METABOLIC STATUS OF LATE GESTATION BEEF COWS AND NEONATAL CALVES

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By

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

METABOLIC STATUS OF LATE GESTATION BEEF COWS AND NEONATAL CALVES

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A candidate for the degree of Master of Science,

And hereby certify that in their opinion it is worthy of acceptance.

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DEDICATION

To my parents

Cary and Connie Larson

Thank you for your unconditional love,

believing in me, and

instilling in me a strong work ethic

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METABOLIC STATUS OF LATE GESTATION BEEF COWS AND NEONATAL CALVES

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ABSTRACT

Two studies were conducted to investigate the changes in beef cattle metabolic status over time. In the first study, the effects of grazing stockpiled tall fescue (STF) versus feeding tall fescue hay during late gestation on nutrient availability for fetal development were investigated over 2 yr. Blood urea nitrogen and plasma glucose were greater in cows grazing STF during late gestation. In yr 1, NEFA was greater on d 56 of study and in yr 2 was greater on d 35, 77, and 99 of study in STF cows. Cows grazing STF tended to have greater thyroxine on d 77 in yr 1. Serum triiodothyronine was less on d 0 but greater on d 99 in cows grazing STF. Cortisol concentrations were greater in STF cows on d 77. Calf birth weight was positively correlated with prepartum maternal BUN and NEFA prior to calving. Results indicate that cows grazing STF had altered metabolic status, which may impact fetal development and calf performance. The objective of the second study was to investigate changes in neonatal calf circulating metabolites and blood chemistry, to determine relationships of metabolites among sampling times, and to determine the relationship between calf vigor and blood chemistry. Circulating plasma glucose, serum BUN, NEFA, and a complete chemistry profile demonstrated changes during the first 72 h postnatally. Sampling times were related at limited hours. Time to stand was correlated with serum BUN, NEFA, and albumin at select sampling hours. In conclusion, neonatal calf metabolic status changes during the first 72 h therefore a consistent blood sampling time is necessary and may be related to vigor after colostrum intake.

REVIEW OF LITERATURE

INTRODUCTION

Developmental programming is the theory that the maternal environment during gestation has effects on fetal growth and development, which may have lasting impacts of postnatal growth, health, and performance on offspring (Barker et al., 1993; Reynolds et al., 2010). In order for the maternal environment to provide for proper fetal development, the dam must adapt to changes during gestation and in preparation for lactation. These adaptations to improve conditions for the maternal and fetal systems include cardiovascular, respiratory, organ size, metabolic, and metabolic hormone changes. One of the major contributors to proper adaptation during pregnancy is maternal nutrition, especially during late gestation when the majority of fetal growth occurs (Robinson et al., 1977). The dam's nutrient requirements are exponentially increasing and other maternal adaptations that happen during pregnancy occur at this time.

The fetus also undergoes changes during the prenatal period, which are necessary for neonatal survival. The maternal environment may have consequences on critical fetal developmental windows, which may impact changes during gestation that prepare the fetus for the extrauterine environment after calving. Understanding the changes and critical periods of fetal development may impact growth, development, and subsequent health and performance of the offspring. The objective of this review is to discuss the adaptation the maternal environment undergoes during pregnancy including physiological changes, the impact of maternal nutrition during gestation on these adaptations, and

changes the offspring undergoes during the neonatal period for optimal health and survival in ruminants.

MATERNAL ADAPTATION TO PREGNANCY

Normal Changes During Pregnancy

Cardiovascular Adaptations. The most dramatic changes and adaptations during pregnancy occur in the maternal cardiovascular system. The system is critical and important for the transport of nutrients from the maternal system to the placenta, ultimately reaching the fetus during gestation (Thornburg et al., 2006). Cardiovascular adaptations begin within a few weeks of conception and continues through parturition and involve remodeling of the maternal circulation (Thornburg et al., 2006). The maternal cardiovascular system adapts to pregnancy in multiple ways. First, there is an increase in total blood volume (Metcalfe and Parer, 1966). Although there was no change in blood volume per kg of total BW, pregnant ewes had an increase in blood volume after fetal and uterine contents were removed compared with the total blood volume in non-pregnant ewes (Metcalfe and Parer, 1966). Barcroft et al. (1939) conducted a similar study in ewes and measured blood volume changes throughout gestation. In this study, there was an average increase in blood volume of 24% at term compared with 10 d after breeding (Barcroft et al., 1939). This increase in blood volume is essential for 2 main purposes: 1) to allow more efficient maternal and fetal exchange of gases, nutrients, and metabolites to support fetal growth and development and 2) to reduce the impact of blood loss during parturition (Thornburg et al., 2006).

Maternal heart rate also increases through gestation. This may be due to the increase in the transport of blood to maternal organs and to the uteroplacenta (Metcalfe

and Parer, 1966). Heart rate increased by 28% in pregnant compared with non-pregnant ewes throughout gestation. Pregnant ewes had a maximum heart rate of 108 beats/min during the final 30 d of pregnancy and declined to a postpartum heart rate of 84 beats/min (Metcalfe and Parer, 1966). Rovinsky and Jaffin (1966) suggested that the increase in heart rate during gestation has a positive relationship with fetal mass. Ewes carrying a single fetus had a 21% increase in heart rate compared with non-pregnant ewes. Ewes carrying twin fetuses had a 42% increase in heart rate compared with non-pregnant ewes (Rovinsky and Jaffin, 1966).

Cardiac output is the volume of blood pumped by the heart per min and has been shown to increase progressively in sheep (Metcalfe and Parer, 1966; Rosenfeld, 1977) and goats (Hoversland et al., 1974) through pregnancy. During early gestation, the majority of the increase in cardiac output is directed toward nonreproductive tissues. As gestation progresses and placental vascularization develops, the rate of blood flow to other maternal tissues return to the nonpregnant levels but increases to the placenta and mammary gland (Stock and Metcalfe, 1994). Cardiac output has also been shown to increase in rats during gestation. The change in cardiac output tended to increase in pregnant compared with nonpregnant rats by mid-gestation and increase further when measured during late gestation (Gilson et al., 1992).

Systolic and diastolic blood pressures of pregnant sheep did not change during gestation, however, blood pressures of non-pregnant ewes increased when measured during the same period (Metcalfe and Parer, 1966). Pregnant ewes had a 16% increase in systolic blood pressure and a 9% increase in diastolic blood pressure postpartum compared with prepartum values (Metcalfe and Parer, 1966). The increase was still less

however, than non-pregnant ewe blood pressure values. This change is blood pressure may be caused by the increase in total blood volume during gestation.

Cardiovascular adaptations during normal pregnancy allow uterine blood flow to increase more than 10-fold throughout gestation (Resnik and Brink, 1978). There is little resistance to blood flow in the vasculature of the placenta during normal pregnancy for most species (Vonnahme et al., 2013a), and this provides adequate nutrient and gas exchange between maternal and fetal circulation. The mechanisms involved in uterine blood flow during pregnancy have been better demonstrated in sheep than in any other species (Stock and Metcalfe, 1994). During early pregnancy in sheep, the amount of oxygen extracted from the blood in the uterine circulation is low (Reynolds et al., 1983). As gestation progresses, oxygen extraction increases with the exponential growth and demands of the fetus (Stock and Metcalfe, 1994). The variety of adaptations that the cardiovascular system undergoes throughout pregnancy is essential for the transport of nutrients and gas exchange from the maternal to the fetal system. This ensures proper fetal growth and development. Blood volume, heart rate, cardiac output, blood pressure, and uterine blood flow all have crucial roles in the dam's adaptation to pregnancy.

Uteroplacental Adaptation. Uteroplacental blood flow is directly related to the efficiency of placental nutrient transport (Reynolds and Redmer, 1995). The transport of nutrients and waste between the dam and fetus is conducted at the site of placentomes where the fetal cotyledons attach to the caruncles on the uterine wall. In order to maximize the nutrients that are delivered to the uteroplacenta and available to the fetus, adequate vascularization of the placenta is critical (Vonnahme and Lemley, 2011; Vonnahme et al., 2013a). In sheep and cattle, the majority of placental growth occurs

during early to mid-gestation. The placenta reaches maximum weight by d 90 in sheep (Ferrell et al., 1976; Reynolds and Redmer, 1995; Redmer et al., 2004). From mid- to late gestation in sheep, capillary area increases (Borowicz et al., 2007; Funston et al., 2010b). In cattle, however, this increase in capillary area is not the same and instead the placentome mass increases in order to provide more nutrient exchange during late gestation when the fetal nutrient demand is at the greatest demand (Vonnahme et al., 2007).

Blood flow to the placenta increases throughout pregnancy, and by late gestation more than 83% of the total blood flow is directed to the fetal cotyledonary vasculature in the ewe (Makowski et al., 1968; Rosenfeld, 1977). Similar results were reported in rats, where cardiac output to the uterus was increased during gestation in pregnant females compared with non-pregnant controls (Ahokas et al., 1984). Near the end of gestation in the ewe, blood vessels dilate to accommodate the high nutrient demand of the fetus. The total placental blood flow doubles without additional growth in vasculature (Teasdale, 1976). Normal fetal umbilical blood flow increases throughout most of gestation at the same rate as fetal growth. This results in constant blood flow per unit of fetal weight during normal pregnancy (Reynolds and Redmer, 1995). Studies conducted by Anderson et al. (2005, 2006) determined the impacts of restricted blood flow on the uterus during late gestation in rats. A sham procedure was used to create a reduced uteroplacental perfusion pressure model. Placental weight, individual fetal weight, and litter size were reduced when blood flow was reduced. This indicated that a fetal growth restriction occurred. Ultimately this may have had an impact on the cardiovascular function in both F_1 and F_2 offspring (Anderson et al., 2005; Anderson et al., 2006). The work

demonstrates the importance of placental blood flow during pregnancy that is crucial for nutrient delivery from the placenta to the fetus to optimize fetal growth and development.

Respiratory System Adaptations. Oxygen consumption increases throughout gestation due to the demand by the maternal and fetal tissues. In order to meet these demands, maternal blood must increase in the concentration of oxygen available, the respiratory system therefore must adapt to pregnancy (Thornburg et al., 2006). In rats there were minimal changes in the respiratory system during early to mid-gestation, with the exception of a decrease in arterial-mixed venous oxygen concentrations measured during mid-gestation (Gilson et al., 1992). A decrease in the oxygen concentration from the mixed venous blood indicates that the tissues in the body have taken up the oxygen from the blood to meet the maternal tissue and fetal requirements (Gilson et al., 1992). By late gestation, blood oxygen content was even less in pregnant rats compared with nonpregnant females (Gilson et al., 1992). This increase in oxygen consumption is a main contributor to the increase in cardiac output during late gestation (Gilson et al., 1992). Additionally, the thoracic cavity increases in size to adapt to the increase in lung volume. This increases respiration in most species to meet oxygen delivery requirements (Thornburg et al., 2006). The adaptation of the maternal respiratory system to maximize oxygen availability is critical during pregnancy to meet both maternal and fetal tissue demands.

Organ Size Adaptations. Maternal organs adapt to pregnancy in order to meet metabolic needs during gestation (Stock and Metcalfe, 1994). Heart and kidney mass was not different at d 90 or 130 of gestation in pregnant ewes, however lung and liver mass increased by d 130 of gestation compared with d 90 of gestation. The change in liver

mass is likely due to the major role it plays in maternal metabolism and adaption during pregnancy (Scheaffer et al., 2004). Spleen mass decreased from d 90 to 130 of gestation which may be due to the increased shortage or the use of platelets and white blood cells through gestation (O'Neill, 1985). Rosenfeld (1977) reported a decrease in spleen mass in ewes as gestation progressed with no change in blood flow (Rosenfeld, 1977). Although there was no difference in total mass of the digestive tract from d 90 to 130 of gestation, small intestinal mass increased and total internal organs increased even when uterine contents were excluded in ewes from d 90 to 130 (Scheaffer et al., 2004). Total digestive tract weights in cattle have been reported to increase from d 125 compared with d 245 pregnant cows, however small and large intestinal weights were not affected by d of gestation (Meyer et al., 2010). Similar studies reported no difference in small or large intestinal mass during gestation (Scheaffer et al., 2001). Liver weights have been reported to not be affected by d of gestation. Pancreatic mass increased with d of gestation. As a proportion of empty body weight, however, both liver and pancreatic mass increased during gestation (Meyer et al., 2010). Heart mass has been reported to increase in pregnant heifers at d 200 of gestation. This may be due to the changes and adaptations within the cardiovascular system (Scheaffer et al., 2001). The adaptation of organs are crucial for nutrient delivery and blood flow to maternal tissues and the fetus especially during late gestation.

Metabolic Adaptations. Circulating metabolites are used as an indicator of the dam's nutritional and metabolic status. From a production standpoint, reproducing females have their greatest nutrient requirement during lactation, followed by late gestation (NRC, 2000). During the last 90 d of pregnancy in cattle, the dam's energy and

protein requirements increase at a similar rate of fetal growth (NRC, 2000). In litter-bearing ruminant species such as sheep and goats, nutrient requirements increase as fetal count increases (NRC, 2007). The dam may alter metabolism in response to the energy demands of pregnancy due to the increase in nutrient demands especially during mid- to late gestation (Wood et al., 2013). The alterations in maternal metabolism may be determined by circulating metabolites that determine nutrient availability.

Ammonia concentrations increase when there are an excess of available amino acids relative to energy (Hammond, 1992). The unused ammonia gets absorbed and enters the portal blood to the liver, where it is detoxified in the small intestine, kidney and enters the urea cycle producing urea. Urea then enters the blood stream through the hepatic vein, resulting in an increase of blood urea nitrogen **(BUN)** (Hammond, 1992). Bispham et al. (2003) measured circulating BUN concentrations in ewes when NRC nutrient requirements were met from mid- to late gestation and determined that BUN concentrations decreased in pregnant ewes (Bispham et al., 2003). Additionally, circulating BUN was determined in pregnant and non-pregnant beef cows during mid- to late gestation (Wood et al., 2013). After 56 d of a feeding period initiated between d 150 to 165 of gestation, pregnant cows had greater BUN concentrations compared with non-pregnant cows (determined between 4 and 5 weeks prior to parturition; (Wood et al., 2013)). There was no difference in nutrient intake between pregnant and non-pregnant cows (Wood et al., 2013). The increase in circulating BUN may, therefore, be due to the deficiency of energy available compared with protein when demands for energy in the pregnant state are increased.

Glucose is a major energy source used for both maternal tissues as well as fetal growth and development during pregnancy (Doornenbal et al., 1988b). In most species, there is little variation in glucose concentrations associated with gluconeogenesis. This indicates that the uptake and metabolism of glucose is closely regulated (Doornenbal et al., 1988b). In pregnant ruminants, less than 10% of the total glucose utilized is absorbed as glucose in the small intestine. Most of the glucose that is available to tissues is therefore supplied by gluconeogenesis (Otchere et al., 1974; Young, 1976). This may explain why glucose concentrations are closely regulated and have little variation in circulating glucose concentrations. Additionally, ruminants are insulin sensitive, which also helps control the uptake and metabolism of glucose (Reynolds et al., 1990).

There was little variation in blood glucose concentrations during early gestation in beef cattle (Doornenbal et al., 1988b). During mid-gestation, however, there was an increase in glucose concentrations, which may be explained by weaning and the end of lactation. Lactose is a dimer of galactose and glucose. Ending lactation may reduce glucose demand and increase circulating glucose concentrations (Doornenbal et al., 1988b). Linden et al. (2014) compared plasma glucose concentrations in pregnant verses non-pregnant cows and heifers from 7 to 1 wk prepartum. They reported that non-pregnant cows and heifers had greater glucose concentrations compared with pregnant cows and heifers during this period of gestation (Linden et al., 2014). During late gestation, the fetal demand for glucose increases by nearly 50% (Bell, 1995). Despite this, Vernon et al. (1981) measured glucose concentrations in ewes and reported no changes throughout gestation (Vernon et al., 1981). Other studies have also reported no

change in plasma glucose in the uterine artery and fetal circulation throughout gestation (Reynolds et al., 1990).

Circulating non-esterified fatty acid (**NEFA**) concentrations are an indicator of fat mobilization. An increase in circulating NEFA concentrations is an adaptation to pregnancy in most species (Pethick et al., 1983). The change in NEFA may be independent of energy status (Petterson et al., 1994). Serum NEFA concentrations in pregnant ewes increased from d 100 to 135 of gestation, even when nutrient requirements were met (Vernon et al., 1981). Freetly and Ferrell (2000) reported no change in NEFA concentrations in ewes based on litter size. Concentrations of NEF in arterial, portal venous, and hepatic venous blood in pregnant ewes increased from the time of breeding to 6 ± 1 d prior to parturition (Freetly and Ferrell, 2000). These data may also be influenced by feed intake, as it was noted that feed intake decreased in late gestation. The decrease in feed intake may have resulted in increased NEFA due to increased lipolysis in adipose tissue (Freetly and Ferrell, 2000). At d 150 to 165 of gestation, NEFA concentrations were similar for pregnant and non-pregnant beef cows, however by d 194 of gestation, pregnant cows had greater NEFA concentrations compared with non-pregnant cows. This increase may be due to the greater amount of fat being catabolized during pregnancy in pregnant cows that have increased requirements during gestation (Wood et al., 2013). The increase in NEFA during pregnancy may be related to the increase in the production of ketones in the maternal tissues (Pethick et al., 1983). In the study conducted by Wood et al. (2013), circulating beta-hydroxybutyrate (**BHBA**) was greater in pregnant cows during mid- to late gestation compared with non-pregnant cows. This indicates a more ketogenic metabolic state during pregnancy (Wood et al., 2013).

Metabolic Hormone Adaptations. Metabolic hormones adapt to meet maternal and fetal demands during gestation. A reduction in maternal cortisol concentrations during pregnancy may promote lipolysis and decrease maternal carbohydrate oxidation (Bispham et al., 2003). Cortisol concentrations were stable from d 67 through the end of gestation (Lemley et al., 2014). This is in agreement with other studies indicating no differences in cortisol concentrations between ewes on different nutrient intakes during gestation (Vonnahme et al., 2013b). Ambient temperatures are thought to impact cortisol concentrations, however, in a study conducted in pregnant heifers fed to meet nutrient requirements (Andreoli et al., 1988), there was no difference in cortisol concentrations when pregnant heifers were either exposed to winter conditions or housed in thermoneutral conditions during late gestation. Circulating cortisol then remained relatively stable until the last month of gestation when their concentrations began to decrease (Andreoli et al., 1988). These results may suggest that conditions were not severe enough to affect cortisol concentrations.

Maternal GH during gestation is important due to its influence on fetal development (Oberbauer, 2015). Placental GH may become biologically active as a lactogen, however the exact function of placental GH remains unknown but may indirectly alter maternal metabolism, impacting fetal growth (Mirlesse et al., 1993). The increase in administration of GH during gestation has been demonstrated to negatively impact birth weight, indicating a reduction in fetal growth in calves (Oldenbroek et al., 1993), lambs (Wallace et al., 2001), and mice (Oberbauer, 2015). Reynolds et al. (1990) suggested that plasma glucose concentrations increase during mid- to late gestation due to the increase in GH and decrease in insulin concentrations. No

correlations, however, were found between GH and glucose concentrations in cattle (Reynolds et al., 1990). The increase in GH pulse frequency during gestation may decrease hepatic GH receptors and cause a decrease in circulating IGF-1 concentrations which may negatively impact fetal bone growth and density (Breier, 1999). Maternal circulating IGF-1 and estrogen concentrations, however, increase during pregnancy. This may be due to an increase in placental IGF-1 and estrogen being secreted into the maternal circulation (Challier et al., 1986; Lemley et al., 2014).

Circulating concentrations of insulin decrease during pregnancy, especially during late gestation in sheep fed to meet nutrient requirements (Vonnahme et al., 2013b; Lemley et al., 2014). The decrease in insulin may be due to the increase in glucose demand by the fetus and maternal tissues during late gestation. Insulin secretion decreased in pregnant rats compared with non-pregnant females which may be due to the increased sensitivity and response of the islets of Langerhans in pregnant rats compared with non-pregnant females (Green and Taylor, 1972).

Prolactin concentrations increase during pregnancy. During the first 3 mo of pregnancy, there were low concentrations of circulating prolactin (3 to 18 ng/mL). By d 100 of gestation, prolactin concentrations increased to between 150 to 200 ng/mL in ewes 3 d prior to parturition (Kann and Denamur, 1974). Prolactin during gestation seems to be necessary to prepare the mammary gland for lactation following parturition (Humphrey et al., 1983).

