 7.0</td>
<td>28.1 ± 4.1</td>
<td>28.1 ± 3.7</td>
<td>25.1 ± 6.8</td>
<td>26.7 ± 6.7</td>
</tr>
<tr>
<td>Precipitation, cm</td>
<td>0.02 ± 0.02</td>
<td>0.07 ± 0.22</td>
<td>0.07 ± 0.23</td>
<td>0.002 ± 0.01</td>
<td>0.13 ± 0.41</td>
<td>0.24 ± 0.61</td>
<td>0.35 ± 1.64</td>
<td>0.33 ± 0.84</td>
</tr>
<tr>
<td>Calf sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heifers, n</td>
<td>26</td>
<td>22</td>
<td>23</td>
<td>26</td>
<td>32</td>
<td>24</td>
<td>17</td>
<td>45</td>
</tr>
<tr>
<td>Bulls, n</td>
<td>20</td>
<td>31</td>
<td>27</td>
<td>29</td>
<td>17</td>
<td>18</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Parity of dams</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous, n</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>18</td>
<td>13</td>
<td>--</td>
<td>18</td>
<td>--</td>
</tr>
<tr>
<td>Multiparous, n</td>
<td>46</td>
<td>53</td>
<td>50</td>
<td>38</td>
<td>37</td>
<td>42</td>
<td>14</td>
<td>57</td>
</tr>
<tr>
<td>Dam BW, kg</td>
<td>690 ± 73</td>
<td>678 ± 71</td>
<td>663 ± 78</td>
<td>624 ± 81</td>
<td>595 ± 79</td>
<td>704 ± 85</td>
<td>624 ± 98</td>
<td>666 ± 75</td>
</tr>
<tr>
<td>Dam body condition score(^2)</td>
<td>5.8 ± 0.5</td>
<td>5.2 ± 0.5</td>
<td>5.2 ± 0.6</td>
<td>5.2 ± 0.5</td>
<td>5.2 ± 0.7</td>
<td>5.5 ± 0.5</td>
<td>5.5 ± 0.5</td>
<td>5.4 ± 0.9</td>
</tr>
<tr>
<td>Dam age, yr</td>
<td>6.1 ± 2.4</td>
<td>5.6 ± 2.8</td>
<td>5.3 ± 2.5</td>
<td>4.2 ± 2.8</td>
<td>4.1 ± 2.4</td>
<td>4.3 ± 1.2</td>
<td>3.0 ± 1.1</td>
<td>5.1 ± 1.8</td>
</tr>
<tr>
<td>Dam age range, yr</td>
<td>3 - 12</td>
<td>3 - 13</td>
<td>3 - 14</td>
<td>2 - 15</td>
<td>2 - 12</td>
<td>3 - 7</td>
<td>3 - 10</td>
<td>2 - 5</td>
</tr>
</tbody>
</table>

\(^1\) Description of total number of animals, excluding data from pairs that had twining or still-births, from each experiment that was used in this study.

\(^2\) BCS evaluated on scale 1 to 9 (1 = emaciated, 9 = obese).
Table 3.2 Number of calves\(^1\) included in analysis for determination of calving season effects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fall(^2)</th>
<th>Spring(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to stand(^4), n</td>
<td>103</td>
<td>67</td>
</tr>
<tr>
<td>Calf sex, % male</td>
<td>33.0</td>
<td>56.7</td>
</tr>
<tr>
<td>Calf birth weight, n</td>
<td>177</td>
<td>202</td>
</tr>
<tr>
<td>Calf sex, % male</td>
<td>34.5</td>
<td>52.5</td>
</tr>
<tr>
<td>Calf size measures(^5), n</td>
<td>170</td>
<td>99</td>
</tr>
<tr>
<td>Calf sex, % male</td>
<td>35.3</td>
<td>53.5</td>
</tr>
<tr>
<td>Glucose, urea nitrogen, non-esterified fatty acid, triglycerides(^4,6), n</td>
<td>89</td>
<td>63</td>
</tr>
<tr>
<td>Calf sex, % male</td>
<td>30.3</td>
<td>54.0</td>
</tr>
<tr>
<td>Chem panel(^4,6), n</td>
<td>43</td>
<td>63</td>
</tr>
<tr>
<td>Calf sex, % male</td>
<td>39.5</td>
<td>54.0</td>
</tr>
<tr>
<td>Rectal temperature(^4,6), n</td>
<td>79</td>
<td>52</td>
</tr>
<tr>
<td>Calf sex, % male</td>
<td>27.8</td>
<td>55.8</td>
</tr>
<tr>
<td>48 h total protein(^4), n</td>
<td>125</td>
<td>124</td>
</tr>
<tr>
<td>Calf sex, % male</td>
<td>30.4</td>
<td>57.3</td>
</tr>
</tbody>
</table>

\(^1\) Excludes twin or still-born calves across all experiments.
