HEPATIC ENERGY METABOLISM IN EARLY LACTATION DAIRY COWS

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In the period immediately following calving, feed intake is insufficient to support the high milk production of early lactation, resulting in a state of negative energy balance. This state of negative energy balance results in many metabolic adaptations such as the increased mobilization of adipose tissue, manifested as the release of non-esterified fatty acids into circulation to be metabolized by the liver and other tissues and incorporated into milk fat in the mammary gland. Propionate that is produced via fermentation of starch in the rumen is the main precursor for hepatic glucose production, and higher feed and energy intake postpartum generally results in lower circulating non-esterified fatty acids and has been associated with improved health, performance, and less severe postpartum negative energy balance. The objectives herein were to: 1) evaluate the effects of different energetic nutritional strategies on postpartum production and metabolism; 2) evaluate the impact of these nutritional strategies on liver metabolism; 3) evaluate temporal changes in liver metabolism through the transition to lactation and the relationships between hepatic energy substrates; and 4) evaluate the association between the degree of early lactation inflammation with production and metabolism. Cows that were fed diets with greater propiogenic capacity during the postpartum period had improvements in production and metabolism, and increased in vitro conversion of propionate to glucose in the

liver. Overall, alterations in fatty acid metabolism that lead to increased triglyceride accumulation during the transition period appear to impair postpartum hepatic gluconeogenesis, and cows that had elevated inflammation in the first week postpartum exhibited a diverse range of production responses, indicating that there is a large degree of variation in individual adaptation.

BIOGRAPHICAL SKETCH

Maris McCarthy was born and raised in Arroyo Grande, California. She earned her Bachelor's degree in Dairy Science with minors in Agricultural Business and Spanish from Cal Poly San Luis Obispo in 2009. Upon leaving the Central Coast, she moved to Delaware to work on her Master's degree at the University of Delaware with Dr. Tanya Gressley, where she studied the effect of by-product feeds on apparent total tract digestibility and production performance in lactating dairy cows. After completing of her Master's in 2011, Maris began work on her PhD with Dr. Tom Overton in the Animal Science department at Cornell working in the area of fresh cow nutrition and energy metabolism. Maris is defending her thesis in May 2015 and will be starting a position as a Technical Consultant with Elanco Animal Health in California at the end of July 2015.

Dedicated to my father, Kevin James McCarthy, who is the hardest working person I've ever met and who continues to inspire me more than he knows.

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CHAPTER 1

INTRODUCTION

Many studies have evaluated the effect of prepartum nutrition on postpartum lactation performance and its associated metabolic changes, although surprisingly there have been relatively few studies that have evaluated the effects of postpartum nutrition on these metabolic adaptations and their effects on production performance.

After parturition the nutrients required for milk synthesis utilize a large portion of maternal nutrients (Ingvartsen and Andersen, 2000), and this adaptation to lactation also requires homeorhetic changes of the immune system as well. In the period immediately following calving, dry matter intake (**DMI**) is insufficient to support the high milk production of early lactation and results in a state of negative energy balance (**EB**) that usually begins a few days before calving and reaches the greatest deficit about 2 wk after parturition. This state of negative EB results in the increased mobilization of adipose tissue, manifested as the release of non-esterified fatty acids (**NEFA**) into circulation to be metabolized by the liver and other tissues and incorporated into milk fat in the mammary gland. Higher DMI postpartum generally results in lower circulating NEFA and has been associated with improved health, performance, and less severe postpartum negative EB (Ingvartsen and Andersen, 2000).

Optimizing DMI during this postpartum period is especially important to provide sufficient energy to support milk production. Due to the increased glucose demand for milk lactose synthesis, liver glucose production nearly doubles within 11 days of calving as compared to prepartum glucose output (Reynolds et al., 2003).

Propionate that is produced via fermentation of starch in the rumen is the main precursor for hepatic glucose production (Drackley et al., 2001). Although there is a large increase in hepatic utilization of amino acids, lactate, and glycerol postpartum, propionate is still quantitatively the greatest contributor to liver gluconeogenesis at about 60% of precursor supply (Reynolds et al., 2003). Because of this increased demand for glucose, hepatocytes should have the capacity to direct additional propionate supply towards glucose synthesis during this early postpartum period (Drackley et al., 2001).

Because hepatic energy requirements increase dramatically at the onset of lactation (Reynolds et al., 2003) and adipose mobilization is increased (Vernon, 2005), NEFA are most likely the predominant oxidative fuel for the liver (Reynolds et al., 2003). During states of negative EB, the hormone fibroblast growth factor 21 has been shown to stimulate oxidation of fatty acids, ketogenesis, and gluconeogenesis in the liver of mice (Badman et al., 2007) and may be important in the metabolic adaptations of early lactation dairy cows as well (Schlegel et al., 2012; Schoenberg et al., 2011b). Recent work with early lactation animals has shown propionate infusion to be more hypophagic in animals with higher liver acetyl CoA concentrations, which is indicative of higher NEFA mobilization (Stocks and Allen, 2012; 2013). As such there appear to be many complex interactions in the utilization and regulation of energy substrates postpartum.

The objective of this literature review is to highlight the current knowledge of nutritional influences on energy metabolism in early lactation. Specific attention will be dedicated to describing the regulation of gluconeogenic mechanisms and their impact on feed intake regulation in the transition dairy cow.

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CHAPTER 2

LITERATURE REVIEW

HEPATIC ENERGY METABOLISM AND STRATEGIES TO OPTIMIZE PRODUCTIVE PERFORMANCE OF THE TRANSITION COW

METABOLIC CHANGES DURING THE TRANSITON PERIOD

The transition period is generally defined as the three weeks preceding and following parturition (Drackley, 1999), and there are many shifts in metabolism and regulatory changes that occur during this period (Bauman and Currie, 1980). In the periparturient period fetal growth does not place a large demand on maternal nutrient supply until the third trimester of gestation, upon which there is a large increase in fetal mass along with the concurrent increase in mammary cell proliferation in preparation for colostrum and milk synthesis (Bauman and Currie, 1980). In dairy cattle, the occurrence of metabolic disorders is most frequent after calving (LeBlanc et al., 2006) when the metabolic demand of lactation nearly doubles energy requirements during the immediate postpartum period (Drackley et al., 2001).

Negative energy balance of early lactation

In the period immediately following calving, DMI is insufficient to support the high milk production of early lactation and results in a state of negative EB that usually begins a few days before calving and reaches the greatest deficit about 2 wk after parturition (Ingvartsen and Andersen, 2000). This state of negative EB results in the increased mobilization of adipose tissue and NEFA to meet the energetic needs of the cow. Higher DMI postpartum results in lower circulating NEFA and has been associated with improved health, performance, and less severe postpartum negative EB (Ingvartsen and Andersen, 2000).

About three quarters of all disease in dairy cows occurs within the first month after calving (LeBlanc et al., 2006), and cows that experience a greater negative EB postpartum are at greater risk for developing metabolic disorders (Ospina et al., 2010).

When metabolites that are associated with negative EB, such as β-hydroxybutyrate (**BHBA**) and NEFA, are above certain pre- and postpartum thresholds they are associated negative downstream outcomes such as increased risk of disease, as well as decreased milk yield and reproductive performance (Ospina et al., 2013). Elevated BHBA and NEFA have also been shown to have negative effects on immune function (Overton and Waldron, 2004). Hoeben (1997) showed that BHBA added in vitro had inhibitory effects on blood neutrophil activity. The concentration of NEFA in the blood may also affect immune function, as in vitro NEFA addition decreased immune cell activity (Ster et al., 2012). Hammon et al. (2006) also reported a negative correlation between neutrophil killing activity and NEFA concentrations during the first week after calving. The effects of negative EB may be partly responsible for the higher susceptibility to local and systemic infections that are observed during the postpartum period.

The state of negative EB experienced during the periparturient period may also increase immunosuppression because an immune disturbance can increase the maintenance energy requirement that is needed to help counteract disease. In human patients that suffered from severe sepsis or major trauma, it was reported that resting energy expenditure increased to 40% above normal and continued to be elevated after 3 weeks from onset of illness (Plank and Hill, 2000). The energetic cost of immune activation has not yet been well studied in cows and further investigation is warranted.

The severity of negative EB during early lactation is highly correlated with time to first ovulation, and there is an increase in the number of days to ovulation in cows that have greater early lactation negative EB (Butler, 2000; Grummer et al., 2010).

Ovulation of the first dominant follicle generally occurs between days 16 to 20 postpartum. However, if the first follicle is not ovulated and a new follicular wave is initiated, or if the follicle becomes cystic, the cow won't be able to ovulate again until between 40 to 50 d postpartum, greatly increasing the length of time to pregnancy (Butler, 2000). The risk of being anovular was greatly increased in cows that had a BCS of less than 2.75 at d 70 postpartum, and the delay in ovulation was associated with greater negative EB from decreased DMI in early lactation (Santos et al., 2009). Cows that had less BCS loss from calving to d 70 postpartum (indicating better DMI and subsequent EB) also had better conception rates compared to animals with greater BCS loss (Santos et al., 2009). While negative EB is a normal part of the transition to lactation, excessive negative EB can have many negative downstream effects on production, disease incidence, and reproduction.

Changes in glucose needs and utilization in the transition to lactation

The metabolic demand of lactation nearly doubles energy requirements during the immediate postpartum period and creates a state of negative EB (Drackley et al., 2001). One of the main increases in this metabolic demand of lactation is the need for increased hepatic glucose output to support lactose synthesis in the mammary gland (Bell and Bauman, 1997). At 4 d postpartum the glucose utilization of the mammary gland is estimated to be 2 times greater than the glucose utilization of the gravid uterus during late gestation (Bell, 1995). The mammary gland requires 72 g of glucose to produce 1 kg of milk (Kronfeld, 1982). If a cow were to produce 15 kg of milk 2 days postpartum, the mammary gland would need to take up over 1 kg/d of glucose to meet this demand in comparison to the estimated 670 g/d of glucose needed by the late term

gravid uterus (Bell, 1995). This rapid increase in glucose demand for milk synthesis requires a very careful orchestration of the metabolic utilization of nutrients for other tissues (Bauman and Currie, 1980). During early lactation there is a decrease in insulin sensitivity and responsiveness in the peripheral tissues (Kahn, 1978). Adiponectin, the insulin sensitizing hormone produced by white adipose tissue, is decreased during this period and may play a role in the hormonal adaptation of early lactation that promotes insulin resistance (Giesy et al., 2012). This insulin resistance results in an increase in liver gluconeogenesis as well as a decrease in glucose utilization in the peripheral tissues, which thereby spares glucose for milk lactose synthesis (Bell and Bauman, 1997). To accommodate this increase in glucose demand and utilization, the liver in turn increases its metabolic activity (Reynolds et al., 2003; 2004). The liver can utilize propionate, amino acids, lactate, and glycerol for gluconeogenesis. Propionate that is produced from ruminal fermentation is quantitatively the greatest contributor to gluconeogenesis. However, postpartum there is a large increase in the liver's utilization of the other gluconeogenic precursors to help meet the animal's early lactation glucose needs (Reynolds et al., 2003).

Changes in protein metabolism in transition to lactation

During early lactation visceral tissue growth is likely constrained temporarily and may provide a source of amino acids that can be catabolized for gluconeogenesis and milk protein synthesis (Bell et al., 2000). However, the majority of the catabolized amino acids come from muscle protein degradation and the redistribution of these tissue proteins into tissues that support lactation (Andrew et al., 1994). This decrease in muscle protein synthesis and increased release of amino acids are needed to meet the mammary

amino acid and liver gluconeogenesis requirements (Bell and Bauman, 1997; Bell et al., 2000). Amino acids can contribute up to 30 percent of the liver gluconeogenic substrate during early lactation and are important in meeting the postpartum glucose needs of the cow (Reynolds et al., 2003). A study by Komaragiri et al. (1997) demonstrated that by week 5 postpartum cows had mobilized about 21 kg of protein. They saw no effect of increasing rumen undegradable protein on body tissue mobilization, which suggests that tissue mobilization is not limited by dietary protein and not ameliorated by increasing protein supply (Komaragiri and Erdman, 1997). A similar study by the same research group demonstrated similar protein mobilizations regardless of a dietary fat treatment (Komaragiri et al., 1998). These data suggest that large muscle tissue mobilization is obligatory as part of the normal transition to lactation, however, excessive insulin resistance during early lactation may increase tissue protein mobilization (Bell et al., 2000). Cows that are overweight at parturition have much greater NEFA mobilization and experience greater insulin resistance in early lactation (Dann et al., 2006; Douglas et al., 2006). A decrease in insulin signaling will increase signaling that inhibits muscle synthesis and lead to an increase in muscle degradation (Glass, 2010), although early lactation muscle protein mobilization still has not been very well studied in dairy cows.

Changes in fatty acid metabolism in the transition to lactation

The synthesis of milk fat postpartum is one of the major energy strains on the cow's metabolism, and at 4 d postpartum the fatty acid utilization of the mammary gland is estimated to be about 4 times greater than the fatty acid utilization of the gravid uterus in late gestation (Bell, 1995). This energy strain that milk fat synthesis places on the cow has led some research groups to try to intentionally decrease milk fat content by

feeding conjugated linoleic acid (**CLA**) to reduce the energy requirements of the cow and alleviate some of the negative EB during early lactation (Bernal-Santos et al., 2003; von Soosten et al., 2012). However, CLA treatment was unable to decrease milk fat until after wk 3 of lactation, suggesting that the mobilization and use of NEFA, and the synthesis of milk fat by the mammary gland is obligatory during early lactation, although the mechanism for this still remains unknown (Bernal-Santos et al., 2003).

The state of insulin resistance in the peripheral tissues that is experienced postpartum helps to spare glucose for mammary gland lactose synthesis. Because of this glucose sparing mechanism, there is a decrease in adipose tissue lipogenesis and an increase in lipolysis of the adipose tissue (Bell and Bauman, 1997). During the periparturient period this increase in lipolysis increases the circulating pool of NEFA in the blood stream, and NEFA uptake by the liver increases proportionally as well (Reynolds et al., 2003). When lipid mobilization is extreme, uptake of NEFA by the liver can exceed the rates of oxidation and export as very-low density lipoproteins, leading to liver triglyceride accumulation and increased risk for fatty liver and other disorders such as ketosis, retained placenta, and mastitis (Goff and Horst, 1997; Drackley, 1999). Excess liver triglyceride accumulation has been shown to decrease the ability of the hepatocyte to synthesize urea (Strang et al., 1998), and the consequent increase in liver ammonia may decrease glucose synthesis from propionate (Overton et al., 1999). Therefore, excess hepatic triglyceride accumulation in transition cows has the ability to negatively impact gluconeogenesis.

Changes in splanchnic metabolism during the transition period

During the periparturient period the tissues of the splanchnic bed (the

gastrointestinal tract, pancreas, spleen, and liver) undergo many metabolic adaptations to accommodate the increase in nutrient flow in early lactation. Splanchnic metabolism can be measured by the catheterization of the arterial blood and the venous blood that drains the liver (hepatic portal vein and vena cava), and blood flow is calculated utilizing the Fick principle. For this catheterization technique, the net release of a nutrient across the splanchnic tissues represents the availability of that nutrient for other body tissues, such as the mammary gland, while the net removal of a nutrient represents the nutrient utilized by the liver, such as propionate that is used for glucose synthesis in the liver (Drackley et al., 2006). Another method of measuring gross nutrient removal by the liver is to utilize isotopic labeling of a specific nutrient, such as propionate to trace the metabolism of that nutrient (e.g. incorporation of the isotopic label into glucose). Although, one shortcoming of this method is that it does not account for nutrient use during absorption (Reynolds, 2002).

Postpartum there is a large increase in blood flow and nutrient supply that is available to the liver (Reynolds et al., 2003). In the immediate postpartum period there is very little difference in the weight of the liver compared to prepartum, although liver weight does increase by d 22 of lactation in response to increased DMI (Reynolds et al., 2004). However, immediately postpartum liver oxygen uptake nearly doubles from prepartum oxygen uptake which suggests that there is a large increase in liver metabolic activity during this postpartum period (Reynolds et al., 2003; 2004), especially considering the negligible increase in liver mass. These changes in activity will be discussed in detail in the following section.

LIVER FUNCTION AND ACTIVITY IN TRANSTION COWS

Gluconeogenesis

In the early postpartum period renal gluconeogenesis can account for up to 15 percent of glucose output; however, because hepatic gluconeogenesis is quantitatively the most important source of glucose synthesis, it will be the focus of the remainder of this discussion. At 11 d postpartum, liver glucose output increases 310 mmol/h compared to 9 d prepartum (Reynolds et al., 2003). The flux of nutrients and gluconeogenic substrate that are delivered to the liver depend on the liver mass, the rate of blood flow, rate of transfer of substrate across the cell membrane, as well as the intracellular rates of metabolism (Drackley et al., 2001). Changes in these variables in early lactation increase the flux of gluconeogenic substrates that are available to the liver. The changes in liver mass immediately after parturition are very modest and only account for a small part of the increase in glucose output (Reynolds et al., 2004). In early lactation blood flow through the liver is greatly increased and can reach rates of nearly 3000 L/h (Drackley et al., 2006), increasing the rate of nutrient delivery to the liver. It is estimated that by d 21 postpartum glucose demand increases 1500 g/d from prepartum needs (Drackley et al., 2001). Because relatively little glucose is absorbed from the small intestines of ruminants, the majority of the increase in glucose needs are met by gluconeogenesis in the liver.

Gluconeogenesis is regulated mainly by changes in activity of the enzymes that catalyze key steps in the gluconeogenic pathway, including glucose 6-phosphatase, fructose 1,6-bisphospatase, pyruvate carboxylase (**PC**), and phosphoenolpyruvate carboxykinase (**PEPCK**). Glucose 6-phosphatase (**G6Pase**) is responsible for the last

step in the production of glucose by gluconeogenesis, and is required for the transport of glucose from the liver cell to other tissues. G6Pase activity occurs in the endoplasmic reticulum and the rate of this reaction depends primarily upon the concentration of substrate. When citric acid cycle intermediates and oxygen are sufficient, the ATP levels in the cell are high and AMP levels are low, leading to high fructose 1,6-bisphosphatase activity. The elevated activity of fructose 1,6-bisphosphatase ensures sufficient substrate for G6Pase. When there is a buildup of acetyl-CoA in the mitochondria, PC activity is increased, as the increase in acetyl-CoA buildup signals an increased need for oxaloacetate. However, PEPCK is the rate-determining step for gluconeogenesis. The main precursors that are used for hepatic gluconeogenesis are propionate, amino acids, lactate, and glycerol, and their utilization will be discussed further in the following sections.

Propionate. Propionate is quantitatively the most important gluconeogenic precursor contributing about 60 to 74 percent of gluconeogenic precursor (Drackley et al., 2001), and is derived mainly from dietary starch fermentation in the rumen. The ruminal fermentation of starch will be discussed in more detail in a later section, but briefly propionate is a 3 carbon volatile fatty acid (**VFA**) that is produced from the breakdown of starch and simple carbohydrates by amylolytic bacteria in the rumen. Around 40 to 60 percent of the ruminal propionate that is produced is absorbed into the portal blood (Bergman et al., 1966), while the remainder is either metabolized or converted to other metabolites prior to absorption. About 90 percent of the propionate in the portal blood stream is removed from circulation and metabolized by the liver, so there is very little propionate circulating in the arterial blood. In the liver, propionate

can be either oxidized to supply energy for the liver tissue or utilized as a substrate for gluconeogenesis.

The ability of the liver to convert propionate to glucose is responsive to propionate supply, and there is a positive linear relationship between increased propionate supply and liver glucose output (Dijkstra et al., 2005). Propionate is metabolized by mitochondrial propionyl-CoA carboxylase and methylmalonyl-CoA mutase. Methylmalonyl-CoA mutase is a key enzymatic control point for gluconeogenesis from propionate, and vitamin B12 is a necessary coenzyme for this reaction, as supplemental vitamin B12 has been shown to enhance gluconeogenesis from propionate in ovine liver slices (Peters and Elliot, 1983).

Although propionate metabolism is not directly dependent on pyruvate carboxylase, the activity of PC is critical in providing a pool of oxaloacetate for gluconeogenesis and TCA cycle activity (Aiello and Armentano, 1987). After the propionate carbon enters the TCA cycle at succinyl-CoA, it is eventually converted to oxaloacetate and can then be metabolized by PEPCK to phosphoenolpyruvate and then glucose (Aschenbach et al., 2010). PEPCK is a key regulatory enzyme in the gluconeogenic pathway (Croniger et al., 2002), and the PEPCK gene promoter has been shown to be positively regulated by propionate (Hazelton et al., 2008). There are two isoforms of PEPCK in animals, one that resides in the mitochondria (**PEPCK-M**) and one in the cytosol (**PEPCK-C**). The mRNA expression of the cytosolic form is most often reported in the literature because its levels are regulated by hormone and diet, whereas the mitochondrial form does not appear to be controlled by these factors (Croniger et al., 2002). However, the presence of both mitochondrial and cytosolic

forms of the PEPCK enzyme in bovine liver may provide increased flexibility of substrate metabolism for gluconeogenesis (White et al., 2012). It has also been proposed that PEPCK-C is required for gluconeogenesis from amino acids, whereas PEPCK-M is more needed for gluconeogenesis from lactate (Aschenbach et al., 2010). Cytosolic PEPCK mRNA expression slowly increases postpartum, although cytosolic NADH may not limit gluconeogenesis from propionate. At least 60% of propionate flux to glucose is dependent on PEPCK-M formed in ruminant hepatocytes (Aiello and Armentano, 1987), so this slow increase in PEPCK-C expression it may not be as restricting to gluconeogenesis in ruminants as in nonruminants (Greenfield et al., 2000). Previous work indicated that PEPCK-M mRNA was not altered during the transition to lactation (Agca et al., 2002). Recently cell cultures of bovine kidney cells with a 1 mM fatty acid cocktail (similar to the fatty acid profile exhibited at calving) suggested that PEPCK-M activity is increased in response to calving, however, the combination and concentration of fatty acids required to evoke a PEPCK-M response in vitro may not be physiologically relevant in the cow (White et al., 2012).

In contrast to data from nonruminants, feed restriction does not increase the PEPCK-C mRNA abundance (Velez and Donkin, 2005) and postpartum increases in PEPCK-C expression are usually delayed until DMI increases (Karcher et al., 2007). Karcher et al. (2007) saw an increase in mRNA expression of PEPCK-C at d -14 and 1 relative to parturition in cows treated with monensin compared with control cow PEPCK-C expression, although there were no significant differences in DMI between treatments. These data would suggest that the increased propionate supply to the liver with monensin treatment increased PEPCK-C activity.

The ratio of PC to either PEPCK-C or PEPCK-M may provide insight into the potential precursor preference to support gluconeogenesis (White et al., 2012). If PC activity is unchanged and PEPCK-C activity is increased, glucose production from alanine will be enhanced. If PC activity is unchanged and PEPCK-M activity is increased, glucose production from lactate will be enhanced. If PC activity is increased but no change in activity of either PEPCK isoform occurs, the rate of glucose production is unchanged and the oxidative capacity of the TCA cycle will increase because of an increase in the oxaloacetate pool (White et al., 2012).

In experiments using labeled isotopes, the [1-^{14}C]propionate label randomizes in the TCA cycle such that every mole of [1-^{14}C]propionate that is directed toward oxaloacetate would yield 0.5 moles of radiolabeled CO_2 and 0.5 moles of radiolabeled glucose (Knapp et al., 1992). Therefore an increase in this ratio of labeled glucose to CO_2 would suggest an increase in the efficiency of utilization of propionate for gluconeogenesis. It appears that in the early lactation cow when there is an increase in propionate supply to the liver that there is an increased propensity to convert the propionate to glucose rather than oxidize it (Drackley et al., 2001). The rates of gluconeogenesis from [1-^{14}C]propionate in early lactation liver slices are increased compared to rates in liver slices from the same cows once they have reached mid lactation (Aiello et al., 1989). Drackley et al. (2001) saw a positive correlation between animal carbohydrate intake in the immediate postpartum period with the efficiency of [1-^{14}C]propionate conversion to glucose in liver biopsy slices.