Thyroid hormones play a vital role in energy metabolism and are used as an indicator of available nutrients (Fowden and Forhead, 2004). Gestational hyperthyroidism occurs when thyroid hormone concentrations are too high during

pregnancy that can lead to preeclampsia, premature labor, perinatal death, and low birth weight (McNabb and King, 1993; Fowden and Forhead, 2004). Thyroxine concentrations decreased during gestation when pregnant ewes were fed to meet NRC nutrient requirements (Vonnahme et al., 2013a). The active form of thyroid hormone, triiodothyronine (T_3), however, has been shown to remain steady through gestation when ewes were fed to meet NRC nutrient requirements (Lemley et al., 2014). No difference in T_3 concentrations were reported in pregnant compared with non-pregnant beef cows (Wood et al., 2013). Thyroid hormones also play a key role in stimulating the production of a number of placental hormones necessary for the maintenance and adaptation to pregnancy (Maruo et al., 1991).

Effects of Altered Nutrition During Gestation on Metabolic Adaptation

The ruminant dam is at risk for being exposed to a variety of stressors that can affect adaptation to pregnancy. This may impact fetal growth and development and the maternal performance both pre- and postpartum (Arnott et al., 2012). The stressors that may be placed on the dam include nutrition, social stresses due to management practices, and thermal stress if maintained outside of the thermoneutral zone (Arnott et al., 2012). Maternal nutritional status during gestation is a major factor in the development and function of the fetal organ systems. Nutrition is important for offspring growth and development and may impact postnatal health and performance (Wu et al., 2006). Studies during gestation have been used to assess the effects of nutrition during early, mid-, or late gestation on maternal and offspring performance. There are limited data on the metabolic impacts of the dam, especially in beef cattle. Although maternal nutrient intake is known to influence fetal growth and development (Godfey and Barker, 2000; Wu et

al., 2006; Caton and Hess, 2010), the impact maternal nutrient intake on the metabolic status of the dam is less clear.

Blood urea nitrogen was decreased in nutrient restricted ewes compared with control ewes fed to meet nutrient requirements, and increased in overnourished ewes compared with control ewes due to the increase in nutrient availability by late gestation (Vonnahme et al., 2013b). Gunn et al. (2014) measured BUN in pregnant beef heifers fed either to meet nutrient requirements or to meet energy requirements but exceed CP requirements in late gestation. Around d 248 of gestation, heifers fed the high protein diet had greater BUN compared with heifers on the control diet (Gunn et al., 2014). These results are not surprising because as protein intake increases, BUN concentrations are also expected to increase (Radunz et al., 2010; Gunn et al., 2014), especially during pregnancy when nutrient demand increases (NRC, 2000).

Although plasma glucose concentrations are directly associated with nutrient intake (Vizcarra et al., 1998), in ruminants, less than 10% of the total glucose utilized is absorbed as glucose in the small intestine; therefore, most of the glucose that is available to tissues is supplied by gluconeogenesis (Otchere et al., 1974; Young, 1976). A study conducted in pregnant goats during late gestation demonstrated that a 40% restriction of protein or energy decreased plasma glucose concentrations compared with goats fed to meet nutrient requirements, which may impact fetal growth and development (He et al., 2015). This decrease in circulating glucose due to nutrient restriction is also consistent with previous studies using the ewe as a model (Yuen et al., 2002).

Nutrient restriction during pregnancy has been reported to increase circulating NEFA concentrations (Luther et al., 2007a; Luther et al., 2007b; Lekatz et al., 2010a,

2011; Vonnahme et al., 2013b). In a study conducted by Vonnahme et al. (2013), NEFA concentrations increased in pregnant ewes. After nutritional treatments were applied, as nutritional intake increased, NEFA concentrations decreased (Vonnahme et al., 2013b). As there are greater nutrients available, the breakdown of adipose tissue for energy should decrease, as it is evident in overnourished sheep models. Additionally, an increase in nutrient availability may have increased adiposity in ewes therefore the amount of adipose tissue broken down during gestation may not be as severe.

Adaptations to pregnancy require hormonal responses to stress which is necessary for normal fetal organ maturation and for preparing the fetus for the extrauterine environment (Challis et al., 2001). As expected, cortisol concentrations decreased in ewes that were restricted and increased in ewes that were overnourished from mid- to late gestation compared to control ewes fed to meet nutrient requirements (Lemley et al., 2014). The reduction in cortisol in ewes that are nutrient restricted during gestation is likely due to the decrease in maternal cortisol secretion, which may impact other metabolic hormones during pregnancy (Bispham et al., 2003).

One of the main determinants of thyroid hormone concentrations is nutrient intake, especially energy intake during late gestation (Symonds, 1995). Circulating triidothyronine (T_3) was greater in over nourished ewes compared with restricted and control ewes fed to meet nutrient requirements during gestation. Additionally, thyroxine (T_4) concentrations were decreased in restricted ewes on d 81, 95, 109, 123, and 137 compared with control ewes and increased in over nourished ewes on d 137 and 144 of gestation compared with control ewes (Lemley et al., 2014). Other ewe studies have reported an increase in T3 and T4 between restricted and control fed ewes beginning by d

61 and continuing through gestation (Vonnahme et al., 2013b). An increase in thyroid hormone concentrations in over nourished ewes during gestation has been reported in other studies (Wallace et al., 1997) due to the increase in available energy.

Maternal plane of nutrition has been shown to effect the somatotropic axis in both cattle and sheep (Breier, 1999). Ewes that were nutrient restricted beginning on d 40 of gestation had increased GH concentrations on d 137 of gestation compared with control ewes fed to meet nutrient requirements(Lemley et al., 2014). Additionally, ewes that were over nourished had decreased GH at d 109 and 137 of gestation compared with ewes fed to meet nutrient requirements. However, IGF-1 on d 109 of gestation was decreased in restricted ewes compared with control fed ewes, whereas on d 123, 137, and 144 of gestation, IGF-1 was increased in over nourished ewes. (Lemley et al., 2014). Nutrient restriction causes a reduction of hypothalamic somatostatin secretion, increase in GH pulse frequency, and decrease in growth hormone receptors, resulting in reduced somatotropic negative feedback and decreased circulating IGF-1 (Breier, 1999).

In a study by Lents et al. (2005), pregnant beef cows either received high treatment with ad libitum access to a high-energy diet in a dry lot, or grazed native grass pasture and received moderate (1.6 kg), low (1.1 kg), or very low (0.5 kg) of beginning on d 112 of gestation. At d 180 of gestation, insulin was greatest for cows on the high treatment compared with cows on all other treatments but IGF-1 concentrations were not influenced by treatment. At d 221 of gestation, insulin concentrations were decreased with reduced nutrient intake (Lents et al., 2005). High cows had greater IGF-1 compared with moderate or very low, but was not different from low fed cows (Lents et al., 2005). Nutrient restriction has been demonstrated to decrease IGF-1 concentrations

(Houseknecht et al., 1988; Rutter et al., 1989; Richards et al., 1995) and insulin (Trenkle, 1978; Richards et al., 1989; Bossis et al., 1999) in cattle due to a decrease in nutrient intake and may have a more severe impact on pregnant females due to the increased demands during pregnancy.

Prolactin during gestation is necessary to prepare the mammary gland for lactation (Humphrey et al., 1983) and physiological adaptations occur during pregnancy to maintain an increase in prolactin throughout lactation and is achieved by activating the prolactin receptor by lactogenic hormones produced by the placenta (Grattan, 2001). The importance of prolactin has been demonstrated to be just as important as GH in maintaining milk yield in goats (Knight et al., 1990). Ewes that were nutrient restricted had decreased prolactin concentrations at d 121 of gestation compared with control fed ewes, and ewes overnourished during mid- to late gestation had increased concentrations beginning on d 123 and throughout the remainder of gestation (Lemley et al., 2014). Stress has been shown in increase prolactin release (Humphrey et al., 1983), however the degree of nutrient restriction may not be severe enough to induce stress and change maternal prolactin concentrations during gestation. Each of these metabolic adaptations are necessary for the maternal and fetal environment and the demands of pregnancy.

The dam adapts to pregnancy in many ways that are critical for the metabolic adaptations and changes the maternal system undergoes. The changes in nutrient intake may have consequences on fetal development and postnatal performance if the maternal system does not properly adapt to pregnancy, however, the fetus must also adapt in order to maximize fetal growth and development during gestation which may have lasting impacts of offspring health and performance.

FETAL AND NEONATAL DEVELOPMENT AND METABOLISM

Developmental Programming

Nearly 70% of fetal growth occurs during the last one-third of gestation (Robinson et al., 1977). The lack of nutrients available during this critical time period may negatively impact the fetal environment because growth is dependent upon the dams nutritional, hormonal, and metabolic status (Desai and Hales, 1997). The maternal environment during gestation may have lasting impacts on fetal growth and development. Based on research in the area of developmental programming, pre- and postnatal changes have demonstrated to affect long-term offspring health and performance (Barker, 2004). The timing in which the developing fetus is affected by the consequences of the maternal environment may have different impacts on the offspring (Fowden et al., 2006). Maternal nutritional status during gestation is a major factor in the development and function of the fetal organ systems and is important for offspring growth and development which may impact postnatal health and performance (Wu et al., 2006).

Effects of the maternal environment on offspring, also referred to as the "Barker hypothesis" or "fetal programming," is not a new concept. Continued review in this area of study has been conducted in biomedical and animal research models. "Developmental programming" is a better term due to the impacts of the maternal environment on the fetus during gestation as well as postnatally, having "programmed" impacts on her offspring later in life (Caton and Hess, 2010). The programming that takes place in utero has impacts on fetal growth at birth as well as efficiency and body composition later in life (Greenwood et al., 2000; Wu et al., 2006; Caton et al., 2007; Larson et al., 2009). The fetus undergoes development and changes throughout gestation, with critical periods

when the fetus is vulnerable to maternal stressors (Nathanielsz, 2006). The developmental windows are times in fetal life which can have consequence on future growth, development, and performance if the fetus is impacted by a poor maternal environment during those time periods (Fowden et al., 2006).

Cardiovascular Adaptation and Placental Development

The transfer of nutrients from the maternal system to the fetus via the placenta is essential for growth and development. Early to mid-gestation, is the period of time in which placental development occurs. In order for nutrient transfer to occur, the fetal circulatory system must adapt to changes to maximize organ maturation and postnatal survival. To determine the distribution of normal blood flow and changes during fetal development, blood flow was measured in fetal lambs at different stages during pregnancy beginning on d 60 of gestation until term (Rudolph and Heymann, 1970). Umbilical blood flow was measured relative to fetal weight. There were no differences throughout gestation, indicating umbilical blood flow increases at the same rate in which fetal weight increases. Additionally, cardiac output, which measures the volume of blood pumped by the heart per min, was not different through gestation when measuring output of both ventricles on a fetal weight basis, again, increasing as fetal weight increased. The distribution of cardiac output changed, however, within the fetal organs throughout gestation. Output to the placenta decreased as gestation progressed from 47-50% in the 60-85 d fetuses to about 40% near term. Cardiac output to the intestines increased from 2.7-3.1% in younger fetuses to 5.6-5.9% after d 120. The spleen received only 1% of cardiac output. There was a tendency to decrease output to the kidneys as gestation progressed. There were no differences in cardiac output to the fetal brain during

gestation. Cardiac output to the fetal lungs, however, increased during gestation as fetal weight increased (Rudolph and Heymann, 1970). These changes in cardiac output may be a way to prepare the fetus for the extrauterine environment. Within this same study, blood gas levels in the umbilical artery and vein did not change throughout gestation, however, others have measured a gradual decrease in umbilical vein oxygen saturation by term (Barcroft et al., 1940).

A restriction in placental blood flow inhibits overall fetal growth, especially during the time of exponential growth during late gestation (Vonnahme et al., 2013a). Many of the major determinants of fetal growth are the size, transport, metabolic, and endocrine functions of the placenta (Bell et al., 1998). Livestock species have an epitheliochorial placentation which has 6 layers that separate the maternal blood from the fetal blood. These layers increase the surface area between the maternal endometrium and fetal membranes, increasing the areas of attachment (Dantzer, 1984). In ruminants, these areas of attachment are between the caruncles on the uterine wall and the fetal cotyledons forming a placentome, which serves as the site of nutrient and gas exchange between the dam and fetus (Vonnahme et al., 2013a). Change in capillary area density is associated with blood flow (Borowicz et al., 2007) and occurs during the last two-thirds of gestation (Funston et al., 2010b). In sheep, capillary area density of the placenta increases from 200 to 400% in size during this time period (Borowicz et al., 2007). Changes in capillary area density differ in cattle compared to sheep with density in caruncular tissue decreasing and cotyledonary tissue increasing during mid- to late gestation in cattle (Vonnahme et al., 2007) and remain relatively unchanged in the ewe (Borowicz et al., 2007). In cattle, placental weight and calf birth weight are highly correlated. In twin-bearing species, it is

common for growth restriction to occur because the total weight of the placenta is increased with multiple fetuses but the mass per fetus is reduced, resulting in a placental insufficiency (Redmer et al., 2004). Unlike sheep, the placenta of cattle may continue to increase in mass until term (Vonnahme et al., 2013a). It is less clear if fetal growth in cattle is regulated by the placenta in the same way as it is in sheep (Ferrell, 1989). In a study conducted by Rasby et al. (1990), cows were fed to achieve a thin BCS of 4 or a moderate BCS of 6 between d 145 and 195 of gestation and were fed to maintain weight and body condition between d 195 and 259 of gestation. There was no difference in fetal weight at d 259 of gestation between these treatments, but thin cows had an increase in chorioallantois and cotyledon growth. The larger placenta may have offset any differences in fetal growth when measured during gestation, which may be due to the increase in nutrient transport available with increased number or size placentomes. Thin cows in this study also had increased maternal estrogen concentrations. Estrogen is produced by the placenta and may be related to the greater mass of cotyledons (Rasby et al., 1990). Other studies have reported reduced calf birth weights that are associated with reduced prepartum circulating estrogen concentrations (Collier et al., 1982; Guilbault et al., 1985). Estrogen causes an increase in uterine blood flow (Huckabee et al., 1970; Ford, 1985) which may increase the nutrients available to be transferred to the fetus. Fetal growth is not only dependent on the increase in nutrients extracted from the blood, but also the increase in the amount of blood that is able to reach the fetal system from the maternal system through placental size and nutrient transfer capacity (Reynolds and Redmer, 1995; Reynolds et al., 2010).

Organ Size and Maturation.

If organogenesis is impacted by environmental stressors, such as poor nutrient intake of the dam, there may be a permanent reduction in the functionality of the organs that may not be expressed until later in the offspring's life (Fowden et al., 2006). Additionally, because fetal organs grow and mature at different rates, the timing of environmental stress can have consequences on the structure and function of the organ later in life. Organ maturation occurs during late gestation while maximum fetal growth occurs from mid- to late gestation. Although the fetus may be relatively developed in size, early parturition may lead to less mature vital organs and may negatively impact the offspring during the neonatal period or later in life (Fowden et al., 2006).

Fetal organ growth is dependent upon the nutritional, hormonal, and metabolic status of the dam, which can have negative consequences on the offspring especially when fetal nutrient availability is not adequate (Desai and Hales, 1997). In a study conducted by Meyer et al. (2013), lambs born to nutrient restricted ewes (d 40 of gestation to term) had decreased BW, total empty gastrointestinal, stomach complex, and liver masses at 20 d of age compared with lambs born to ewes fed to meet nutrient requirements. Lambs in this study were artificially reared and fed a similar colostrum and milk replacer postnatally to ensure that offspring differences would be due to gestational treatment differences and not due to differences in colostrum or milk quality. From these data, researchers concluded that maternal nutrition during gestation may impact fetal organ development, which may impact the function of the neonatal gastrointestinal tract possibly affecting offspring growth and performance (Meyer et al., 2013). In similar studies, measurements of more organ masses were decreased in late-term fetal lambs due to nutrient restriction of the dam during mid- to late gestation (Reed et al., 2007; Lemley

et al., 2012). The lack of differences in some organ masses measured in 20-d old lambs may be due to an increase in maturation during the final days of gestation and in early postnatal life in lambs born to ewes that were restricted during mid- to late gestation.

Fetal organ maturation begins around 75 to 80% of gestation and increases until term (Fowden et al., 2006) and may continue postnatally. Nutrient delivery has an important role in organ maturation. The development of the small intestine during the perinatal period is especially important as it undergoes morphological changes to increase the absorption of nutrients, secrete digestive enzymes, and undergoes cell proliferation to prepare for the extrauterine environment (Sangild et al., 2000). Organ maturation in pigs continues to occur postnatally with amnionic fluid, cortisol, and colostrum intake having a major role in continuing this process (Burrin et al., 1997). In a sheep study conducted by Trahair and Sangild (2000), colostrum was infused into fetal lambs on d 117 to 119 of gestation. There was an increase in organ growth within fetuses that were infused, including an increase in growth of the gastrointestinal system (Trahair and Sangild, 2000). These studies demonstrate the importance of colostrum to the neonate for continued organ maturation which may impact future postnatal growth and efficiency.

Fetal Growth

Birth weight is used as a measure of final fetal growth. In most livestock species, there is an increased chance of survival postnatally when offspring are born at an above average birth weight compared with those that are born at a below average birth weight (Wu et al., 2006), as long as dystocia is not at risk due to larger offspring. Low birth weight has many postnatal consequences including an increased risk of neonatal morbidity and mortality (Hammer et al., 2011), intestinal and respiratory dysfunctions,

and decreased postnatal growth (Wu et al., 2006). Additionally, when there is no difference in birth weight postnatal performance may still be compromised (Funston et al., 2010a). Results from studies determining the impact of nutrient restriction during pregnancy on fetal growth are inconsistent. A number of studies indicated a reduction in birth weight (Corah et al., 1975; Bellows and Short, 1978; Houghton et al., 1990; Freetly and Ferrell, 2000) and a number of studies indicated no difference in birth weight (Stalker et al., 2006; Martin et al., 2007; Funston et al., 2010b). These conflicting data may be due to differences in the timing and extent of nutrient restriction as well as age, breed, and other environmental factors. During gestation, nutrient partitioning often favors the fetus at the expense of the dam (Barcroft et al., 1940). The period of time during gestation when the dam cannot provide enough nutrients to the fetus may depend on the stage of gestation and fetal development (Fowden et al., 2006) and may impact offspring vigor postnatally.

Offspring Vigor

Colostrum intake is essential for newborn livestock. Consumption of colostrum depends on the ability to stand, find the udder, and suckle; a trait known as "vigor." Lamb vigor was determined in a study by Dwyer et al. (2003). Lambs born to ewes with less mobilized back fat had increased vigor compared to lambs born to ewes mobilizing more back fat (Dwyer, 2003). Birth weight may be a factor affecting vigor. Low birth weight calves are less vigorous at birth and take longer to consume colostrum. Lambs born to ewes with less mobilized back fat gave birth to heavier lambs which were more active during the neonatal period compared with their counterparts (Dwyer, 2003).

Neonatal vigor also may be influenced by cold and heat stress, however further investigation is necessary to determine how environmental factors affect vigor.

Metabolic Changes

Offspring undergo many changes before and after birth which may be due to maternal nutrition during gestation or changes in metabolite and metabolic hormone concentrations over time. Much of the perinatal metabolite and metabolic hormone data are measured postnatally. Blood samples obtained prior to colostrum intake are similar to the prenatal metabolic status of the fetus. Colostrum intake is essential in newborn livestock not only for the nutrients, but as a source of immunoglobulins, peptides, hormones, growth factors, nucleotides, and enzymes (Blum and Hammon, 2000) that are critical due to the epitheliochorial placenta of livestock species which prevents the maternal transfer of proteins to the fetus (Bell and Ehrhardt, 2002). Colostrum intake will initiate many physiological events which may lead to many metabolic changes during the neonatal period (Blum and Hammon, 2000).

Blood Urea Nitrogen. A primary indicator of protein deficiency in ruminant neonates is circulating BUN (Tzou et al., 1991). Plasma protein concentrations were greater in calves that had nursed compared with calves that had not nursed within 2 h postnatally (Lents et al., 1998). Additionally, plasma proteins increased from 24 to 48 h postnally then remained similar through d 14 (Lents et al., 1998). Fetuses from ewes restricted to 60% of nutrient requirements during mid- to late gestation had reduced BUN concentrations compared to fetuses from ewes fed to meet nutrient requirements. This decrease in fetal BUN may be due to the decrease in amino acids available to be degraded by the fetus (Lekatz et al., 2011). In a study conducted by Bull et al. (1991), BUN

concentrations were decreased in calves born to heifers that were nutrient restricted during the last 150 d of gestation compared to calves born to heifers offered adequate protein and energy (Bull et al., 1991). These changes in calf BUN concentrations indicate that maternal nutrition during gestation impacts perinatal nutrient supply.