\(^2\) Calving date range: September 1 – November 3; Average calving date: September 19.
\(^3\) Calving date range: January 28 – April 20; Average calving date: February 13.
\(^4\) Excludes calves with calving difficulty score > 1 (1 = unassisted, 5 = cesarean-section) across all experiments.
\(^5\) Calf size measures include shoulder to rump length, heart girth, abdominal girth, and cannon circumference.
\(^6\) Includes calves with data point for at least 4 of the 5 time points, and excludes calves tubed colostrum during first 48 h postnatal across all experiments.
Table 3.3 Effects of calving season on dam BW, body condition score, age and prepartum metabolites

<table>
<thead>
<tr>
<th>Variables</th>
<th>Season</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepartum BW(^4), kg</td>
<td>Fall(^2)</td>
<td>0.59</td>
</tr>
<tr>
<td>Peripartum body condition score(^5)</td>
<td>Spring(^3)</td>
<td></td>
</tr>
<tr>
<td>Dam age, yr</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Prepartum metabolites(^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>63.7 ± 2.0</td>
<td>0.98</td>
</tr>
<tr>
<td>Non-esterified fatty acids, μEq/L</td>
<td>63.7 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>368 ± 71</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Urea nitrogen, mg/dL</td>
<td>397 ± 16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>289 ± 15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.5 ± 2.9</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>8.5 ± 2.9</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SEM presented for measures.
\(^2\) Calving date range: September 1 – November 3; Average calving date: September 19.
\(^3\) Calving date range: January 28 – April 20; Average calving date: February 13.
\(^4\) Measured at 12 ± 9.4 d [SD] prior to calving.
\(^5\) Determined at 9.1 ± 14.4 d [SD] prior to calving on scale 1 to 9 (1 = emaciated, 9 = obese).
\(^6\) Jugular blood samples collected at 11.2 ± 8.0 d [SD] prior to calving.
Table 3.4. Effects of calving season on calf size, vigor, and 48-h serum total protein

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fall(^2)</th>
<th>Spring(^3)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation length(^4), d</td>
<td>280 ± 1.4</td>
<td>279 ± 1.3</td>
<td>0.52</td>
</tr>
<tr>
<td>Calf Size(^5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>33.9 ± 0.9</td>
<td>36.1 ± 0.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Shoulder to rump length, cm</td>
<td>58.2 ± 0.8</td>
<td>59.0 ± 1.2</td>
<td>0.58</td>
</tr>
<tr>
<td>Heart girth, cm</td>
<td>73.6 ± 1.3</td>
<td>76.3 ± 1.9</td>
<td>0.25</td>
</tr>
<tr>
<td>Abdominal girth, cm</td>
<td>69.6 ± 2.5</td>
<td>75.3 ± 3.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Cannon circumference, cm</td>
<td>12.1 ± 0.3</td>
<td>12.7 ± 0.4</td>
<td>0.25</td>
</tr>
<tr>
<td>Ponderal index(^6), kg/m(^3)</td>
<td>176 ± 8</td>
<td>176 ± 12</td>
<td>0.97</td>
</tr>
<tr>
<td>Heart girth:Length(^7)</td>
<td>1.27 ± 0.02</td>
<td>1.30 ± 0.03</td>
<td>0.40</td>
</tr>
<tr>
<td>Time to stand(^8), min</td>
<td>23.4 ± 1.8</td>
<td>28.8 ± 2.1</td>
<td>0.05</td>
</tr>
<tr>
<td>48-h serum total protein, g/dL</td>
<td>6.65 ± 0.2</td>
<td>6.62 ± 0.2</td>
<td>0.91</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SEM presented for measures.