Amino acids. All amino acids, except for the completely ketogenic leucine and lysine, are able to make a net contribution to hepatic glucose synthesis. In a state of

negative EB alanine and glutamine are released from the muscle tissues into the circulation and can be taken up by the liver and catabolized for gluconeogenesis. As such, alanine and glutamine are the largest amino acid contributors to liver glucose output, contributing about 40 to 60 percent of the gluconeogenic potential from amino acids (Bergman and Heitmann, 1978), and during early lactation amino acids contribute about of 30 percent of the gluconeogenic substrate (Reynolds et al., 2003). In liver slices at day 1 postpartum Overton et al. (1998) observed a nearly 200% increase in alanine conversion to glucose compared to conversion rates at 21 d prepartum, indicating that there likely is a large utilization of amino acids immediately postcalving to help support postpartum glucose needs.

Because the gluconeogenic pathway withdraws a molecule of oxaloacetate from the citric acid cycle without replenishing the oxaloacetate pool, if the oxaloacetate were not replenished the cycle would stop functioning. The catabolism of amino acids is very important to the citric acid cycle in that it produces intermediates of the cycle that can be converted to oxaloacetate and provide additional cycle intermediates without derailing the system. Alanine is catabolized to pyruvate and then enters the TCA cycle as oxaloacetate. Glutamine is catabolized to glutamate and ammonia and the glutamate then enters the TCA cycle as α-ketoglutarate. Pyruvate carboxylase is one of the rate limiting enzymes in gluconeogenesis from amino acids (alanine, cysteine, glycine, serine, and threonine) as well as lactate. The activity of PC is critical in providing a pool of oxaloacetate for gluconeogenesis and TCA cycle intermediates. In the immediate postpartum period Greenfield et al. (2000) observed an increase in the mRNA abundance of PC at 1 d postpartum compared with 28 d prepartum and 28 d postpartum.

This immediate increase in PC mRNA abundance postcalving reinforces the important gluconeogenic contribution of amino acid in the early postpartum period.

Lactate and glycerol. Lactate utilization for gluconeogenesis in the early lactation animal mostly represents a recycling of carbon that is formed during the partial oxidation of glucose by the peripheral tissues. In animals fed highly fermentable diets lactate from ruminal fermentation can be absorbed into the blood stream, however, in the transition cow there is relatively little ruminal lactate contribution to the gluconeogenic lactate pool.

When glucose is oxidized anaerobically in the skeletal muscle lactate is produced. Lactate dehydrogenase is the enzyme responsible for converting pyruvate to lactate. During early lactation there is increased expression of lactate dehydrogenase in the skeletal muscle, and down regulation of the aerobic mitochondrial citric acid cycle enzymes, which would allow glucose to be completely oxidized to CO_2. This indicates that during early lactation the increase in skeletal lactate production indirectly supports gluconeogenesis (Kuhla et al., 2011). The increased liver PC mRNA abundance that seen in the early postpartum period (Greenfield et al., 2000) increases conversion of lactate to pyruvate and subsequently glucose.

Glycerol is released from adipose tissue triacylglycerol when the glycerol backbone is cleaved from the fatty acids upon release into circulation in the blood stream. During early lactation when cows are mobilizing large amounts of adipose tissue, glycerol can make significant contributions to liver gluconeogenesis. Data from Reynolds et al. (2003) showed glycerol contributions of almost 10 percent of gluconeogenic precursor at 11 d postpartum. However, because glycerol contribution

depends on the amount of adipose tissue mobilized, the gluconeogenic contribution of glycerol is variable.

Hormonal regulation of gluconeogenesis

Gluconeogenesis is regulated mainly by the changes in the activity of the enzymes that catalyze key steps in the gluconeogenic pathway, however, hormonal regulation by insulin, glucagon, somatotropin, and cortisol controls these changes in the transition dairy cow (Drackley et al., 2001).

Insulin. Propionate is a potent insulin secretogague, likely because propionate production increases rapidly following meal consumption. In turn insulin decreases liver glucose release into the blood stream through its inhibitory effects on gluconeogenesis. Insulin action increases the proportion of propionate that is used as a substrate for gluconeogenesis (Brockman, 1990). Insulin stimulates protein and triglyceride anabolism and thus decreases the amount of circulating amino acids, lactate, and glycerol, which decreases their availability for gluconeogenesis. The decrease in availability of other gluconeogenic precursors increases the percentage of glucose that is derived from propionate. Therefore, in early lactation the low circulating insulin concentrations and peripheral tissue insulin resistance may help to attenuate the gluconeogenic contribution from other glucose precursors and increase liver glucose output. When insulin sensitizing agent thiazolidinedione (**TZD**) was administered to transition dairy cows, the TZD treated cows tended to have more negative glucose area under the curve during an insulin challenge than controls, suggesting that TZD treated cows had greater responses to insulin (Schoenberg et al., 2011a).

Glucagon. In cell cultures from preruminant calf hepatocytes, glucagon

stimulated conversion of propionate to glucose (Donkin and Armentano, 1995) and glucagon has also been demonstrated to increases the catabolism of muscle protein to support glucoses production (Brockman and Bergman, 1975). When exogenous glucagon was infused in dairy cows, plasma glucose was increased in a dose dependent manner (Hippen et al., 1999).

The insulin:glucagon ratio also decreases in the immediate postpartum period (Greenfield et al., 2000). This relationship of decreased insulin concentration and increased glucagon concentrations in early lactation could explain the concurrent increased mRNA abundance of PEPCK-C with progressing lactation (Greenfield et al., 2000) as insulin decreased mRNA and activity of PEPCK enzyme by regulating the expression of the gene (O'Brien et al., 1990).

Somatotropin. Knapp et al. (1992) observed increased rates of conversion of [1-^{14}C]propionate to glucose in liver slices from cows treated with somatotropin, although rates of conversion for [1-^{14}C]alanine, [4-^{14}C]aspartate and [U-^{14}C]glutamate to glucose were not affected by somatotropin treatment. These data suggest that the increased somatotropin that occurs around parturition (Bell, 1995) would also increase the rate of propionate conversion to glucose. Postpartum cows decrease whole body glucose oxidation to conserve glucose for milk lactose synthesis (Bennink et al., 1972) and this increase in glucose conservation is likely also driven by the increases in somatotropin at calving (Grum et al., 1996).

Cortisol. Glucocorticoids are steroid hormones that are produced in the adrenal cortex and are permissive for the actions of other hormones such as glucagon. In monogastrics glucocorticoids are secreted in response to physical or mental stress and

one of their functions is to suppress insulin signaling. In ruminants cortisol concentrations increase shortly before parturition, peak at calving, and quickly decline a few days after calving (Bell, 1995), potentially contributing to the decreased in insulin concentration and insulin resistance postpartum. Glucocorticoids are also released in response to decrease blood glucose and help to increase the expression of G6Pase and increase liver glucose release (Onuma et al., 2009).

Metabolism of fatty acids

During the weeks immediately following parturition, the increased glucose demand requires homeorhetic adaptations to support both peripheral tissue metabolism as well as the increased demands of the mammary gland (Bauman and Currie, 1980). One such adaptation is the increased lipolysis of adipose tissue and the release of NEFA as an oxidative substrate for the liver.

NEFA are taken up and metabolized by the liver in proportion to their concentration in the blood stream (Reynolds et al., 2003). These NEFA can be oxidized to generate reducing equivalents or ketone bodies, reesterified and exported as triglycerides in very low density lipoproteins (**VLDL**), or they can accumulate within the liver as stored triglycerides.

In the liver NEFA can be oxidized to CO_2 in the mitochondria and generate ATP in the respiratory chain reaction. The entry of NEFA into the mitochondria is regulated by carnitine palmitolytransferase I (**CPT-I**) and is a major control point in the metabolism of NEFA (Drackley, 1999). Short and medium chain fatty acids do not require carnitine transport to enter the mitochondria; however, long chain fatty acids require CPT-I to penetrate the mitochondrial membrane (Guzmán and Geelen, 1993).

CPT-I translocates fatty acids from the cytosol into the mitochondria, and is a central regulatory point for determining the oxidative flux of fatty acids within the liver (Drackley, 1999). When carnitine was added to media in an in vitro liver slice experiment, rates of oxidation of [1-^{14}C]palmitate doubled compared to controls (Drackley et al., 1991), further indicating the importance of carnitine and CPT-I as regulatory points.

Malonyl-CoA formation is a key regulatory step in fatty acid biosynthesis and the production of malonyl-CoA inhibits CPT-I activity so that there isn't a futile cycling of substrate (Guzmán and Geelen, 1993). Methylmalonyl-CoA is one of the intermediate products of propionate metabolism and is also inhibitory to CPT-I activity (Drackley et al., 2001). However, during early lactation these inhibitory mechanisms may be decreased. In rodent models the inhibition of CPT-I by malonyl-CoA is diminished when insulin concentrations decrease, such as during early lactation in the dairy cow, which may increase NEFA transport into the mitochondria as well as oxidation (Zammit, 1996). Carnitine sensitivity to inhibition of malonyl-CoA has also been shown to decrease during starvation (Guzmán and Geelen, 1993), and may be applied to the negative EB of early lactation as well.

During times of increased NEFA supply to the liver, the peroxisomal β-oxidation pathway can be induced and can account for almost 50 percent of β-oxidation capacity from palmitate (Grum et al., 1996). Peroxisomal β-oxidation is not subject to regulation by CPT-I and may help the liver cope with the excess NEFA uptake that occurs around parturition, although one caveat is that there is an increased release of energy as heat. Peroxisomal β-oxidation can use a wider variety of substrates and is

especially active towards very long chain fatty acids (Guzmán and Geelen, 1993) which can be partially oxidized and then transferred to the mitochondria for further metabolism.

The partial oxidation of fatty acids to ketone bodies provides substrate for the peripheral tissues, thereby sparing glucose for the mammary gland. Ketone bodies are a water soluble intermediate metabolite and can be used as a fuel source by many organs (heart, kidney, skeletal muscle, mammary gland, and gastrointestinal tract) as well as a substrate for mammary fatty acid synthesis. Liver ketogenesis is regulated by the amount of substrate to the liver, the activity of CPT-I to promote the entry of NEFA into the mitochondria, and the intramitochondrial activity of 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase) which is the rate limiting step in converting acetyl-CoA to ketones. HMG-CoA synthase is inhibited by the succinylation of succinyl-CoA. When there is a large supply of propionate, the pool of succinyl-CoA increases and leads to the inhibition of HMG-CoA synthase (Drackley et al., 2001) and decreased ketone body synthesis.

The bovine liver has a limited capacity for triglyceride export compared to monogastric animals, and the export of triglycerides is a rate limiting step in hepatic NEFA metabolism. Some lipid accumulation in the liver during the periparturient period seems to be a normal adaptation to lactation (Grum et al., 1996); however, excess liver triglyceride accumulation has been shown to decrease the ability of the hepatocyte to synthesize urea (Strang et al., 1998). The consequent increase in liver ammonia may decrease glucose synthesis from propionate (Overton et al.,, 1999), and excess hepatic triglyceride accumulation in transition cows has a negative impact on gluconeogenesis.

The proportion of [1-^{14}C]palmitate that was oxidized by liver slices in vitro increased and the proportion of esterified [1-^{14}C]palmitate decreased in early lactation as compared to other stages of lactation (Drackley et al., 1991). However, in another liver slice experiment evaluating dry cow feeding strategies, the capacity of the liver tissue to convert [1-^{14}C]palmitate to esterified products was increased during the early lactation period whereas total oxidation was only slightly increased (Litherland et al., 2011). Liver triglyceride content was negatively correlated with tissue oxidation of [1-^{14}C]palmitate but positively correlated with esterified products in the same study. The excessive accumulation of triglycerides in the liver postpartum negatively impacts gluconeogenesis and the metabolic capacity of the liver tissue.

Hormonal regulation of fatty acid utilization

The oxidation of NEFA provides ATP that is needed for gluconeogenesis. During the early postpartum period when there is an increase in NEFA mobilization as well as an increased demand for liver glucose output, it is likely that there is an increase in NEFA oxidation by the liver (Reynolds et al., 2003). Fatty acids are also known to be regulators of several gluconeogenic genes, including PC (Jitrapakdee et al., 2006, 2008), cytosolic and mitochondrial PEPCK (Duplus et al., 2002; Jump et al., 2005, 2008), and G6Pase (Jump et al., 2008). The β-oxidation of NEFA produces acetyl-CoA, which is an activator of PC, and can serve to help maintain oxaloacetate and TCA cycle intermediates in the mitochondria (Chow and Jesse, 1992).

One possible mechanism for the hepatic regulation in the early lactation cow is the Randle cycle that is discussed in human medicine, but rarely discussed in dairy cows (De Koster and Opsomer, 2013). The Randle cycle suggests that when there is a large

supply of fatty acids available that the oxidation of the fatty acids inhibits the use of glucose as a substrate for cellular metabolism in skeletal muscle, heart, liver, and pancreas. Applying this theory to the early lactation dairy cow, when there is a large supply of NEFA and a large demand for glucose, would help to explain the mechanism for the liver's ability to substantially increase the gluconeogenic capacity. To a large extent fatty acid utilization by the liver is controlled by hormonal regulation.

Insulin. Lipogenesis and lipolysis in the adipocyte are both influenced by insulin. Insulin has been shown to decrease CPT-I activity in isolated sheep hepatocytes (Chow and Jesse, 1992). However, the decrease in insulin along with the rise in glucagon concentrations in early lactation decreases acetyl-CoA synthase which decreases the malonyl-CoA concentration leading to an increase in CPT-I activity and fatty acid oxidation (Vernon, 2005). Faulkner and Pollock (1990) observed that lipid metabolism in pregnant and lactating sheep was affected by a state of insulin resistance. Adiponectin, the insulin sensitizing hormone produced by white adipose tissue, is decreased during this period and may play a role in the hormonal adaptation of early lactation that promotes insulin resistance (Giesy et al., 2012).

Glucagon. When exogenous glucagon was administered to early lactation dairy cows it was shown to increase clearance of stored triglycerides and prevent triglyceride accumulation (Hippen et al., 1999). Glucagon also stimulates ketone body formation (Guzmán and Geelen, 1993). The increase in glucagon concentration in early lactation may assist with lipid metabolism by increasing ketone production and preventing excess triglyceride accumulation in the liver.

FGF21. During states of negative EB hormone fibroblast growth factor 21

(**FGF21**) has been shown to stimulate β-oxidation of fatty acids, ketogenesis, and gluconeogenesis in the liver of mice (Badman et al., 2007) and may be important in the metabolic adaptations of early lactation dairy cows as well (Schlegel et al., 2012; Schoenberg et al., 2011b). It is a relatively novel hormone and has not yet been well studied in dairy cows; however there is a larger body of work studying FGF21 in other mammals. Whereas FGF21 has been shown to induce β-oxidation of fatty acids, ketogenesis, and gluconeogenesis, it does not stimulate glycogenolysis, suggesting that it is more important during prolonged fasting and starvation (Kliewer and Mangelsdorf, 2010). In rodents the liver expression of FGF21 has been shown to be upregulated during fasting and rapidly suppressed by refeeding (Badman et al., 2007). In dairy cows, liver FGF21 mRNA abundance is barely detectable prepartum, is greatly increased at 1 week postpartum, and decreases as lactation progresses (Schlegel et al., 2012; Schoenberg et al., 2011b). Feed restriction in late lactation cows also increased the expression of FGF21, indicating that negative EB is a major contributor to increased FGF21 expression in early lactation (Schoenberg et al., 2011b). FGF21 is upregulated by PPAR-α, which in turn is activated during NEFA release, similar to in early lactation (Badman et al., 2007).

Inflammation and liver metabolism

During the immediate postpartum period, there is a large increase in inflammation (Bradford and Farney, 2010) and an increase in plasma acute phase protein concentration (Bionaz et al., 2007). When insulin concentrations begin to increase a few weeks postpartum, the rate of NEFA release from adipose tissue is down regulated because there is a reduction in lipase activity that is insulin dependent

(Sordillo and Raphael, 2013). When NEFA mobilization is excessive, NEFA may interfere with this feedback response on lipase through increased expression of tumor necrosis factor α (**TNF- α**). One hypothesis is that TNF- α may interfere with lipase activity and thus an appropriate reduction in NEFA release is not obtained, resulting in accumulation of plasma NEFA and increased metabolic stress (Sordillo and Raphael, 2013).

Inflammation is a necessary component of the initial immune response to recognize pathogens and recruit additional immune cells to fight off infection. Cytokines that are produced by immune cells can elicit an inflammatory response and include the release of acute phase proteins from the liver, such as haptoglobin. In human subjects the systemic inflammation that is experienced during obesity is characterized by the production of proinflammatory cytokines (i.e. TNF- α), and over conditioned cows exhibit similar increases in TNF- α (Sordillo and Raphael, 2013).

During early lactation, when there is a large mobilization of adipose tissue, there is a large influx of NEFA by the liver (Reynolds et al., 2003). One way that the liver keeps up is by increasing peroxisomal oxidation. This increases the total oxidative capacity of the hepatocyte; however, hydrogen peroxide is also produced as an initial metabolite, which can increase reactive oxygen accumulation when there is a large supply of NEFA available (Sordillo et al., 2009). During the postpartum period there are changes in the fatty acid composition of NEFA, which can then alter the fatty acid composition of immune cells. Postpartum there is a decrease in some of the polyunsaturated fatty acids, such as eicosapentaeoic acid (**EPA**) and docosahexaenoic acid (**DHA**). DHA is a ligand for PPAR and can downregulate inflammatory reactions

in some cells, including monocytes and endothelial cells, while saturated fatty acids like palmitate and stearate, are able to enhance some of the proinflammatory pathways (de Heredia et al., 2012). In vitro cell culture work has shown that increases in stearic acid concentrations similar to those seen at calving may play a role in inhibition of PEPCK-C mRNA expression (White et al., 2012), and recent studies have indicated that it may be possible to alter plasma fatty acid profiles in dairy cows by altering dietary fatty acid composition (Douglas et al., 2007; Petit et al., 2007). Thus, the changes in lipid content of the immune cells as a result of adipose mobilization in early lactation may have the ability to affect the postpartum inflammatory response (Sordillo and Raphael, 2013).

STRATEGIES TO INCREASE PROPIONATE PRODUCTION

As previously stated, ruminal propionate is the largest contributor to liver glucose release (Reynolds et al., 2003). By increasing ruminal propionate production, the amount of glucogenic precursor that is available to the liver is increased, which should increase the energy status of the cow postpartum. The three strategies to increase ruminal propionate that will be discussed here will be: increased dietary starch content, the increased ruminal fermentability of the starch, and the addition of an ionophore to the diet.

Dietary Starch

Non-structural carbohydrates (**NSC**) are the principle source of energy for the lactating dairy cow, and are found inside the cells of plants. NSC are more easily digested than structural carbohydrates and are made up of sugars, starches, organic acids, and other carbohydrates. This section will focus on starch because dairy ration

formulations in the United States typically contain much higher percentages of starch than any other NSC.

Starch is a heterogeneous polysaccharide that is composed of two types of α-glucans, amylose and amylopectin (Tester et al., 2004). Specifically, starch is composed of an insoluble linear polymer of glucose bound by α-1, 4 linkages with varying degrees of branching resulting from α-1, 6 bonds at each branch point. Amylose is a long, linear α-glucan containing around 99% 1,4 α- and 1% 1,6 α-linkages (Tester et al., 2004). Amylopectin is a much larger molecule than amylose and is a heavily branched structure that is made of about 95% 1,4 α- and 5% 1,6 α- linkages (Tester et al., 2004).

Cereal grains are the main sources of starch in the diets of lactating dairy cows and are made up of a pericarp (outer covering), a germ (embryo), and the endosperm. The pericarp and germ regulate water uptake, but contain little starch, as the majority of the grain's starch is stored in the endosperm (Kotarski et al., 1992). Starch makes up 50 to 100% of NSC in most feedstuffs; however, the digestibility of starch varies greatly among feedstuffs (NRC, 2001.). Starch provides approximately 50% of the energy found in corn silage and 75% of the energy in corn grain (calculated from NRC, 2001).

Ruminal and postruminal digestion of starch. The first site of starch digestion is in the rumen where the starch is fermented by the rumen microbes (Kotarski et al., 1992). The process of starch digestion in the rumen involves α-amylase and isoamylase that are produced by rumen bacteria. The α-amylase randomly cleaves internal α-1,4 linkages of the polymer backbone and releases maltodextrins (low molecular weight oligosaccharides produced from starch hydrolysis by amylolytic bacteria), while

isoamylase cleaves the α-1,6 linkages of the amylopectin branch points (Tricarico et al., 2008).

The rumen bacteria with the greatest capacity for starch digestion are *Ruminobacter amylophilus* and *Streptococcus bovis*, followed by *Prevotella ruminicola* and some *Butyrivibrio fibrisolvens* strains (Tricarico et al., 2008). In order to hydrolyze starch, bacteria must either actively secrete amylase or produce surface associated amylases to hydrolyze starch for transport into the bacterial cell (Kotarski et al., 1991).

While protozoa and fungi are known to contribute to ruminal starch digestion, their roles are still not clearly defined (Tricarico et al., 2008). Ciliated protozoan concentrations tend to increase with an increase in grain feeding, and their populations range from 0 to 10^9 /L (Kotarski et al., 1992). In grain fed animals, protozoa can slow overall starch hydrolysis rates by ingesting a sufficient quantity of bacteria to decrease ruminal fermentation rates, as well as by ingesting starch granules and decreasing the accessibility of these substrates for bacterial fermentation (Kotarski et al., 1992).

The primary VFA resulting from rumen fermentation are acetate, propionate, and butyrate, with lactate sometimes produced as an end product during times of excessive fermentation (Allen, 2000). Propionate is the main end product of starch fermentation. Between 40 to 60 percent of the ruminal propionate produced is absorbed into the portal blood (Bergman et al., 1966), the rest is metabolized or converted to other metabolites during or prior to absorption and transport into the portal blood. About 90 percent of the propionate in the portal blood is removed from circulation by the liver, where it can be either oxidized or utilized as a substrate for gluconeogenesis as previously discussed.

Lower tract digestion and absorption of starch in the cow is relatively low because of the extensive ruminal fermentation and disappearance before digesta enters the hindgut (Van Soest, 1994). Although starch is digested more efficiently in the small intestines, starch digestion in the rumen is more beneficial than postruminal digestion of starch because ruminal digestion also increases the microbial protein outflow from the rumen where it is absorbed in the small intestines (DeFrain et al., 2005). During early lactation, postruminal starch infusion resulted in an increase in postruminal glucose absorption (Reynolds et al., 2001). However, this increased glucose supply was used with very high efficiency for fat deposition in the omental and mesenteric fat (Reynolds et al., 2001). Thus, increasing postruminal starch supply does not necessarily increase whole body glucose availability during this early lactation period.

Factors affecting starch digestion. Carbohydrates have the most variable rates of ruminal degradation among dietary nutrient classes and degradability is impacted by particle size and processing (Allen, 1997). Digestion of starch is dependent on the amount of starch present in the ration. The rate and extent of starch digestion in the rumen, in turn, influences the composition of VFA that are produced, rumen pH, and the amount of starch available for post ruminal digestion (Kotarski et al., 1992).