Gluconeogenesis. Glucose is the major energy source for fetal growth and development of vital organs during pregnancy (Doornenbal et al., 1988b). Once the maternal and fetal glucose concentrations are stable, uptake of glucose may decrease due to the maternal and fetal gradient transfer of glucose being reached. However, when glucose demands are not met, gluconeogenesis in the fetus occurs especially during late gestation when requirements are greatest (Dalinghaus et al., 1991). Gluconeogenesis is dependent on lactate and amino acids in the fetal liver of sheep (Lekatz et al., 2010b). During late gestation, glycogen is stored in the fetal liver and gluconeogenesis takes place even if glucose concentrations are not limiting (Lekatz et al., 2011). Even when the fetus experiences environmental stressors such as intrauterine growth restriction (IUGR), Lekatz et al. (2010) indicated that those fetuses are still capable of gluconeogenesis because similar plasma glucose concentrations were observed in IUGR lambs and lambs of ewes fed to meet their nutrient requirements (Lekatz et al., 2010b). Fetal liver glycogen has been determined to be unaltered by maternal level of nutrition during gestation (Luther et al., 2007a; Lekatz et al., 2011). However, when there is greater liver glycogen produced in the fetus, there is a greater chance of neonatal survival (Lekatz et al., 2010b). The rate of gluconeogenesis in the fetus may be determined by glucose available through placental transport and glucose production necessary through the demand of glucose, resulting in similar glucose levels (Dalinghaus et al., 1991). Despite

this, glucose produced by the fetus of ruminants is minimal therefore the placental supply of glucose from the maternal circulation is critical for proper growth and development (Lager and Powell, 2012).

Circulating Glucose. Neonatal metabolites were measured in calves within 24 h and on 2, 3, 4, 6, 8, and 14 d of age. Consumption of colostrum prior to the first sampling time influenced glucose concentrations within the same study. Calves that had nursed tended to have greater plasma glucose concentrations at the sampling within 24 h of birth compared to calves that had not nursed and glucose concentrations increased from 24 to 48 h postnatally then remained similar through the remainder of the sampling period (Lents et al., 1998). Circulating blood glucose concentrations have been reported to increase during the first 24 h postnatally when calves were born into a cold environment (Edwards and Silver, 1970). Following colostrum intake at 6 and 12 h postnatally, glucose concentrations increased in calves which may be due to the slow breakdown of lactose and absorption of glucose into circulation as neonatal ruminants are most similar to monogastrics due to a lack of a functioning rumen. These data illustrate the importance of colostrum intake as soon as possible after birth due to the importance of glucose as an energy source.

Glucose concentrations increased during the first 30 min after birth, but then quickly decreased until colostrum intake 6 h after birth. However, some calves had decreased plasma glucose concentrations immediately after birth until colostrum feeding (Daniels et al., 1974). The change in glucose concentrations in calves may indicate liver glycogen content or glycogenolysis differs in individual calves and may not be fully developed at the time of birth. Additionally, the increase in blood glucose immediately

after birth may be due to stress response at birth and during the adaptation to extrauterine environment.

Glucose is converted to fructose if not metabolized in the fetal system (Alexander et al., 1955) by the placenta and in allantoic and amnionic fluids and is a readily available fetal energy source (Battaglia and Meschia, 1978). Circulating fructose concentrations in both lambs and calves quickly declined during the first 6 h which may be due to the important role fructose plays in energy metabolism in the neonatal ruminant due to the high concentrations during the first few hours of life as an energy source (Daniels et al., 1974).

Circulating NEFA. Circulating NEFA concentrations were measured in calves prior to colostrum intake in a study by Lents et al. (1998). At that time, NEFA was increased compared to concentrations later in the neonatal period due to the nutrients available in colostrum and milk (Lents et al., 1998). Additionally, NEFA concentrations were greater in calves that had not nursed compared to calves that had nursed (Lents et al., 1998). Vonnahme et al. (2013) reported that 60% nutrient restriction in the ewe increased NEFA by 54% in the maternal circulation; however, fetal NEFA concentrations from those restricted dams only increased by 6% (numerical difference only) during gestation (Vonnahme et al., 2013b). These studies indicate the importance of colostrum intake after birth to the neonate for energy availability, therefore will decrease lipid mobilization and NEFA concentrations during the neonatal period.

Metabolic Hormones. Growth restriction during gestation has impacts on offspring neonatal metabolic hormone concentrations, which may be due to an increase growth rate and adiposity during the perinatal period that alters the secretion and

metabolism of thyroid hormones (De Blasio et al., 2006). In a study conducted by Davicco et al. (1982), circulating T3 and T4 concentrations in Holstein x Friesian calves increased from birth to 6 h, decreased until d 7 and remained stable until 30 d postnatally, however in Charolais and Salers calves there were no changes in neonatal T3 or T4 concentrations (Davicco et al., 1982). In neonatal beef calves, IGF-1 was reduced in calves that had nursed within 2 h postnatally and remained the same until between 4 and 6 d of age. Insulin concentrations were greater in calves that had nursed (Lents et al., 1998).

Circulating T4 concentrations have been shown to be positively correlated with birth weight (Mellor et al., 1977; De Blasio et al., 2006), and placental or growth restriction during gestation has been suggested to increase the activation of T3, which may lead to compensatory neonatal growth that may occur after exposure to intrauterine restriction (De Blasio et al., 2006). At birth, lambs born to ewes restricted to 60% of nutrient requirements beginning on d 40 of gestation had decreased T3 concentrations compared to ewes offered 100% and 140% of nutrient requirements (NRC, 2007). There was no difference in neonatal serum T4 or cortisol concentrations between maternal nutritional planes at the time of birth (Camacho et al., 2012). By 24 h of age, there was no difference in T3 concentrations, however T4 concentrations in lambs born to restricted ewes increased and cortisol concentrations decreased compared to lambs born to over nourished ewes. Concentrations of T4 were also increased in adequately fed ewes compared to over nourished ewes. From d 3 to 19, there was no difference in T3, T4, or cortisol concentrations between maternal nutritional planes (Camacho et al., 2012). Neonatal survival is most vulnerable during the first few hours of life, and cortisol and

thyroid hormones may be essential for survival which may be due to the impact they have on growth patterns and physiological functions.

The perinatal period is a time in which the fetus will undergo changes in order to prepare for the postnatal environment. These adaptions include changes in circulation and placental development, organ development and maturation, and metabolic changes due to maternal nutrition during gestation and changes in concentrations over time.

SUMMARY AND CONCLUSIONS

In conclusion, the dam will adapt in various ways during pregnancy including cardiovascular, respiratory, and metabolic alterations necessary for the maternal and fetal demands of pregnancy. Proper maternal nutrition is critical during gestation to provide for proper adaptation. Despite this, little is known about the impacts gestational nutrition has on the metabolic status of beef cows.

Additionally, the fetus undergoes changes to support growth and development. These adaptations that occur at various developmental windows as well as throughout gestation include circulatory system and placental changes, organ development and maturation, fetal growth that may impact offspring vigor, and metabolite and metabolic hormone changes. Research in this area is lacking the changes that occur during the neonatal period during the first 72 h postnatally in beef calves, which is a critical time for calve survivability.

CHAPTER 2

Changes in Late Gestation Beef Cow Circulating Metabolite and Metabolic Hormone Concentrations Due to Feeding Stockpiled Tall Fescue Versus Tall Fescue Hay

ABSTRACT

The objective was to investigate the effects of grazing stockpiled tall fescue (STF) versus feeding summer-baled hay during late gestation on nutrient availability for fetal development. Within the 2-yr study, multiparous, spring-calving crossbred beef cows (yr 1: n = 48; yr 2: n = 56) either strip-grazed STF pasture (n = 4/yr; 12.3% CP, 63.9% NDF; DM basis), or received ad libitum tall fescue hay (6.9% CP, 66.6% NDF; DM basis) fed in uncovered drylots (n = 4/yr) until calving. Jugular blood samples were obtained from cows on d 0 (baseline; d 188 ± 2 of gestation), 20, 35, 56, 77, and 99 (remaining prepartum cows). Effects of forage system, sampling day, year, and all interactions were included in the model. Sampling day was considered a repeated effect. Calf date of birth was included in the model when $P < 0.25$; pasture or pen was the experimental unit. Correlations were determined between calf birth weight and the last prepartum maternal metabolite or hormone concentration. The forage system x day x year interaction affected ($P < 0.001$) NEFA and tended to affect thyroxine ($P = 0.06$). In year 1, NEFA was greater ($P < 0.001$) on d 56 in cows grazing STF than cows consuming hay. In year 2, NEFA tended to be greater ($P = 0.09$) on d 35 and was greater ($P < 0.001$) on d 77 and 99 in cows grazing STF. Cows grazing STF tended to have greater ($P = 0.06$) thyroxine on d 77 in year 1, but there were no differences ($P \geq 0.18$) in year 2. There was a forage system x day interaction ($P < 0.01$) for serum blood urea nitrogen (BUN), cortisol, and triiodothyronine. After d 0, BUN was greater ($P < 0.001$) in cows consuming STF on all

days measured. Cortisol was greater (P = 0.003) on d 35 and tended to be greater (P = 0.10) on d 99 in cows grazing STF. Triiodothyronine was less (P = 0.03) on d 0, but greater (P = 0.004) on d 99, in cows grazing STF. Cows grazing STF tended to have greater (P = 0.08) glucose than cows consuming hay. Calf birth weight was positively correlated with prepartum maternal BUN (r = 0.31, P = 0.002) and NEFA (r = 0.12, P = 0.005), but not glucose, cortisol, triiodothyronine, or thyroxine ($P \geq 0.15$). Results indicate that forage systems alter metabolic status, which may impact fetal development and subsequent calf performance.

INTRODUCTION

Maternal nutrition during gestation plays a key role in pre- and postnatal growth, where nutrient restriction may impact fetal development and potentially have long-term consequences on offspring (Godfey and Barker, 2000; Wu et al., 2006). Nutrient requirements of beef cows exponentially increase during the last third of gestation. About 70% of fetal growth is occurs during this time period making up over 30% of the dam's total nutrient requirements (NRC, 2000). The offspring prenatally may be sensitive to late gestation nutrition of the dam due to the increased demand for nutrients. Previous beef cow research during late gestation has demonstrated that CP supplementation of low quality forages (Funston et al., 2010a; Bohnert et al., 2013) or the type of energy source provided (Radunz et al., 2012)during late gestation may impact fetal growth and postnatal performance. Less is known about the impact forage systems common to the lower Midwest have on fetal development.

Cow-calf producers in the lower Midwest often feed poor quality hay or allow cows to graze stockpiled tall fescue during the winter months, which for a spring-calving beef herd is during late gestation. Using these forage systems, a 2-yr study in our lab has reported that calves born to cows consuming tall fescue hay during late gestation had a 10% decrease in fetal growth, measured as birth weight, compared with calves born to cows grazing stockpiled tall fescue with greater nutrient intake (Niederecker, 2015). What remains unclear is whether the late gestation cow metabolic status can be used as an indicator of fetal growth due to the impact of nutrient intake has on the dam's metabolic status. Our overall hypothesis was that cows grazing stockpiled tall fescue would have increased nutrient intake, improved metabolic status of the dam, improved fetal growth, and improved calf performance compared with cows consuming summer-baled hay. The specific objectives of this study were to investigate the effects of grazing stockpiled tall fescue versus feeding summer-baled hay during late gestation on circulating metabolites and metabolic hormones in beef cows and to determine the relationship of maternal metabolites and hormones prior to calving with calf birth weight.

MATERIALS AND METHODS

The University of Missouri Animal Care and Use Committee approved animal care and use in this study.

Animal Management and Forage Systems

A 2-yr study was conducted using multiparous, spring-calving, crossbred beef cows (yr 1: n = 48, initial BW = 678 ± 12 [SEM throughout] kg, initial BCS = 5.8 ± 0.1, initial age = 5.7 ± 0.3 yr; yr 2: n = 56, initial BW = 640 ± 10 kg, initial BCS = 5.6 ± 0.1,

initial age = 4.9 ± 0.4 yr) during late gestation at the University of Missouri Beef Research and Teaching Farm, as described in Niederecker et al. (2015). Cows were exposed to bulls (≤ 50 cows per bull) beginning 10 d after a fixed-time AI 7-d CoSynch + CIDR estrus synchronization protocol in the spring prior to the study initiation. The calving season was 46 d and 79 d in yr 1 and 2, respectively. Cows were allocated by BW, BCS, age, service sire (n = 4/yr), and expected calving date (AI due date: yr 1 = February 8, 2014; yr 2 = February 7, 2015) to 1 of 2 forage systems (Table 2.1). Cows either strip-grazed endophyte-infected stockpiled tall fescue (**STF**) or received endophyte-infected tall fescue hay (**HAY**) with 4 replications per forage system in each year. The study was initiated on November 15, 2013 (yr 1) and November 14, 2014 (yr 2) on d 188 ± 2 of gestation (both yr).

Stockpiled tall fescue pastures were approximately 4.05 ha each. Forage accumulation occurred from mid-August of both years, when 45 kg of N/ha was applied, until study initiation. Forage was strip-grazed with a goal forage utilization of 75 to 80% of the total available forage. Rye haylage (yr 1: 59.7% NDF, 36.6% ADF, 12.3% CP; yr 2: 54.4% NDF, 29.6% ADF, 15.1% CP; all DM basis) was substituted for 8 d during the study in yr 1 when weather conditions such as excessive snow or ice prevented grazing. Cows grazing STF which had not calved by d 99 of study consumed rye haylage after d 77 of study until the time of calving after d 99. Rye haylage had a similar nutrient composition to STF, making it a viable alternative until conditions improved.

As described in Niederecker et al. (2015), STF pastures were sampled 3 times in yr 1 (d -7, 62, and 131 of study) and 4 times in yr 2 (d 5, 29, 62, and 119 of study) to obtain pre- and post-grazing forage samples. Estimated DMI of STF was calculated as the

difference in pre-grazing and post-grazing forage yield using pasture area and days grazed.

Cows in the HAY forage system were confined in 18 x 61 m uncovered dry lots that were well-drained and limestone based. Summer-baled, endophyte-infected tall fescue-based grass hay was fed in round bale ring feeders equipped with cone-shaped chains on a 9.1 x 9.1 m concrete pad. Each bale was individually weighed and core sampled for nutrient analysis prior to feeding. Estimated DMI of HAY was calculated as total bale weight divided by the total number of animals per pen. Waste of 18% was estimated using results from similar hay feeders (Moore and Sexten, 2015).

In both forage systems, cows had free excess to water and a mineral and vitamin supplement (Gold Star® MFA Breeder 12 Mineral: MFA, Columbia, MO) to provide the recommended 56.7 $g \cdot animal^{-1} \cdot d^{-1}$.

Cows were monitored for calving throughout the calving period. After parturition, calves were weighed, tagged, and sex was determined within 8 h of birth.

Late Gestation Blood Collection

Jugular blood samples were obtained from cows in both years on d 0 (baseline of the study; d 188 ± 2 of gestation), 20 (d 208 of gestation), 35 (d 223 of gestation), 56 (d 244 of gestation), and 77 (d 265 of gestation), of the study, as well as d 99 (d 287 of gestation; yr 1: HAY: n = 10, STF: n = 10, yr 2: HAY: n = 7, STF: n = 10) of study on remaining prepartum cows. At each sampling time, blood samples were collected into 4 blood collection 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% K_3 EDTA [10 mL draw; Covidien, Mansfield, MA], and 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). Plasma tubes were inverted as directed and placed on ice immediately following collection, and serum tubes were allowed to clot prior to placing on ice. Samples were centrifuged within 4 h of collection at 1500 x g at 4°C for 30 min. Serum or plasma was then pipetted into 2 mL microcentrifuge tubes and stored at -20°C until analysis.

Metabolite and Hormone Analyses

Serum samples collected on all sampling days were analyzed for blood urea nitrogen (**BUN**) using a commercially available urea nitrogen kit (Urea Nitrogen Procedure Number 0580; Stanbio Laboratory, Boerne, TX) based on the diacetylmonoxime method. Samples were read in duplicate in 96-well polystyrene plates (Corning Inc., Corning, NY) on a microplate reader (Biotek Synergy™ HT, Biotek® Instruments Inc., Winooski, VT) at 520 nm. The intraassay and interassay CV were 3.42% and 8.64%, respectively.

Glucose concentration was determined in plasma samples (collected in treated tubes described above) collected on all sampling days using the Infinity™ glucose hexokinase commercially available kit (Cat. # TR15421, Fisher Diagnostics, Middletown, VA) based on the glucose-6-phospate dehydrogenase method. Samples were read in duplicate in 96-well plates as described above at 340 nm. The intraassay and interassay CV were 3.97% and 6.94%, respectively.

Serum concentrations of NEFA from all sampling days were measured using a modified procedure of the NEFA C kit (Wako Pure Chemical Industries, Ltd, Osaka,

Japan), using the acyl-CoA synthetase-acyl-CoA oxidase method. Samples were read in duplicate in 96-well plates as described above at 550 nm. The intraassay and interassay CV were 4.06% and 6.79%, respectively.

Serum total triiodothyronine (**T_3**) and thyroxine (**T_4**) concentrations were determined in d 0, 35, 77, and 99 of study serum samples in duplicate using an Immulite 1000 (Siemens, Los Angeles, CA) and commercially available kits (Cat. #LKT31 [T_3] and KLT41 [T_4]; Siemens, Los Angeles, CA) following the manufacturer protocol. The intraassay and interassay CV were 9.72% and 9.46%, respectively for T_3, and 6.18% and 9.71%, respectively for T_4.

Serum cortisol concentration was determined in d 0, 35, 77, and 99 of study serum samples in duplicate using an Immulite 1000 (Siemens, Los Angeles, CA) and components of commercial kits (Cat. #LKCO1; Diagnostic Products Corp., Los Angeles, CA). The intraassay and interassay CV were 7.50% and 2.55%, respectively.

Statistical Analysis

Cows were removed from the study due to failure to calve (yr 1: n = 1; yr 2: n = 1), twin pregnancy (yr 2: n = 1), or calving outside of a 60-d calving window (yr 2: n = 3), resulting in 47 cows included in yr 1 and 51 cows in yr 2.

Data were analyzed using the MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC) with late gestation pasture or drylot as the experimental unit. Effects of forage system, sampling day, year, and all interactions were included in the model. Sampling day was analyzed as a repeated measure using the best-fit covariance structure. Calf date of birth was included in the model when $P < 0.25$. Correlations were determined between calf birth weight and the last prepartum maternal metabolite or hormone concentration

collected prior to calving. In the absence of the highest order interactions ($P > 0.10$), significant main effects are reported; otherwise, highest order interactive means are discussed (Table 2.2).

RESULTS AND DISCUSSION

Responses of cow performance as well as calf birth weight and growth through weaning, to forage system during late gestation has been previously reported (Niederecker, 2015). Briefly, there was a forage system x year interaction for overall prepartum BW change where cows consuming HAY in year 2 tended to gain more BW than STF during late gestation, but may be due to decreased digestibility of the HAY, resulting in greater fill. There were no differences in overall prepartum cow BW change during yr 1. There were no forage system x year interactions for prepartum BCS change. Overall, cows consuming STF lost less condition prepartum than HAY cows. There were no forage system x year interactions or forage system main effects for prepartum RF changes. Maternal circulating metabolites and metabolic hormones during late gestation presented here aid in understanding the physiological mechanisms involved in these overall production differences between STF cows being able to consume more nutrients during late gestation indicating an increased nutritional plane compared to cows consuming HAY. Although cow performance did not change much, fetal growth was decreased in calves born to cows consuming HAY during late gestation.

Maternal Circulating Metabolites

Blood Urea Nitrogen. There was a forage system x day interaction ($P < 0.001$) for serum BUN concentrations in cows during late gestation (Figure 2.1). On d 0, at the

onset of the study, there was no difference ($P = 0.77$) in serum BUN concentrations between cows in either forage system. However, by d 20 and throughout the remainder of gestation, serum BUN concentrations were increased ($P < 0.05$) in cows grazing STF compared with cows consuming HAY.

The greater CP content of STF, being nearly twice that of the HAY, resulted in an increase in ammonia nitrogen absorbed in the portal blood system either from digestible protein degraded in the rumen or passed to the abomasum and small intestine to ultimately be absorbed and deaminanted (Hammond, 1992). Ruminal degradable protein may be broken down to ammonia in the rumen and is maximized when the protein and available energy ratio is optimized. Within this study, BUN concentrations may have been greater in STF cows due to the excess of nitrogen relative to energy in the rumen, increasing the ruminal ammonia concentration, which when unused, is converted to urea and circulates in the blood or is excreted (Hammond, 1992). It has been previously reported that when circulating BUN concentrations in beef cattle are less than 7 mg/dL, protein is deficient compared with energy available (Hammond, 1992). This indicates that cows consuming HAY in this study may have been deficient in protein from d 35 of study and through the remainder of gestation, which may have been due to decreased nutrient intake compared to cows grazing STF.