\(^2\) Calving date range: January 28 – April 20; Average calving date: February 13.
\(^3\) Calving date range: September 1 – November 3; Average calving date: September 19.
\(^4\) Calculated for cows that conceived by artificial insemination.
\(^5\) Calves were weighed and measured at 8.9 ± 10.8 h [SD] of age.
\(^6\) Ponderal index = calf birth weight (kg)/shoulder to rump length (m)\(^3\).
\(^7\) Ratio of heart girth (cm):shoulder to rump length (cm).
\(^8\) Defined as time from birth to calf standing on all 4 legs for a minimum of 5 consecutive seconds.
Table 3.5. Effects of calving season and sampling hour on neonatal calf blood chemistry during the first 48 h postnatal\(^1\)

<table>
<thead>
<tr>
<th>Variable(^2)</th>
<th>Season</th>
<th>P-values</th>
<th>Season</th>
<th>Hour</th>
<th>Season × Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall(^3)</td>
<td>Spring(^4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>84.3 ± 4.9</td>
<td>95.6 ± 6.8</td>
<td>0.18</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Non-esterified fatty acids, μEq/L</td>
<td>425 ± 30</td>
<td>560 ± 41</td>
<td>0.008</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>283 ± 17</td>
<td>398 ± 22</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Urea nitrogen, mg/dL</td>
<td>10.4 ± 1.6</td>
<td>7.3 ± 2.2</td>
<td>0.23</td>
<td>&lt; 0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>2.56 ± 0.1</td>
<td>2.49 ± 0.1</td>
<td>0.66</td>
<td>&lt; 0.001</td>
<td>0.31</td>
</tr>
<tr>
<td>Globulin, g/dL</td>
<td>3.68 ± 0.1</td>
<td>3.59 ± 0.1</td>
<td>0.56</td>
<td>&lt; 0.001</td>
<td>0.06</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>2.46 ± 0.05</td>
<td>2.24 ± 0.05</td>
<td>0.003</td>
<td>&lt; 0.001</td>
<td>0.40</td>
</tr>
<tr>
<td>Total Protein, g/dL</td>
<td>6.12 ± 0.2</td>
<td>5.82 ± 0.1</td>
<td>0.15</td>
<td>&lt; 0.001</td>
<td>0.06</td>
</tr>
<tr>
<td>Sodium, mEq/L</td>
<td>143 ± 0.8</td>
<td>140 ± 0.8</td>
<td>0.009</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Phosphorus, mg/dL</td>
<td>7.27 ± 0.08</td>
<td>7.49 ± 0.07</td>
<td>0.05</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Magnesium, mg/dL</td>
<td>2.63 ± 0.03</td>
<td>2.47 ± 0.03</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Chloride, mEq/L</td>
<td>101 ± 0.98</td>
<td>98 ± 0.97</td>
<td>0.006</td>
<td>&lt; 0.001</td>
<td>0.31</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>11.3 ± 0.1</td>
<td>10.9 ± 0.1</td>
<td>0.03</td>
<td>&lt; 0.001</td>
<td>0.20</td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>5.29 ± 0.05</td>
<td>5.39 ± 0.05</td>
<td>0.16</td>
<td>&lt; 0.001</td>
<td>0.43</td>
</tr>
<tr>
<td>Bicarbonate, mEq/L</td>
<td>26.6 ± 0.6</td>
<td>28.6 ± 0.6</td>
<td>0.03</td>
<td>0.002</td>
<td>0.48</td>
</tr>
<tr>
<td>Anion gap, mEq/L</td>
<td>20.1 ± 0.4</td>
<td>19.1 ± 0.4</td>
<td>0.10</td>
<td>&lt; 0.001</td>
<td>0.20</td>
</tr>
<tr>
<td>Direct bilirubin, mg/dL</td>
<td>0.28 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.007</td>
<td>&lt; 0.001</td>
<td>0.89</td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>0.69 ± 0.03</td>
<td>0.74 ± 0.03</td>
<td>0.15</td>
<td>&lt; 0.001</td>
<td>0.64</td>
</tr>
<tr>
<td>Aspartate</td>
<td>47.9 ± 1.5</td>
<td>50.9 ± 1.3</td>
<td>0.12</td>
<td>&lt; 0.001</td>
<td>0.06</td>
</tr>
<tr>
<td>Gamma-glutamyl transferase, U/L</td>
<td>911 ± 93</td>
<td>1,088 ± 76</td>
<td>0.14</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatine kinase, U/L</td>
<td>185 ± 23</td>
<td>258 ± 19</td>
<td>0.02</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SEM presented for measures.