Of the common grains fed to ruminants, oats are the most digestible and least vitreous grain, followed by wheat, barley, and corn, with sorghum being the least digestible and most vitreous (NRC, 2001). Most grain processing methods increase the rate of starch fermentation and ruminal starch digestibility. Cereal processing methods use heat, moisture, and mechanical methods to break down the endosperm and expose the starch granule, which creates varying degrees of starch gelatinization and increases

ruminal digestibility (Kotarski et al., 1992). Decreasing particle size also increases the rate of starch digestion (NRC, 2001). Because the starch in cereal grains is embedded in a protein matrix inside the endosperm, either the physical or chemical disruption of the protein matrix is required for starch digestion by microbes in the rumen (Kotarski et al., 1992) Ruminal starch availability is an important factor affecting rate of digestion by ruminal microbes. High moisture corn increases ruminal starch digestibility compared to dry ground corn and decreased the acetate:propionate ratio (Oba and Allen, 2003a,b). In corn grain of differing endosperm types, floury corn grain increased ruminal propionate and decreased the acetate:propionate ratio in comparison to more vitreous corn, indicating an increased ruminal fermentability (Taylor and Allen, 2005). When cows were fed steam flaked corn in early lactation ruminal propionate numerically increased, and animals had increased milk yields compared to cows fed cracked corn (Dann et al., 1999). Feeding a more ruminally fermentable starch source increases ruminal propionate production and increases the substrate available for hepatic gluconeogenesis.

Monensin

Monensin is an ionophore that has been in use since the late 1960's in feeding poultry, beef cattle, and more recently dairy cattle, and is administered for benefits in improved feed efficiency, control of coccidiosis and bloat, and the reduction of metabolic disorders. In the United States, monensin is commercially available as a sodium salt (Rumensin 90; Elanco Animal Health, Greenfield, IN). This product is added to the diet and consumed by the animal. In some countries, monensin can be administered as a controlled release capsule, which is an oral bolus that is slowly

released in the rumen.

Monensin mode of action. Monensin is bacteriostatic agent against mainly gram-positive bacteria. Gram-positive bacteria are more susceptible to ionophores because they have a thinner cell wall than gram negative bacteria (Russell and Strobel, 1989). The oxygen atoms in the monensin are positioned throughout the molecule to create a cavity to trap cations. The monensin is able to attach to the bacterial wall and facilitates the movement of cations across the cell membrane. This monensin induced cation gradient reduces intracellular K^+ concentrations, while increasing intracellular Na^+ and H^+ concentrations and lowering intracellular pH (Russell and Strobel, 1989). This change in the cellular concentration of H^+ and Na^+ forces the bacteria to utilize the proton ATPase and sodium ATPase pumps to expel H^+ and Na^+ from the cell at the expense of 1 ATP per ion (McGuffey et al., 2001). The increased maintenance cost for the gram-positive bacteria fosters a ruminal environment in which gram-negative bacteria have a competitive advantage, and ruminal propionate is produced mainly by gram-negative bacteria (Van Maanen et al., 1978).

Monensin in transition cow health. While the approved Food and Drug Administration label claim for monensin in the United States is for increased feed efficiency, the benefits of monensin in the transition cow are seen in improved energy metabolism.

In beef animals and cows in established lactation, monensin has been shown to modulate feed intake. Published intake prediction equations suggest between a 4 to 10% decrease in feed intake when feedlot cattle are administered monensin (Fox et al., 1988; NRC, 2001). Because monensin stimulates greater ruminal propionate production, it is

possible that in feedlot animals and cows in established lactation this reduction in feed intake is a chemical response to the hepatic oxidation of propionate (Allen, 2000). However, in early lactation animals, transition period monensin supplementation has been shown to either increase postpartum DMI (Schroeder et al., 2009), decreased intermeal interval (Mullins et al., 2012), or have no effect on intake (Van der Werf et al., 1998; Phipps et al., 2000), indicating that monensin supplementation likely affects intake differently during the transition period.

Glucogenic effects of monensin. Monensin selectively modifies the rumen environment and promotes increased propionate production (Armentano and Young, 1983; Van Maanen et al., 1978). This increase in propionate supply then increases hepatic gluconeogenesis (Aiello and Armentano, 1987), which explains increases in blood glucose that have been observed with monensin supplementation (Duffield et al., 2008a). In the transition cow, the increase in glucose supply is one of the primary benefits of monensin. However, when propionate kinetics were measured during the periparturient period using isotopic tracers, monensin did not affect ruminal propionate production (Markantonatos et al., 2009). Arieli et al. (2001) did not observe changes in postpartum blood glucose concentration when monensin was fed prepartum, but observed an increase in glucose pool size using isotopic tracers, suggesting increased uptake of glucose by peripheral tissues in response to monensin. The lack of effect on ruminal propionate production in the transition cow suggests that effects of monensin in transition cows may extend beyond gluconeogenic flux, although more research in this area is needed.

Phosphoenolpyruvate carboxykinase is one of the rate-determining

gluconeogenic enzymes (Greenfield et al., 2000; Agca et al., 2002), and monensin supplementation has been shown to increase gene expression of cytosolic PEPCK during the immediate periparturient period (Karcher et al., 2007). Likely, monensin increases ruminal propionate production, which up regulates PEPCK to increase hepatic gluconeogenesis (Donkin et al., 2009), however, if ruminal propionate is not altered during the transition period (Markantonatos et al., 2009), it is possible that monensin may affect metabolism in a different way than by increasing propionate.

The meta-analyses of Duffield et al. (2008a) included a number of studies with transition cows and indicated that monensin significantly decreased blood concentration of ketone bodies and NEFA, and increased blood glucose and urea. The effects of monensin associated with the greater propionate supply result in improvements in energy status as seen in reductions in NEFA and BHBA. Across the trials summarized, monensin decreased circulating NEFA concentrations postpartum and cows that were treated with monensin maintained better BCS than control cows (Duffield et al., 2008b). Sauer et al. (1989) added monensin to transition period diets at either 0, 15, or 30 g/ton of DM and reported that 50% of control cows had clinical or subclinical ketosis, whereas the incidence was reduced to 33 and 8% in the groups receiving 15 and 30 g/ton, respectively. The group of cows fed the high dose of monensin also had significantly lower blood BHBA compared to the control cows (Sauer et al., 1989). In the meta-analysis of Duffield et al. (2008a) data showed reductions of 13.4% for BHBA and observed decreases in ketone bodies that may be explained by better energy balance status from increased propionate supply.

The Duffield et al. (2008a) data set showed that the relative risks of ketosis,

displaced abomasum, and mastitis also were decreased with monensin administration. The decreased risk for metabolic disorders with monensin supplementation are likely related to energy status, which is improved through greater provision of propionate. However, the ability of monensin to decrease incidence of infectious mastitis is surprising. Crawford et al. (2005) observed that heifers that received monensin prepartum and remained clinically healthy throughout the transition period had lower serum haptoglobin levels than control heifers, and that heifers that had a clinical disorder during the transition period had higher serum haptoglobin levels during the first week postpartum if they had received monensin prepartum compared to controls. These data suggest that monensin treated heifers perhaps had improved ability to mobilize early pro-inflammatory acute phase proteins and that healthy animals treated with monensin had lower subclinical disease compared to control animals. This could imply that the improved energy status with monensin treatment allows the immune system greater ability to fight off infections such as mastitis, although further investigation is needed.

In summary, by feeding diets with a higher propiogenic capacity there is the potential to enhance ruminal propionate production. This increase in propionate provides more gluconeogenic substrate to the liver and has the potential to improve energy status in the transition cow.

INTAKE REGULATION

In the last 3 wk prior to calving some of the many metabolic adaptations that occur in the periparturient dairy cow include the reduction in glucose utilization by peripheral tissues and increased hepatic gluconeogenesis (Bell and Bauman, 1997). These adaptations occur while DMI is generally decreased in the weeks prior to calving

(Ingvartsen and Andersen, 2000; Hayirli et al., 2002). Many different factors interact to affect feed intake, including diet, management, environment, level of production, and the physiological state of the animal (Ingvartsen and Andersen, 2000). Intake regulation is a complex orchestration of many physical and chemical signals, and the mechanisms controlling intake regulation are still under study.

Total feed intake is determined by the size and frequency of meals that the animal consumes (Allen and Piantoni, 2013). Meal size is determined by eating rate and meal length, which in turn is determined by satiety. The frequency at which meals are consumed is affected by the time interval between meals, and is determined by hunger. Feeding behavior is controlled by the brain in the hypothalamus and receives signals from the liver, gut, and also from metabolites and hormones.

The signals involved in feed intake regulation are additive and involve multiple signals including distention from rumen fill, gut peptides, hormones, and oxidation of fuels. Signals from the distention of the rumen from physical fill are likely to dominate in the control of feed intake during periods of high milk production, as increased NDF content of feed has been shown to slow rate of digestion, passage, and feed intake in animals where fill limits intake (Allen, 2000). However, in early lactation physical fill does not likely play a large role in the regulation of feed intake.

In the last few weeks prior to parturition a gradual decrease in feed intake occurs that continues into early lactation, with the lowest intake occurring at calving (Ingvartsen and Andersen, 2000). It was previously thought that the physical compression from the gravid uterus was involved in this decreased intake in late gestation (Coppock et al., 1972). However some of this decrease in intake from

decreased rumen volume is partly compensated by an increase in the rate of passage out of the rumen (Gunter et al., 1990). If rumen volume was the sole factor limiting intake in transition then the increased space in the abdominal cavity after calving should allow for a rapid increase in DMI. However, postpartum DMI increases rather slowly compared to the increase in milk production (Ingvartsen and Andersen, 2000), indicating that to some extent during early lactation the energy requirements for milk production drive intake.

Parabiotic experiments with laboratory animals have shown that there are circulating factors that affect animal feed intake. In an early parabiotic experiments with rats, a lesion created in the ventromedial hypothalamus of one rat led to obesity in the lesioned rat and hypophagia and weight loss in the other rat (Hervey, 1959). Subsequent research suggests that hormones, gut peptides, and hepatic oxidation of fuels affect the feeding behavior of animals, and that these signals are carried to the brain centers.

Leptin is a hormone that is produced mainly by adipose tissue, and plasma leptin is positively correlated with body reserves (Ingvartsen and Andersen, 2000). Leptin acts on the receptors in the hypothalamus to inhibit appetite, while in early lactation plasma leptin is decreased which may be partly responsible for the postpartum increase in DMI (Ingvartsen and Andersen, 2000). Energy balance controls the expression of the leptin receptor in the ruminant hypothalamus and it seems that hypoinsulinemia in early lactation increases hepatic leptin receptor abundance and may be a mechanism to attenuate leptin action during decreased leptin abundance (Thorn et al., 2008).

The reproductive hormones may also play an important role in the regulation of feed intake in cows although these are not yet well understood. Intravenous injection of

estradiol decreased both milk yield and DMI in lactating dairy cows (Grummer et al., 1990), while progesterone has been reported to block the effects of estrogen (Muir et al., 1972).

Gut peptides may also potentially contribute to the control of feed intake, although most of the work studying gut peptides had been conducted in nonruminant species, ghrelin, cholecystokinin (**CCK**), and glucagon like peptide 1 (**GLP-1**) may play roles in DMI regulation in dairy cows. Ghrelin is the "hunger" peptide and stimulates feeding behavior (Allen and Piantoni, 2013). Allen and Bradford (2008) saw an increase in ghrelin concentrations in early lactation animals in negative EB but not at other stages of lactation. Pigs that are fed on a regular meal schedule have been shown to have increased concentrations of ghrelin before a meal but animals that have ad libitum access to feed do not have feeding associated ghrelin increases (Reynolds et al., 2010). CCK is mainly secreted from the duodenum and jejunum (Ingvartsen and Andersen, 2000), and ingestion of fat and protein are particularly potent stimulators of CCK secretion. The direct effect of CCK suggest that food in the intestine causes the release of CCK which acts on the CCK receptors in the vagus nerve to feedback to the brain CCK receptors and decrease feed intake. Intracerebroventricular injection of a CCK-antiserum caused a significant hyperphagia in sheep, suggesting that CCK may play a role in intake regulation in ruminants (Della-Fera and Baile, 1984).

Glucagon like peptide-1 is secreted from the small intestine in response to nutrient and neurohumoral stimulation and helps to stimulate insulin secretion during a meal (Strader and Woods, 2005). GLP-1 inhibits gastrointestinal motility and reduces secretion, increases gastric emptying, and regulates transit of nutrients through the tract.

In rats, central administration of GLP-1 reduced feed intake in a dose dependent manner (Thiele et al. 1997). Because GLP-1 stimulates insulin secretion and reduces food intake, it has been suggested as a therapeutic agent in the treatment of obesity and type 2 diabetes in humans. GLP-1 has been shown to increase insulin release under normoglycemic conditions in young Holstein steers (ThanThan et al., 2012), although very little work has been conducted in ruminants.

Fatty acid oxidation in the brain and the liver have been suggested as signals in intake regulation (Ingvartsen and Andersen, 2000) as intravenous infusion of long chain fatty acids has been shown to cause hypophagia in rats and sheep (Carpenter and Grossman, 1983; Vandermeerschen-Doizé and Paquay, 1984). In rats subcutaneous injections of BHBA caused hypophagia (Fisler et al., 1995), however the liver cannot oxidize BHBA beyond acetoacetate so hypophagic effect of BHBA may be via central mechanism since an intercerebroventricular infusion reduced intake in rats (Arase et al., 1988). The mechanisms by which BHBA could trigger anorexic signaling in the brain may be diverse, however, BHBA may enter the neuron and be oxidized and thorough the generation of ATP, trigger anorexic signaling. Laeger et al. (2013) observed increased BHBA concentrations in cerebrospinal fluid in early lactation dairy cows, which may potentially act as central signals and suppress DMI during early lactation.

The β-oxidation of fatty acids in the liver is inhibited by mercaptoacetate, an acyl-CoA dehydrogenase activity depressant. When mercaptoacetate was injected into the peritoneum, to inhibit β-oxidation of fatty acids, feed intake in rats was increased, but only when the rate of fatty acid oxidation was high (Singer-Koegler et al, 1996). This is similar to early lactation when NEFA mobilization is high and oxidation of

NEFA is also likely to be greater than at other stages of lactation. Choi et al. (1997) used mercaptoacetate injections to study intake regulation in nonpregnant dairy heifers fed either low or high fat diets and determined that mercaptoacetate was not a suitable model for studying intake regulation in ruminants because it was associated with a decrease in intake. However, while mercaptoacetate injection increased plasma NEFA concentration post injection, the total NEFA concentrations were still very low (maximum 180 μmol/L at 2 h post injection). Because the rates of fatty acid oxidation were likely low in this model, this may have inhibited any effect of mercaptoacetate on increasing feed intake. Further study of mercaptoacetate is warranted in early lactation cows to evaluate the role of β-oxidation of fatty acids on feeding behavior.

Allen et al. (2009) proposed that liver energy status may serve to regulate DMI. Increased propionate and fatty acid supply to the liver (such as when a high starch diet is fed, or when mobilization of adipose tissue is high) is thought to reduce intake through increased hepatic oxidation (Allen et al., 2009; Allen and Piantoni, 2013). When oxidative fuel metabolism by the liver exceeds energy requirements, the brain is signaled to reduce DMI. This hepatic oxidation theory would suggest that feeding diets high in fermentable starch and oxidative capacity (such as feeding monensin) during early lactation would decrease DMI via propionate signaling.

Modulation of DMI by propionate during very early lactation is less likely than at other phases of lactation because NEFA likely are the predominant oxidative fuel for liver during this period (Reynolds et al., 2003), and any hypophagic effect of propionate would depend upon NEFA supply to the liver (Stocks and Allen, 2012). There is a positive correlation between liver capacity to convert propionate to glucose and fat free

NE_L intake (proxy for carbohydrate intake) in cows at d 1 and 21 post calving that does not exist either before calving or at peak lactation (Drackley et al., 2001). This would suggest that the liver has the capacity to direct additional propionate toward glucose. Hepatic energy requirements increase dramatically at the onset of lactation (Reynolds et al., 2003). The first point is supported by recent work (Stocks and Allen, 2012; 2013), in which it was determined that the hypophagic effects of propionate increased when hepatic acetyl CoA concentrations are higher, as they would be if cows were mobilizing large amounts of adipose tissue with the corresponding uptake of NEFA by the liver. Stocks and Allen (2012; 2013) suggest that increased hepatic acetyl CoA that likely leads to greater NEFA oxidation in the liver decreases intake in early lactation (Allen and Piantoni, 2013). While this mechanism is likely, the role of oxidation of NEFA in feed intake regulation of early lactation cows has not yet been quantified and more work is needed.

The release of cytokines by the immune system can also cause hypophagia in animals, with release of the acute phase inflammatory responses such as TNF-α, interleukin-1-β (**IL-1**), and interleukin-6 (**IL-6**) being the most potent hypophagic stimulators. TNF-α reduces intake in rodents when injected into the peritoneum. In ruminants TNF-α also decreases rumen motility and may be a contributing factor to observed decreases in feed intake. Peripheral administration of IL-1 has been shown to reduce intake in rats.

Intake regulation is a complex area of study and especially in the early lactation animal it is likely that a plethora of regulatory factors are at play in the decrease in DMI during this time period.

TRANSTION COW FEEDING STRATEGIES

Effects of prepartum nutrition and management programs on postpartum outcomes

Prepartum dietary plane of nutrition has a large impact on the degree of negative EB that the animal experiences postpartum. It was previously thought that feeding a high carbohydrate diet prepartum was necessary for the development of rumen papillae to adequately handle the increased rates of ruminal fermentation postpartum (Dirksen et al., 1985). However, the postpartum metabolic consequences of this type of high carbohydrate prepartum diet outweigh the ruminal adaptation benefits (Overton and Waldron, 2004). Higher carbohydrate diets have been shown to increase prepartum DMI. However, overfeeding cows prepartum, especially during the far off dry period, can lead to increased BCS gain and decreased postpartum DMI (Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010). This subsequent decrease in DMI during early lactation can lead to an increase in NEFA mobilization as well as increased insulin resistance and incidence of metabolic disorders, such as ketosis and fatty liver (Grummer et al., 2010). In a liver slice experiment, the over feeding during the far off dry period decreased in vitro liver capacity for oxidation of [1-^{14}C]palmitate and increase esterification, leading to a greater likelihood for fatty liver accumulation (Litherland et al., 2011). Restricting animal energy intake during the dry period has been shown to improve postpartum metabolic status and the incidence of postpartum health disorders by reducing postpartum NEFA mobilization (Douglas et al., 2006). However, in the group-fed housing that is today's industry standard, restricting energy intake for a group of animals is difficult to implement, as there is large variation among individual cows. Utilizing a high straw dry cow diet controls energy intake without restricting

DMI, and has been shown to produce similar decreases in postpartum adipose tissue mobilization as restricting prepartum feed intake (Janovick and Drackley, 2010; Janovick et al., 2011).

Postpartum nutrition and management programs

Most transition cow nutrition studies have focused on prepartum dietary strategies and their effect on postpartum performance, whereas relatively few have evaluated the effects of postpartum nutrition on metabolic adaptations and their effects on production performance.

During early lactation, postruminal starch infusion resulted in an increases in postruminal glucose absorption (Reynolds et al., 2001) leading to recommendations for feeding starch sources with greater postruminal digestibility (Allen et al., 2009). Larsen and Kristensen (2009) observed that a daily abomasal glucose infusion (1500 g/d) in postpartum dairy cows increased glucogenic status and decreased NEFA levels. In a subsequent study by the same research group utilizing NaOH-treated wheat grain to decrease ruminal starch digestibility resulted in increased small intestinal digestibility (Larsen and Kristensen, 2012). Feeding a glucogenic diet (containing the NaOH treated wheat grain) induced the higher glucogenic status from increased glucose release from splanchnic tissues compared to a more ketogenic diet (Larsen and Kristensen, 2012). However, in another study increased postruminal glucose supply was used with very high efficiency for fat deposition in the omental and mesenteric fat (Reynolds et al., 2001). Thus, increasing postruminal starch supply does not necessarily increase whole body glucose availability during the early lactation period.

When a high and low energy diet was fed pre and postpartum as a 2 × 2 factorial

design, cows fed the higher energy postpartum diet had a faster increase in milk production, as well as and higher DM and energy intakes in the first 20 d of lactation compared with cows fed the low energy postpartum diet (Rabelo et al., 2003). Cows fed the higher energy diet had greater ruminal propionate concentrations and increased plasma concentrations of glucose and insulin, indicating better energy status likely from increased propionate supply for gluconeogenesis (Rabelo et al., 2005). When a control release capsule of monensin (335 mg/d) was administered with a high energy postpartum diet, increased plasma glucose and decreased plasma BHBA and NEFA were observed compared with control cows (Arieli et al., 2008). Weekly milk yield tended to be higher in monensin treated cows as well, although DMI was not reported in this study (Arieli et al., 2008).

When monensin was fed to transition cows (400 mg/d) decreased plasma BHBA were observed along with decreased pre- and postpartum intermeal intervals (Mullins et al., 2012). There was an increase in liver mRNA abundance of CPT-I postpartum, although no effects of monensin treatment on milk production, PEPCK, or other plasma metabolites were observed (Mullins et al., 2012). This increase in CPT-I expression is interesting as that it indicates that monensin may modulate hepatic NEFA metabolism, although there are very few reports on the effects of monensin on NEFA metabolism in the liver.

In a recent study with early postpartum cows Dann and Nelson. (2011) reported that cows fed a low starch (21%) diet had higher DMI with no difference in milk yield compared to cows fed a medium starch (23%) diet, whereas cows fed a high starch (26%) diet had lower DMI and milk yield, suggesting that reducing dietary starch in

early lactation may increase DMI and milk yield.

The previous lack of research in the area of early lactation nutrition strategies is currently under study as the early lactation period can have long term effects on the health and productivity of the cow, although there is still a lack of consensus as to the best feeding strategy during early lactation.

RESEARCH OBJECTIVES

The transition period is a critical time in the lactation cycle of a dairy cow and can have lasting effects on downstream production performance. Thus gaining a better understanding of the metabolic changes that occur during this period related to energy partitioning and metabolism will serve to influence our ability to implement management strategies that will ensure successful transitions to lactation. A variety of nutritional strategies to optimize health conditions in cows during the transition period have been discussed; however, the main focus of this dissertation will be energy metabolism in early lactation with a specific focus on propionate and fatty acid metabolism by the liver.

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CHAPTER 3

PERFORMANCE OF EARLY LACTATION DAIRY COWS AS AFFECTED BY DIETARY STARCH AND MONENSIN SUPPLEMENTATION

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ABSTRACT

The objective of this study was to evaluate the impact of postpartum dietary starch content and monensin supplementation throughout the periparturient period and into early lactation on production performance of dairy cows during early lactation. Prior to parturition primiparous (n=21) and multiparous (n=49) Holstein cows were fed a common controlled energy close up diet with a daily topdress of either 0 or 400 mg/d monensin. From d 1 to 21 postpartum, cows were fed a high starch (HS; 26.2% starch, 34.3% NDF, 22.7% ADF, 15.5% CP) or low starch (LS; 21.5% starch, 36.9% NDF, 25.2% ADF, 15.4% CP) total mixed ration (TMR) with a daily topdress of either 0 mg/d monensin (Con) or 450 mg/d monensin (Mon), continuing with prepartum topdress assignment. From d 22 through 63 postpartum cows were fed HS and continued with their assigned daily topdress. Interactions of starch content and Mon supplementation were not significant for any of the variables measured. Cows fed HS from wk 1 to 3 postpartum had higher early lactation milk yields (starch × week interaction) compared to LS cows, but HS cows also had lower percentages of milk fat, true protein, lactose, and total solids during the same period, resulting in similar yields of energy corrected milk (ECM) between starch treatments. Cows fed HS had higher early lactation dry matter intake (DMI; starch × week interaction) and lost less body condition score during wk 1 to 3, contributing to improved energy balance postpartum. There was no effect of starch treatment on apparent total tract dry matter or starch digestibilities assessed during d 18 to 19 (± 2) postpartum, although cows fed the LS diet had greater apparent total tract NDF digestibility compared to cows fed the HS diet. Cows fed Mon had higher DMI and higher milk yields during the first 9 wk of lactation. However, all cows

had similar yields of ECM because of trends for lower milk fat content during early lactation. In part because of similar yields of ECM between these treatments and higher DMI for cows fed Mon, ECM/DMI during the first 9 wk of lactation was not affected by Mon treatment. There was no effect of Mon treatment on apparent total tract dry matter, NDF, or starch digestibilities. Overall, cows fed more propiogenic diets in early lactation (high starch or monensin) had increased milk yield and DMI during the immediate postpartum period, indicating that diets with greater propiogenic capacity do not have detrimental effects on early lactation DMI.