In agreement with the current study, research in pregnant ewes and heifers has indicated that as nutritional plane increases, BUN increases during late gestation (Vonnahme et al., 2013a; Gunn et al., 2014). Another study in ewes resulted in nutritional intake not impacting BUN concentrations during late gestation, however these results may have been due to protein catabolism that occurred when protein was restricted

(Lekatz et al., 2010a). Within the current study, the increase in BUN and maternal metabolic status resulted from the increase in nutrient intake and CP content in the STF forage system compared with cows consuming HAY with less CP and nutrient intake.

Glucose. There tended to be a main effect ($P = 0.08$) of forage system for cow plasma glucose, in which cows grazing STF tended to have greater plasma glucose compared with cows consuming HAY (Figure 2.2). Additionally, there was a main effect of day ($P < 0.001$) where plasma glucose concentrations increased ($P = 0.002$) from d 0 to 20, remained similar ($P = 0.56$) from d 20 to 35, and increased ($P < 0.001$) from d 35 to 56. Glucose concentrations decreased ($P < 0.001$) from d 56 to 77 of study then remained similar ($P = 0.80$) through d 99.

During late gestation, uptake of glucose by the uterus accounts for 30-50% of an increase in the maternal glucose supply with the majority taken up by the placenta through facilitated diffusion by GLUT-1 and GLUT-3 transporters (Bell and Bauman, 1997). In ruminants, on average less than 10% of the total glucose utilized is absorbed as glucose in the small intestine, therefore most of the glucose that is available to tissues is supplied by gluconeogenesis (Otchere et al., 1974; Young, 1976). The timing of the changes in plasma glucose within this study is less clear, however Doornenbal et al. demonstrated that glucose concentrations decreased during late gestation in beef cattle (Doornenbal et al., 1988a), which may be due to the increased demand and uptake of glucose by the uterus as the primary nutrient source for fetal growth (Bell and Bauman, 1997). The increase in nutrient intake in cows grazing STF is likely to have increase glucose concentrations during late gestation compared to cows consuming HAY.

Nonesterified Fatty Acids. There was a forage system x day x year interaction ($P < 0.001$) for serum NEFA concentrations (Figure 2.3). In yr 1, cows grazing STF had greater ($P < 0.001$) circulating NEFA concentrations on d 56 than cows consuming HAY but were similar ($P \geq 0.20$) on all other sampling days. In yr 2, cows grazing STF tended to have greater ($P = 0.10$) serum NEFA concentrations on d 35 and had greater ($P < 0.001$) NEFA on d 77 and 99 of study compared with cows consuming HAY.

We hypothesized that cows consuming HAY would have increased NEFA concentrations compared with cows grazing STF due to the HAY forage system having a decrease in nutrient intake. Circulating NEFA generally increase when fat stores are mobilized in order to meet energy demands, thus increasing if requirements through the plane of nutrition are not met. Contrary to this study, nutrient restriction during pregnancy has been reported to increase circulating NEFA concentrations in ewes (Luther et al., 2007a; Luther et al., 2007b; Lekatz et al., 2010a, 2011; Vonnahme et al., 2013a). There may be a number of reasons why NEFA concentrations were not different between forage systems during much of gestation in both years. At the onset of the study, the average BCS was 5.7, indicating a greater amount of lipids could be mobilized. Cows grazing STF may have had an increase in locomotion in order to consume nutrients as well as a greater distance to walk on blood collection days. Additionally, the exposure to cold stress during the winter months of late gestation may have impacted NEFA concentrations of both forage systems by increasing fat mobilization in order to meet energy demands of thermoregulation.

At the onset of lactation when there is likely to be a negative energy balance, NEFA concentrations may increase to negate the impacts of the high nutrient demand

that is not being met by nutritional intake. Within this study, postpartum NEFA concentrations were greater in cows consuming HAY during late gestation compared with cows grazing STF at 7 d after calving (data not shown).

Maternal Circulating Metabolic Hormones

Thyroid Hormones. There was a forage system x day interaction for cow serum T_3 concentrations during late gestation (Figure 2.4). At the onset of the study, serum T_3 was greater ($P = 0.03$) for cows consuming HAY during late gestation, but at d 35 and 77, serum T_3 was not different ($P \geq 0.21$) between either forage system. On d 99 of study, cows grazing STF had greater ($P = 0.004$) serum T_3 concentrations compared with cows consuming HAY, however only a subset of cows had not yet calved by d 99 of study and were sampled which may have impacted these results. In a previous study, T_3 concentrations were reduced in ewes restricted in nutrient intake during mid- to late gestation compared to ewes fed to meet nutrient requirements (Ward et al., 2008), indicating the variation in maternal T_3 concentrations during gestation.

Additionally, there tended to be a forage system x day x year interaction ($P = 0.06$) for cow serum T_4 concentrations (Figure 2.5). In yr 1, cows grazing STF tended to have greater ($P = 0.06$) serum T_4 on d 77 of study, but were not different ($P \geq 0.18$) between forage systems on any sampling day in yr 2. This may indicate that these forage systems may have minimal impacts on thyroid hormone function. There was a forage system x day interaction ($P = 0.02$) for serum T_4:T_3 (Figure 2.6). The ratio of T_4 to T_3 was not different ($P = 0.45$) on d 0 of study, however, on d 35 the ratio of T_4 to T_3 was greater ($P = 0.04$) in cows grazing STF. The ratio of T_4 to T_3 was not different ($P = 0.52$) on d 77 of study, but on d 99 cows consuming HAY had a greater ($P = 0.03$) ratio of T_4

to T_3. This increase on d 35 of study in STF cows may be due to the greater T_3 concentrations in cows consuming STF as discussed earlier, however the difference on d 99 may be due to greater conversion to the active form, T_3 in STF cows compared to HAY cows.

From these data, we conclude that the difference in nutrient intake between these forage systems in this study had minimal impacts on maternal thyroid hormones during late gestation. Thyroid hormones play an important role in fetal development (McNabb and King, 1993) and determining available energy (Fowden and Forhead, 2004): however, Symonds (1995) has reported that as gestation progresses in sheep, the maternal thyroid hormone is less important and therefore reduced (Symonds, 1995). In another study, ewes that were restricted to 60% of nutrient requirements during late gestation had similar thyroid hormone concentrations to control fed ewes during late gestation (Lekatz et al., 2010a), which is in agreement to the results of the current study.

Cortisol. There was a forage system x day interaction ($P = 0.01$) for circulating cortisol concentrations (Figure 2.7). There was no difference ($P = 0.42$) in serum cortisol between forage systems at the onset of the study; however, by d 35 serum cortisol concentrations were greater ($P = 0.003$) in cows grazing STF compared with cows consuming HAY. Serum cortisol concentrations were not different ($P = 0.47$) on d 77 between forage systems, but at d 99 serum cortisol tended to be greater ($P = 0.10$) in cows grazing STF compared with cows consuming HAY.

Studies conducted in sheep have reported similar results in which as nutrient intake increased, circulating cortisol increased (Lemley et al., 2014). The reduction of maternal cortisol during nutrient restriction may be due to the decrease in maternal

cortisol secretion, which may promote lipolysis and maximize nutrient supply to the growing fetus during late gestation (Bispham et al., 2003). Other studies have reported that cortisol concentrations increase during nutrient restriction because of the metabolic role of cortisol, including increasing gluconeogenesis, lipolysis, and protein catabolism (Guyton, 1991) which would increase circulating cortisol concentration. However, prolonged exposure to nutrient restriction has been reported to reduce cortisol concentrations gradually over time (Sticker et al., 1995).

Maternal Prepartum Metabolite and Hormone Correlations with Calf Birth Weight

Partial correlation coefficients between the last prepartum maternal metabolite or hormone concentrations and calf birth weight are shown in Table 2.3. Serum BUN had a moderate positive correlation with calf birth weight (Figure 2.8) and serum NEFA had a weak positive correlation with calf birth weight (Figure 2.9); however, all other metabolites and hormones analyzed (plasma glucose, T_3, T_4, and cortisol) were not correlated ($P \geq 0.15$) with calf birth weight.

These correlations indicate that as maternal prepartum serum BUN and NEFA concentrations increased in the current study, calf birth weight increased. The positive correlations of maternal serum BUN and NEFA with calf birth weight may be due to a greater transfer of nutrients to the fetus, resulting in an increase in nutrient availability. Another possibility may be the increased nutrient intake of the dam or mobilization of fat resulting in increased fetal growth and heavier calves (Abeni et al., 2004). resulting in a greater supply for nutrient availability and an increase in body fat reserves being mobilized to meet the demands during gestation. Circulating NEFA concentrations have

been reported to be greater during the last 4 wk of gestation in cows that calved to larger calves compared with cows that calved medium and light calves (Guedon et al., 1999).

CONCLUSION

In summary, throughout the forage system period, serum BUN and plasma glucose concentrations were greater in cows grazing STF. There were variable effects of forage system on circulating NEFA, T_3, T_4, and cortisol concentrations during late gestation. Additionally, pre-calving serum BUN and NEFA concentrations were positively correlated with calf birth weight. Results suggest that late gestation forage system impacts cow metabolic status, which may influence fetal growth and development and calf performance.

IMPLICATIONS

This study indicates the metabolic changes beef cattle undergo due to changes in forage systems during late gestation. Cows consuming poor quality hay during late gestation had decreased metabolic concentrations compared to cows grazing STF which indicates the advantages of increased nutrient intake during late gestation. Further research is necessary to determine the metabolic impacts forage system has on calves due to the metabolic changes of the dam during pregnancy. Additionally, more metabolites and hormones need to be analyzed to determine what other changes occur due to changes in nutrient intake during late gestation.

Table 2.1. Nutrient composition, digestibility, and yield of stockpiled tall fescue and tall fescue hay offered to beef cows during late gestation in both years

Variable	STF[1], yr 1	HAY[2], yr 1	STF[1], yr 2	HAY[2], yr 2
DM, %	45.5	91.5	44.7	85.7
DM basis				
CP, %	12.1	6.2	12.4	7.6
NDF, %	61.4	64.9	66.4	68.3
ADF, %	33.4	36.6	37.8	38.3
Ash, %	8.5	9.7	13.7	9.0
IVTD[3], %	61.4	59.4	64.1	57.7
Yield, kg/ha	4,668	--	5,622	--
Forage utilization, %	80.4	--	70.8	--

[1]Average STF includes pre-grazing forage samples from all 4 pastures throughout the study in each year

[2]Average HAY includes core samples collected from each hay bale prior to feeding

[3]In vitro true digestibility

Table 2.2. Effects of forage system, d, and yr on cow circulating metabolites and metabolic hormones during late gestation

	P-value[1]					
Item	Forage System	Day	Forage System x Day	Year	Forage System x Year	Forage System x Day x Year
Serum BUN	< 0.001	0.58	***< 0.001***	0.02	0.61	0.28
Plasma Glucose	***0.08***	***< 0.001***	0.61	0.03	0.28	0.23
Serum NEFA	0.01	< 0.001	< 0.001	0.42	0.003	***< 0.001***
Serum T3	0.69	< 0.001	***< 0.001***	0.38	0.60	0.63
Serum T4	0.94	< 0.001	0.31	0.70	0.63	***0.06***
Serum Cortisol	0.12	0.001	***0.006***	0.89	0.36	0.54

[1]Probability values for forage system, day, year, and their interactions. Bold, italic *P*-values are greatest order interactions with Forage System in the model or main effects for each item ($P < 0.10$).

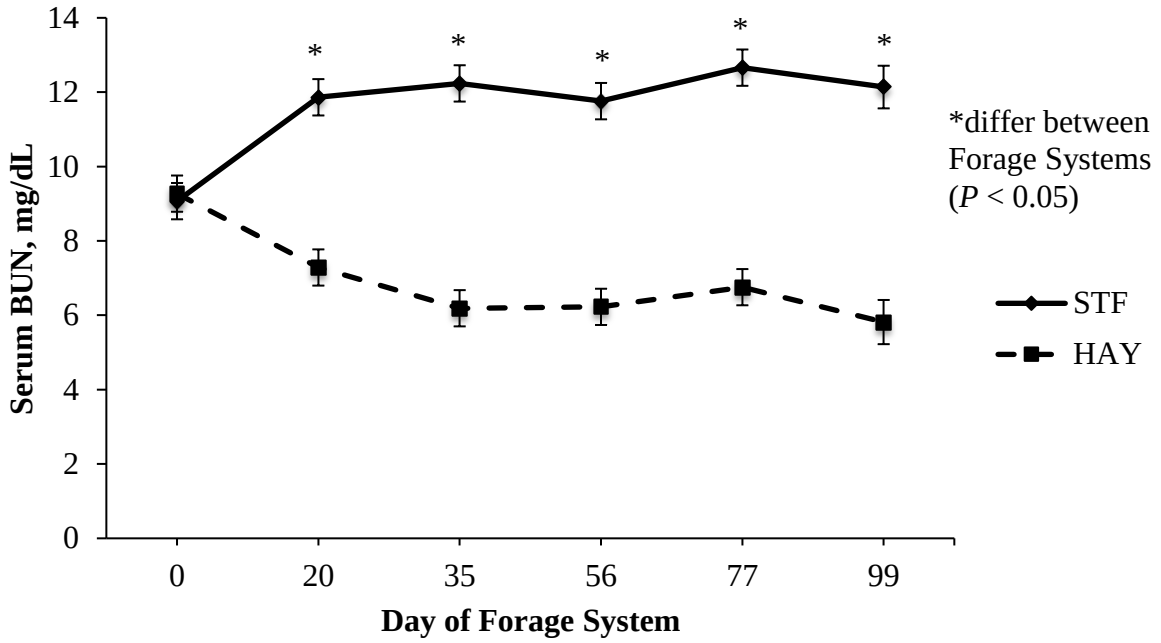
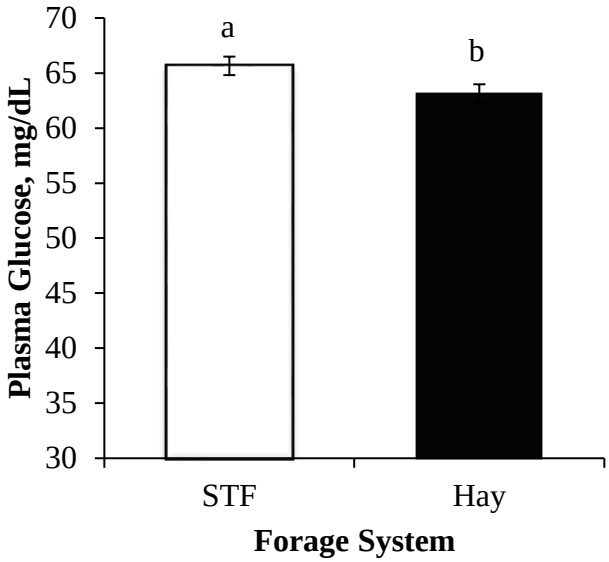


Figure 2.1. Effects of feeding stockpiled tall fescue versus tall fescue hay during late gestation on cow serum blood urea nitrogen (BUN) concentrations.

Forage systems started on d 188 ± 2 of gestation. There was a forage system x day interaction ($P < 0.001$) for serum BUN. Least squares means ± SEM are presented.

A.



B.

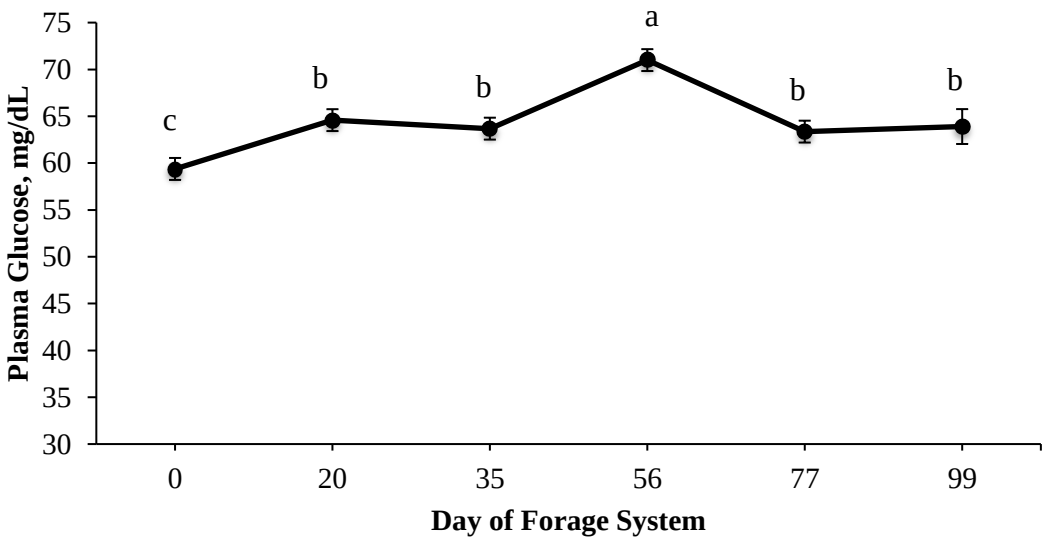
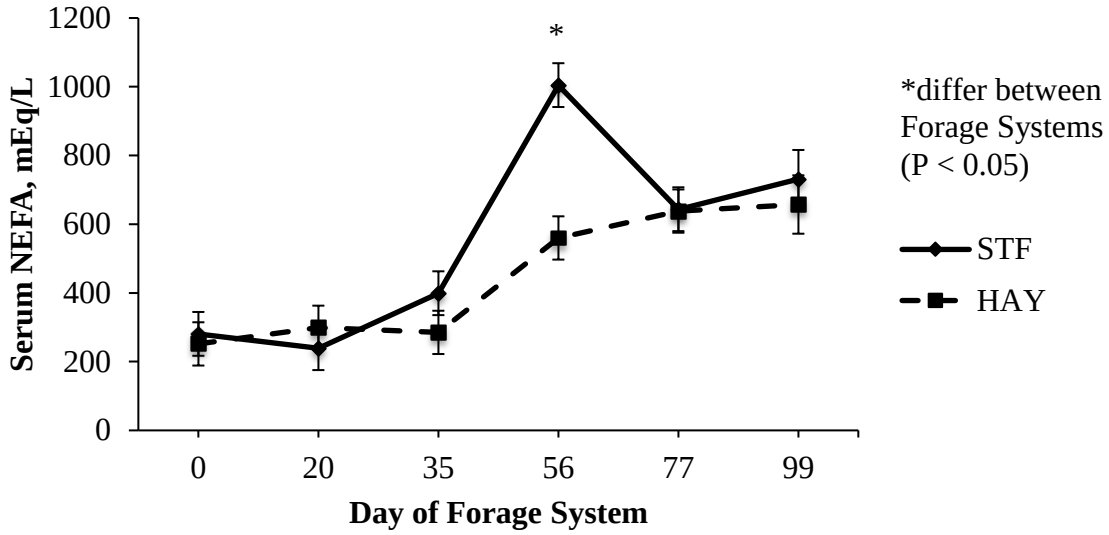


Figure 2.2. Effects of feeding stockpiled tall fescue versus tall fescue hay during late gestation on cow plasma glucose concentrations.

Forage systems started at d 188 ± 2 of gestation. There was not a forage system x day interaction ($P = 0.61$). Concentrations of plasma glucose during late gestation tended to have a main effect of forage system ($P = 0.08$; A). Concentrations of plasma glucose during late gestation had a main effect of day ($P < 0.001$; B). Least squares means ± SEM are presented. [a,b,c]items lacking a common superscript differ ($P < 0.05$).

A.



B.

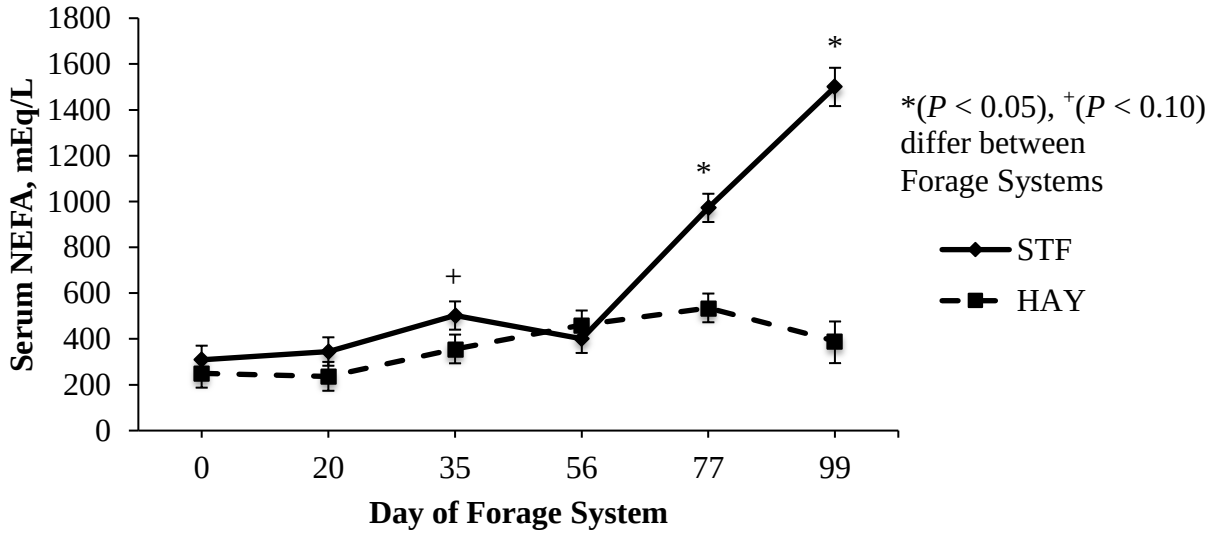


Figure 2.3. Effects of feeding stockpiled tall fescue versus tall fescue hay during late gestation on cow serum NEFA concentrations.