\(^2\) Jugular blood samples collected at 0 (after standing and prior to suckling), 6, 12, 24, and 48 h postnatal. Calves needed to have a blood sample for at least 4 of the 5 time points to be included in the analysis.
\(^3\) Calving date range: September 1 – November 3; Average calving date: September 19.
\(^4\) Calving date range: January 28 – April 20; Average calving date: February 13.
Table 3.6. Partial correlation coefficients (r) and associated P-values between 48-h calf serum total protein, time to stand, and calf size from calves born in the fall or spring

<table>
<thead>
<tr>
<th>Fall Variable</th>
<th>Time to stand, min</th>
<th>Calf birth weight, kg</th>
<th>Shoulder to rump length, cm</th>
<th>Heart girth, cm</th>
<th>Abdominal girth, cm</th>
<th>Cannon circumference, cm</th>
<th>Ponderal index, kg/m^3</th>
<th>Heart girth:Length^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 h total protein, g/dL</td>
<td>0.12 (P = 0.27)</td>
<td>0.24 (P = 0.006)</td>
<td>0.12 (P = 0.17)</td>
<td>0.21 (P = 0.02)</td>
<td>0.15 (P = 0.09)</td>
<td>0.12 (P = 0.20)</td>
<td>0.003 (P = 0.97)</td>
<td>0.04 (P = 0.69)</td>
</tr>
<tr>
<td>Time to stand, min</td>
<td>--</td>
<td>0.21 (P = 0.03)</td>
<td>0.07 (P = 0.47)</td>
<td>0.13 (P = 0.21)</td>
<td>0.05 (P = 0.66)</td>
<td>0.15 (P = 0.14)</td>
<td>0.02 (P = 0.86)</td>
<td>0.005 (P = 0.96)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spring Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 h total protein, g/dL</td>
</tr>
<tr>
<td>Time to stand, min</td>
</tr>
</tbody>
</table>

1 Calving date range: September 1 – November 3; Average calving date: September 19.
2 Defined as time from birth to calf standing on all 4 legs for a minimum of 5 consecutive seconds.
3 Ponderal index = calf birth weight (kg)/shoulder to rump length (m)^3.
4 Ratio of heart girth (cm):shoulder to rump length (cm).
5 Calving date range: January 28 – April 20; Average calving date: February 13.
Figure 3.1. Drylot calving pen layout (18 × 61 m) for all experiments (Duncan and Meyer, 2019). Hay feeders with cone chains were placed on 9.1 × 9.1 m concrete pads; feed bunks were 9.8 m per pen; each shed was split between 2 pens and were closed off unless during inclement weather during spring-calving experiments. During fall-calving experiments, gates were hung from the front of each shed to provide creep shade for calves.
Figure 3.2. Effects of calving season on rectal temperature of neonatal beef calves. Least squares means ± SEM are presented. Open circles (○) represent calves born in the spring and solid circles (●) represent calves born in the fall. There was a calving season × hour interaction ($P \leq 0.009$). *Calving season means within hour differ ($P \leq 0.05$). abc Means differ ($P \leq 0.05$) for spring-born calves across hours. xyz Means differ ($P \leq 0.05$) for fall-born calves across hours.
Figure 3.3. Effects of calving season on serum energy metabolites glucose (Panel A), non-esterified fatty acid (NEFA; Panel B), and triglycerides (Panel C) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Open circles (○) represent calves born in the spring and solid circles (●) represent calves born in the fall. There was an interaction of season × hour for glucose (P < 0.001), NEFA (P < 0.001), and triglycerides (P < 0.001). *Calving season means within hour differ (P ≤ 0.05). abcd Means differ (P ≤ 0.05) for spring-born calves across hours. vwxyz Means differ (P ≤ 0.05) for fall-born calves across hours.