Keywords: Early lactation, starch, monensin

INTRODUCTION

In the period immediately following calving, DMI is insufficient to support the high milk production of early lactation, resulting in a state of negative energy balance (**EB**) that usually begins a few days before calving and reaches the greatest deficit about 2 wk after parturition (Butler, 2000). This state of negative EB results in the increased mobilization of adipose tissue, manifested as the release of NEFA into circulation to be metabolized by the liver and other tissues and incorporated into milk fat in the mammary gland. Higher DMI postpartum generally results in lower circulating NEFA and has been associated with improved health, performance, and less severe postpartum negative EB (Ingvartsen and Andersen, 2000).

Optimizing DMI during the periparturient period is especially important to provide sufficient available energy to support milk production. Because of the increased glucose demand for milk lactose synthesis, hepatic glucose production nearly doubles within 11 d of calving compared to prepartum hepatic glucose output (Reynolds et al.,

2003). Propionate that is produced via fermentation of starch in the rumen is the main precursor for hepatic glucose production (Aschenbach et al., 2010). Monensin is an ionophore that has also been shown to increase ruminal propionate production (Armentano and Young, 1983), likely from changes in the populations of gram-positive bacteria along with changes in the metabolism of gram-negative bacterial populations in the rumen that occur with ionophore treatment (McGuffey et al., 2000).

Allen et al. (2009) proposed that hepatic energy status is a major regulator of DMI in dairy cows. When hepatic oxidative fuel supply (propionate and NEFA) exceeds hepatic energy requirements, the brain is signaled via the vagal afferent nerve to reduce DMI (Allen et al., 2009). This hepatic oxidation theory would suggest that feeding diets that promote greater ruminal propionate production (e.g., high in fermentable starch, monensin supplementation) during early lactation could be hypophagic and thus further reduce DMI during this period of negative EB. If the hepatic oxidation theory applies to the early lactation period, then reducing dietary starch content or fermentability may increase DMI by reducing propionate production in the rumen (Allen et al., 2009). Recent work conducted using early lactation animals has shown propionate infusion to be more hypophagic in animals with higher liver acetyl CoA concentrations (Stocks and Allen, 2012, 2013), which would occur with higher NEFA mobilization. Because liver energy requirements increase dramatically at the onset of lactation (Reynolds et al., 2003) concurrent with increased adipose mobilization (Ingvartsen and Andersen, 2000), we speculate that NEFA are most likely the predominant hepatic oxidative substrate during this period. Thus, the hypophagic effect of propionate is likely to be reduced in the immediate postpartum period because of these large increases in hepatic energy

demands at the onset of lactation (Reynolds et al., 2003).

The efficacy of monensin to decrease periparturient negative EB associated health disorders, improve energy metabolism, and enhance lactation performance has been demonstrated (Duffield et al., 2008a, b, c). However, based upon the concepts presented in the hepatic oxidation theory, it is of interest to determine whether effects of monensin on performance of postpartum cows are independent of dietary starch content, as both likely will increase supply of propionate. The objectives of this study were to evaluate the effects of dietary starch content during the immediate postpartum period on intake and production, and to evaluate the effects of peripartal monensin supplementation in conjunction with these diets of differing starch content on DMI, production, feed efficiency, and EB. We hypothesized that increasing starch content during the immediate postpartum period and feeding monensin throughout the periparturient period and into early lactation would enhance milk production and improve energy metabolism without detrimental effects on DMI, and that the effects of monensin on performance would be independent of postpartum dietary starch content.

MATERIALS AND METHODS

Animals and treatments

All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee and the experiment was conducted from March to October 2012. The study was a completely randomized design with randomization restricted to balance for expected calving date of primiparous and multiparous cows and previous lactation 305-d mature-equivalent milk production for multiparous cows. A 2 × 2 factorial arrangement of postpartum treatments was utilized with early lactation

period feeding strategy [high starch (**HS**) vs. low starch (**LS**) diet during the first 21 d postpartum] and postpartum monensin supplementation [0 mg/d (**Con**) or 450 mg/d (**Mon**); Rumensin; Elanco Animal Health, Greenfield, IN] as the variables of interest. In addition, cows that received Mon during the postpartum period were fed Mon (400 mg/d) initiated on 1 d between d 21-28 before expected parturition (average treatment of 25 d; minimum of 14 d on treatment before actual parturition was required for inclusion in the data set). It is our experience that farms that feed monensin typically feed monensin throughout the entire transition period and into established lactation, which is why we chose to continue either monensin or control treatments throughout the entire trial period, in addition monensin treatment was initiated during the prepartum period to allow time for ruminal adaptation prior to calving. The HS and LS dietary treatments reflect common feeding strategies on commercial farms and were designed specifically to evaluate whether starch content of the diet fed during the early postpartum period affected DMI and cow performance.

A total of 80 cows were enrolled in the study and the final data set included a total of 70 animals (HS + Con primiparous n = 5, multiparous n = 13; HS + Mon primiparous n = 5, multiparous n = 13; LS + Con primiparous n = 6, multiparous n = 13; LS + Mon primiparous n = 5, multiparous n = 10). A total of 10 cows were removed from the experiment for reasons not related to experimental treatments (6 calved prior to having a minimum 2-wk period on the dry period treatment, 3 calved with twins, 1 was removed from data set for being an outlier with milk production that was 3 standard deviations below the mean). Lactating cows were dried off at least 45 d (average 53 d dry period length) prior to expected parturition, and moved to the experimental tie-stall

barn approximately 28 d prior to expected parturition where they began consuming the experimental close up dry cow diet (Table 3-1).

Diet formulation, nutrient composition, and feeding

Diets were balanced using the Cornell Net Carbohydrate and Protein System (CNCPS version 6.1, Cornell University, Ithaca, NY) and were fed for ad libitum intake. The HS and LS experimental diets were fed from parturition until d 21 postpartum, after which on d 22 all cows were fed the HS diet until the end of the study at d 63 of lactation.

The ingredient composition of the diets is shown in Table 3-1. All formulated diets were typical of the Northeast and Upper Midwest regions of the U.S. Prepartum all cows were fed a controlled energy ration based on corn silage, wheat straw, and a concentrate mix. Forage compositions are presented in Table 3-2. Postpartum diets were formulated on the basis of a lactation diet in which BMR corn silage was the predominant forage, with smaller amounts of wheat straw and legume silage. The concentrate portion of the HS diet was based on corn grain (20.97% of diet DM). For the LS diet corn grain (10.29% of diet DM) was partially replaced with citrus pulp (7.15% of diet DM) and soy hulls (3.58% of diet DM).

Mean composition (± standard deviations) of the TMR and topdress pellets are presented in Table 3-3. The topdress pellets were analyzed as single samples; therefore, standard deviations are not given. The basal HS and LS diets were formulated to contain 28.0 and 21.0% starch, respectively, whereas the analyzed starch contents of the basal HS and LS diets were 26.2 and 21.5%. The calculated composition of the total diet (TMR + topdress) is presented in Table 3-4. The calculated starch contents, accounting for the contribution of the topdress pellets, of the HS and LS diets were 25.5 and 20.9

% starch, respectively. The analyzed and calculated starch content of the HS diet was lower than expected; however, the difference between the two diets is meaningful from an application standpoint.

The topdress pellets were 33.6% soybean meal, 33.2% wheat middlings, and 33.2% canola meal, and were formulated to contain either 0 g/metric ton (Con) or 473 g/metric ton Mon. The Mon topdress fed was targeted to provide 400 mg/d prepartum and 450 mg/d postpartum and was fed as a daily topdress at rates of 0.85 kg DM/d prepartum and 0.95 kg DM/d postpartum. The concentration of Mon in the topdress was verified by Covance Laboratories (method 997.04; AOAC International, 2006; Greenfield, IN). The mean assayed concentration of Mon was 461 g/metric ton. No Mon was detected in the Con topdress (assayed concentration was below the level of detection; < 1.0 g/metric ton). Cows continued to receive assigned topdress treatments through wk 9 postpartum.

Cows were fed once daily for ad libitum intake at 0700 h. Refusals were removed daily before feeding, weighed, and recorded. All ingredients were sampled weekly for determination of DM content by drying at 55˚C for 48 h and values were used weekly to adjust ration formulation. Water was available ad libitum.

Data Collection, Sampling Procedures, and Analytical Methods

Samples of all TMR and ration ingredients were obtained weekly and composited at 4-wk intervals for analysis. Samples were analyzed for chemical composition using wet chemistry techniques for CP (method 990.03; AOAC International, 2006), NDF using α-amylase and sodium sulfite (Van Soest et al., 1991), ADF (method 973.18; AOAC International, 2006), lignin (Goering and Van Soest,

1970), 30 h NDF digestibility (Goering and Van Soest, 1970), starch (Hall, 2009), sugar (Dubois et al., 1956), ether extract (method 2003.05; AOAC International, 2006), ash (method 942.05; AOAC International, 2006), neutral detergent insoluble CP, acid detergent insoluble CP, soluble CP (Krishnamoorthy et al., 1982), and minerals (method 985.01; AOAC International, 2006) at a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD). Dry matter intake was calculated from the amounts of feed offered and refused, together with the corresponding DM value for the TMR, and weekly means of daily DMI, starch, and NDF intakes were calculated for statistical analysis.

All cows were weighed once weekly and BCS were assigned for all cows weekly by 2 individuals using a 5 point system (Wildman et al., 1982). The scores were averaged prior to statistical analysis. Daily observations and general health records were maintained throughout the study.

All cows were milked 2 times daily for the 9-wk lactation phase of the trial and daily milk yield was measured electronically. Daily milk yield was the sum of the 2 milkings, and weekly means of daily production were calculated prior to statistical analysis. Milk samples were collected from 2 consecutive milkings obtained over a 24-h period once per week. Individual milk samples were sent to a commercial laboratory for analysis of milk composition (Dairy One, Ithaca, NY). Samples were analyzed for contents of fat, true protein, lactose, total solids, MUN using mid-infrared analysis (AOAC International, 2006; method 972.160), and SCC by an optical fluorescent method (AOAC International, 2006; method 978.26) and SCS was calculated as SCS = log2 (SCC/100) + 3. Weekly yields of milk components were calculated, as well as

yields of 3.5% FCM = (0.432×milk kg) + (16.216×fat kg) (Dairy Records Management Systems, 2014) and ECM = (0.327×milk kg) + (12.95×fat kg) + (7.65×true protein kg) as described by Tyrrell and Reid (1965).

Prepartum and postpartum energy balance calculations were determined according to NRC (2001) equations. Weekly values for prepartum calculated energy balance were determined as follows:

Prepartum NE_L (Mcal/d) balance = energy intake (Mcal of NE_L/d) – [maintenance requirement (Mcal of NE_L/d) + pregnancy requirement (Mcal of NE_L/d)], where energy intake (Mcal/d) = weekly DMI average (kg/d) × diet NE_L (Mcal/kg of DM); maintenance requirement (Mcal) = week metabolic BW (MBW; $kg^{0.75}$) × 0.08 (Mcal/$kg^{0.75}$ per d); and pregnancy requirement (Mcal) = (0.00318 × d of gestation – 0.0352) × (1/0.218).

Weekly values for postpartum calculated energy balance were determined as follows:

Postpartum NE_L (Mcal/d) balance = energy intake (Mcal of NE_L/d) – [maintenance requirement (Mcal of NE_L/d) + lactation requirement (Mcal of NE_L/d)], where energy intake (Mcal/d) = weekly DMI average (kg/d) × diet NE_L (Mcal/kg of DM); maintenance requirement (Mcal/d) = week MBW ($kg^{0.75}$) × 0.08 (Mcal/ $kg^{0.75}$ per d); and lactation requirement (Mcal/d) = milk yield (kg/d) × [(0.0929 × fat percentage) + (0.0563 × true protein percentage) + (0.0395 × lactose percentage)].

Total tract nutrient digestibilities of DM, NDF, and starch were determined using 240-h undigestible NDF as an internal marker and nutrient concentrations in the orts-adjusted diet and feces. Two fecal grab samples were collected from a subset of

cows (primiparous n = 14, multiparous n = 33) at 24-h intervals on 2 consecutive days between d 18 and 19 (± 2) postpartum. Daily orts samples were collected for each cow and daily treatment TMR samples were collected during the fecal sampling period. Treatment TMR, fecal, and ort samples were composited by the 2 d sampling period (TMR samples) or cow (orts and fecal samples) and the composite samples were analyzed for DM, NDF, starch, and 240-h undigestible NDF (Goering and Van Soest, 1970) at a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD).

Statistical analysis

Statistical analyses were performed using SAS software (version 9.2; SAS Institute Inc., Cary, NC). Prepartum and postpartum data were analyzed separately. Postpartum data were analyzed as a completely randomized design with a 2 × 2 factorial arrangement of treatments. Fixed effects included starch content, Mon treatment, parity, time (week), and all 2 way interactions. A prepartum covariate (data collected week prior to enrollment on prepartum topdress assignment) was used for all DMI, starch and NDF intakes, BW, BCS, and EB analyses. The random effect was cow nested within starch and Mon treatment. Postpartum data were analyzed separately as wk 1 to 3 (dietary treatment period) and wk 1 to 9 (duration of experiment). Data measured over time were subjected to ANOVA using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). For variables with measurements repeated over time, four covariance structures were tested: compound symmetry, heterogeneous compound symmetry, first-order autoregressive, and heterogeneous first-order autoregressive and the covariance structure that resulted in the smallest Akaike's

information criterion was used (Littell et al., 1996). Data not analyzed over time were subjected to ANOVA using the MIXED procedure of SAS (Littell et al., 1996). Fixed effects included starch content, Mon treatment, parity, and all 2 way interactions. The random effect was cow nested within starch and Mon treatment. Degrees of freedom were estimated by using the Kenward-Roger option in the model statement.

RESULTS

Prepartum DMI, BW, BCS, and EB

Effects of Mon treatment during the prepartum period on prepartum weekly DMI, BW, BCS and EB are shown in Table 3-5. There was no effect of prepartum Mon treatment on DMI (average 12.5 ± 0.2 kg/d), BW (679 ± 4 kg), or BCS (3.55 ± 0.03). Overall, animals maintained similar DMI as a % of BW prepartum; however, as parturition approached cows fed Mon had slightly lower DMI as a % of BW compared to Con cows (Mon × week interaction; $P = 0.002$). Primiparous cows fed Mon gained 8 kg less precalving than primiparous cows fed Con (Mon × parity interaction; $P < 0.001$). Cows fed Mon tended to have a greater decrease in BCS prepartum compared to Con ($P = 0.07$), and had generally decreasing EB as parturition approached, expressed as both Mcal/d and as a percentage of requirement, whereas changes were less in Con cows (Mon × week interaction; $P = 0.009$ and $P = 0.006$ respectively).

Milk production and composition

Milk production and composition results are reported in Table 3-6. Interactions of starch content in the early postpartum diet and Mon treatment were not significant for any of the variables measured; therefore, results are presented and discussed as the main effects of starch and Mon. Overall effects of starch content on milk yield during

early lactation were not significant during wk 1 to 3 or wk 1 to 9; however, treatment × week interactions existed during both periods because cows fed the HS diet had increased milk production in early lactation (Figure 3-1A; $P < 0.001$). Further evaluation of the patterns of milk yield during wk 1 to 3 for cows fed different starch content using daily milk yield data rather than weekly means suggest that cows fed the HS diet tended to have higher overall milk yield (31.1 vs. 29.2 kg/d; $P = 0.10$; data not shown).

Cows fed HS postpartum diets had lower percentages of milk fat ($P = 0.01$) and true protein ($P = 0.05$) than cows fed LS during wk 1 to 3 postpartum (Table 3-6); however, these effects were not significant when evaluated over the 9-wk postpartum period. Percentages of lactose ($P = 0.05$), and total solids ($P = 0.009$) also were decreased during wk 1 to 3 in cows fed HS postpartum diets compared to those fed LS; these effects were also significant when evaluated over the 9-wk postpartum period ($P = 0.03$ for both variables). A starch × parity interaction ($P = 0.03$) for lactose percentage during wk 1 to 9 suggested that these decreases were more pronounced in primiparous cows fed HS than multiparous cows (5.08% lactose for primiparous cows fed LS vs. 4.78% for primiparous cows fed HS). There was no difference in lactose yields during wk 1 to 9 for primiparous cows although lactose yields tended to be higher in multiparous cows fed HS compared to LS (starch × parity interaction; $P = 0.08$; 2.06 kg/d of lactose for multiparous cows fed HS vs. 1.52 kg/d of lactose for multiparous cows fed LS). Overall, concentrations of MUN and SCS were not affected by treatment during either postpartum period evaluated; however, starch × wk interactions existed during both wk 1 to 3 ($P = 0.04$) and wk 1 to 9 ($P = 0.05$) for SCS, although differences

appeared to be slight in both cases.

Despite the differences in milk component percentages during wk 1 to 3 for cows fed diets of different starch content, effects of starch content on overall yields of milk fat, true protein, lactose, total solids were not significant during either wk 1 to 3 or 1 to 9 (Table 3-6). Although overall yields of milk and milk components were not affected by starch content; interactions or trends for interactions of starch content × week existed during wk 1 to 3 for yields of milk and milk components (true protein yield, lactose yield, total solids yield, 3.5% FCM, and ECM) such that component yields were lower during the early postpartum period for cows fed the HS diet. While trends for interactions of starch content × week existed for yields of lactose and total solids over the 9 wk postpartum period, these effects were small and similar to those detected during wk 1 to 3.

Even though the overall effects of Mon administration on milk yield during wk 1 to 3 were not significant, when evaluated from wk 1 to 9 postpartum cows fed Mon produced 2.2 kg/d more milk than Con (Figure 3-1B; $P = 0.05$). Trends for Mon × week interactions during wk 1 to 3 for both milk yield ($P = 0.07$) and lactose yield ($P = 0.06$) suggested that yields of each increased more as lactation progressed for cows fed Mon compared to those fed Con. Cows fed Mon tended to have lower percentages of fat ($P = 0.10$) and lactose ($P = 0.09$) during wk 1 to 3 postpartum; however, effects of Mon treatment on percentages of true protein and total solids, as well as yields of fat, true protein, lactose, total solids, 3.5% FCM, and ECM during wk 1 to 3 were not significant. During wk 1 to 9, cows fed Mon had lower percentages of lactose in milk ($P = 0.03$); however, percentages of other components, yields of milk components, and 3.5% FCM

and ECM were not affected by treatment. Cows fed Mon had higher MUN during wk 1 to 3 and wk 1 to 9 ($P = 0.007$ and $P = 0.02$, respectively). There was no difference between Mon treatments for SCS.

Postpartum DMI, BW, BCS, milk production efficiency, and EB

Postpartum DMI, BW, BCS, milk production efficiency, and EB for cows fed varying content of starch and Mon are presented in Table 3-7. All cows had similar overall DMI expressed as kg/d during the early lactation period (Figure 3-2A), however interactions of starch content × week for DMI (expressed either as kg/d or as a percentage of BW) suggested that cows fed HS had a faster increase in DMI during wk 1 to 3 [$P = 0.04$ (kg/d) and $P = 0.01$ (% of BW)]; this difference resulted in a similar interaction from wk 1 to 9 when expressed as a % of BW (Figure 3-3A; $P < 0.001$). There was a starch × week interaction for both wk 1 to 3 ($P < 0.001$) and wk 1 to 9 ($P = < 0.001$) for starch intake and cows fed HS diets had higher early lactation starch intakes. Cows fed HS tended to have increased NDF intake during wk 1 to 3 compared to cows fed LS ($P = 0.08$) and there was a starch × week interaction ($P = 0.004$) for wk 1 to 9 suggesting that HS cows had greater early lactation NDF intake likely because HS cows had greater early lactation DMI compared to cows fed LS. There was no effect of dietary starch content on postpartum BW, BW change, or BCS.

Primiparous cows fed the HS postpartum diet lost less BCS compared to those fed LS during the first 3 wk postpartum (-0.37 for LS primiparous cows vs. -0.01 for HS primiparous cows; starch × parity interaction; $P = 0.01$). Milk production efficiency during both wk 1 to 3 and wk 1 to 9, calculated either as milk yield per unit of DMI ($P = 0.04$ for both time periods) or ECM yield per unit of DMI ($P = 0.002$ and 0.006,

respectively), was increased in cows fed the LS diets. This increased calculated milk production efficiency is likely because cows fed the LS diet had decreased DMI postpartum and likely were mobilizing more adipose tissue during wk 1 to 3 rather than reflecting a true increase in feed efficiency for cows fed the LS diet. Cows fed HS diets postpartum had less severe negative EB compared to LS cows ($P < 0.001$). There was an interaction of starch × week during wk 1 to 9 for EB and cows fed LS had a greater degree of negative EB postpartum compared to cows fed HS when expressed as either Mcal/d (Figure 3-4A; $P < 0.001$) or as a percentage of requirements ($P < 0.001$).

Overall cows fed Mon had a 1.8 kg/d higher DMI than Con cows during wk 1 to 3 and a 1.1 kg/d higher DMI during wk 1 to 9. This increase in DMI was driven by an interaction of Mon × week for both wk 1 to 3 ($P = 0.009$) and wk 1 to 9 (Figure 3-2B; $P < 0.001$) such that cows fed Mon had a faster increase in DMI during early lactation. There was a Mon × week interaction ($P = 0.04$) for starch intake during wk 1 to 3, and cows fed Mon had greater starch intake compared to Con cows as lactation progressed, likely from the increased DMI observed in Mon cows. There was also a Mon × week interaction ($P = 0.05$) for NDF intake during wk 1 to 3, and cows fed Mon had greater NDF intake compared to Con cows as lactation progressed, again likely from increased DMI in Mon cows. Cows fed Mon tended to have greater BW change during wk 1 to 3 (-36 vs. -24 kg; $P = 0.08$). While there was no overall effect of Mon treatment on DMI as a % of BW or BCS during the postpartum period, an interaction of Mon × parity ($P = 0.006$) for BCS change existed during wk 1 to 9 and primiparous cows fed Mon lost slightly less BCS (-0.57 for Con vs. -0.31 for Mon) and multiparous cows fed Mon lost slightly more BCS (-0.34 for Con cows vs. -0.52 for Mon cows).

An interaction of Mon × parity and a trend for this interaction were evident for milk/DMI ($P = 0.04$) and ECM/DMI ($P = 0.07$), such that primiparous cows fed Mon had slightly lower feed efficiency than Con, whereas efficiencies were similar between treatments for multiparous cows (data not shown). The higher DMI and trends for lower milk fat content for primiparous cows fed Mon likely contributed to this effect. Primiparous cows fed Mon tended to have less negative EB in early lactation compared to primiparous Con cows as they met a higher percentage of energy requirements in wk 1 to 3 than Con primiparous cows (Mon × parity; $P = 0.06$; 73.9 vs. 63. 4 % of requirement); however, differences between treatments for multiparous cows were not significant (average 77.0% of requirement).