Forage systems started at d 188 ± 2 of gestation. There was a forage system x day x year interaction ($P < 0.001$) for serum NEFA concentrations during late gestation. Serum NEFA concentrations are presented for yr 1 (A) and yr 2 (B). Least squares means ± SEM are presented.

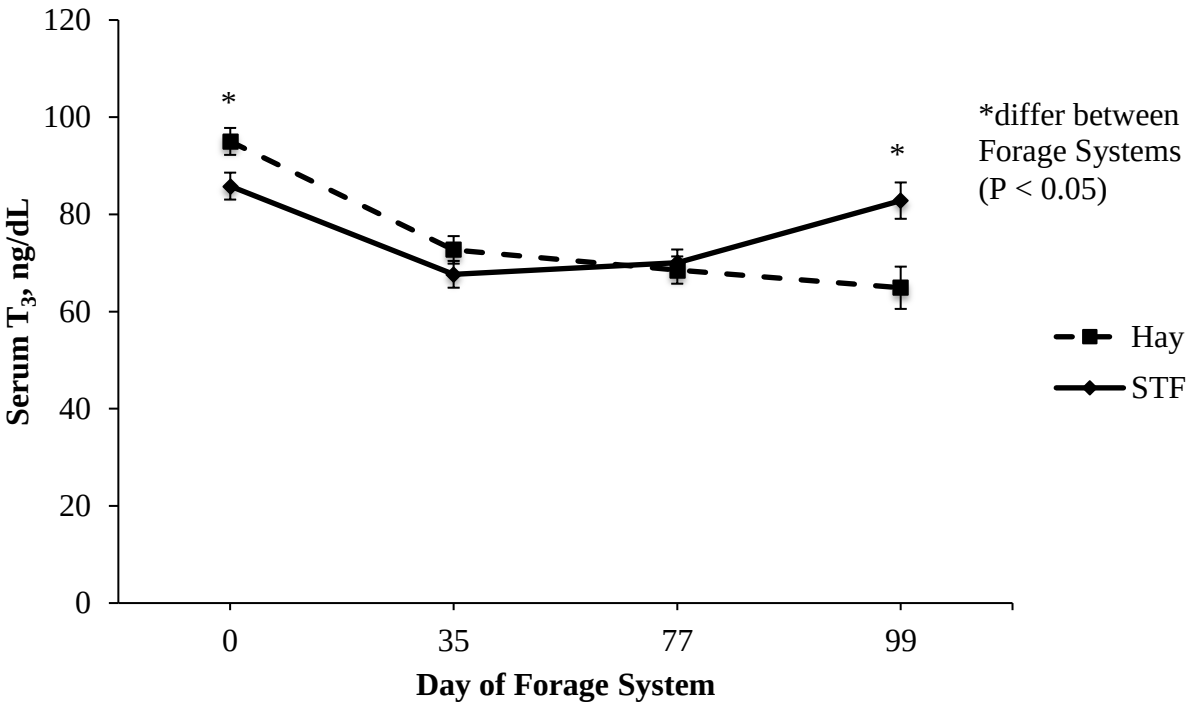
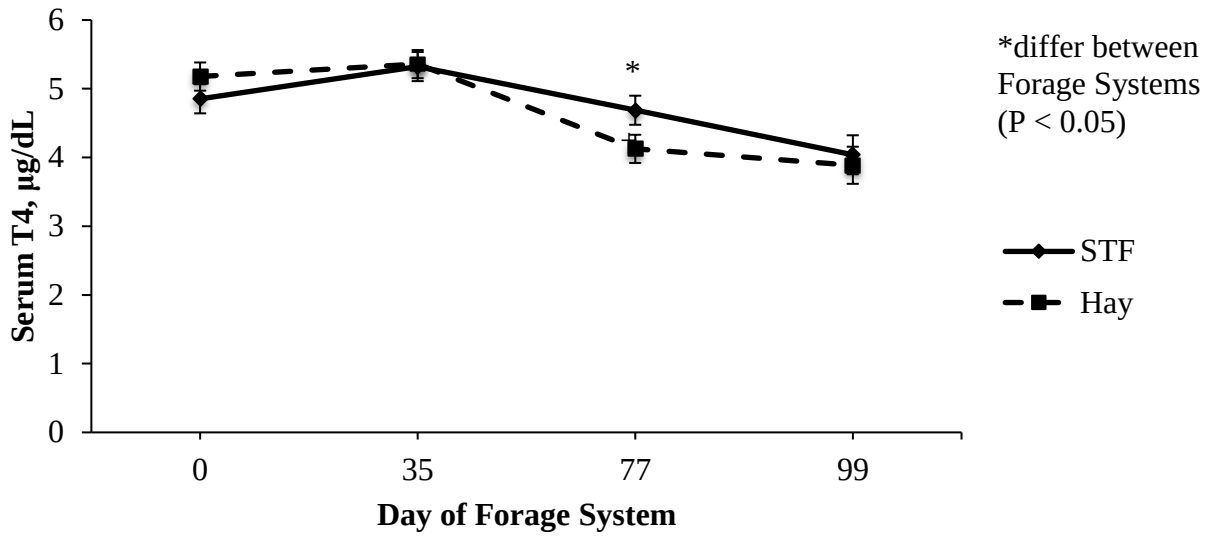


Figure 2.4. Effects of feeding stockpiled tall fescue versus tall fescue hay during late gestation on cow serum triiodothyronine (T_3) concentrations.

Forage systems started at d 188 ± 2 of gestation. There was a forage system x day interaction ($P < 0.001$) for serum T_3 concentrations. Least squares means ± SEM are presented.

A.



B.

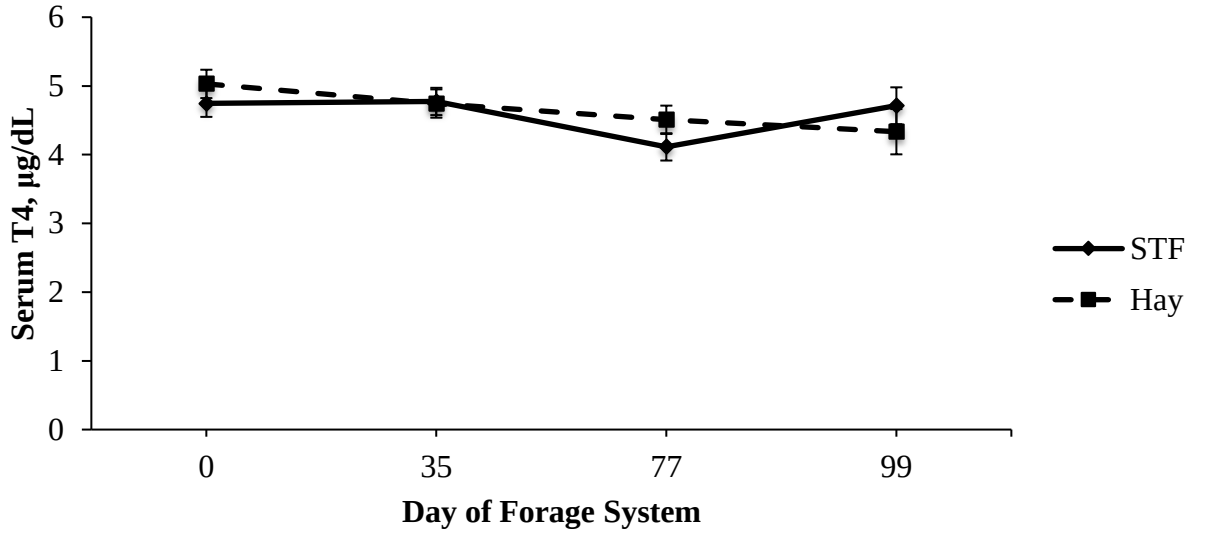


Figure 2.5. Effects of feeding stockpiled tall fescue versus tall fescue hay during late gestation on cow serum thyroxine (T_4) concentrations.

Forage systems started at d 188 ± 2 of gestation. There tended to be a forage system x day x year interaction ($P = 0.06$) for serum T_4 concentrations during late gestation. Serum T_4 concentrations are presented (A) in yr 1 and (B) in yr 2. Least squares means ± SEM are presented.

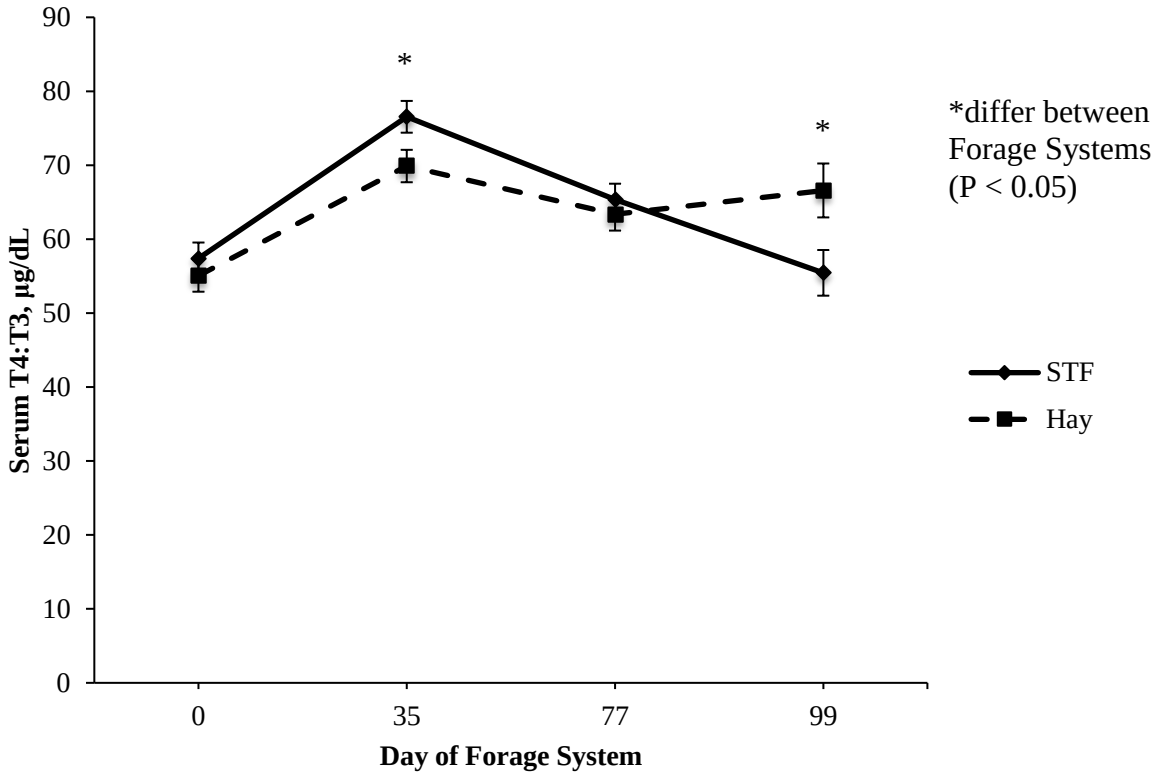


Figure 2.6. Effects of feeding stockpiled tall fescue versus tall fescue hay during late gestation on cow serum triiodothyronine (T_3):thyroxine (T_4) concentrations.

Forage systems started at d 188 ± 2 of gestation. There was a forage system x day interaction ($P = 0.02$) for serum T_4:T_3 concentrations during late gestation. Least squares means ± SEM are presented.

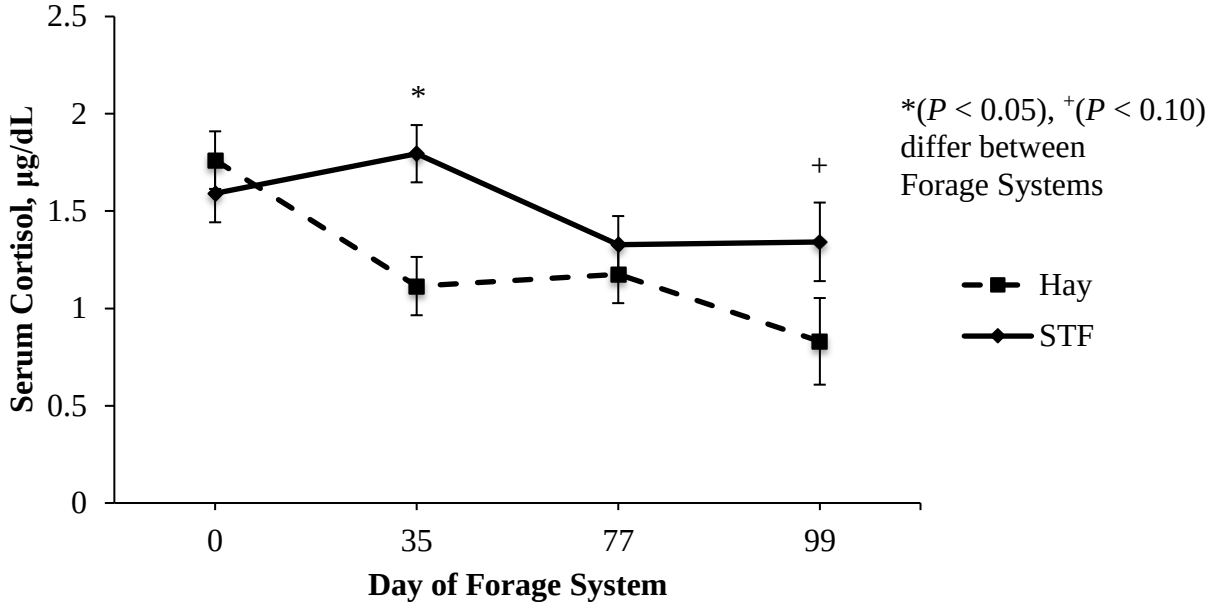


Figure 2.7. Effects of feeding stockpiled tall fescue (STF) versus tall fescue hay during late gestation on cow serum cortisol concentrations.

Forage systems started at d 188 ± 2 of gestation and continued through calving. There was a forage system x day interaction ($P < 0.006$) for serum cortisol concentrations. Least squares means ± SEM are presented.

Table 2.3. Partial correlation coefficients between final prepartum maternal metabolite or hormone concentration and calf birth weight

Maternal metabolite or hormone[1]	Partial correlation coefficient with calf birth weight (r)	*P*-value
BUN	0.31	0.002
Glucose	-0.07	0.47
NEFA	0.12	0.005
T_3	-0.05	0.41
T_4	0.08	0.15
Cortisol	0.04	0.46

[1]Last prepartum maternal circulating metabolite or hormone concentration. Average d prepartum sample was collected prior to calving were 6.6 ± 1.0 d (yr 1) and 8.4 ± 0.6 d (yr 2).

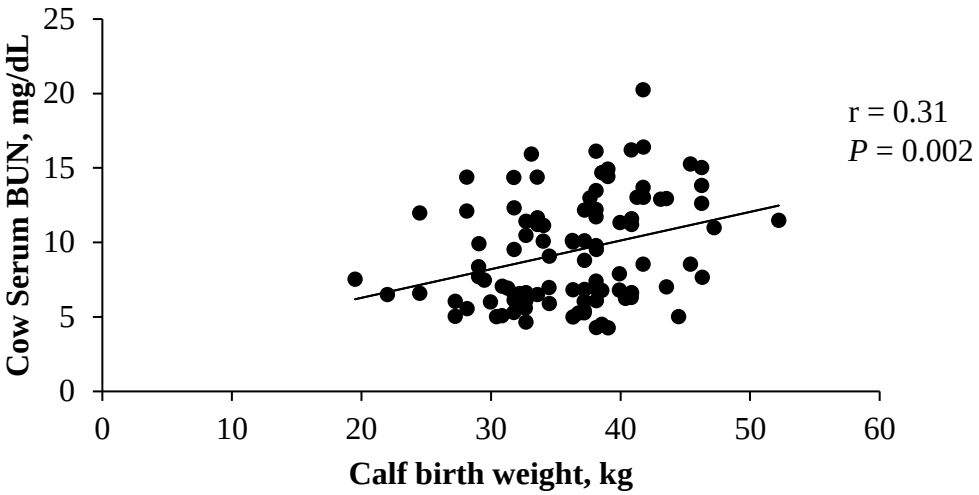


Figure 2.8. Last prepartum maternal serum blood urea nitrogen (BUN) concentration correlation with calf birth weight.

Average d prepartum sample was collected prior to calving were 6.6 ± 1.0 d (yr 1) and 8.4 ± 0.6 d (yr 2).

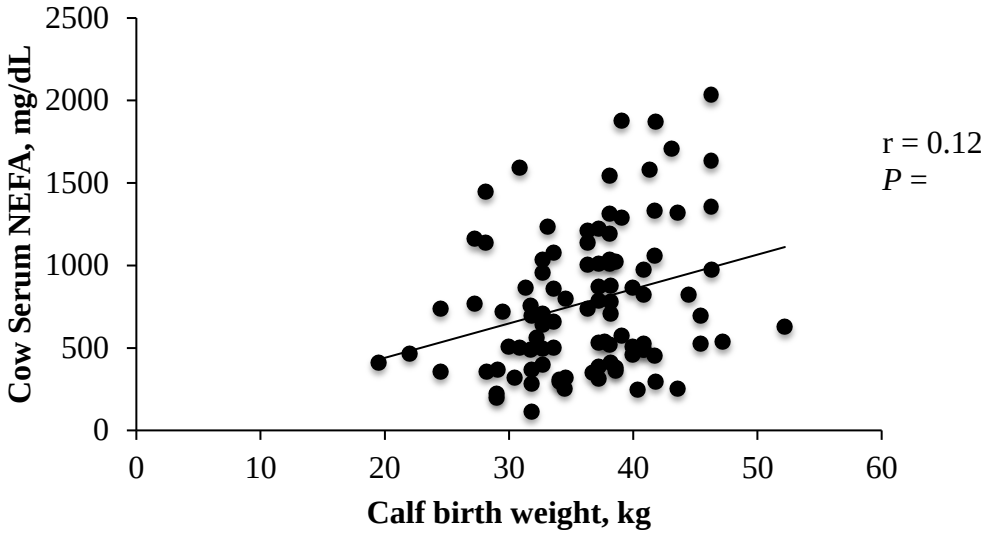


Figure 2.9. Last prepartum maternal serum nonesterified fatty acid (NEFA) concentration correlation with calf birth weight.

Average d prepartum sample was collected prior to calving were 6.6 ± 1.0 d (yr 1) and 8.4 ± 0.6 d (yr 2).

CHAPTER 3

Neonatal beef calf blood chemistry and its relationship with calf vigor

ABSTRACT

To determine changes in neonatal calf blood chemistry during the first 72 h of life and its relationship with calf vigor, 66 beef cows and heifers (average age = 4.4 ± 0.5 yr; average BCS = 5.2 ± 0.1; average calving date = September 11, 2015) were monitored during calving. Calf vigor was assessed by determining the time of birth until the time the calf successfully stood for 5 consecutive seconds (time to stand; n = 30). Jugular blood samples were obtained from 8 bull and 16 heifer calves from this subset at 0 (after standing, but before suckling), 6, 12, 24, 48, and 72 h postnatally for chemistry analysis. Serum fructose was determined in 0 h samples only. Data were analyzed using the MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). Data were analyzed using sampling hour as a repeated effect. The correlations within circulating metabolite concentrations were determined among sampling hours. Correlations were determined between calf time to stand metabolites and metabolic hormones at each sampling hour and serum fructose at 0 h. Correlations were determined between calf time to stand and serum chemistry profile at 0 h. Circulating glucose, blood urea nitrogen (BUN), total protein, and globulin concentrations increased ($P < 0.001$) from 0 to 24 h. Serum magnesium, total and direct bilirubin, aspartate aminotransferase, gamma-glutamyl transpeptidase, and creatine kinase increased ($P < 0.001$) during the first 12 h, then decreased from 24 to 72 h of age. Serum creatinine decreased ($P < 0.001$) from 0 to 72 h, whereas serum albumin, calcium, and anion gap increased ($P < 0.001$) from 24 to 48 h. Serum sodium and chloride had only slight changes (P < 0.001) from 0 to 72 h of age.