Figure 3.4. Effects of calving season on serum protein metabolites urea nitrogen (urea N; Panel A), globulin (Panel B), and total protein (Panel C) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Open circles (○) represent calves born in the spring and solid circles (●) represent calves born in the fall. There was an interaction of season × hour for urea N (P = 0.02) and tendency for globulin (P = 0.06) and total protein (P = 0.06). *Calving season means within hour differ (P ≤ 0.05), + tend to differ (P < 0.10). abcd Means differ (P ≤ 0.05) for spring-born calves across hours. wxyz Means differ (P ≤ 0.05) for fall-born calves across hours.
Figure 3.5. Effects of calving season on serum electrolytes including sodium (Panel A), phosphorus (Panel B), and magnesium (Panel C) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Open circles (○) represent calves born in the spring and solid circles (●) represent calves born in the fall. There was an interaction of season × hour for sodium (P < 0.001), phosphorus (P < 0.001), and magnesium (P = 0.01). *Calving season means within hour differ (P ≤ 0.05). a,b,c,d Means differ (P ≤ 0.05) for spring-born calves across hours. v,w,x,y,z Means differ (P ≤ 0.05) for fall-born calves across hours.
Figure 3.6. Effects of calving season on serum metabolic enzymes including aspartate aminotransferase (AST; Panel A) and creatine kinase (CK; Panel B) of neonatal beef calves during the first 48 h postnatal. Least squares means ± SEM are presented. Open circles (○) represent calves born in the spring and solid circles (●) represent calves born in the fall. There was a tendency for a season × hour interaction ($P = 0.06$) for AST and a season × hour interaction ($P = 0.004$) for CK. *Calving season means within hour differ ($P \leq 0.05$), †tend to differ ($P < 0.10$). abcde Means differ ($P \leq 0.05$) for spring-born calves across hours. xyz Means differ ($P \leq 0.05$) for fall-born calves across hours.
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Appendix Figure 1. Effect of hour ($P \leq 0.02$) on neonatal beef calf hematology including white blood cells (WBC; Panel A), mean corpuscular volume (MCV; Panel B), mean
corpuscular hemoglobin (MCH; Panel C), mean corpuscular hemoglobin concentration (MCHC; Panel D), and platelets (Panel E) during the first 48 h (data from chapter 2). Least square means ± SEM are presented. \textit{abcede} Means differ \((P \leq 0.05)\) across hours.
Appendix Figure 2. Effect of hour ($P < 0.001$) on serum protein metabolites creatinine (Panel A) and albumin (Panel B) of spring- and fall-born neonatal beef calves during the first 48 h postnatal (data from chapter 3). Least squares means ± SEM are presented. $^{abcde}$ Means differ ($P \leq 0.05$) across hours.
Appendix Figure 3. Effect of hour ($P < 0.001$) on serum electrolytes including chloride (Panel A), calcium (Panel B) and potassium (Panel C) of spring- and fall-born neonatal beef calves during the first 48 h postnatal (data from chapter 3). Least squares means ± SEM are presented. $^{abc}$ Means differ ($P \leq 0.05$) across hours.
Appendix Figure 4. Effect of hour ($P < 0.002$) on serum bicarbonate (Panel A) and anion gap (Panel B) of spring- and fall-born neonatal beef calves during the first 48 h postnatal (data from chapter 3). Least squares means ± SEM are presented. $^{abcd}$ Means differ ($P \leq 0.05$) across hours.
Appendix Figure 5. Effect of hour ($P < 0.001$) on serum direct bilirubin (Panel A) and total bilirubin (Panel B) of spring- and fall-born neonatal beef calves during the first 48 h postnatal (data from chapter 3). Least squares means ± SEM are presented. $^{abcd}$ Means differ ($P \leq 0.05$) across hours.
Appendix Figure 6. Effect of hour \((P < 0.001)\) on serum gamma-glutamyl transferase of spring- and fall-born neonatal beef calves during the first 48 h postnatal (data from chapter 3). Least squares means ± SEM are presented. \(^{abcde}\) Means differ \((P \leq 0.05)\) across hours.