Apparent total tract digestibility

Results for the apparent total tract digestibilities for DM, NDF, and starch measured from d 18 and 19 (± 2) postpartum for cows fed the different treatments are presented in Table 3-8. There was no effect of starch content on apparent total tract digestibility for digestibility of either DM or starch. Cows fed LS had higher apparent total tract digestibility of NDF compared to cows fed HS diet ($P <0.001$). This is likely because of the increased inclusion of high-NDF byproduct feeds that typically have high NDF digestibility in the LS diet. There was no effect of peripartal Mon treatment on measures of apparent total tract digestibility for DM, NDF, or starch.

Table 3-1. Ingredient composition of the basal diets (% of DM)

	Prepartum	Postpartum[1]	
		HS	LS
Corn silage, processed	42.14	–	–
BMR corn silage[2]	–	38.50	38.50
Wheat straw	21.75	11.54	11.55
Legume silage	–	9.62	9.62
Shelled corn, finely ground	4.28	20.97	10.29
Citrus pulp	7.23	1.01	7.15
Corn germ meal	–	2.52	5.56
Soybean hulls	7.08	–	3.58
Soybean meal	5.27	5.87	3.86
Canola meal	4.63	2.73	2.08
Blood meal	1.05	1.94	1.93
Amino Plus[3]	1.78	1.70	2.34
Energy Booster 100[4]	–	0.77	0.96
Calcium carbonate	1.53	1.12	0.82
Sodium bicarbonate	–	0.86	0.85
Soy Chlor[5]	1.33	–	–
Salt	0.16	0.42	0.41
Calcium sulfate	0.73	0.17	0.17
Magnesium oxide	0.20	0.15	0.15
Magnesium sulfate, 9.9%	0.61	–	–
Selenium 0.06%	0.04	0.05	0.05
Mono dicalcium phosphate	–	0.02	0.07
Trace mineral premix[6]	0.02	0.03	0.03
Vitamin A, D, E premix[7]	0.05	0.02	0.02
Vitamin E premix[8]	0.005	–	–
Zinc sulfate	0.002	–	–
Copper sulfate	0.0004	0.001	0.001

[1]HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]BMR= brown mid-rib corn silage.
[3]AGP Inc., Omaha, NE.
[4]Milk Specialties Global; Carpentersville, IL.
[5]West Central, Ralston, IA.
[6]Contained 30,317 mg/kg of Cu, 136,466 mg/kg of Mn, 3,393 mg/kg of Co, 3,040 mg/kg of I, and 153,916 mg/kg of Zn.
[7]Contained 30,464 IU/kg of Vitamin A, 5,862 IU/kg of Vitamin D, and 93,784 IU/kg of Vitamin E.
[8]Contained 510,750 IU/kg of Vitamin E.

Table 3-2. Nutrient composition of corn silage, brown mid-rib (BMR) corn silage, legume silage, and wheat straw (± SD[1])

Item	Corn silage	BMR corn silage	Legume silage	Wheat straw
DM, % as fed	32.7 ± 1.9	31.3 ± 0.8	41.0 ± 4.3	93.5 ± 1.9
CP, %	8.5 ± 0.5	8.6 ± 0.3	20.4 ± 1.8	4.7 ±1.3
ADF, %	22.7 ± 0.7	24.6 ± 0.9	32.3 ± 3.2	55.8 ± 2.7
NDF, %	38.1 ± 1.4	39.5 ± 1.1	39.2 ± 4.5	79.9 ± 1.8
30 h NDFD, %[2]	21.4	26.6	17.1	33.1
30 h NDFD, % of NDF[2]	55.4	67.3	43.3	41.2
Sugar, %[2]	1.3	1.5	3.7	2.1
Starch, %[2]	33.1	30.6	1.5	0.9
Fat, %[2]	4.4	3.6	3.4	0.5

[1]Chemcial composition was analyzed on 6 composite samples.
[2]Chemcial composition for these analyses were determined on 1 sample obtained from a subsample of the 6 composites.

Table 3-3. Chemical composition of experimental diets (± SD[1])

	Prepartum diet	Postpartum diet[2]		Topdress pellet[3]	
Item		HS	LS	Con[4]	Mon[5]
DM, %	50.7 ± 2.4	48.3 ± 2.7	48.0 ± 3.2	93.2 ± 1.0	93.7 ± 1.2
CP, %	13.0 ± 0.8	15.5 ± 1.2	15.4 ± 0.8	37.5	37.0
ADF, %	28.2 ± 1.2	22.7 ± 1.2	25.2 ± 1.2	11.1	12.9
NDF, %	42.9 ± 2.0	34.3 ± 1.5	36.9 ± 1.5	22.6	21.3
30 h NDFD[6], %	–	18.9 ± 1.2	20.7 ± 1.1	–	–
30 h NDFD[6], % of NDF	–	55.1 ± 2.0	56.1 ± 1.4	–	–
Sugar, %	4.9 ± 0.8	3.5 ± 0.6	4.5 ± 0.4	10.6	11.3
Starch, %	17.4 ± 1.2	26.2 ± 1.2	21.5 ± 1.0	13.1	13.8
Fat, %	2.6 ± 0.2	4.0 ± 0.2	2.2 ± 0.6	2.4	2.5
Ca, %	1.28 ± 0.16	0.94 ± 0.09	1.01 ± 0.04	0.51	0.6
P, %	0.30 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.97	0.99
Mg, %	0.41 ± 0.04	0.28 ± 0.02	0.3 ± 0.03	0.48	0.48
K, %	1.12 ± 0.13	1.12 ± 0.09	1.18 ± 0.08	1.67	1.70
S, %	0.37 ± 0.04	0.21 ± 0.09	0.22 ±0.01	0.46	0.44
Na, %	0.12 ± 0.02	0.47 ±0.08	0.46 ± 0.05	0.07	0.07
Cl, %	0.37 ± 0.01	0.44 ± 0.04	0.44 ± 0.03	0.07	0.09
NE_L, Mcal/kg	1.48 ± 0.03	1.64 ± 0.02	1.56 ± 0.03	1.72	1.74

[1]Chemcial composition was analyzed on 6 composite samples of the prepartum diet, 7 composite samples of the high starch postpartum diet, and 6 composite samples of the low starch postpartum diet.

[2]HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).

[3]The composition of the topdress was 33.6% soybean meal, 33.2% wheat middlings, and 33.2% canola meal.

[4]Con = control topdress, formulated to supplement 0 mg/d monensin.

[5] Mon = monensin topdress, formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.

[6]NDFD = NDF digestibility.

Table 3-4. Calculated diet composition during the prepartum and postpartum periods, including both TMR and topdress, for cows fed high starch (HS) or low starch (LS) diets during weeks 1 to 3 postpartum.

		Postpartum[1]	
Item	Prepartum	HS	LS
DM, %	53.6	51.1	50.9
CP, %	14.7	16.8	16.8
ADF, %	27.1	22.1	24.4
NDF, %	41.5	33.6	35.9
30 h NDFD[2], %	–	18.9	20.7
30 h NDFD[2], % of NDF	–	55.1	56.1
Sugar, %	5.3	4.0	4.9
Starch, %	17.1	25.5	20.9
Fat, %	2.6	3.9	2.2
Ca, %	1.23	0.91	0.98
P, %	0.35	0.38	0.38
Mg, %	0.41	0.29	0.31
K, %	1.16	1.15	1.21
S, %	0.38	0.23	0.24
Na, %	0.12	0.46	0.44
Cl, %	0.35	0.42	0.41
NE_L, Mcal/kg	1.50	1.65	1.57
Metabolizable energy, Mcal/kg DM[3]	2.16	2.50	2.45
Total metabolizable protein available, g/d[3]	1,150	1,660	1,548
Metabolizable protein, g/kg DM[3]	92.74	106.41	104.59
Carbohydrate fermentability, % of DM[3]	41.9	41.1	40.7
NDF fermentability, % of DM[3]	15.5	11.5	13.6
Starch fermentability, % of DM[3]	15.2	21.9	17.1

[1]HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]NDFD = NDF digestibility.
[3]Values predicted in CNCPS (v 6.1) using actual feed intakes of 12.4 kg/d DMI for dry cows, 15.6 kg/d DMI for cows fed the high starch diet, and 14.8 kg/d DMI for cows fed the low starch diet.

Table 3-5. Prepartum DMI, BW, and BCS as affected by monensin supplementation beginning during the prepartum period

	Prepartum diet[1]			*P*-values					
Item	Con[2]	Mon[3]	SEM	Mon	Parity	Wk	Mon × Wk	Mon × Parity	Parity × Wk
DMI, kg/d	12.3	12.6	0.2	0.37	<0.001	<0.001	0.39	0.87	0.08
DMI, % of BW	1.76	1.79	0.05	0.65	<0.001	<0.001	0.002	0.99	0.20
BW, kg	680	678	4	0.71	0.18	<0.001	0.56	0.76	0.42
BW change, kg[4]	21	21	13	0.84	<0.001	–	–	<0.001	–
BCS	3.56	3.53	0.03	0.40	0.003	0.001	0.78	0.34	0.07
BCS change[4]	-0.02	-0.12	0.04	0.07	0.13	–	–	0.11	–
Energy balance, Mcal/d	5.00	5.11	0.54	0.88	<0.001	<0.001	0.009	0.18	0.35
Energy balance, %	140.44	140.62	4.60	0.98	<0.001	<0.001	0.006	0.16	0.20

[1]Con = control topdress and Mon = monensin topdress.
[2]Formulated to supplement 0 mg/d monensin.
[3]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[4]Body weight and BCS change were calculated as the difference between BW (or BCS) at the initiation of monensin treatment and wk -1 prepartum.

Table 3-6. Milk yield and composition for cows fed either high or low starch diets during the first 3 wk postpartum and control or monensin treatments throughout the periparturient period and into early lactation

	Diet[1]			Topdress[2]			*P*-values[5]							
Item	HS	LS	SEM	Con[3]	Mon[4]	SEM	S	M	P	S×M	S×Wk	M×Wk	S×P	M×P
Milk yield, kg/d														
wk 1 to 3	31.0	29.8	0.9	29.8	31.0	0.9	0.35	0.32	<0.001	0.45	0.002	0.07	0.57	0.63
wk 1 to 9	36.3	36.0	0.8	35.1	37.3	0.8	0.81	0.05	<0.001	0.43	<0.001	0.19	0.33	0.71
3.5% FCM, kg/d														
wk 1 to 3	34.7	36.3	1.2	35.9	35.1	1.3	0.36	0.64	<0.001	0.93	0.04	0.98	0.58	0.77
wk 1 to 9	37.1	37.8	1.0	37.0	38.0	1.0	0.61	0.52	<0.001	0.63	0.22	0.44	0.37	0.74
ECM, kg/d														
wk 1 to 3	34.7	36.7	1.2	35.9	35.4	1.2	0.24	0.80	<0.001	0.97	0.01	0.93	0.45	0.93
wk 1 to 9	36.9	37.6	1.0	36.8	37.8	1.0	0.59	0.47	<0.001	0.51	0.19	0.48	0.44	0.55
Fat, %														
wk 1 to 3	4.38	5.01	0.17	4.90	4.48	0.18	0.01	0.10	0.03	0.74	0.46	0.42	0.55	0.98
wk 1 to 9	3.76	3.97	0.09	3.96	3.77	0.09	0.11	0.13	0.002	0.52	0.16	0.69	0.97	0.52
Fat, kg														
wk 1 to 3	1.32	1.44	0.06	1.41	1.34	0.06	0.14	0.34	<0.001	0.91	0.11	0.97	0.67	0.86
wk 1 to 9	1.33	1.37	0.04	1.35	1.35	0.05	0.48	0.93	<0.001	0.95	0.20	0.61	0.56	0.96
True protein, %														
wk 1 to 3	3.31	3.84	0.20	3.59	3.57	0.20	0.05	0.94	0.58	0.33	0.17	0.74	0.98	0.95
wk 1 to 9	2.92	3.10	0.09	3.01	3.01	0.09	0.12	0.96	0.29	0.32	0.22	0.90	0.47	0.75
True protein, kg														
wk 1 to 3	0.99	1.07	0.05	1.02	1.04	0.05	0.25	0.85	<0.001	0.79	0.09	0.44	0.35	0.81
wk 1 to 9	1.03	1.06	0.03	1.02	1.06	0.04	0.53	0.33	<0.001	0.76	0.15	0.64	0.62	0.33
Lactose, %														
wk 1 to 3	4.60	4.83	0.08	4.81	4.62	0.09	0.05	0.09	0.51	0.79	0.30	0.20	0.46	0.81
wk 1 to 9	4.82	4.93	0.04	4.93	4.82	0.04	0.03	0.03	<0.001	0.88	0.08	0.69	0.03	0.69
Lactose, kg														
wk 1 to 3	1.43	1.44	0.05	1.42	1.44	0.05	0.89	0.77	<0.001	0.58	0.04	0.06	0.23	0.90
wk 1 to 9	1.75	1.78	0.04	1.73	1.79	0.04	0.62	0.32	<0.001	0.35	0.09	0.36	0.08	0.70
Total Solids, %														
wk 1 to 3	13.31	14.76	0.40	14.35	13.71	0.40	0.009	0.24	0.13	0.96	0.16	0.59	0.18	0.66
wk 1 to 9	12.42	12.97	0.17	12.88	12.52	0.18	0.03	0.13	0.002	0.76	0.08	0.88	0.46	0.86

Table 3-6. Continued

Total Solids, kg														
wk 1 to 3	4.04	4.27	0.15	4.17	4.13	0.15	0.26	0.84	<0.001	0.90	0.02	0.69	0.39	0.94
wk 1 to 9	4.44	4.55	0.11	4.44	4.55	0.12	0.49	0.51	<0.001	0.44	0.21	0.63	0.26	0.49
MUN, mg/dL														
wk 1 to 3	11.6	10.9	0.4	10.5	11.9	0.4	0.16	0.007	0.05	0.86	0.44	0.46	0.89	0.59
wk 1 to 9	11.7	11.5	0.4	11.0	12.2	0.4	0.59	0.02	0.23	0.42	0.21	0.37	0.98	0.49
SCS														
wk 1 to 3	3.38	3.28	0.30	3.55	3.11	0.31	0.81	0.30	0.72	0.45	0.04	0.34	0.60	0.89
wk 1 to 9	2.41	2.36	0.29	2.50	2.27	0.30	0.91	0.58	0.07	0.86	0.05	0.28	0.40	0.75

[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, and P = parity.

Table 3-7. Postpartum DMI, BW, BCS, efficiency and energy balance for cows fed either high or low starch diets during the first 3 wk postpartum and control or monensin treatments throughout the periparturient period and into early lactation

	Diet[1]			Topdress[2]			*P*-values[5]							
Item	HS	LS	SEM	Con[3]	Mon[4]	SEM	S	M	P	S×M	S×Wk	M×Wk	S×P	M×P
DMI, kg/d														
wk 1 to 3	15.6	14.8	0.4	14.3	16.1	0.4	0.21	0.004	<0.001	0.77	0.04	0.009	0.24	0.74
wk 1 to 9	19.8	19.1	0.3	18.9	20.0	0.3	0.13	0.02	<0.001	0.66	0.32	<0.001	0.99	0.67
DMI, % of BW														
wk 1 to 3	2.67	2.41	0.06	2.48	2.60	0.06	0.006	0.20	<0.001	0.71	0.01	0.29	0.84	0.25
wk 1 to 9	3.37	3.22	0.05	3.24	3.35	0.05	0.03	0.11	<0.001	0.47	<0.001	0.66	0.98	0.99
Starch intake, kg/d														
wk 1 to 3	3.90	3.22	0.12	3.51	3.62	0.11	0.001	0.49	<0.001	0.45	<0.001	0.04	0.51	0.44
wk 1 to 9	5.29	5.03	0.12	5.13	5.19	0.12	0.10	0.73	0.005	0.21	<0.001	0.39	0.96	0.69
NDF intake, kg/d														
wk 1 to 3	5.61	5.21	0.16	5.34	5.49	0.16	0.08	0.50	<0.001	0.42	0.26	0.05	0.93	0.47
wk 1 to 9	7.03	6.77	0.16	6.88	6.92	0.16	0.25	0.86	0.001	0.25	0.004	0.33	0.72	0.63
BW, kg														
wk 1 to 3	614	605	4	609	609	4	0.12	0.93	0.07	0.32	0.85	0.99	0.36	0.49
wk 1 to 9	602	595	4	597	600	4	0.21	0.55	0.02	0.47	0.85	0.85	0.21	0.60
BW change, kg[6]														
wk 1 to 3	-27	-33	5	-24	-36	6	0.37	0.08	0.34	0.30	–	–	0.99	0.29
wk 1 to 9	-21	-29	7	-19	-31	7	0.39	0.20	0.48	0.80	–	–	0.58	0.15
BCS														
wk 1 to 3	3.19	3.18	0.04	3.20	3.17	0.04	0.98	0.50	<0.001	0.32	0.90	0.64	0.99	0.70
wk 1 to 9	3.01	3.02	0.04	3.03	3.01	0.04	0.86	0.75	<0.001	0.56	0.96	0.66	0.67	0.67
BCS change[6]														
wk 1 to 3	-0.21	-0.33	0.04	-0.30	-0.24	0.04	0.04	0.29	0.23	0.32	–	–	0.01	0.16
wk 1 to 9	-0.39	-0.48	0.05	-0.46	-0.42	0.05	0.23	0.59	0.91	0.76	–	–	0.33	0.006
Milk/DMI														
wk 1 to 3	1.95	2.10	0.05	2.05	2.03	0.06	0.04	0.59	0.84	0.48	0.51	0.95	0.30	0.04
wk 1 to 9	1.84	1.95	0.03	1.88	1.90	0.03	0.04	0.67	0.97	0.17	0.53	0.95	0.28	0.21
ECM/DMI														
wk 1 to 3	2.20	2.50	0.07	2.45	2.26	0.07	0.002	0.05	0.47	0.98	0.66	0.76	0.45	0.07
wk 1 to 9	1.90	2.05	0.04	1.99	1.97	0.04	0.006	0.69	0.14	0.26	0.13	0.58	0.28	0.65

Table 3-7. Continued

Energy Balance, Mcal/d														
wk 1 to 3	-6.76	-11.83	0.84	-10.06	-8.53	0.83	<0.001	0.19	0.74	0.89	0.27	0.52	0.73	0.32
wk 1 to 9	-2.05	-4.50	0.46	-3.46	-3.08	0.46	<0.001	0.55	<0.001	0.20	<0.001	0.65	0.39	0.92
Energy Balance, %														
wk 1 to 3	79.97	65.68	1.95	70.09	75.55	1.93	<0.001	0.05	0.003	0.98	0.12	0.57	0.41	0.06
wk 1 to 9	94.35	87.00	1.13	89.76	91.59	1.15	<0.001	0.25	<0.001	0.22	<0.001	0.68	0.15	0.36

[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, and P = parity.
[6]Body weight and BCS change were calculated as the difference between body weight (or BCS) in wk 1 postpartum and either wk 3 or wk 9 postpartum.

Table 3-8. Apparent total tract digestibility of DM, NDF, and starch during early lactation (d 18 and 19, ± 2) for cows fed either high or low starch diets during the first 3 weeks postpartum and control or monensin treatments throughout the periparturient period and into early lactation

	Diet[1]			Topdress[2]			*P*-values[5]					
Item	HS	LS	SEM	Con[3]	Mon[4]	SEM	S	M	P	S×M	S×P	M× P
DM digestibility, %	61.3	63.9	1.2	63.3	61.9	1.2	0.13	0.38	0.23	0.43	0.66	0.42
NDF digestibility, %	37.0	47.8	1.5	43.9	41.0	1.4	<0.001	0.15	0.87	0.81	0.33	0.27
Starch digestibility, %	95.4	96.3	0.5	95.9	95.8	0.5	0.15	0.87	0.07	0.45	0.51	0.52

[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, and P = parity.

Figure 3-1. Least squares means for milk yield (kg/d) for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data was analyzed separately as wk 1 to 3 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs. low (21.5%) starch fed during wk 1 to 9. The *P* values for the overall effects of starch content were 0.35 during wk 1 to 3 and 0.81 during wk 1 to 9. The *P* values for the interaction of starch content × week were 0.002 for wk 1 to 3 and < 0.001 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). The *P* values for the overall effect of monensin were 0.32 for wk 1 to 3 and 0.05 for wk 1 to 9. The *P* values for the interaction of monensin × week were 0.07 for wk 1 to 3 and 0.19 for wk 1 to 9.

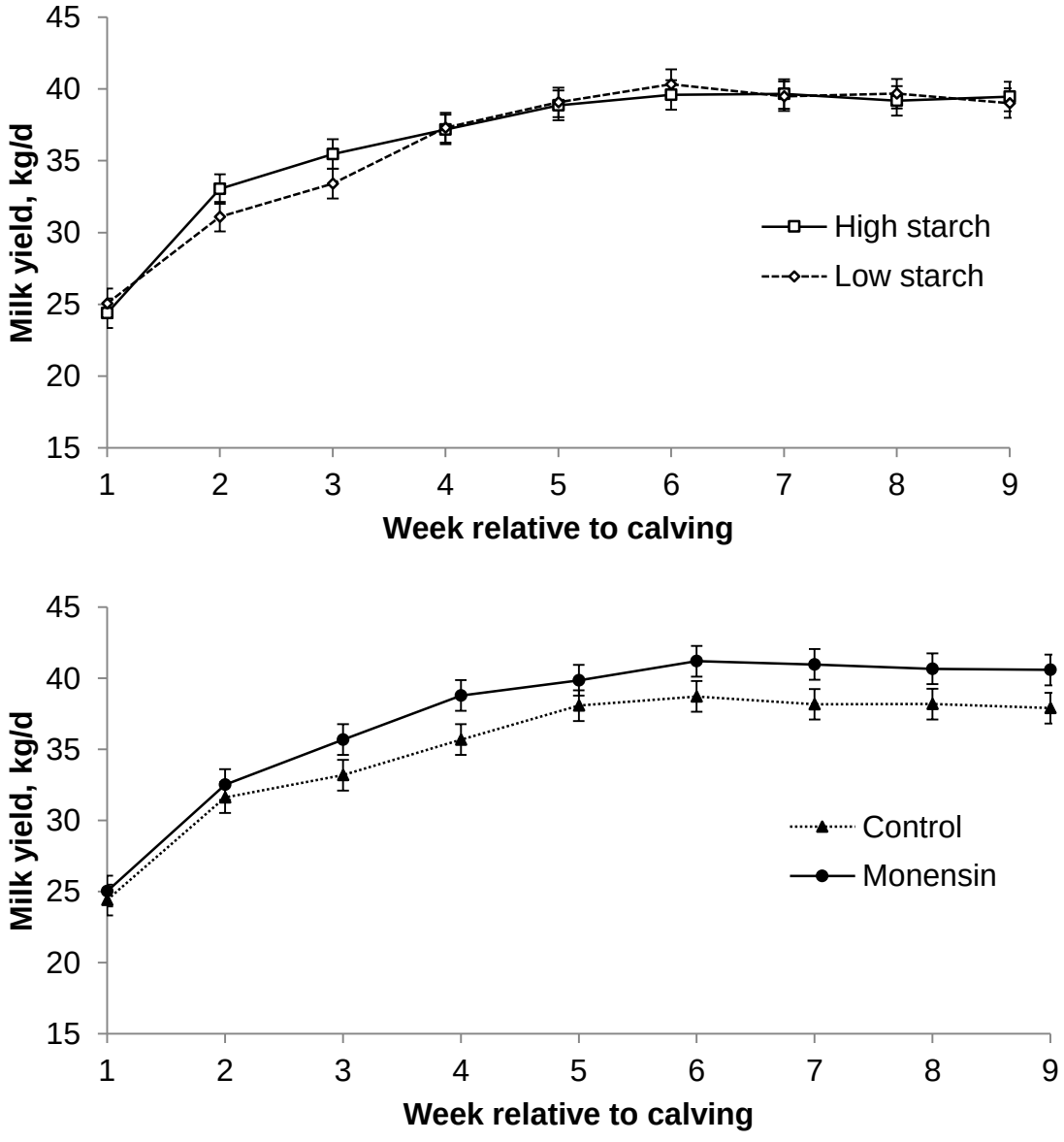
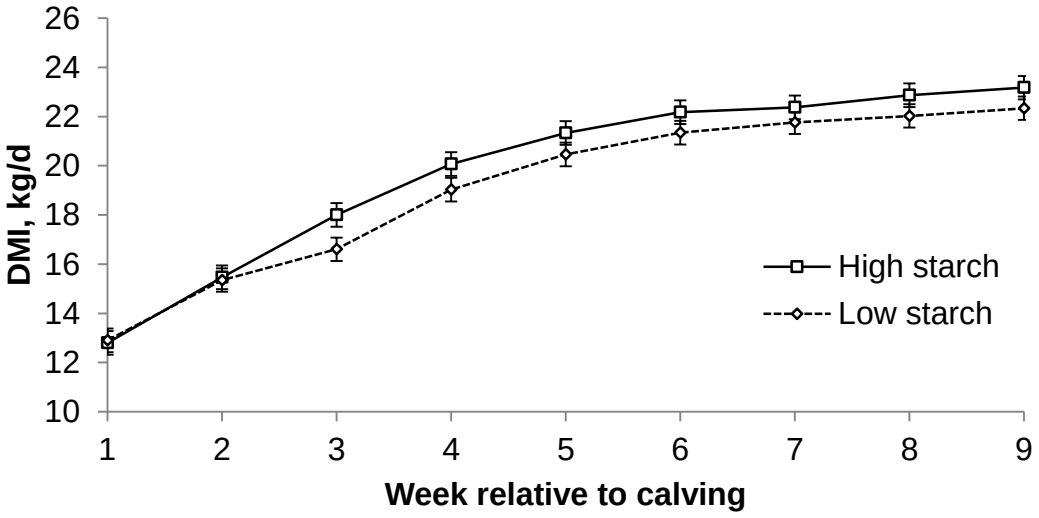


Figure 3-2. Least squares means for DMI (kg/d) for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data was analyzed separately as wk 1 to 3 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs low (21.5%) starch fed during wk 1 to 9. The *P* value for the overall effects of starch content was 0.21 for wk 1 to 3 and 0.13 for wk 1 to 9. The *P* value for the interaction of starch content × week was 0.04 for wk 1 to 3 and 0.32 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). Pre and postpartum effects of monensin were analyzed separately. The *P* value for the overall effect of monensin during the prepartum period was 0.37. The *P* value for the interaction of monensin × week prepartum was 0.39. The *P* value for the overall effect of monensin postpartum was 0.004 for wk 1 to 3 and 0.02 for wk 1 to 9. The *P* values for the interaction of monensin × week postpartum was 0.009 for wk 1 to 3 and < 0.001 for wk 1 to 9.