Serum BUN, total protein, globulin, and plasma glucose at 0 h were correlated ($P \leq 0.10$) with few other sampling times. However, samples collected at 6 h had positive correlations ($P \leq 0.10$) with samples from 12 to 48 h. In general, there were a greater number of positive relationships ($P \leq 0.10$) in metabolites from consecutive sampling times. Time to stand was negatively correlated ($P \leq 0.04$) with serum NEFA and albumin and positively correlated ($P \leq 0.05$) with serum BUN at select sampling hours. Serum anion gap, phosphorus, and creatine kinase at 0 h were correlated ($P \leq 0.05$) with time to stand. In conclusion, neonatal calf metabolic status changes during the first 72 h; therefore a consistent blood sampling time is necessary for determination of blood chemistry. Pre-suckling circulating metabolites appear to be poor predictors of vigor, but several neonatal metabolites after colostrum intake were related to vigor in this study.

INTRODUCTION

Calf survival is of great economic value for cow-calf producers, whether they sell their calf crop at weaning or retain ownership. In 2007, 6.4% of beef calves were either born dead or died prior to weaning (USDA-APHIS, 2010). Of that 6.4%, 55.4% were born alive, however one-third died within 24 h of birth and one-third died between 24 h and 3 wk of age (USDA-APHIS, 2010). A previous study has reported that over 50% of calf death loss occurs during the first 48 h of life (Kroger et al., 1967), suggesting that the neonatal period is a critical time for calf survival. It has been determined in dairy cattle that weak or small calves have an increased risk of mortality (Martin et al., 1975). Maternal CP restriction during late gestation has also been reported to impact neonatal calves' ability to maintain body temperature during cold conditions, which may be due to

an inadequate amount of energy stores available during the neonatal period (Carstens et al., 1987) and may impact calf metabolic status.

Concentrations of circulating metabolites indicate nutrient availability to the calf pre- and postnatally, which may impact neonatal survivability. Data from our lab have demonstrated that maternal nutrition during late gestation may alter calf metabolites at 48 h of age (Niederecker, 2015). However, there are limited data providing the normal blood chemistry reference values or changes over time in neonatal beef calves. Additionally, limited research has been conducted in beef calves to determine the metabolic status affects or can be used to determine calf vigor, which may impact the likelihood of calf survival. The objectives of this study were to determine neonatal suckling beef calf metabolic status and blood chemistry over time, evaluate the relationships of neonatal sampling times for the analysis of circulating metabolites, and determine the relationships of circulating metabolites with calf vigor.

MATERIALS AND METHODS

The University of Missouri Animal Care and Use Committee approved animal care and use in this study.

Animal Management and Sample Collection

A total of 66 AI-bred, fall-calving Angus-cross and purebred Hereford beef cows and heifers were monitored during calving beginning September 5, 2015 at the University of Missouri Beef Research and Teaching Farm. Cows and heifers were confined in 18 x 61 m dry lots that were well-drained and limestone based during the time of calving and for a minimum of 72 h postpartum. Tall fescue haylage (39% DM; 12.7% CP, 61.6%

NDF, and 36.5% ADF on DM basis) was fed in round bale ring feeders on a 9.1 x 9.1 m concrete pad to prevent mud accumulation around the feeders. Each bale was core sampled prior to feeding for nutrient analysis. Animals had free excess to water and a mineral and vitamin supplement (MLS #12 MINERA-LIX, Midcontinent Livestock Supplements, Inc., Moberly, MO).

A trained individual was present throughout the calving period for data collection. To quantify calf vigor in calves, calf time of birth and time to stand were recorded (Dwyer, 2003). Time to stand was defined as when the calf was standing on all 4 legs for a minimum of 5 consecutive seconds. Calves were weighed, tagged, and sex was determined within 6 h of birth.

Neonatal Blood Collection

Calf blood samples were obtained from a subset of 24 calves (17 crossbred, 7 Hereford; 8 bull calves, 16 heifer calves; average cow age = 4.4 ± 0.5 yr; average cow BCS = 5.2 ± 0.1; average calving date = September 11, 2015). Jugular blood samples were obtained from calves at 0, 6, 12, 24, 48, and 72 h postnatally. Blood samples collected at 0 h were obtained prior to suckling but after standing. At each sampling time, blood samples were collected into 4 blood collection 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% K_3 EDTA [10 mL draw; Covidien, Mansfield, MA], and 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). Plasma tubes were inverted as directed and placed on ice immediately following collection, and serum tubes were allowed to clot

prior to placing on ice. Samples were centrifuged at 1500 x g at 4°C for 30 min. Serum or plasma was then pipetted into 2 mL microcentrifuge tubes and stored at -20°C until analysis.

Blood Chemistry and Metabolite Analyses

Calf serum was refrigerated and transported to the University of Missouri Veterinary Medical Diagnostic Laboratory for a complete chemistry profile analysis. Creatinine, sodium, chloride, bicarbonate, anion gap, albumin, total protein, globulin, calcium, phosphorus, magnesium, total bilirubin, direct bilirubin, aspartate aminotransferase, gamma-glutamyl transpeptidase, and creatine kinase concentrations were determined using a Beckman Coulter AU 400e Chemistry System (Beckman Coulter Inc., Brea, CA). Samples were analyzed on the day of collection or refrigerated when collected on evenings or weekends until analysis.

Serum calf postnatal samples were analyzed for blood urea nitrogen (**BUN**) using a commercially available urea nitrogen kit (Urea Nitrogen Procedure Number 0580; Stanbio Laboratory, Boerne, TX) based on the diacetylmonoxime method. Samples were read in duplicate in 96-well polystyrene plates (Corning Inc., Corning, NY) on a microplate reader (Biotek Synergy™ HT, Biotek® Instruments Inc., Winooski, VT) at 520 nm. The intraassay and interassay CV were 2.84% and 3.38%, respectively.

Glucose concentration was determined in plasma samples (collected in treated tubes described above) collected on all sampling days using the Infinity™ glucose hexokinase commercially available kit (Cat. # TR15421, Fisher Diagnostics, Middletown, VA) based on the glucose-6-phospate dehydrogenase method. Samples

were read in duplicate in 96-well plates as described above at 340 nm. The intraassay and interassay CV were 4.15% and 4.39%, respectively.

Serum concentrations of NEFA were determined in calf postnatal samples using a modified procedure of the NEFA C kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan), using the acyl-CoA synthetase-acyl-CoA oxidase method. Samples were read in duplicate in 96-well plates as described above at 550 nm. The intraassay and interassay CV were 4.09% and 8.16%, respectively.

Serum fructose concentrations were determined in 0 h calf postnatal samples using the EnzyChrom^{TM} Fructose assay kit (EFRU-100, BioAssay Systems, Hayward, CA). Samples were read in duplicate in 96-well plates as described above at 565 nm. Samples were ran over 2 plates. The intraassay and interassay CV were 2.81% and 19.6%, respectively.

Statistical Analysis

Data were analyzed using the MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). Data were analyzed using sampling hour as a repeated effect. The correlations between each sampling hour pair were determined within each metabolite for BUN, glucose, total protein and globulin. Correlations were determined between calf time to stand and 1) serum BUN, 2) plasma glucose, 3) serum NEFA, 4) serum albumin, 5) serum total protein, and 6) serum globulin at each sampling hour and 7) serum fructose at 0 h. Correlations were determined between calf time to stand and serum chemistry profile at 0 h.

RESULTS AND DISCUSSION

To our knowledge, there have not been any other studies conducted in neonatal beef calves to determine changes in the blood chemistry of calves during the first 72 h of life. Much of the bovine data may have been less intensive measurements or collected in neonatal dairy calves. Dairy calves are different in many ways from neonatal beef calves; the major contributor being that they are meal-fed compared with beef calves that are allowed to suckle at will throughout the day.

Neonatal Circulating Metabolites

Protein-related Metabolites. Protein-related metabolites are presented in Figure 3.1. Circulating serum BUN increased ($P < 0.001$) from 0 to 24 h postnatally with BUN concentrations not different ($P \geq 0.10$) at 12 h with 6 or 24 h concentrations, then was similar ($P \geq 0.10$) for the remainder of the neonatal sampling period. Total protein and globulin concentrations increased ($P < 0.001$) from 0 to 24 h postnatally, decreased ($P \leq 0.02$) from 24 to 48 h, then were similar ($P \geq 0.12$) from 48 to 72 h in total protein or tended to decrease ($P = 0.09$) from 48 to 72 in globulin concentrations. Serum albumin and creatinine concentrations decreased ($P \leq 0.01$) from 0 to 24 h after birth. There was an increase ($P < 0.001$) in albumin concentrations from 24 until 48 h then concentrations remained similar ($P = 0.18$) from 48 to 72 h, however creatinine continued to decrease ($P < 0.001$) throughout the sampling period.

The increase in circulating protein concentrations during the neonatal period is dependent on the time and amount of colostrum and immunoglobulins present (Blum and Hammon, 2000). Concentrations of total protein and globulin increased until 24 h postnatally, indicating colostrum intake during that time period and immunoglobulin

absorption. Albumin concentrations decreased during the early neonatal period, which is in agreement with previous research in dairy calves, where plasma albumin concentrations decrease after the initial colostrum intake and increasing after d 2 (Rauprich et al., 2000), similar to our data with concentrations of circulating albumin increasing from 24 to 48 h postnatally. Albumin aids in maintaining homeostasis and is an important carrier protein that binds to many molecules including long-chain fatty acids, steroids, calcium, and magnesium (Fanali et al., 2012).

The increase in serum BUN during the neonatal period may be due to the increase in protein intake available through consumption of colostrum and milk compared to protein degradation and amino acid deamination prenatally (Hammon et al., 2002). This increase in serum BUN concentrations is not likely to have occurred due to renal dysfunction due to the decrease in serum creatinine during this same time period, which is similar to the relationships reported in neonatal dairy calves during the first 3 d postnatally (Hammon et al., 2002). Dehydration of muscle creatinine will increase the amount of circulating creatinine when muscle mass increases (Perrone et al., 1992). The decrease in creatinine may be due to the result of improved postnatal kidney function, as the improvement in urinary clearance has been reported in neonatal beef calf studies (Egli and Blum, 1998).

Energy-related Metabolites. Plasma glucose concentrations (Figure 3.2) increased ($P \leq 0.04$) from 0 through 24 h postnatally but were not different ($P \geq 0.10$) at 48 h and through the remainder of the neonatal sampling time. Circulating NEFA (Figure 3.2) increased ($P < 0.001$) from 0 until 6 h postnatally, remained similar ($P = 0.18$) from

6 to 12 h, and then decreased ($P \leq 0.02$) throughout the remainder of the sampling times to concentrations similar to at birth.

The increase in plasma glucose is to be expected due consumption of colostrum and milk. Prior to colostrum intake, newborn calves are characterized as having relatively low plasma glucose levels (Egli and Blum, 1998). The increase in circulating glucose may be due to the fact that calves will consume small portions during much of the day, not allowing for glucose concentrations to decrease. These data are in agreement with previous research in which following colostrum intake, circulating glucose levels increased in dairy calves during the first 24 h postnatally and may be due to the slow breakdown of lactose and absorption of glucose into circulation (Edwards and Silver, 1970). The increase in glucose concentrations may also be due to glucagon, which plays a major role in gluconeogenesis in neonates, and increases as colostrum intake increases (Ward Platt and Deshpande, 2005).

There was great variation in NEFA concentrations between calves at 0 h (SEM = 66.9) postnatally which may be due to the different reactions to the stress of parturition and using energy stores resulting in an increase in lipid mobilization prior to colostrum intake. Studies conducted in neonatal dairy calves have reported a decrease in NEFA concentrations 4 h after birth (Rauprich et al., 2000), however the time in which calves consumed milk during this study was unknown. The increase in NEFA during the first 12 h postnatally may be due to the calf's adaption to the extrauterine environment and the increase in related stress of the calving process, as decreased NEFA after 12 h indicates less fat being mobilized for use as an energy source (Lents et al., 1998). When colostrum was withheld during the first 24 h postnatally, NEFA concentrations increased in calves

(Blum and Hammon, 2000). In the current study, calf intake of colostrum and milk was not controlled and the decrease in NEFA concentrations after 12 h may indicate greater nutrients available due to more frequent suckling.

Total and Direct Bilirubin. Total bilirubin and direct bilirubin concentrations (Figure 3.3) increased ($P \leq 0.04$) from 0 until 12 h postnatally and remained similar ($P \geq 0.34$) from 12 to 24 h, then decreased ($P \leq 0.01$) from 24 to 72 h postnatally. Bilirubin is the end product of heme catabolism in mammals and has a beneficial role as an antioxidant (Stocker et al., 1987). The decrease after 24 h may be due to the destruction of fetal red blood cells in the liver and spleen (Egli and Blum, 1998).

Electrolytes. Changes in circulating electrolyte concentrations are presented in Figure 3.4. Potassium concentrations decreased ($P = 0.02$) from 0 to 6 h postnatally, then remained similar ($P \geq 0.81$) through 24 h. From 24 to 48 h, potassium increased ($P = 0.001$), but then remained similar ($P = 0.58$) from 48 to 72 h. Serum sodium was similar ($P = 0.80$) from 0 to 6 h postnatally, then decreased ($P < 0.001$) from 6 to 12 h, then remained similar ($P = 0.21$) from 12 to 24 h. Concentrations of sodium tended to increase ($P = 0.07$) from 24 to 48 h, then decreased ($P < 0.001$) from 48 to 72 h. Serum chloride was similar ($P = 0.11$) from 0 to 6 h, then tended to decrease ($P = 0.09$) from 6 to 12 h postnatally. Chloride increased ($P = 0.02$) from 12 to 24 h, then remained similar from 24 to 48 h until decreasing ($P < 0.001$) from 48 to 72 h. Anion gap tended to decrease from 0 to 6 h and then decreased ($P \leq 0.05$) through 24 h. Anion gap tended to increase ($P = 0.09$) from 24 to 48 h, but did not change ($P = 0.30$) from 48 to 72 h postnatally. Bicarbonate concentrations did not change ($P \geq 0.18$) throughout the neonatal sampling period.

Changes in electrolyte concentrations during the neonatal period seem to be tightly regulated. A study conducted by Piccione et al. (2010), demonstrated no significant changes in electrolyte concentrations in beef calves during the first week postnatally, suggesting that they are not influenced by age (Piccione et al., 2010). Serum anion gap concentrations decreased from 0 to 24 h postnatally. An increase in anion gap is caused by metabolic acidosis and in animals and humans the increase may occur due to cold exposure, restricted intake, and impaired metabolism or renal excretion (Hoffman et al., 1992). In a study in neonatal foals, an increased anion gap was associated with neonatal death (Hoffman et al., 1992). There was no mortality in the current study and all calves sampled were healthy, which may be indicated by the decrease in anion gap over the neonatal period.

Other Minerals. Changes in circulating mineral concentrations are presented in Figure 3.5. Calcium concentrations tended to increase ($P = 0.07$) from 0 to 6 h then decreased ($P < 0.001$) from 6 through 24 h. Calcium then increased ($P < 0.001$) from 24 to 48 h and remained similar ($P = 0.30$) from 48 to 72 h postnatally. Phosphorous concentrations decreased ($P < 0.001$) from 0 to 6 h, then remained similar ($P \geq 0.18$) from 6 to 24 h, increased ($P < 0.001$) from 24 to 48 h then remained similar ($P = 0.57$) from 48 to 72 h. Magnesium concentrations increased ($P < 0.001$) from 0 to 12 h postnatally then remained similar ($P = 0.25$) from 12 to 24 h until decreasing ($P < 0.001$) from 24 to 72 h.

There was variation in circulating mineral concentrations during the 72 h neonatal period. Previous studies have reported calcium levels to be high at birth then decrease during the first 28 d of life (Egli and Blum, 1998). Calcium concentrations remained

similar in the current study at 0 and 6 h postnatally, indicating colostrum intake did not impact circulating calcium concentrations. Magnesium concentrations increased from 0 to 12 h, then decreased during the remainder of the sampling period which is in agreement with concentrations measured in postnatal dairy calves measured on d 1 postnatally (Egli and Blum, 1998). Others have reported no significant changes in circulating mineral concentrations in neonatal calves (Zanker et al., 2001).

Enzymes

Aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and creatine kinase (CK) concentrations (Figure 3.6) increased ($P < 0.001$) from 0 to 6 h. Concentrations of AST and GGT continued to increase ($P < 0.001$) until 12 h. Serum AST remained similar ($P = 0.22$) from 12 to 24 h, then decreased ($P < 0.001$) through the remainder of the neonatal sampling time. Serum GGT decreased ($P < 0.01$) from 12 to 24 h, then increased ($P < 0.001$) from 24 to 48 h and remained similar ($P = 0.23$) from 48 to 72 h. Serum CK concentrations remained similar ($P = 0.74$) from 6 to 12 h, then decreased ($P \leq 0.01$) through the remainder of the sampling period.

Increased concentrations of GGT may be used as an indicator of liver disease and AST as heart and skeletal muscle diseases (Zanker et al., 2001), however, serum concentrations of AST, GGT, and CK rapidly increased from 0 to 6 h postnatally, indicating a relationship with colostrum intake. Over 40 enzymes have been identified in bovine colostrum with GGT the most studied and absorbed, readily appearing in circulation in neonatal calves (Zanker et al., 2001). Our data have demonstrated a rapid increase in enzyme concentrations during the early postnatal hours, indicating absorption of these enzymes available through colostrum and their own production of these enzymes during this time. There was a decrease in all enzyme concentrations after 24 h

postnatally. Previous studies in neonatal dairy calves demonstrate GGT absorption is inhibited between 6 and 12 h postnatally (Zanker et al., 2001), while our data suggests inhibition occurs later, after 12 h. These differences may be due to the breed, quality of colostrum, and the time and amount of colostrum intake over this time period. Concentrations of GGT and AST were analyzed in colostrum and milk and indicated enzyme levels were very high in initial colostrum then decreased as time went on in milk (Zanker et al., 2001), indicating a similar relationship of enzyme concentrations in milk and circulation. Serum CK concentrations may have rapidly increased from 0 to 6 h postnatally, which may indicate some muscle damage during parturition (Egli and Blum, 1998), however the impact was not permanent due to the rapid decline of CK after 12 h. Due to the rapid degradation in neonatal circulation, the importance of these enzymes still remains unknown (Blum and Hammon, 2000).

Relationship Among Sampling Times

Correlations of postnatal calf sampling time for serum BUN, plasma glucose, serum total protein, and serum globulin are presented in Table 3.2. Within serum BUN, sampling times had strong positive correlations ($P \leq 0.05$) at consecutive sampling times. Sampling times 12 and 24 h postnatally had or tended to have a moderate or strong correlation ($P < 0.10$) with all sampling h except 0 h. Relationships between sampling h and 48 as well as 72 h had or tended to have a moderate or strong correlation ($P < 0.10$) with all sampling h except 0 and 6 h. Plasma glucose sampling times had a strong positive correlation ($P \leq 0.05$) between 0 and 6 h and 12 and 24 h postnatally. Sampling times of plasma glucose tended to have moderate positive correlations ($P < 0.10$) between 0 and 48 h, 6 and 12 h, 6 and 24 h, and 6 and 48 and tended to have a moderate negative

correlation ($P < 0.10$) between 12 and 72 h postnatally. Total protein sampling times had a strong positive correlation ($P \leq 0.05$) between 6 and 12 h, 12 and 24 h, 12 and 48 h, and 24 and 48 h postnatally. There was a moderate positive correlation ($P \leq 0.05$) between 6 and 24 h and 6 and 48 h, and there tended to be a weak positive correlation ($P < 0.10$) between 6 and 72 h within total protein. There were no relationships ($P > 0.10$) between 0 h samples and any other sampling time within globulin concentrations. There was a strong positive correlation ($P \leq 0.05$) within globulin between 6 and 12 h, 12 and 24 h, 12 and 48 h, 24 and 48 h and 48 and 72 h. Additionally, there was a moderate positive correlation between 6 and 24 h and 6 and 48 h postnatally.

Metabolites at 0 h were correlated with few other sampling times, which may be due the changes in metabolites over time and the difference in pre- and postnatal delivery of nutrients. There was a greater number of positive relationships in metabolites from consecutive sampling times and may be due to the similar intake of colostrum or milk after the initial intake of the postnatal nutrients. Better knowledge of sampling time differences is necessary to determine the relationship of neonatal metabolic status, vigor, and survival. These data demonstrate that a consistent sampling time is necessary due to the changes in metabolites over time in neonatal beef calves. To better determine prenatal metabolite concentrations, samples need to be collected prior to colostrum intake, however, the current study suggests metabolite concentrations are not consistent during the first 72 h, therefore sampling time does matter during the neonatal period.