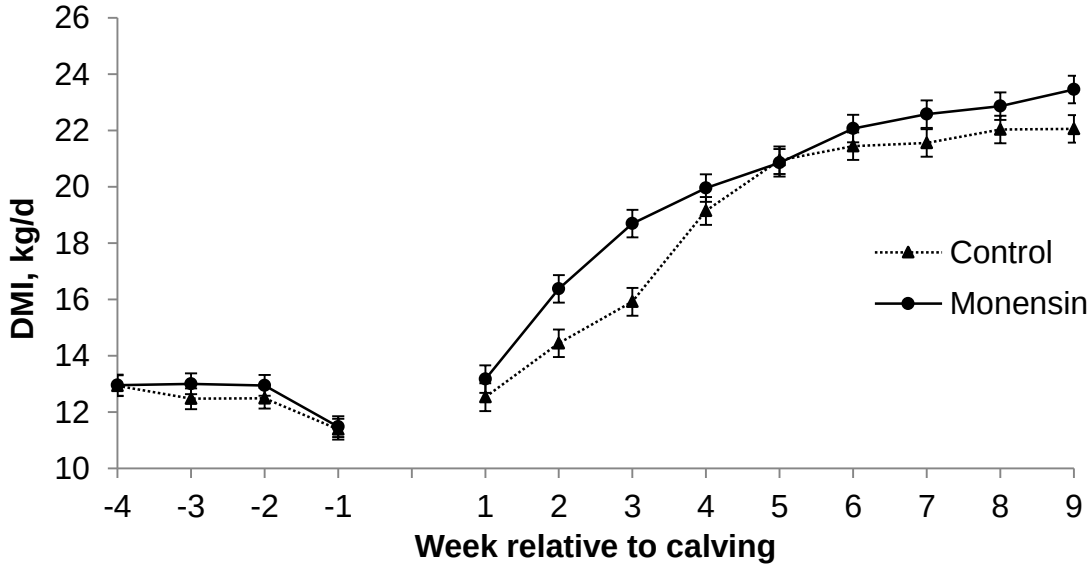
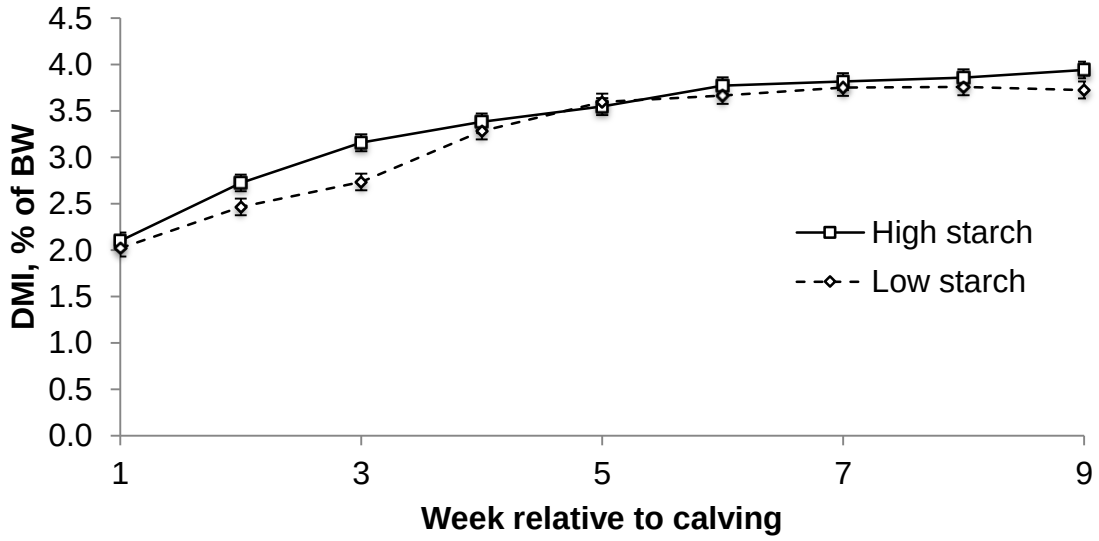


Figure 3-3. Least squares means for DMI expressed as a percentage of body weight for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data was analyzed separately as wk 1 to 3 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs low (21.5%) starch fed during wk 1 to 9. The *P* value for the overall effects of starch content was 0.006 for wk 1 to 3 and 0.03 for wk 1 to 9. The *P* value for the interaction of starch content × week was 0.01 for wk 1 to 3 and < 0.001 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). Pre and postpartum effects of monensin were analyzed separately. The *P* value for the overall effect of monensin during the prepartum period was 0.65. The *P* value for the interaction of monensin × week prepartum was 0.002. The *P* value for the overall effect of monensin postpartum was 0.20 for wk 1 to 3 and 0.11 for wk 1 to 9. The *P* values for the interaction of monensin × week was 0.29 for wk 1 to 3 and 0.66 for wk 1 to 9.



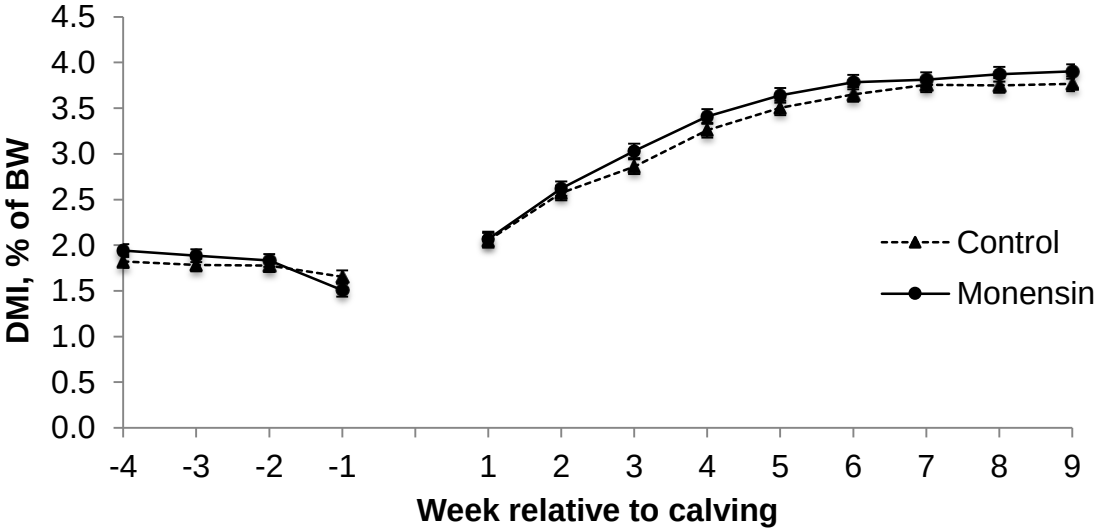
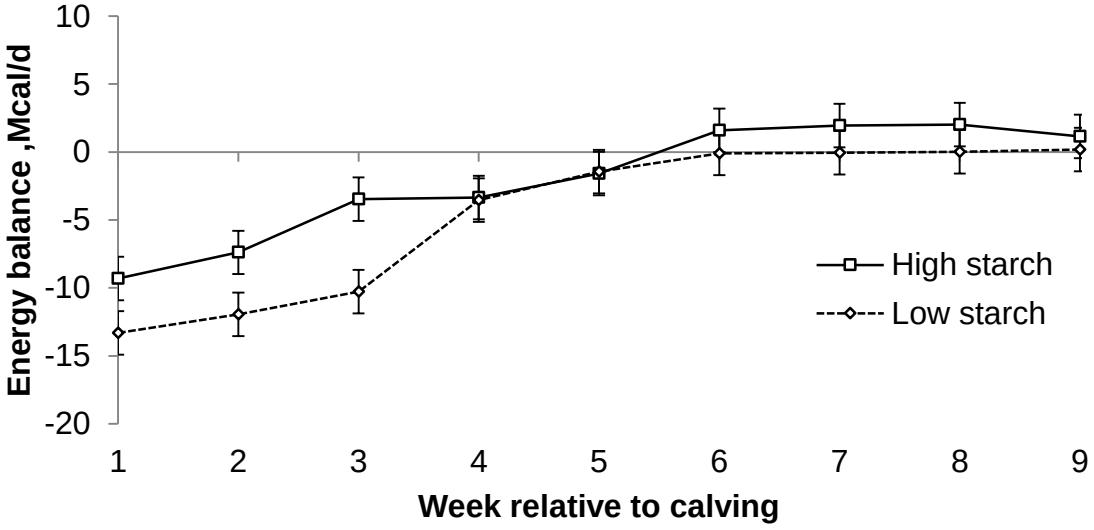
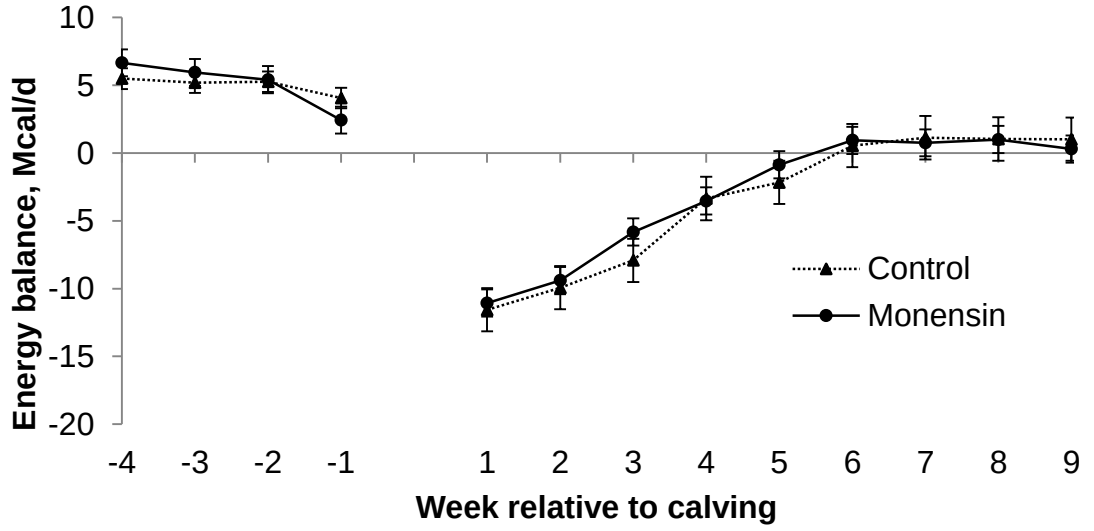


Figure 3-4. Least squares means for energy balance (Mcal/d) for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data was analyzed separately as wk 1 to 3 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs low (21.5%) starch fed during wk 1 to 9. The *P* value for the overall effects of starch content was < 0.001 for both wk 1 to 3 and wk 1 to 9. The *P* value for the interaction of starch content × week was 0.27 for wk 1 to 3 and < 0.001 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). Pre and postpartum effects of monensin were analyzed separately. The *P* value for the overall effect of monensin during the prepartum period was 0.88. The *P* value for the interaction of monensin × week prepartum was 0.009. The *P* value for the overall effect of monensin postpartum was 0.19 for wk 1 to 3 and 0.55 for wk 1 to 9. The *P* values for the interaction of monensin × week was 0.52 for wk 1 to 3 and 0.65 for wk 1 to 9.





DISCUSSION

Our overall hypothesis was that feeding diets with greater propiogenic potential during early lactation would increase milk yield because of increased gluconeogenic capacity. We also hypothesized that these more propiogenic diets would not have hypophagic effects on DMI because of the increased demand for glucose after calving. Postpartum, the estimated glucose requirements of the lactating mammary gland more than doubles compared to glucose requirements of the gravid uterus prepartum (Bell, 1995). These adaptations in glucose demand occur while DMI is generally decreased in the weeks prior to calving (Ingvartsen and Andersen, 2000; Hayirli et al., 2002;) and results in a state of negative EB postpartum. Ruminal propionate is the largest contributor to liver glucose output (Reynolds et al., 2003). Therefore, by increasing ruminal propiogenic substrate supply via dietary feeding strategies (e.g. feeding higher starch diets or monensin), the amount of propionate that is available to the liver is increased (Armentano and Young, 1983; Rabelo et al., 2003). An increase in hepatic glucose output increases the energy that is available to the cow postpartum. In the current study, increasing dietary starch content and the addition of monensin to the diet were the strategies employed to increase hepatic propionate supply after parturition. When the diets were analyzed in the CNCPS software using actual intakes and forage analyses (Table 3-4), the LS diet had a lower starch fermentability compared to the HS diet (17.1% vs. 21.9 % of DM); however, overall carbohydrate fermentability was very similar between the two diets (40.7% vs. 41.1% of DM). It is likely that the decreased starch fermentability in the LS diet slightly reduced the predicted metabolizable energy and metabolizable protein compared to the HS diet.

Over the first 9 wk of lactation overall milk yields were similar between cows fed HS and LS diets, although the cows fed the HS diet had increased milk yields in the immediate postpartum period. In the study of Andersen et al. (2003), cows fed a higher energy propiogenic diet (26.7% starch) had higher milk yields (38.8 kg/d vs. 30.5 kg/d) in the first 8 wk of lactation compared to cows fed a low energy diet (17.8% starch). Similarly, Rabelo et al. (2003) observed that cows fed a higher energy propiogenic postpartum diet (47.2% NFC) had a faster increase in milk yield compared to cows fed a lower energy diet postpartum (41.1% NFC). The increase in milk yield in early lactation seen across these studies is likely a result of greater gluconeogenic precursor supply to the liver that is provided by the higher energy propiogenic diets (with greater concentrate inclusion). In a recent study, cows that were precision-fed to maintain a 5 Mcal/d positive EB in early lactation (via increased % of concentrate offered) had sustained increases in milk yield that extended beyond the period in which the precision-fed cows were consuming a more energy dense diet than control cows, suggesting that higher energy propiogenic diets offered in early lactation have benefits to milk production over the longer term (Maltz et al., 2013).

In the current study, cows fed HS had lower milk component percentages than those fed LS, although milk component yields were not affected. Rabelo et al. (2003) observed that cows fed a high energy diet postpartum tended to have lower milk protein percentages, although other component percentages and yields were not affected by diet, while Andersen et al. (2003) determined that cows fed the high energy density diet had a lower fat percentage, a higher protein percentage, and higher milk lactose content. The effects of a higher energy diet on early lactation milk components have not been

consistent across studies; however, in the case of the current study the lower milk component percentages from HS cows are likely the result of these cows being in less negative EB and mobilizing less BCS during the immediate postpartum period than cows fed LS.

Allen et al. (2009) proposed that liver energy status may serve to regulate DMI and that increased propionate supply to the liver during the early postpartum period would likely decrease DMI. Increased propionate and fatty acid supply to the liver may reduce DMI through increased hepatic oxidation (Allen et al., 2009; Allen and Piantoni, 2013). However, we proposed that the modulation of DMI by propionate during early lactation is less than at other stages of lactation because NEFA likely are the predominant oxidative substrate for liver during this period because they are in such abundant supply (Reynolds et al., 2003), and any hypophagic effect of propionate would depend upon NEFA supply to the liver (Stocks and Allen, 2012, 2013). However, feeding higher energy propiogenic diets in early lactation have been reported to have positive effects on DMI, likely driving increases in milk yield (Andersen et al., 2003; Rabelo et al., 2003) and improved measures of energy metabolism (Andersen et al., 2004; Rabelo et al., 2005) observed in cows fed those diets. In the current study, cows fed HS had faster increases in DMI, as well as starch and NDF intakes during early lactation than cows fed LS. Cows fed the HS diet had lower feed efficiency expressed as milk/DMI and ECM/DMI compared to cows fed LS. However, because both DMI and EB were higher in HS cows immediately postpartum, this decreased feed efficiency is likely a reflection of increased DMI and less BCS mobilization postpartum rather than true reflections of decreased feed efficiency. In the current study, cows fed HS had

reached positive EB by wk 6 postpartum, whereas cows fed LS remained at zero EB from wk 6 through the end of the trial period. Rabelo et al. (2003) reported that cows fed a higher energy diet had greater ruminal propionate concentrations, and both Andersen et al. (2004) and Rabelo et al. (2005) observed increased plasma concentrations of glucose and insulin, and decreased BHBA for cows fed higher levels of starch and NFC, respectively, indicating improved EB concurrent with increased propionate supply for gluconeogenesis.

We hypothesized that we would not see any difference in apparent total tract starch digestibility because starch sources were the same in both diets. Cows fed the LS diet had higher apparent total tract NDF digestibility than those fed HS, which concurs with previous observations that cows fed diets containing higher concentrations of digestible NDF byproducts have increased apparent total tract NDF digestibility (Gencoglu et al., 2010; McCarthy et al., 2013).

Monensin treatment has been shown to increase ruminal propionate production (Armentano and Young, 1983). However, based upon the concepts presented in the hepatic oxidation theory (Allen et al., 2009), one could speculate that responses to monensin may vary depending upon dietary starch content, as both likely will increase supply of propionate. However, in the current study there was no interaction between starch and monensin for any of the production variables measured. The effects of monensin were not dependent on early lactation dietary starch content in this study, as such feeding monensin during the periparturient period and into early lactation would result in these responses regardless of if they are fed as part of a high starch or low starch diet during the early lactation period. Conversely, the effects of feeding a high or low

starch diet results in these responses regardless of if monensin is included in the diet or not. Interestingly, this lack of interaction between starch and monensin treatments on production performance variables has also recently been observed in midlactation cows (Akins et al., 2014).

In the current study, cows fed Mon during the periparturient period had faster increases in milk yield compared to Con and averaged 2.2 kg/d higher milk yield. When a controlled release capsule of monensin (335 mg/d) was administered in conjunction with a high energy postpartum diet, weekly milk yield was higher in monensin-treated cows compared to controls (Arieli et al., 2008). Using data from cows in all stages of lactation, monensin treatment resulted in a 2.3% increase in milk yield (Duffield et al., 2008b), although no effect of monensin treatment on early lactation milk production has also been observed (Mullins et al., 2012; Erasmus et al., 2005).

In the meta-analysis of Duffield et al. (2008b), monensin treatment resulted in an overall 2.3% decrease in DMI using data from cows in all stages of lactation. Supplementation of monensin during the periparturient period has been shown to either have no effect on early lactation intake (Van der Werf et al., 1998; Phipps et al., 2000; Chung et al., 2007) or decrease intermeal interval (Mullins et al., 2012), whereas in the current study, cows fed Mon had increased DMI during both wk 1 to 3 and wk 1 to 9 compared to Con. Regardless, monensin treatment in early lactation does not appear to have a hypophagic effect on DMI likely because of the increased need for propionate to support gluconeogenesis during this time period.

Arieli et al. (2008) observed no differences in milk component percentages between treatments although monensin treated cows had increased component yields.

In the current study, cows fed Mon had lower percentages of milk fat and lactose, although there was no effect of monensin treatment on component yields. The effects of monensin on milk components have been variable across studies (Duffield et al.; 2008b; Arieli et al., 2008; Mullins et al., 2012); however, in the current study the decreased milk component percentages for cows fed Mon are likely the result of these cows mobilizing less adipose tissue due to increased DMI in the immediate postpartum period.

Cows fed monensin in the current study had lower calculated early lactation feed efficiency (ECM/DMI), which is likely a reflection of higher DMI rather than biological differences in feed efficiency. Data from cows at all stages of lactation show small increases in BCS and body weight with monensin treatment (Duffield et al., 2008b), and monensin supplementation had been shown to improve energy metabolism (Duffield et al., 2008a) and reduce incidence of energy related diseases (Duffield et al., 2008c). This is likely because monensin increases ruminal propionate supply to the liver resulting in increased hepatic glucose production and improvements in EB of the cow. In the current study we observed improvements in EB as a percentage of requirement during wk 1 to 3 for cows fed Mon, indicating improvements in EB during very early lactation with Mon treatment.

CONCLUSIONS

In conclusion, cows fed more propiogenic diets in early lactation via increased starch content or monensin inclusion had increased milk yield and DMI during the immediate postpartum period. Cows fed high starch diets had lower fat, true protein and

lactose percentages, and cows fed monensin had lower fat and lactose percentages in early lactation, although there were no differences among treatments in overall milk component yields. Cows fed high starch diets or monensin had less negative EB during the immediate postpartum period. Overall, feeding more propiogenic diets via higher starch content and monensin inclusion favorably affected postpartum production outcomes, increased feed intake, and improved energy balance during the postpartum period.