Relationship of Blood Chemistry and Calf Vigor

Correlation coefficients for circulating metabolite and calf time to stand are presented in Table 3.3. Plasma glucose tended to have a moderate or weak positive

correlation ($P = 0.09$) with time to stand at 72 h postnatally. Time to stand had a moderate or strong positive correlation ($P < 0.01$) with serum BUN concentrations at 6, 12, and 24 h and had a moderate positive correlation ($P = 0.05$) at 48 h. Time to stand had a moderate negative correlation ($P = 0.04$) with serum NEFA concentrations at 24 h and tended to have a moderate negative correlation ($P = 0.07$) with serum NEFA at 72 h. Serum albumin tended to have a moderate negative correlation ($P = 0.06$) with time to stand at 0 h, and had a moderate or strong negative correlation ($P < 0.02$) with time to stand at the remaining sampling times. Serum total protein, globulin, and fructose concentrations were not correlated ($P \geq 0.25$) with time to stand at any sampling times.

Serum BUN concentrations had a moderate positive correlation ($P \leq 0.04$) with calf birth weight at 6 h ($r = 0.55$) and 12 h ($r = 0.51$) postnatally and tended to have a weak positive correlation ($P = 0.10$) at 24 h ($r = 0.36$) postnatally. All other metabolites were not correlated ($P > 0.36$) with calf birth weight during the neonatal period.

Correlations between calf time to stand and serum chemistry profile at 0 h postnatally are presented in Table 3.4. Time to stand had a moderate positive correlation ($P \leq 0.03$) with anion gap, phosphorus, and CK, and tended to have a moderate positive correlation ($P = 0.06$) with aspartate aminotransferase and moderate negative correlation ($P = 0.06$) with albumin. All other serum chemistry measures were not correlated with time to stand.

Neonatal survival is dependent upon colostrum intake and the time it takes the neonate to stand and reach the udder in order to achieve passive transfer of immunity from the dam and obtain the vital nutrients for extrauterine survival (Dwyer, 2003). A study conducted by Vasseur et al. (2009) indicated that calves that were less vigorous at

birth had reduced colostrum intake (Vasseur et al., 2009), which may ultimately have a negative impact on calf health and performance. These relationships in metabolites with the time it took the calf to stand may be used as an indicator of the calving difficulty. Although all claves were presented normally and no dystocia was recorded, the time of birth or stress of the calving process may have caused the relationships with time to stand and certain metabolies. Calf birth weight had a moderate positive correlation ($P = 0.01$) with time to stand, indicating the larger calves took longer to stand. This relationship may be due to the larger calves having greater stores of body fat so colostrum intake for energy was not as crucial compared to the smaller calves. Although calving difficulty was not observed, the larger calves may have encountered greater stress during the calving process or may have been more susceptible to the impacts of heat stress. Larger calves may also need more milk to be consumed in order to change the metabolic status of the neonate. With a decrease in vigor, it may take larger calves longer to consume milk decreasing the metabolic concentrations during the neonatal period. Previous sheep data indicates the opposite in that heavier lambs at birth were more active than their counterparts (Dwyer, 2003). In the current study, pre-suckling circulating metabolites appear to be poor predictors of vigor, but several neonatal metabolites after colostrum intake were related to vigor in this study. Serum BUN was positively correlated with time to stand indicating that the calves that may have been more protein deficient took less time to stand to consume more nutrients for survival at the initial postnatal sampling times and had less excess circulating BUN due to lower concentrations. Additionally, serum albumin was negatively correlated with time to stand, indicating that calves with greater albumin concentrations took less time to stand and may be due to the

improvement in nutrient transfer ability and available energy. Calf vigor may indicate a greater consumption of milk, increasing the metabolic status of the neonate.

CONCLUSION

In summary, neonatal calf metabolic status changes during the first 72 h. Determining normal changes in metabolic concentrations during the neonatal period in healthy beef calves may be used as an indicator of normal reference ranges and assist in determining calf health status. Additionally, the change in metabolite concentrations may be impacted by colostrum intake during the early hours postnatally, indicating the importance of a consistent sampling time and may help determine beef calves consumption of colostrum during the neonatal period to improve immune transfer and postnatal health if colostrum intake was not sufficient. Pre-suckling circulating metabolites appear to be poor predictors of vigor, but several neonatal metabolites after colostrum intake were related to vigor in this study. Metabolites that were related to vigor, however, may be used as an indicator of calving difficulty and may be related to calf birth weight affecting the calving process. Further research is necessary to determine the impacts of maternal nutrition during gestation, suckling time, and the quality of colostrum has on neonatal beef calves metabolic status.

IMPLICATIONS

To our knowledge, changes and determination of reference ranges in chemistry profiles during the first 72 h of life in neonatal calves has not been well studied. Our data in beef calves may be used as an indicator of calf vigor and health during the neonatal period. These data indicate changes occur during this time period and are different from

adult bovine reference ranges, which are currently used in practice. These neonatal calf data may be used within the veterinary diagnostic clinical pathology laboratory as normal reference ranges to identify sick calves, however, a consistent sampling time is necessary and should be taken into consideration, especially if obtaining samples prior to colostrum intake.

The metabolite correlations with calf time to stand may also be used to indicate the calving process and potentially a measure of calving ease. Within this study, smaller calves took less time to stand compared to larger calves. Additionally, there were moderate positive correlations between anion gap, phosphorus, aspartate aminotransferase, and creatine kinase at 0 h with calf time to stand. These correlations indicate that with greater concentrations, the time it took calves to stand was longer. These correlations may be due to the restriction of oxygen the calf would receive while in the birth canal and increased muscle breakdown if the calving process was longer or more difficult, resulting in an increase in the time it would take the calf to stand. Albumin at 0 h, however, was negatively correlated with time to stand, which may indicate that with greater prenatal albumin concentrations, calves are able to stand faster, therefore may be used as an indicator for calf vigor. These data indicate the importance of the chemistry profile in calves, and the potential to help determine calving difficulty based on concentrations at the time of birth and the time it takes the calf to stand to consume colostrum as soon after birth as possible.

Table 3.1. Calf size, time to stand, and gestation length for heifer and bull calves[1]

Variable	Heifer	Bull	P-value
Calf birth BW, kg[2]	32.7 ± 0.8	34.8 ± 1.1	0.13
Crown to rump length, cm[3]	87.8 ± 1.1	86.0 ± 1.4	0.30
Shoulder to rump length, cm[4]	60.3 ± 0.8	60.5 ± 0.9	0.87
Abdominal girth, cm[5]	75.1 ± 1.1	77.1 ± 1.4	0.25
Heart girth, cm[6]	76.0 ± 0.5	77.1 ± 0.6	0.20
Cannon bone circumference, cm[7]	12.9 ± 0.1	13.0 ± 0.2	0.44
Gestation length, d	280 ± 0.6	281 ± 0.8	0.27
Time to stand, min[8]	20.4 ± 3.2	25.3 ± 4.6	0.39

[1]Least squares means ± SEM are presented.
[2]Calf birth weight was measured within 6 h postnatally
[3]Crown to rump length was measured from the poll to the end of the tail head.
[4]Shoulder to rump length was measured from the middle of the withers of the calf to the end of the tail head.
[5]Abdominal girth circumference measured the distance around the navel.
[6]Heart girth circumference measured the distance around the heart girth at the middle of the withers.
[7]Cannon bone circumference was measured on the each calves rear leg at the smallest diameter of the cannon bone.
[8]Time to stand was defined as when the calf was standing on all 4 legs for a minimum of 5 consecutive seconds.

a

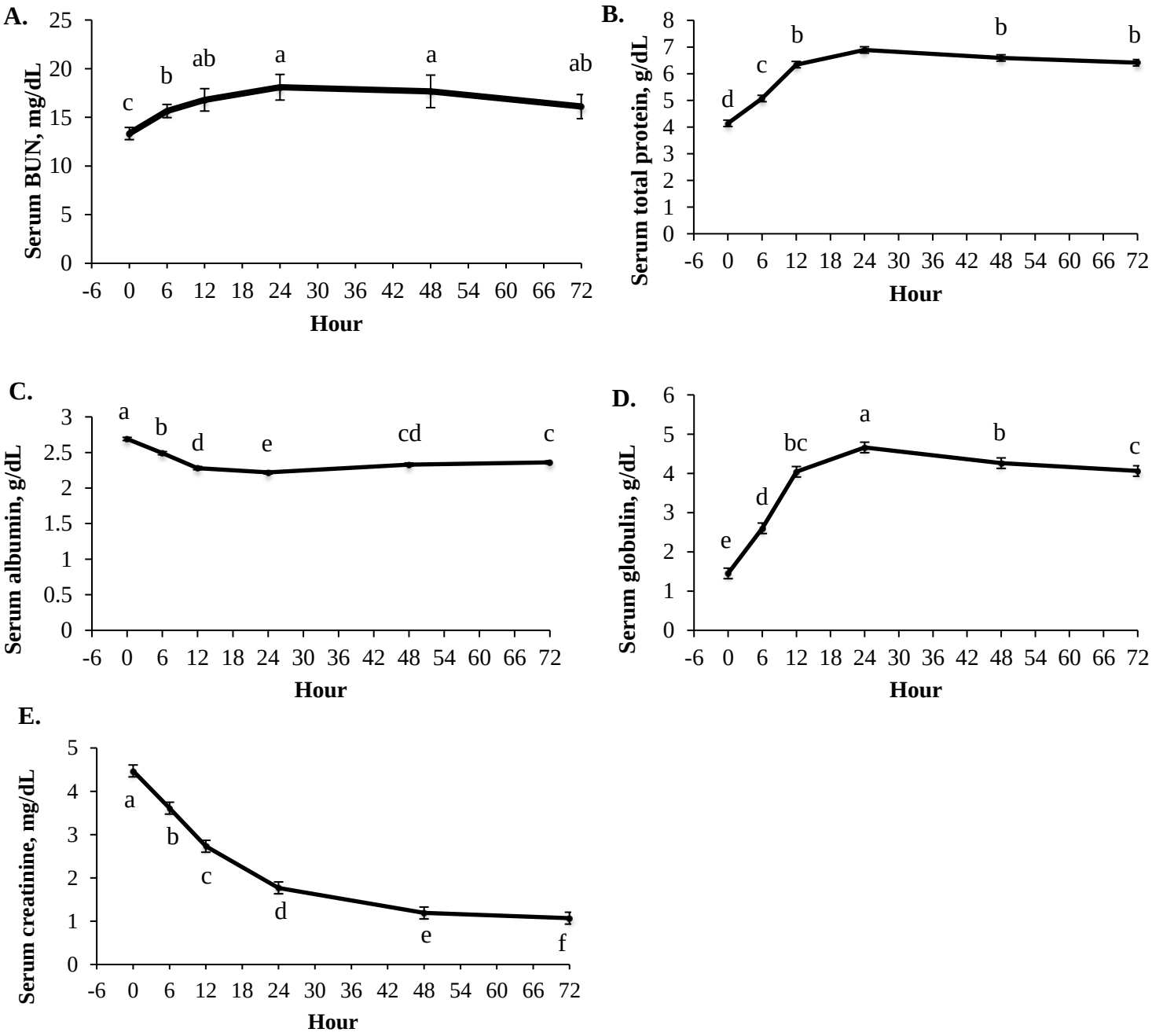


Figure 3.1. Effect of hour on circulating protein metabolite concentrations in beef calves from 0 through 72 h postnatally.

Main effect of hour was significant ($P < 0.001$) for serum (A) blood urea nitrogen (BUN), (B) total protein, (C) albumin, (D) globulin, and (E) creatinine concentrations in beef calves 0 through 72 h postnatally. Least squares means ± SEM are presented.

[a,b,c,d,e]items lacking a common superscript differ by $P < 0.10$.

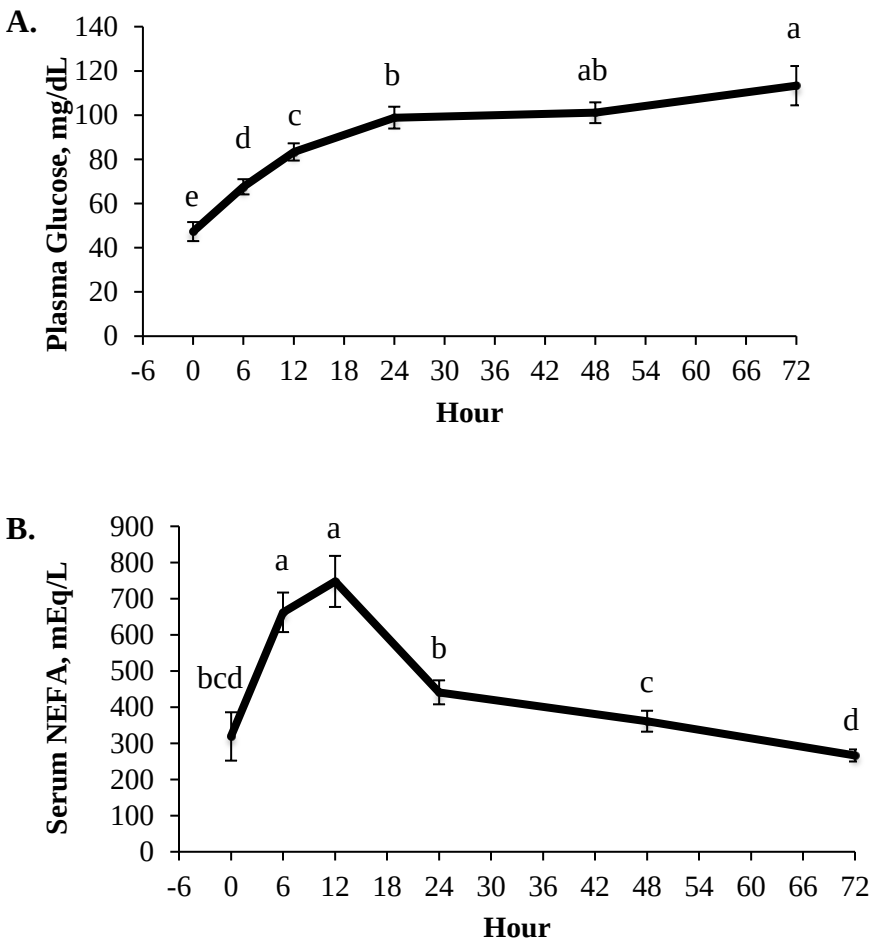


Figure 3.2. Effect of hour on circulating energy metabolite concentrations in beef calves from 0 through 72 h postnatally.

Main effect of hour was significant ($P < 0.001$) for (A) plasma glucose and (B) serum NEFA concentrations in beef calves 0 through 72 h postnatally. Least squares means ± SEM are presented.

[a,b,c,d]items lacking a common superscript differ by $P < 0.10$.

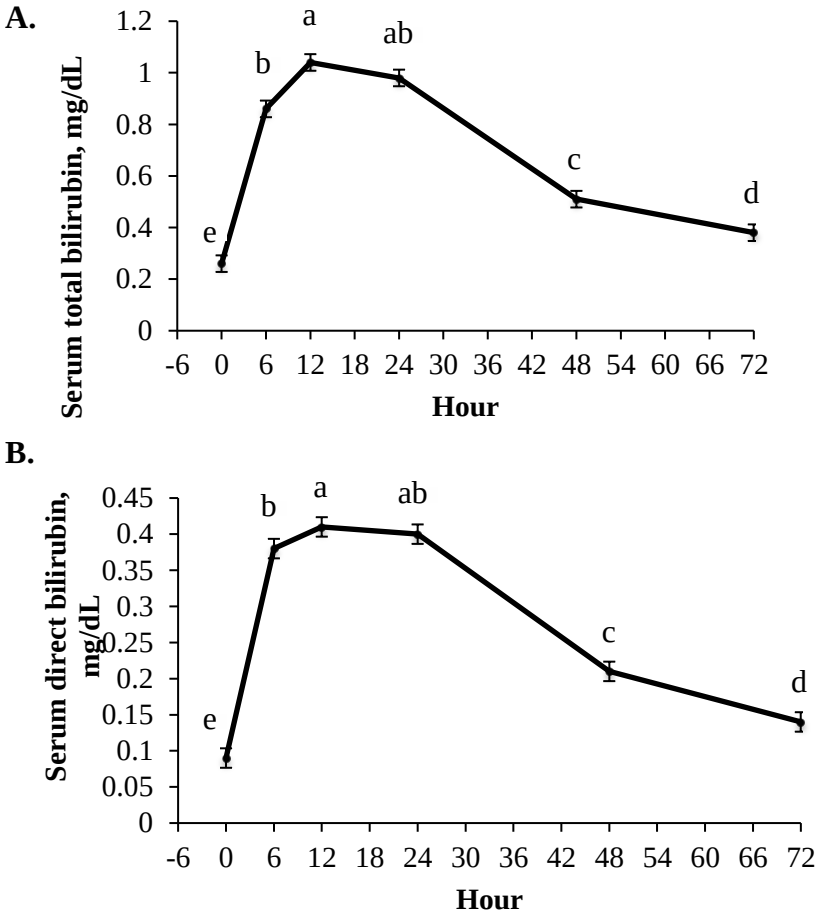


Figure 3.3. Effect of hour on circulating metabolite concentrations in beef calves from 0 through 72 h postnatally.

Main effect of hour was significant ($P < 0.001$) for (A) serum total bilirubin and (B) serum direct bilirubin concentrations in beef calves 0 through 72 h postnatally. Least squares means ± SEM are presented.

[a,b,c,d,e]items lacking a common superscript differ by $P < 0.10$.

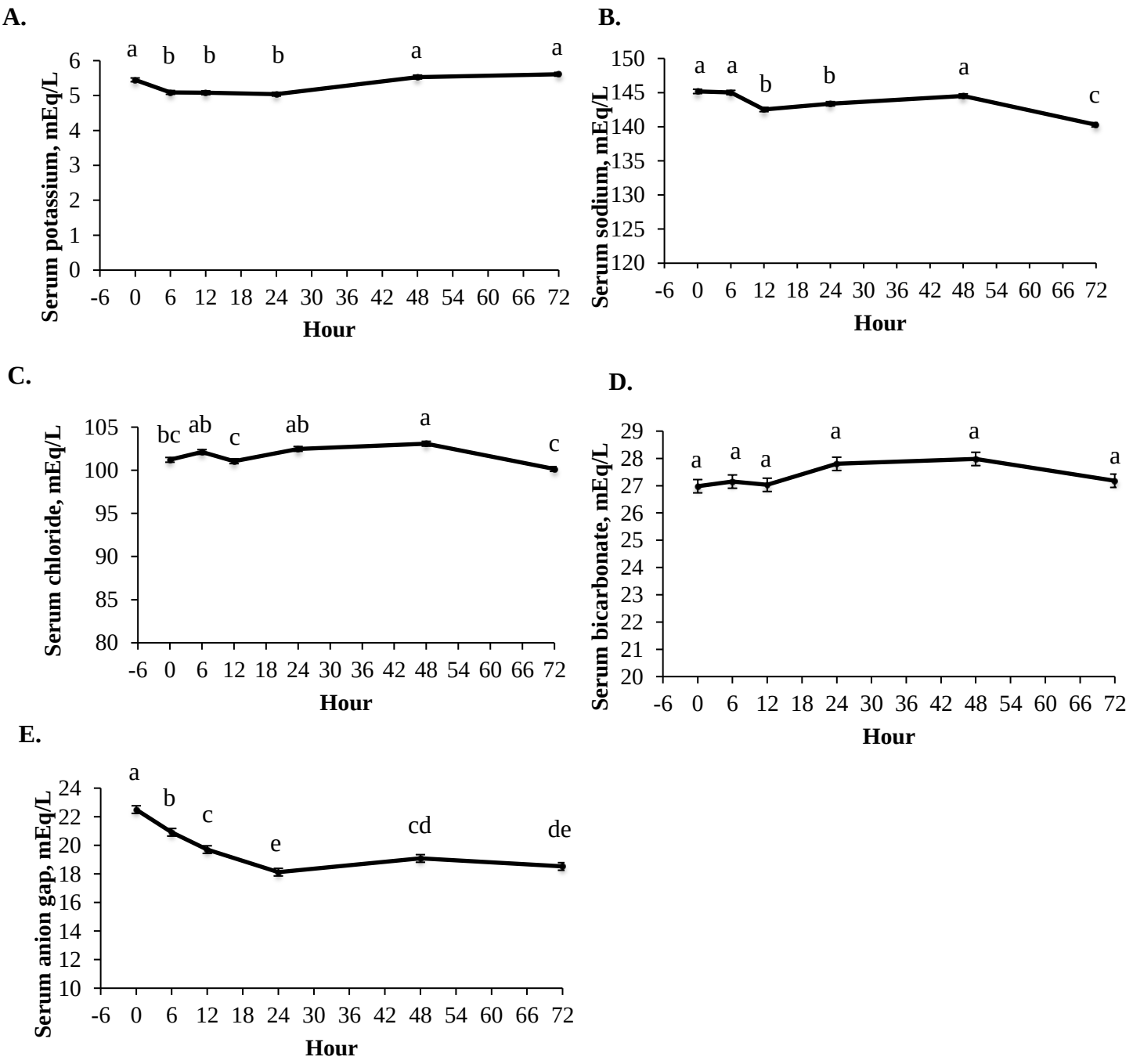


Figure 3.4. Effect of hour on circulating electrolyte and related concentrations in beef calves from 0 through 72 h postnatally.