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CHAPTER 4

METABOLISM OF EARLY LACTATION DAIRY COWS AS AFFECTED BY DIETARY STARCH AND MONENSIN SUPPLEMENTATION

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ABSTRACT

The objective of this study was to evaluate the impact of dietary starch content and monensin (Mon) on metabolism of dairy cows during early lactation. Prior to parturition primiparous (n=21) and multiparous (n=49) Holstein cows were fed a common controlled energy close up diet with a daily topdress of either 0 or 400 mg/d monensin. From d 1 to 21 postpartum, cows were fed a high starch (HS; 26.2% starch, 34.3% NDF, 22.7% ADF, 15.5% CP) or low starch (LS; 21.5% starch, 36.9% NDF, 25.2% ADF, 15.4% CP) total mixed ration (TMR) with a daily topdress of either 0 mg/d monensin (Con) or 450 mg/d monensin (Mon), continuing with prepartum topdress assignment. From d 22 through 63 postpartum all cows were fed HS and continued with assigned topdress treatment until d 63. Cows fed HS had higher plasma glucose and insulin and lower non-esterified fatty acids (NEFA) than cows fed LS during d 1 to 21 postpartum. Cows fed LS had elevated early lactation β-hydroxybutyrate (BHBA) compared to cows fed HS. Cows fed HS had greater insulin resistance and increased plasma haptoglobin in the early lactation period. There was no effect of Mon on postpartum plasma NEFA. Cows fed Mon had higher plasma glucose compared to Con cows, which was driven by a Mon × parity interaction in which primiparous cows fed Mon had greater plasma glucose concentrations than Con. Cows fed Mon had lower plasma BHBA compared to Con, which was contributed to by a Mon × parity interaction in which primiparous cows fed Mon had lower BHBA concentrations than Con. There was no effect of starch treatment on overall liver triglyceride content. Primiparous cows fed Mon had increased liver triglyceride content compared to Con primiparous cows

and multiparous cows fed Mon had decreased liver triglyceride content compared to Con cows. Multiparous cows fed LS with Mon had higher liver glycogen content than multiparous cows fed the LS without Mon, with no effect of Mon treatment for multiparous cows fed HS. There was no effect of starch or Mon treatment on liver capacity to oxidize propionate to CO_2, and effects of starch on gluconeogenesis were not significant. Cows fed Mon tended to have greater capacity to convert propionate to glucose than Con. Mon supplementation increased the ratio of glucose to CO_2, which indicated that cows fed Mon had a greater propensity to convert propionate to glucose. Overall, cows fed more propiogenic diets in early lactation (high starch or monensin) exhibited improved energy metabolism during early lactation.

Keywords: Early lactation, metabolism, starch, monensin

INTRODUCTION

Many postpartum metabolic disorders are a result of insufficient energy intake in the period immediately surrounding parturition. After calving, DMI is insufficient to support the high milk production of early lactation and results in a state of negative energy balance (**EB**), leading to greater mobilization of adipose tissue and the release of NEFA into circulation to be metabolized by the liver (Drackley, 1999). Higher energy intake postpartum results in lower circulating NEFA (Andersen et al., 2004; Rabelo et al., 2005) and has been associated with improved health (Ospina et al., 2010), performance (Andersen et al., 2003; Rabelo et al., 2003), and less severe postpartum negative EB (Ingvartsen and Andersen, 2000).

The fermentation of starch in the rumen favors production of propionate which is the main precursor for hepatic glucose production, and monensin (an ionophore)

supplementation has been shown to increase ruminal propionate production (Armentano and Young, 1983). It has been observed that cows fed higher energy diets postpartum (Andersen et al., 2002, 2004; Rabelo et al., 2005) and monensin during the peripartal period (Duffield et al., 2008a; Arieli et al., 2008) have improvements in postpartum energy metabolism.

The hepatic oxidation theory proposed by Allen et al. (2009) would suggest that feeding diets that promote greater ruminal propionate production (e.g., high in fermentable starch, monensin supplementation) during early lactation would be hypophagic and further exacerbate the state of negative EB due to the increased oxidation of propionate in the liver. However, it has been observed that cows fed more propiogenic diets have increased DMI in early lactation (Andersen et al., 2003; Rabelo et al., 2003; McCarthy et al., 2015). The hypophagic effect of propionate is likely to be reduced in the immediate postpartum period because of the large increases in hepatic energy demands at the onset of lactation (Reynolds et al., 2003). The increase in early lactation NEFA mobilization (Vernon, 2005) and subsequent hepatic uptake of NEFA and mitochondrial β-oxidation of fatty acyl CoA to acetyl CoA provides substantial amounts of oxidative substrate, in addition to propionate, to the tricarboxylic acid (**TCA**) cycle (Drackley et al., 2001).

When there is an increase in propionate supply to the liver, early lactation cows appear to have an increased propensity to convert the propionate to glucose rather than oxidize it (Drackley et al., 2001). The rate of gluconeogenesis from [1-^{14}C]propionate in liver slices from early lactation cows are increased compared to the rate in liver slices from the same cows once they have reached mid lactation (Aiello et al., 1989). Drackley

et al. (2001) saw a positive correlation between carbohydrate intake in the immediate postpartum period with the efficiency of [1-^{14}C]propionate conversion to glucose in liver biopsy slices, which would suggest that the liver has the capacity to direct additional propionate toward glucose during early lactation.

Monensin has been shown to decrease periparturient negative EB associated health disorders and improve energy metabolism (Duffield et al., 2008b; 2008c). However, it is of interest to determine whether effects of monensin in fresh cows are independent of dietary starch content, as both likely will increase supply of propionate, leading to increased hepatic oxidative supply. The objectives of this study were to evaluate the effect of dietary starch content during the immediate postpartum period and monensin inclusion during the periparturient period and into early lactation on metabolic indices related to energy metabolism and in vitro hepatic gluconeogenesis. We hypothesized that increasing starch content during the immediate postpartum period and feeding monensin throughout the periparturient period and into early lactation would increase hepatic gluconeogenesis as well as improve measures of energy metabolism.

MATERIALS AND METHODS

Animals and dietary treatments

All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee and the experiment was conducted from March to October 2012. The experimental design and treatments were described more completely in the companion paper (McCarthy et al., 2015). Briefly, the study was a completely randomized design with randomization restricted to balance for expected calving date

of primiparous and multiparous cows and previous lactation 305-d mature-equivalent milk production for multiparous cows. A 2 × 2 factorial arrangement of postpartum treatments was utilized with early lactation period feeding strategy [high starch (**HS**) vs. low starch (**LS**) diet during the first 21 d postpartum] and monensin supplementation [0 mg monensin/d (**Con**) or 450 mg monensin/d (**Mon**); monensin; Elanco Animal Health, Greenfield, IN] as the variables of interest. In addition, cows that received Mon during the postpartum period were fed Mon (400 mg/d) initiated on 1 d between d 21-28 before expected parturition (average treatment of 25 d; minimum of 14 d on treatment before actual parturition was required for inclusion in the data set). The final dataset included 70 cows (primiparous n = 21, multiparous n = 49). Lactating cows were dried off at least 45 d (average 53 d dry period length) prior to expected parturition, and moved to the experimental tie stall barn approximately 28 d prior to expected parturition where they began consuming the experimental close up dry cow diet.

Diet ingredients are presented in Table 4-1 and nutrient composition are presented in Table 4-2. Procedures and methods for feed sampling and analysis are detailed in McCarthy et al. (submitted). The topdress pellets were formulated to contain either 0 (Con) or 461 g/metric ton Mon and were fed as a daily topdress at rates of 0.85 kg/d prepartum and 0.95 kg/d postpartum. The Mon topdress was targeted to provide 400 mg/d prepartum and 450 mg/d postpartum. Cows continued to receive assigned topdress treatments through d 63 postpartum.

Plasma and Tissue Sampling and Analyses

Blood samples were collected via venipuncture of the coccygeal vessels using heparinized Vacutainer (Becton Dickinson, Franklin Lakes, NJ) tubes 1 h prior to

feeding. Blood samples were collected 1×/wk prepartum beginning the week prior to commencement of prepartum topdress treatments, 3×/wk from calving through 21 d postpartum, and 1×/wk from d 22 to 63. Blood samples were placed on ice immediately following collection and plasma was harvested after centrifugation of the blood at 1300 × g for 15 min at 4°C. Plasma was stored at −20°C until subsequent analysis for glucose, NEFA, BHBA, haptoglobin, and insulin. Plasma concentrations of glucose were determined by enzymatic analysis (glucose oxidase; protocol from Sigma-Aldrich kit 510-A; St. Louis, MO) using commercial products (PGO Enzyme Preparation and o-dianisidine dihydrochloride, Sigma-Aldrich, St. Louis, MO). Plasma concentrations of NEFA were also analyzed by enzymatic analysis (HR Series NEFA HR (2), Wako Pure Chemical Industries, Osaka, Japan). Plasma concentrations of BHBA were analyzed by enzymatic analysis (BHBA dehydrogenase) using commercial products (Nicotinamide adenine dinucleotide and TRIS (hydroymethyl)aminomethane, Sigma-Aldrich; 3-hydroxybutyrate dehydrogenase, Roche Diagnostics Co., Indianapolis, IN). Plasma concentrations of haptoglobin were only determined on d 1 to 15 plasma samples, and were analyzed by enzymatic analysis using a commercial kit that measures haptoglobin-hemoglobin complex by estimated differences in peroxidase activity (Haptoglobin Assay, kit no. TP801, Tridelta Diagnostics Ltd., Morris Plains, NJ). All spectrophotometric measurements were conducted using a Versamax tunable microplate reader (Molecular Devices, Sunnyvale, CA). Inter- and intra-assay variation was maintained at < 10% for all enzymatic assays. Plasma concentrations of insulin were determined by double-antibody RIA (Porcine Insulin RIA cat. no. PI-12K, Linco Research, Millipore, St. Charles, MO), with a reported specificity to bovine insulin of

90%. Inter- and intra-assay coefficients of variation were 13.3 and 13.5%, respectively.

The revised quantitative insulin sensitivity check index (**RQUICKI**), a relative insulin sensitivity measure used in ruminants, was calculated as follows (Holtenius and Holtenius, 2007):

RQUICKI = 1/[log(glucose, mg/dL) + log(insulin, μU/mL) + log(NEFA, mmol/L)],

such that a lower RQUICKI suggests greater insulin resistance.

Liver biopsy, liver metabolic incubations, and liver composition

Liver tissue (3-5 g) was sampled via percutaneous trocar biopsy (Veenhuizen et al., 1991) from cows under local anesthesia on d 7 (± 4 d range; ± 1.6 SD) relative to parturition. Biopsies were obtained from at least 7 multiparous cows and 4 primiparous cows from each treatment. After blotting the liver sample to remove excess blood and connective tissue, a portion of the sample was immersed in ice-cold PBS (0.015 *M*; 0.9% NaCl, pH 7.4) and transported to the laboratory within 45 min of tissue collection. The remaining portion of liver tissue that was collected was snap-frozen in liquid N and stored at -80°C until analysis for triglyceride and glycogen content. Liver triglyceride content was determined using the Folch extraction method (Folch et al., 1957) followed by a colorimetric method based upon the Hantzsch condensation for estimating serum triglyceride (Fletcher, 1968) with modifications described by Foster and Dunn (1973). Glycogen content of liver was determined according to the procedures described in Hawk and Bergeim (1926) with modifications described by Bernal-Santos et al. (2003).

Hepatic capacities for conversion of [1-^{14}C]propionate (1 μCi per flask; American Radiolabeled Chemicals, Inc., St. Louis, MO) in Krebs-Ringer bicarbonate

media (final substrate concentration of 10 m*M*) to CO_2 and glucose were measured in triplicate flasks using tissue slices (60 to 80 mg) according to procedures described by Piepenbrink et al. (2004). Tissue metabolism was terminated using 0.5 mL of 0.75 *M* H_2SO_4 injected into the media at either 0 (blanks) or 120 min of incubation. After termination of tissue metabolism, evolved CO_2 was collected on NaOH-soaked (0.1mL; 30% wt/vol) filter paper in a hanging center well for 1 h in a shaking ice-water bath. After 1 h, flasks were uncapped and the filter paper was removed to a scintillation vial and dried overnight under moving air at 39° C. Ten mL of scintillation cocktail (Scintisafe Econo 2; Fisher Scientific, Pittsburgh, PA) was added to each vial and radioactivity was measured using liquid scintillation spectroscopy (2200 CA Tricarb Liquid Scintillation Analyzer, Packard Instrument Co., Meriden, CT).

After uncapping the flasks and removing the hanging center well for measurement of CO_2, the contents of each flask were processed for gluconeogenesis. An internal standard ([3H] L-glucose, 0.055 μCi per flask; American Radiolabeled Chemicals, Inc., St. Louis, MO) was added to the media, and flasks were neutralized and deproteinized by additions of saturated $Ba(OH)_2$ solution. Radioactive glucose from media supernatants was isolated using an ion-exchange method of Azain et al. (1999) as modified by Piepenbrink et al. (2004). Radioactivity was measured using dual-label liquid scintillation spectroscopy.

Statistical analyses

Statistical computations were performed using SAS software (version 9.2; SAS Institute Inc., Cary, NC). Data were analyzed separately as wk -4 to -1 prepartum, d 1 to 21 (dietary treatment period), and wk 1 to 9 postpartum (duration of experiment). The

3 × wk sampling schedule from d 1 to 21 postpartum resulted in cows being sampled on different d relative to calving, thus 3 d average d intervals were calculated for these analyses. For weeks with multiple blood samples/wk (wk 1 to 3) data were averaged to weekly means prior to wk 1 to 9 analyses. The prepartum model include the fixed effects Mon treatment, parity, and the 2 way interactions. The random effect was cow nested within Mon treatment. Postpartum data were analyzed as a completely randomized design with a 2 × 2 factorial arrangement of treatments. Fixed effects included starch content, Mon treatment, parity, time, and all 2 way interactions. A prepartum covariate (week prior to starting topdress treatment) was used for all plasma glucose, NEFA, BHBA, insulin, and RQUICKI analyses. The random effect was cow nested within starch and Mon treatment. Data measured over time were subjected to ANOVA using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). For variables with measurements repeated over time, four covariance structures were tested: compound symmetry, heterogeneous compound symmetry, first-order autoregressive, and heterogeneous first-order autoregressive and the covariance structure that resulted in the smallest Akaike's information criterion was used (Littell et al., 1996). Data not analyzed over time were subjected to ANOVA using the MIXED procedure of SAS (Littell et al., 1996). Fixed effects included starch content, Mon treatment, parity, and all 2 way interactions. The random effect was cow nested within starch and Mon treatment. Degrees of freedom were estimated by using the Kenward-Roger option in the model statement. Statistical significance was declared at $P \leq 0.05$ and trends were discussed at $0.05 < P < 0.10$, with the exception of the in vitro liver metabolism. Because a subset of cows was used for this analysis, trends were declared at $0.05 < P \leq 0.15$.

Effects of parity, starch, and Mon on occurrence of subclinical ketosis and health outcomes were analyzed by chi-square for each effect using FREQ procedure of SAS, with statistical significance declared at $P \leq 0.05$ and trends discussed at $0.05 < P \leq 0.15$.

RESULTS

Prepartum plasma metabolites

There was no effect of Mon treatment on prepartum plasma metabolite concentrations for glucose, NEFA, BHBA, insulin, or RQUICKI (Table 4-3).

Postpartum plasma metabolites

Postpartum plasma metabolite results are presented in Table 4-4. Cows fed HS had higher plasma glucose concentrations than cows fed LS during d 1 to 21 postpartum ($P = 0.003$) and tended to have higher glucose during wk 1 to 9 ($P = 0.06$). There was a starch × wk interaction (Figure 4-1A; $P = 0.007$) during wk 1 to 9 such that cows that were fed HS had higher plasma glucose concentrations during d 14 through 21 compared to cows fed LS; however, LS cows maintained similar plasma glucose concentrations as HS cows after all cows were switched to the HS diet beginning on d 22 postpartum. Cows fed HS had lower plasma NEFA concentrations during both d 1 to 21 ($P = 0.002$) and wk 1 to 9 ($P = 0.002$) postpartum than cows fed LS. There was an interaction of starch × wk for wk 1 to 9 (Figure 4-2A; $P = 0.01$) such that cows fed HS had lower plasma NEFA concentrations during wk 1, 2, and 3 compared to cows fed LS; however there was no difference between treatments in plasma NEFA concentrations after cows fed LS started consuming the HS diet on d 22 postpartum. There was a starch × parity interaction ($P = 0.04$) for wk 1 to 9 data and primiparous cows fed LS had higher plasma NEFA than primiparous cows fed HS (503.3 vs. 361.3

μEq/L), but plasma NEFA concentrations in multiparous cows were similar between the two diets (average 393.6 μEq/L). During d 1 to 21 cows fed HS had lower plasma BHBA concentrations compared to cows fed LS ($P = 0.03$). There was a starch × time interaction during both d 1 to 21 ($P = 0.04$) and wk 1 to 9 (Figure 4-3A; $P = 0.02$) because cows fed LS had elevated early lactation BHBA concentrations compared to cows fed HS. Cows fed HS had greater insulin concentrations during both d 1 to 21 ($P = 0.008$) and wk 1 to 9 ($P = 0.004$; Figure 4-4A). There was a tendency for a starch × parity interaction ($P = 0.09$) during wk 1 to 9 because primiparous cows fed HS tended to have larger increases in insulin concentrations than multiparous cows (0.32 vs. 0.21 ng/mL) with less difference between primiparous and multiparous cows fed LS (0.24 vs. 0.19 ng/mL). Cows fed HS had lower RQUICKI than cows fed LS during d 1 to 21 ($P = 0.002$) and tended to have lower RQUICKI during wk 1 to 9 (Figure 4-5A; $P = 0.09$). However, there was a starch × Mon interaction ($P = 0.02$) for RQUICKI during d 1 to 21 such that cows fed HS and Mon had a lower RQUICKI compared to cows fed HS without Mon, and no differences for cows fed LS with or without Mon. Cow fed HS had higher haptoglobin concentrations (Figure 4-6A; $P = 0.04$) during d 1 to 15 postpartum compared to cow fed LS.

Cows fed Mon during the transition period had higher plasma glucose concentrations than Con cows during both d 1 to 21 ($P < 0.001$) and wk 1 to 9 (Figure 4-1B; $P = 0.005$). There was a Mon × parity interaction ($P = 0.008$) during d 1 to 21 such that primiparous cows fed Mon had greater increases in plasma glucose concentration than did primiparous Con cows (62.0 vs. 54.2 mg/dL) with less difference in plasma glucose concentrations for multiparous cows fed Mon and Con (54.1 vs. 52.4

mg/dL). Although there was no effect of Mon treatment on postpartum plasma NEFA (Figure 4-2B), cows fed Mon during the transition period had lower plasma BHBA during d 1 to 21 ($P = 0.002$) and tended to have lower plasma BHBA during wk 1 to 9 (Figure 4-3B; $P = 0.06$) compared to Con cows. There was a Mon × parity interaction ($P = 0.03$) during d 1 to 21 such that primiparous cows fed Mon had larger decreases in BHBA than did primiparous Con cows (10.11 vs. 13.99 mg/dL) with less difference in plasma BHBA concentrations for multiparous cows fed Mon and Con (10.04 vs. 11.33 mg/dL). There was a Mon × d ($P = 0.04$) interaction for plasma insulin concentration during d 1 to 21, although differences were inconsistent and variable across the time period studied. Plasma insulin for wk 1 to 9 is shown in Figure 4-4B. While there was no overall effect of Mon treatment on postpartum plasma RQUICKI concentrations (Figure 4-5B), there was a tendency for an interaction of Mon × parity ($P = 0.09$) during d 1 to 21 for RQUICKI, although RQUICKI between Mon and Con primiparous cows were similar (0.46 vs. 0.48) as was RQUICKI between Mon and Con multiparous cows (0.52 vs. 0.51). There were no effects of Mon treatment on postpartum plasma haptoglobin concentrations (Figure 4-6B).

Subclinical ketosis and health outcomes

Subclinical ketosis results are presented in Table 4-5. Feeding HS during early lactation tended to result in fewer cows with 2 or more consecutive days of plasma BHBA greater than 12 mg/dL ($P = 0.15$). Mon treatment resulted in a decreased percentage of cows with 1 or more days with plasma BHBA concentrations greater than 12 mg/dL ($P = 0.02$) and also with 2 or more consecutive days with a plasma BHBA concentration greater than 12 mg/dL ($P = 0.009$) compared to Con cows.

The frequency of occurrence of various health disorders is reported in Table 6. However, because of the small population of cows studied, care should be taken in extrapolating these results. Health event occurrences were not greatly different among dietary treatments, however, cows fed HS tended to have less cases of clinical ketosis ($P = 0.15$). Cows on the Mon treatment had less cases of clinical ketosis ($P = 0.03$), and tended to have incidence greater number of retained placenta and dystocia cases ($P = 0.10$ for both) compared to cows receiving the Con topdress; however, there were only a total of 3 cases of retained placenta and dystocia. The 3 cases of dystocia occurred in primiparous cows ($P = 0.02$; parity effect).

Liver composition

Results for postpartum liver composition are shown in Table 4-7. There was no effect of starch treatment on liver triglyceride content in biopsies taken on d 7 (± 1.6 SD) postpartum. There was a Mon × parity interaction ($P = 0.05$) such that primiparous cows fed Mon during the transition period had increased liver triglyceride content compared to Con primiparous cows (6.1 vs. 3.7% of wet wt.), while multiparous cows fed Mon had decreased liver triglyceride content compared to multiparous Con cows (6.1 vs. 9.4% of wet wt.). For liver glycogen there was a starch × Mon interaction ($P = 0.008$) and cows fed LS with Mon had higher liver glycogen content than cows fed LS without Mon, with no effect of Mon treatment for cows fed HS.

In vitro liver metabolism

Results for in vitro liver metabolism are presented in Table 4-8. Cows fed Mon tended ($P = 0.14$) to have greater capacity to convert [1-^{14}C]propionate to glucose than Con cows. Cows fed Mon had an increased ratio of glucose to CO_2 ($P = 0.05$),

indicating that cows fed Mon had a greater propensity to convert propionate to glucose. There was no effect of starch or Mon treatment on liver capacity to oxidize [1-^{14}C]propionate to CO_2 and effects of starch on liver capacity to convert propionate to glucose and on the ratio of glucose to CO_2 were not significant. Primiparous cows had greater capacity to both oxidize [1-^{14}C]propionate to CO_2 and convert [1-^{14}C]propionate to glucose than did multiparous cows (P = 0.04 and 0.01, respectively).

Table 4-1. Ingredient composition of the basal diets (% of DM)

	Prepartum	Postpartum[1]	
		HS	LS
Corn silage, processed	42.14	–	–
BMR corn silage[2]	–	38.50	38.50
Wheat straw	21.75	11.54	11.55
Legume silage	–	9.62	9.62
Shelled corn, finely ground	4.28	20.97	10.29
Citrus pulp	7.23	1.01	7.15
Corn germ meal	–	2.52	5.56
Soybean hulls	7.08	–	3.58
Soybean meal	5.27	5.87	3.86
Canola meal	4.63	2.73	2.08
Blood meal	1.05	1.94	1.93
Amino Plus[3]	1.78	1.70	2.34
Energy Booster 100[4]	–	0.77	0.96
Calcium carbonate	1.53	1.12	0.82
Sodium bicarbonate	–	0.86	0.85
Soy Chlor[5]	1.33	–	–
Salt	0.16	0.42	0.41
Calcium sulfate	0.73	0.17	0.17
Magnesium oxide	0.20	0.15	0.15
Magnesium sulfate, 9.9%	0.61	–	–
Selenium 0.06%	0.04	0.05	0.05
Mono dicalcium phosphate	–	0.02	0.07
Trace mineral premix[6]	0.02	0.03	0.03
Vitamin A, D, E premix[7]	0.05	0.02	0.02
Vitamin E premix[8]	0.005	–	–
Zinc sulfate	0.002	–	–
Copper sulfate	0.0004	0.001	0.001

[1]HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]BMR= brown mid-rib corn silage.
[3]AGP Inc., Omaha, NE.
[4]Milk Specialties Global; Carpentersville, IL.
[5]West Central, Ralston, IA.
[6]Contained 30,317 mg/kg of Cu, 136,466 mg/kg of Mn, 3,393 mg/kg of Co, 3,040 mg/kg of I, and 153,916 mg/kg of Zn.
[7]Contained 30,464 IU/kg of Vitamin A, 5,862 IU/kg of Vitamin D, and 93,784 IU/kg of Vitamin E.
[8]Contained 510,750 IU/kg of Vitamin E.