Main effect of hour was significant ($P < 0.001$) for serum (A) potassium, (B) sodium, (C) chloride, (D) bicarbonate, and (E) anion gap, concentrations in beef calves 0 through 72 h postnatally. Least squares means ± SEM are presented.

[a,b,c,d]items lacking a common superscript differ by $P < 0.10$.

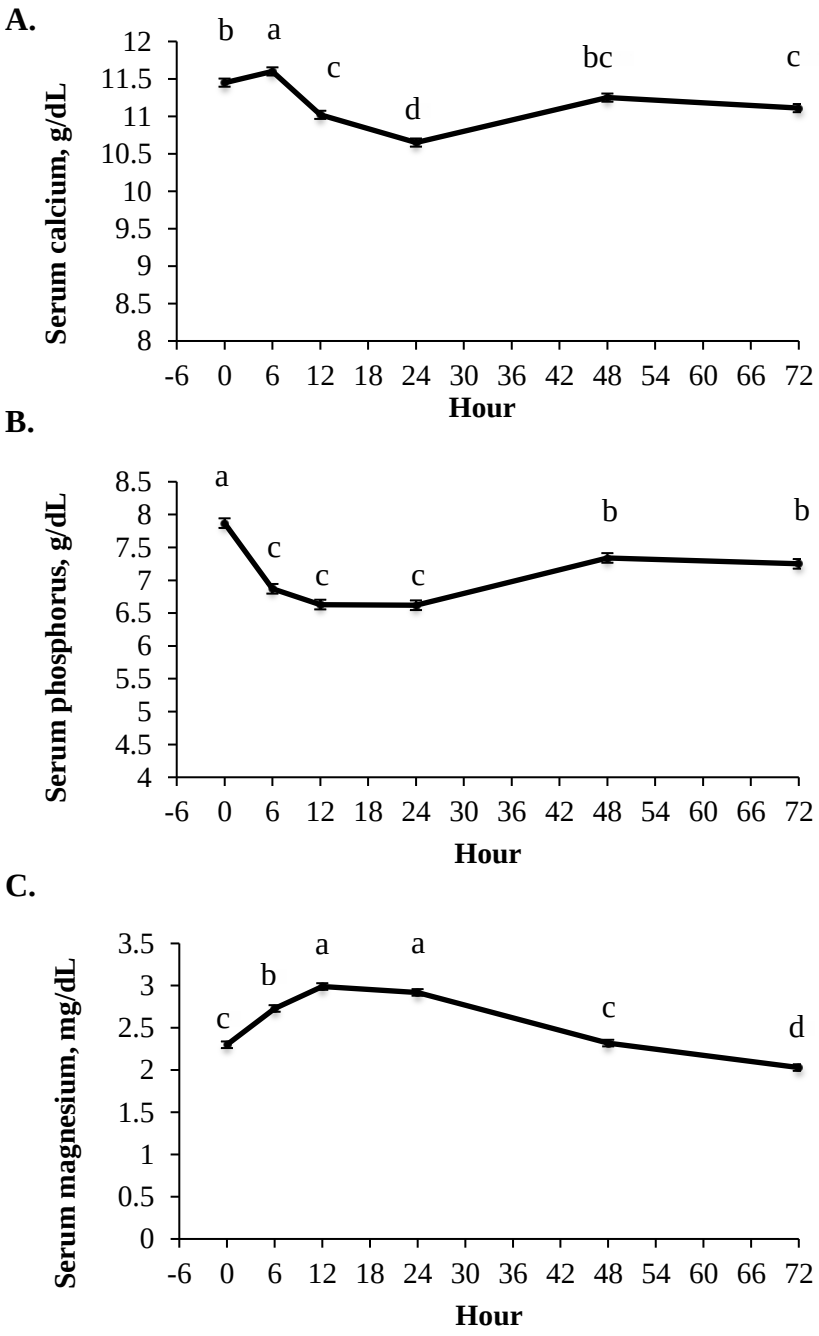


Figure 3.5. Effect of hour on circulating other mineral concentrations beef calves from 0 through 72 h postnatally.

Main effect of hour was significant ($P < 0.001$) for serum (A) calcium, (B) phosphorus, and (C) magnesium concentrations in beef calves 0 through 72 h postnatally. Least squares means ± SEM are presented.

[a,b,c,d]items lacking a common superscript differ by $P < 0.10$.

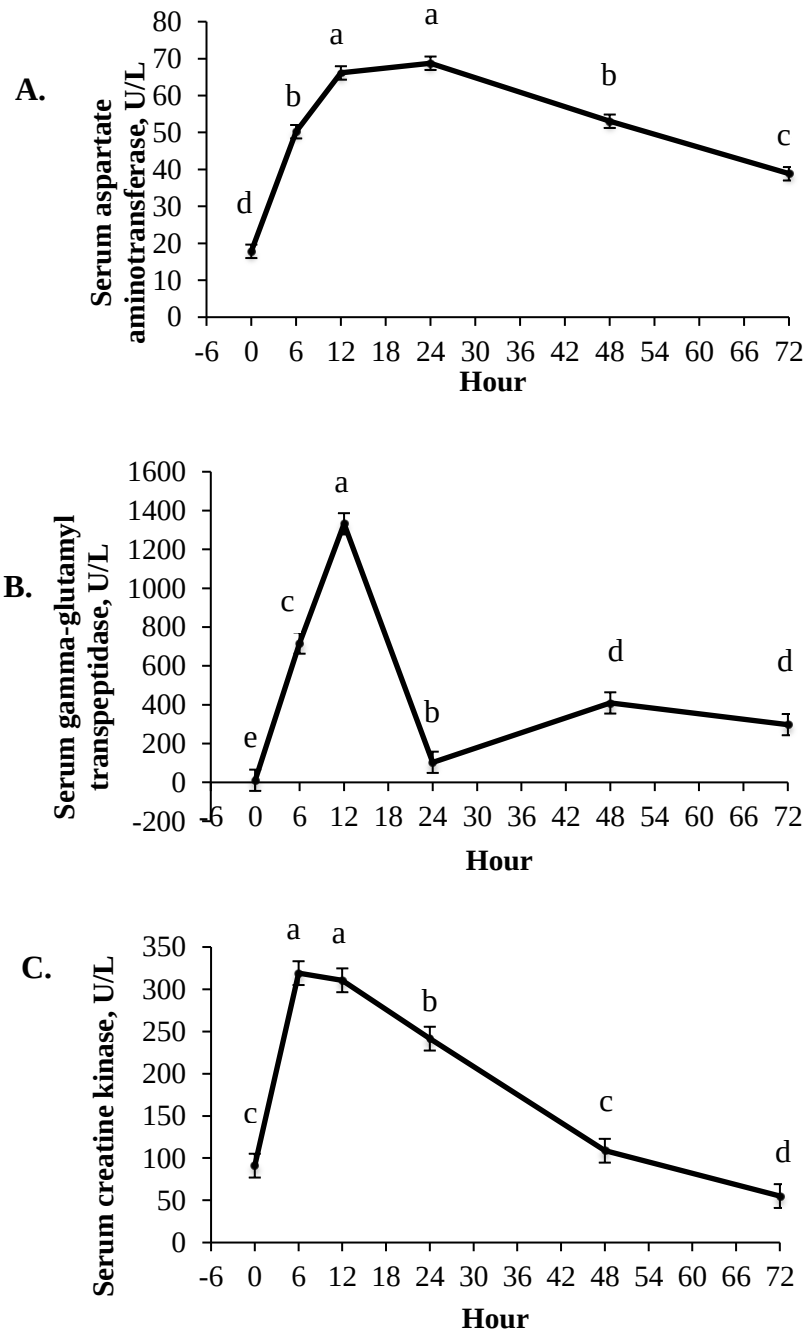


Figure 3.6. Effect of hour on circulating enzyme concentrations in beef calves from 0 through 72 h postnatally.

Main effect of hour was significant ($P < 0.001$) for serum (A) aspartate aminotransferase, (B) gamma-glutamyl transpeptidase, and (C) creatine kinase concentrations in beef calves 0 through 72 h postnatally. Least squares means ± SEM are presented.

[a,b,c,d,e]items lacking a common superscript differ by $P < 0.05$.

Table 3.2. Partial correlation of coefficients of postnatal calf sampling time for serum blood urea nitrogen, plasma glucose, serum total protein, and serum globulin concentrations between sampling hours

Items	0 h	6 h	12 h	24 h	48 h	72 h
Serum BUN, mg/dL						
0 h	--	0.63**	0.30	0.09	0.04	-0.08
6 h	0.63**	--	0.88**	0.57**	0.23	0.11
12 h	0.30	0.88**	--	0.83**	0.52**	0.45*
24 h	0.09	0.57**	0.83**	--	0.75**	0.57**
48 h	0.04	0.23	0.52**	0.75**	--	0.84**
72 h	-0.08	0.11	0.45*	0.57**	0.84**	--
Plasma glucose, mg/dL						
0 h	--	0.69**	-0.07	0.02	0.39*	0.11
6 h	0.69**	--	0.45*	0.43*	0.38*	-0.29
12 h	-0.07	0.45*	--	0.59**	0.04	-0.47*
24 h	0.02	0.43*	0.59**	--	0.34	0.15
48 h	0.39*	0.38*	0.04	0.34	--	0.24
72 h	0.11	-0.29	-0.47*	0.15	0.24	--
Serum total protein, g/dL						
0 h	--	0.19	0.15	0.18	0.03	-0.10
6 h	0.19	--	0.74**	0.45**	0.57**	0.38*
12 h	0.15	0.74**	--	0.84**	0.90**	0.40
24 h	0.18	0.45**	0.84**	--	0.97**	0.10
48 h	0.03	0.57**	0.90**	0.97**	--	0.14
72 h	-0.10	0.38*	0.40	0.10	0.14	--
Serum globulin, g/dL						
0 h	--	-0.12	-0.23	0.18	0.01	0.16
6 h	-0.12	--	0.64**	0.44**	0.52**	0.21
12 h	-0.23	0.64**	--	0.85**	0.90**	0.40
24 h	0.18	0.44**	0.85**	--	0.98**	0.10
48 h	0.01	0.52**	0.90**	0.98**	--	0.71**
72 h	0.16	0.21	0.40	0.10	0.71**	--

**$P \leq 0.05$

*$P \leq 0.10$

Table 3.3 Partial correlation of coefficients of calf time to stand with circulating metabolites in beef calves 0 through 72 h postnatally

	Sampling hour					
Items	0	6	12	24	48	72
Glucose, mg/dL						
r	-0.10	-0.04	0.40	0.33	0.34	0.39*
P-value	0.68	0.88	0.12	0.15	0.13	0.09
BUN, mg/dL						
r	0.19	0.69**	0.70**	0.56**	0.43**	0.18
P-value	0.42	0.001	0.002	0.01	0.05	0.46
NEFA, mEq/L						
r	-0.06	0.04	-0.17	-0.48**	0.03	-0.42*
P-value	0.80	0.86	0.53	0.04	0.89	0.07
Albumin, g/dL						
r	-0.42*	-0.53**	-0.67**	-0.59**	-0.59**	-0.61**
P-value	0.06	0.02	0.004	0.01	0.005	0.004
Total Protein, g/dL						
r	-0.13	-0.20	-0.03	0.19	0.13	0.08
P-value	0.58	0.41	0.92	0.42	0.58	0.75
Globulin, g/dL						
r	0.23	0.03	0.14	0.27	0.22	0.20
P-value	0.31	0.90	0.60	0.25	0.34	0.41
Fructose, µM						
r	-0.23	--	--	--	--	--
P-value	0.32	--	--	--	--	--

**$P \leq 0.05$

*$P \leq 0.10$

Table 3.4. Partial correlation of coefficients of calf time to stand and calf serum chemistry profile measured at 0 h postnatally[1]

	Time to Stand	
Variable	r	*P*-value
Creatinine, mg/dL	-0.18	0.44
Sodium, mEq/L	-0.06	0.80
Potassium, mEq/L	0.15	0.53
Chloride, mEq/L	-0.12	0.60
Anion gap, mEq/L	0.55	0.01**
Albumin, g/dL	-0.42	0.06*
Total protein, g/dL	-0.13	0.58
Globulin, g/dL	0.23	0.31
Calcium, g/dL	-0.32	0.15
Phosphorus, g/dL	0.48	0.03**
Magnesium, mg/dL	-0.08	0.74
Total bilirubin, mg/dL	0.03	0.91
Direct bilirubin, mg/dL	-0.17	0.47
Aspartate aminotransferase, U/L	0.41	0.06*
Gamma-glutamyl transpeptidase, U/L	-0.01	0.95
Creatine kinase, U/L	0.47	0.03**

[1]0 h samples collected after standing but prior to suckling
**$P \leq 0.05$
*$P \leq 0.10$

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Appendix Table 1. Partial correlation of coefficients of birth weight with serum chemistry profile in beef calves 0 through 72 h postnatally

	Sampling hour					
Items	0	6	12	24	48	72
Glucose, mg/dL						
r	-0.21	-0.10	0.21	0.19	0.16	0.03
***P*-value**	0.33	0.67	0.42	0.40	0.46	0.90
BUN, mg/dL						
r	0.29	0.55**	0.51**	0.36*	0.40**	0.23
***P*-value**	0.17	0.01	0.04	0.10	0.05	0.31
NEFA, mEq/L						
r	-0.13	0.25	-0.07	-0.21	0.08	-0.16
***P*-value**	0.56	0.30	0.78	0.35	0.72	0.50
Creatinine, mg/dL						
r	-0.04	-0.13	-0.26	-0.04	0.01	-0.08
***P*-value**	0.84	0.56	0.31	0.86	0.95	0.73
Sodium, mEq/L						
r	-0.008	0.03	-0.26	-0.02	0.42**	-0.01
***P*-value**	0.97	0.88	0.32	0.92	0.03	0.96
Potassium, mEq/L						
r	-0.27	-0.45**	-0.28	-0.05	-0.26	-0.007
***P*-value**	0.20	0.04	0.28	0.81	0.20	0.97
Chloride, mEq/L						
r	-0.10	0.03	-0.13	-0.14	0.32	0.15
***P*-value**	0.66	0.90	0.63	0.53	0.11	0.51
Bicarbonate, mEq/L						
r	-0.15	0.07	-0.08	-0.23	-0.01	-0.42**
***P*-value**	0.48	0.75	0.75	0.29	0.97	0.05
Anion Gap, mEq/L						
r	0.18	-0.18	-0.24	0.35	0.23	0.40*
***P*-value**	0.40	0.42	0.35	0.11	0.27	0.06
Albumin, g/dL						
r	0.01	-0.16	-0.55**	-0.38*	-0.07	-0.28
***P*-value**	0.96	0.50	0.02	0.08	0.73	0.20
Total Protein, g/dL						
r	0.01	0.22	0.40	0.55**	0.33*	0.52**
***P*-value**	0.99	0.34	0.11	0.01	0.10	0.01
Globulin, g/dL						
r	0.02	0.29	0.52**	0.56**	0.32	0.53**
***P*-value**	0.94	0.20	0.03	0.01	0.11	0.01

Calcium, mg/dL						
r	-0.02	-0.01	-0.24	0.17	-0.05	0.29
***P*-value**	0.92	0.95	0.35	0.46	0.81	0.19
Phosphorus, mg/dL						
r	0.40**	0.01	-0.55**	-0.03	-0.12	0.12
***P*-value**	0.05	0.98	0.02	0.89	0.57	0.59
Magnesium, mg/dL						
r	0.17	-0.02	0.30	0.37*	0.37*	0.27
***P*-value**	0.44	0.93	0.23	0.09	0.06	0.23
Total Bilirubin, mg/dL						
r	-0.31	-0.24	-0.25	-0.12	0.11	-0.14
***P*-value**	0.13	0.30	0.33	0.59	0.58	0.53
Direct Bilirubin, mg/dL						
r	-0.17	-0.53**	-0.35	-0.18	0.08	-0.04
***P*-value**	0.42	0.01	0.17	0.43	0.67	0.86
AST, U/L						
r	0.06	0.25	0.02	0.02	0.23	-0.36*
***P*-value**	0.79	0.28	0.94	0.93	0.26	0.10
GGT, U/L						
r	-0.09	-0.03	0.29	0.34	-0.01	0.31
***P*-value**	0.69	0.89	0.27	0.12	0.99	0.16
CK, U/L						
r	0.31	0.13	0.08	0.13	0.23	-0.23
***P*-value**	0.14	0.59	0.76	0.56	0.27	0.31

**$P \leq 0.05$

*$P \leq 0.10$

Appendix Table 2. Partial correlation of coefficients of calf time to stand with serum chemistry profile in beef calves 0 through 72 h postnatally

	Sampling hour					
Items	0	6	12	24	48	72
Glucose, mg/dL						
r	-0.10	-0.04	0.40	0.33	0.34	0.39*
***P*-value**	0.68	0.88	0.12	0.15	0.13	0.09
BUN, mg/dL						
r	0.19	0.69**	0.70**	0.56**	0.43**	0.18
***P*-value**	0.42	0.001	0.002	0.01	0.05	0.46
NEFA, mEq/L						
r	-0.06	0.04	-0.17	-0.48**	0.03	-0.42*
***P*-value**	0.80	0.86	0.53	0.04	0.89	0.07
Creatinine, mg/dL						
r	-0.18	-0.005	0.19	0.25	0.16	-0.07
***P*-value**	0.44	0.98	0.49	0.29	0.49	0.77
Sodium, mEq/L						
r	-0.06	-0.28	-0.53**	-0.37	0.02	-0.17
***P*-value**	0.80	0.25	0.03	0.11	0.95	0.47
Potassium, mEq/L						
r	0.15	-0.03	-0.09	0.04	-0.05	0.09
***P*-value**	0.53	0.89	0.74	0.88	0.84	0.70
Chloride, mEq/L						
r	-0.12	-0.17	-0.35	-0.41*	0.20	0.04
***P*-value**	0.60	0.49	0.18	0.08	0.38	0.87
Bicarbonate, mEq/L						
r	-0.53**	-0.09	-0.20	0.08	-0.001	-0.06
***P*-value**	0.01	0.71	0.47	0.73	0.99	0.81
Anion Gap, mEq/L						
r	0.55**	-0.05	-0.22	-0.27	-0.33	-0.20
***P*-value**	0.009	0.84	0.41	0.24	0.14	0.39
Albumin, g/dL						
r	-0.42*	-0.53**	-0.67**	-0.59**	-0.59**	-0.61**
***P*-value**	0.06	0.02	0.004	0.01	0.005	0.004
Total Protein, g/dL						
r	-0.13	-0.20	-0.03	0.19	0.13	0.08
***P*-value**	0.58	0.41	0.92	0.42	0.58	0.75
Globulin, g/dL						
r	0.23	0.03	0.14	0.27	0.22	0.20
***P*-value**	0.31	0.90	0.60	0.25	0.34	0.41

Calcium, mg/dL						
r	-0.32	-0.43	-0.31	-0.38*	-0.55**	-0.34
***P*-value**	0.15	0.07	0.23	0.10	0.01	0.14
Phosphorus, mg/dL						
r	0.48**	-0.26	-0.39	-0.31	-0.48**	0.10
***P*-value**	0.03	0.28	0.14	0.18	0.03	0.68
Magnesium, mg/dL						
r	-0.08	-0.17	0.23	0.23	0.33	0.10
***P*-value**	0.74	0.48	0.39	0.33	0.15	0.67
Total Bilirubin, mg/dL						
r	0.03	-0.21	-0.39	-0.28	-0.21	-0.25
***P*-value**	0.91	0.40	0.14	0.24	0.36	0.28
Direct Bilirubin, mg/dL						
r	-0.17	-0.60	-0.48	-0.51	-0.24	-0.09
***P*-value**	0.47	0.007	0.06	0.02	0.30	0.69
AST, U/L						
r	0.41	0.34	0.20	0.20	0.13	0.02
***P*-value**	0.06	0.16	0.46	0.39	0.56	0.92
GGT, U/L						
r	-0.01	0.007	0.22	0.33	0.19	0.26
***P*-value**	0.95	0.98	0.40	0.15	0.42	0.27
CK, U/L						
r	0.47	0.27	0.39	0.23	0.18	0.006
***P*-value**	0.03	0.27	0.14	0.33	0.43	0.98

**$P \leq 0.05$

*$P \leq 0.10$