Table 4-2. Chemical composition of experimental diets (± SD[1])

	Prepartum diet	Postpartum diet[2]		Topdress pellet[3]	
Item		HS	LS	Con[4]	Mon[5]
DM, %	50.7 ± 2.4	48.3 ± 2.7	48.0 ± 3.2	93.2 ± 1.0	93.7 ± 1.2
CP, %	13.0 ± 0.8	15.5 ± 1.2	15.4 ± 0.8	37.5	37.0
ADF, %	28.2 ± 1.2	22.7 ± 1.2	25.2 ± 1.2	11.1	12.9
NDF, %	42.9 ± 2.0	34.3 ± 1.5	36.9 ± 1.5	22.6	21.3
30 h NDFD[6], %	–	18.9 ± 1.2	20.7 ± 1.1	–	–
30 h NDFD[6], % of NDF	–	55.1 ± 2.0	56.1 ± 1.4	–	–
Sugar, %	4.9 ± 0.8	3.5 ± 0.6	4.5 ± 0.4	10.6	11.3
Starch, %	17.4 ± 1.2	26.2 ± 1.2	21.5 ± 1.0	13.1	13.8
Fat, %	2.6 ± 0.2	4.0 ± 0.2	2.2 ± 0.6	2.4	2.5
Ca, %	1.28 ± 0.16	0.94 ± 0.09	1.01 ± 0.04	0.51	0.6
P, %	0.30 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.97	0.99
Mg, %	0.41 ± 0.04	0.28 ± 0.02	0.3 ± 0.03	0.48	0.48
K, %	1.12 ± 0.13	1.12 ± 0.09	1.18 ± 0.08	1.67	1.70
S, %	0.37 ± 0.04	0.21 ± 0.09	0.22 ±0.01	0.46	0.44
Na, %	0.12 ± 0.02	0.47 ±0.08	0.46 ± 0.05	0.07	0.07
Cl, %	0.37 ± 0.01	0.44 ± 0.04	0.44 ± 0.03	0.07	0.09
NE_L, Mcal/kg	1.48 ± 0.03	1.64 ± 0.02	1.56 ± 0.03	1.72	1.74

[1]Chemcial composition was analyzed on 6 composite samples of the prepartum diet, 7 composite samples of the high starch postpartum diet, and 6 composite samples of the low starch postpartum diet.

[2]HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).

[3]The composition of the topdress was 33.6% soybean meal, 33.2% wheat middlings, and 33.2% canola meal (DM basis).

[4]Con = control topdress, formulated to supplement 0 mg/d monensin.

[5] Mon = monensin topdress, formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.

[6]NDFD = NDF digestibility.

Table 4-3. Prepartum plasma metabolites as affected by monensin supplementation beginning during the prepartum period

	Prepartum diet[1]			*P*-values[4]					
Item	Con[2]	Mon[3]	SEM	Mon	Parity	T	Mon × T	Mon × Parity	Parity × T
Glucose, mg/dL	64.3	66.4	1.1	0.17	0.48	0.18	0.79	0.73	0.44
NEFA μEq/L	197.0	216.1	10.5	0.19	0.02	<0.001	0.13	0.77	0.69
BHBA mg/dL	8.00	7.73	0.21	0.34	0.07	0.49	0.60	0.15	0.48
Insulin, ng/mL	0.24	0.24	0.02	0.99	0.001	<0.001	0.24	0.59	<0.001
RQUICKI[5]	0.48	0.48	0.01	0.94	<0.001	0.48	0.19	0.91	0.72

[1]Con = control topdress and Mon = monensin topdress.
[2]Formulated to supplement 0 mg/d monensin.
[3]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[4]T = time.
[5]Revised quantitative insulin sensitivity check index (RQUICKI) = 1/[log(glucose) + log(insulin) + log(NEFA)].

Table 4-4. Postpartum plasma metabolites for cows fed either high or low starch diets during the first 3 wk postpartum and control or monensin treatments throughout the periparturient period and into early lactation

	Diet[1]			Topdress[2]			*P*-values[5]							
Item	HS	LS	SEM	Con[3]	Mon[4]	SEM	S	M	P	S×M	S×T	M×T	S×P	M×P
Glucose, mg/dL														
d 1 to 21	57.5	53.9	0.85	53.3	58.1	1.0	0.003	<0.001	<0.001	0.34	0.19	0.76	0.31	0.008
wk 1 to 9	59.3	57.0	0.9	56.4	59.9	0.9	0.06	0.005	0.01	0.32	0.007	0.48	0.73	0.18
NEFA μEq/L														
d 1 to 21	533.1	696.6	35.9	643.9	585.8	35.4	0.002	0.25	0.42	0.23	0.13	0.29	0.37	0.43
wk 1 to 9	386.0	456.9	19.8	412.6	413.2	19.6	0.002	0.98	0.20	0.34	0.01	0.21	0.04	0.45
BHBA mg/dL														
d 1 to 21	10.51	12.22	0.57	12.66	10.08	0.58	0.03	0.002	0.10	0.35	0.04	0.41	0.56	0.03
wk 1 to 9	9.20	9.78	0.40	10.02	8.80	0.48	0.29	0.06	0.02	0.24	0.02	0.15	0.92	0.16
Insulin, ng/mL														
d 1 to 21	0.26	0.19	0.02	0.22	0.23	0.02	0.008	0.88	<0.001	0.43	0.20	0.04	0.17	0.33
wk 1 to 9	0.27	0.21	0.01	0.24	0.24	0.01	0.004	0.97	<0.001	0.14	0.14	0.12	0.09	0.13
RQUICKI[6]														
d 1 to 21	0.48	0.51	0.01	0.49	0.49	0.01	0.002	0.69	<0.001	0.02	0.95	0.21	0.29	0.10
wk 1 to 9	0.51	0.53	0.01	0.53	0.51	0.01	0.09	0.29	0.003	0.11	0.92	0.38	0.74	0.03
Haptoglobin, g/L														
d 1 to 15	1.03	0.77	0.09	0.93	0.88	0.11	0.04	0.70	0.66	0.27	0.48	0.37	0.41	0.56

[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, P = parity, and T = time.
[6]Revised quantitative insulin sensitivity check index (RQUICKI) = 1/[log(glucose) + log(insulin) + log(NEFA)].

Table 4-5. Percentage of cows with elevated BHBA (≥ 12 mg/dL) during d 1 to 21 as affected by dietary treatment

Percentage of cows, %	Diet[1]		Topdress[2]		Parity		*P*-values[5]		
	HS	LS	Con[3]	Mon[4]	Primi	Multi	S	M	P
≥ 1 d of BHBA ≥ 12 mg/dL	63.9	67.7	78.4	51.5	66.7	65.3	0.74	0.02	0.91
≥ 2 d of BHBA ≥ 12 mg/dL	44.4	61.8	67.6	36.4	57.1	51.0	0.15	0.009	0.64

[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, and P = parity.

Table 4-6. Health events for cows fed either high or low starch diets during the first 3 wk postcalvingpostpartum and control or monensin treatments throughout the periparturient period and into early lactation [1]

[1]Health events for cows that remained in the final data set.

	Diet[1]		Topdress[2]		Parity		*P*-values[5]		
Item[3]	HS	LS	Con[3]	Mon[4]	Primi	Multi	S	M	P
Hypocalcemia	2	0	1	1	0	2	0.26	0.51	0.49
Ketosis	6	3	8	1	3	6	0.15	0.02	0.29
Displaced abomasum	0	0	0	0	0	0	0.99	0.99	0.99
Retained placenta	2	1	0	3	1	2	0.39	0.10	0.45
Dystocia	2	1	0	3	3	0	0.39	0.10	0.02
Mastitis	4	3	3	4	2	5	0.29	0.27	0.33
Total health disorders	16	8	12	12	9	15			

[2]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch), Con = control topdress (formulated to supplement 0 mg/d monensin) and Mon = monensin topdress (formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum).

[3]Disorders defined as: hypocalcaemia = cows that had decreased body temperature and were unable to stand were treated with calcium dextrose; ketosis = cows that had rapid decreases in milk production and DMI were checked with Precision Xtra blood meter, readings ≥ 2.6 mmol/L were treated with dextrose and propylene glycol; displaced abomasum = movement of the fourth compartment of the stomach to a location on the right or left side of the cow and detected by auscultating a “ping” sound with finger percussion; retained placenta = cows that retained afterbirth for > 24 hours; dystocia = cows that had a calving ease score of > 3; mastitis = cows with abnormal milk, or inflamed quarter were treated with antibiotics.

Table 4-7. Liver composition at 7 d (± 1.6 SD) postpartum for cows fed either high or low starch diets during the first 3 wk postpartum and control or monensin treatments throughout the periparturient period and into early lactation

	Diet[1]			Topdress[2]			*P*-values[5]					
Item	HS	LS	SEM	Con[3]	Mon[4]	SEM	S	M	P	S×M	S×P	M×P
Liver triglycerides, % of wet wt.	6.68	5.97	1.09	6.56	6.08	1.13	0.63	0.75	0.07	0.61	0.67	0.05
Liver glycogen, % of wet wt.	0.90	0.80	0.12	0.77	0.93	0.13	0.52	0.33	0.03	0.008	0.26	0.63

[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, and P = parity.

Table 4-8. Conversion of [1-^{14}C]propionate, µmol/(g wet wt × h) to CO_2 and glucose µmol/(g wet wt × h) on d 7 (± 1.6 SD) postpartum for cows fed varying content of starch and monensin throughout the periparturient period and into early lactation

	Diet[1]			Topdress[2]			*P*-values[5]					
Item	HS	LS	SEM	Con[3]	Mon[4]	SEM	S	M	P	S×M	S×P	M× P
CO_2	6.13	6.40	0.37	6.38	6.16	0.37	0.58	0.66	0.04	0.82	0.29	0.69
Glucose	4.04	4.40	0.31	3.90	4.53	0.31	0.40	0.14	0.01	0.39	0.22	0.30
Glucose:CO_2 Ratio	0.66	0.67	0.04	0.61	0.72	0.05	0.97	0.05	0.40	0.58	0.79	0.30

[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, P = parity, and I = insulin.

Figure 4-1. Least squares means for plasma glucose (μEq/L) for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data were analyzed separately as d 1 to 21 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs. low (21.5%) starch fed during wk 1 to 9. The *P* values for the overall effects of starch content were 0.003 during d 1 to 21 and 0.06 during wk 1 to 9. The *P* values for the interaction of starch content × time were 0.19 for d 1 to 21 and 0.007 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). The *P* values for the overall effect of monensin were 0.17 prepartum, <0.001 for d 1 to 21, and 0.005 for wk 1 to 9. The *P* values for the interaction of monensin × time were 0.79 prepartum, 0.76 for d 1 to 21, and 0.48 for wk 1 to 9.

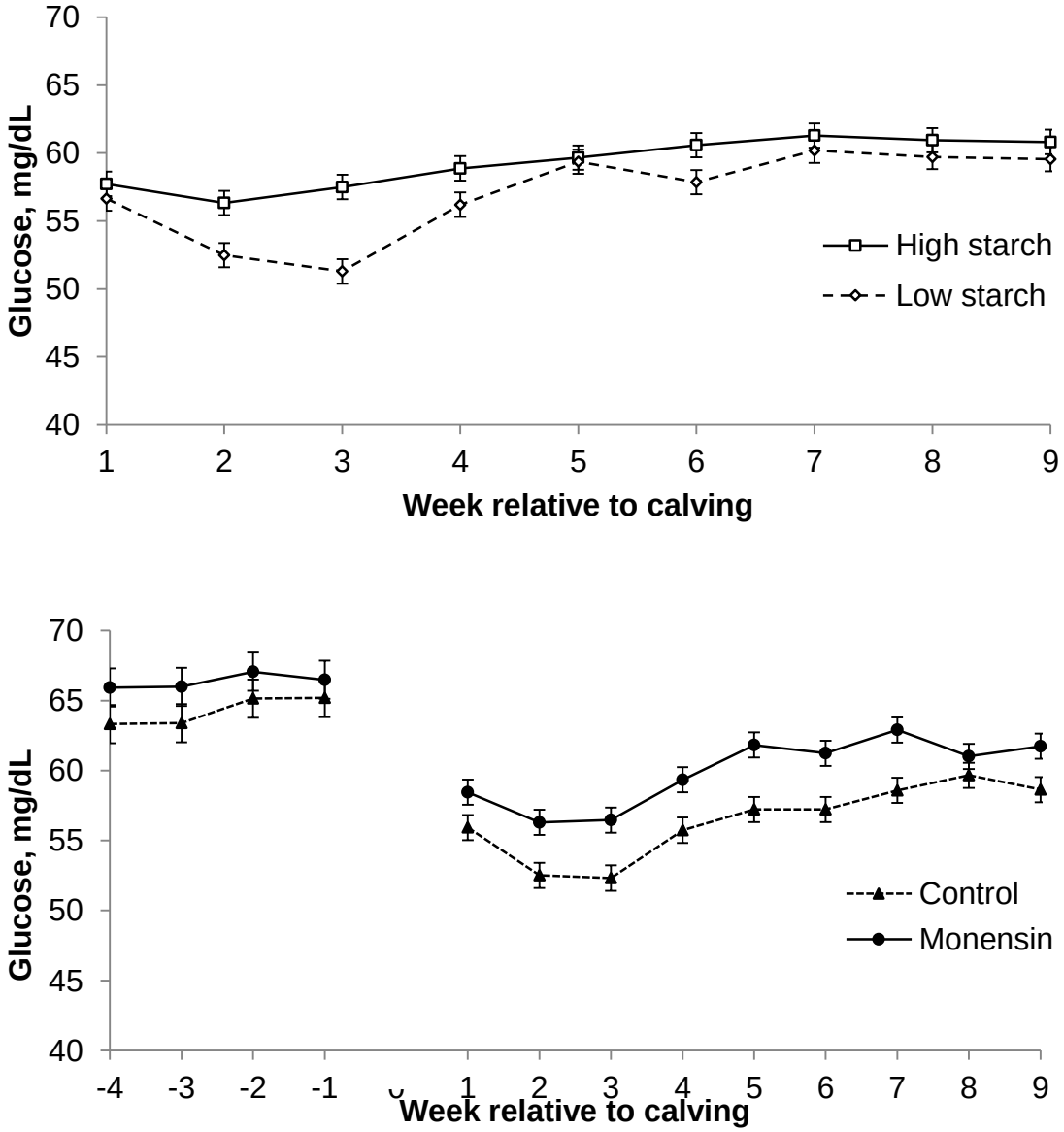
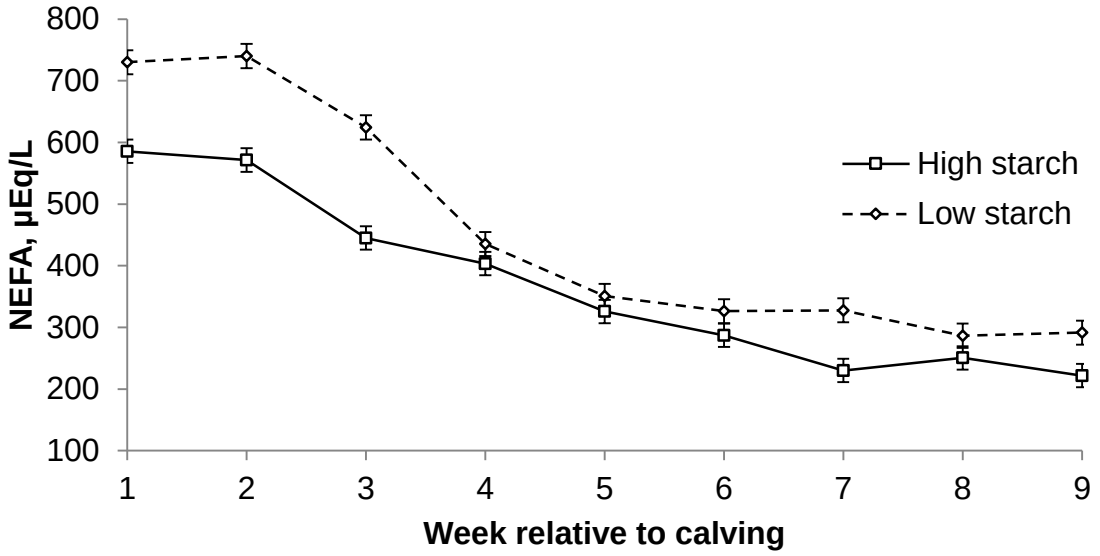


Figure 4-2. Least squares means for plasma NEFA (mg/dL) for cows fed different

starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data were analyzed separately as d 1 to 21 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs. low (21.5%) starch fed during wk 1 to 9. The *P* values for the overall effects of starch content were 0.002 during d 1 to 21 and 0.002 during wk 1 to 9. The *P* values for the interaction of starch content × time were 0.13 for d 1 to 21 and 0.01 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). The *P* values for the overall effect of monensin were 0.19 prepartum, 0.25 for d 1 to 21, and 0.98 for wk 1 to 9. The *P* values for the interaction of monensin × time were 0.13 prepartum, 0.29 for d 1 to 21, and 0.21 for wk 1 to 9.



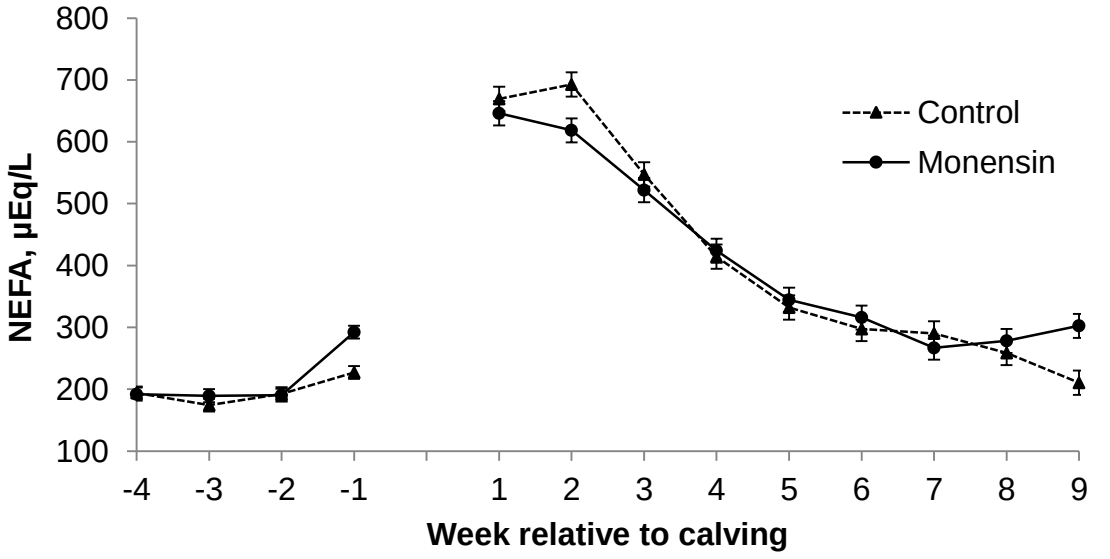
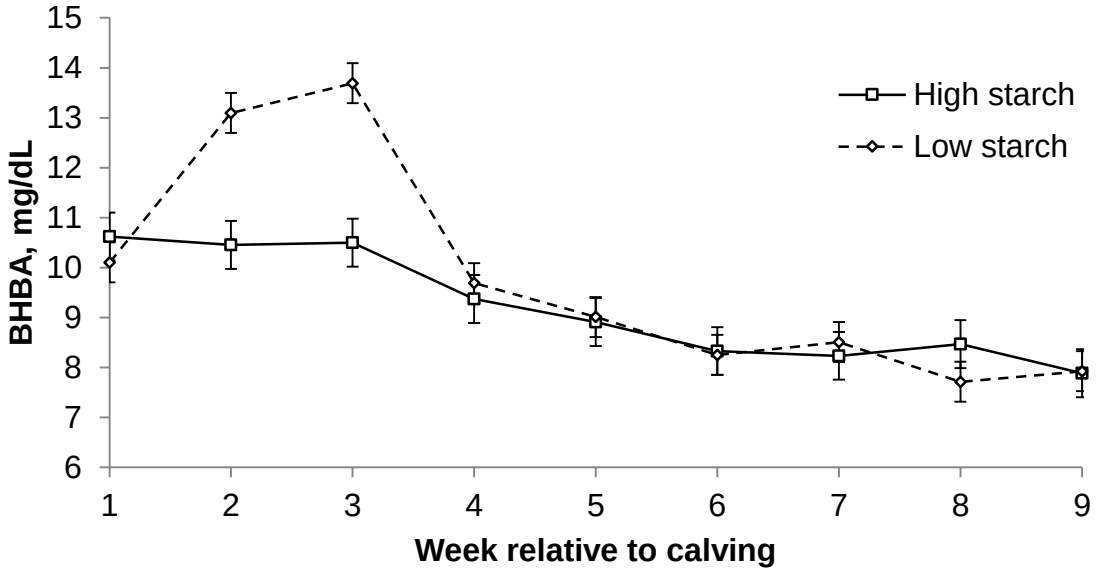


Figure 4-3. Least squares means for plasma BHBA (mg/dL) for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data were analyzed separately as d 1 to 21 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs. low (21.5%) starch fed during wk 1 to 9. The *P* values for the overall effects of starch content were 0.03 during d 1 to 21 and 0.29 during wk 1 to 9. The *P* values for the interaction of starch content × time were 0.04 for d 1 to 21 and 0.02 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). The *P* values for the overall effect of monensin were 0.34 prepartum, 0.002 for d 1 to 21, and 0.06 for wk 1 to 9. The *P* values for the interaction of monensin × time were 0.60 prepartum, 0.41 for d 1 to 21, and 0.15 for wk 1 to 9.



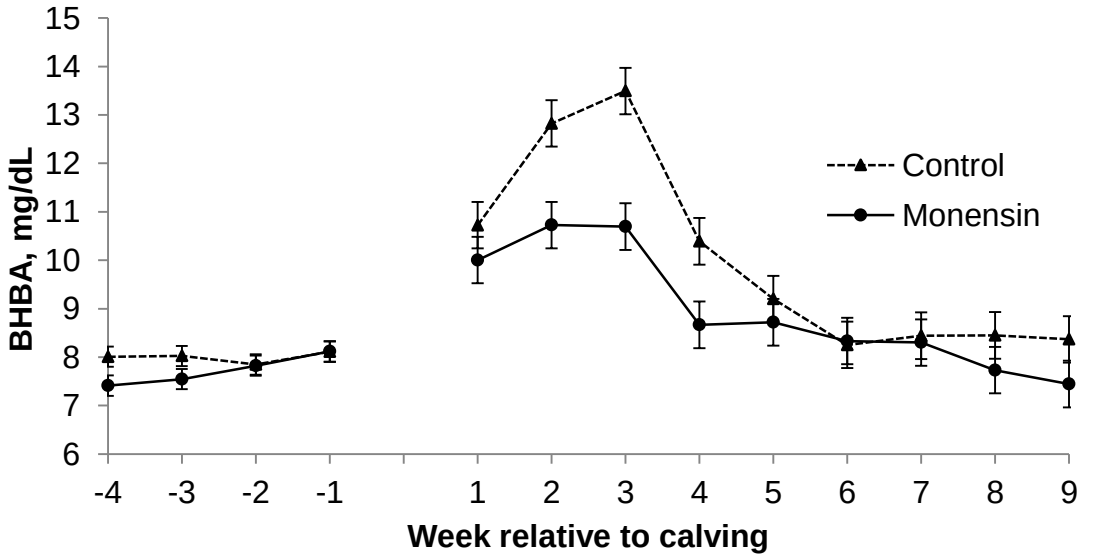
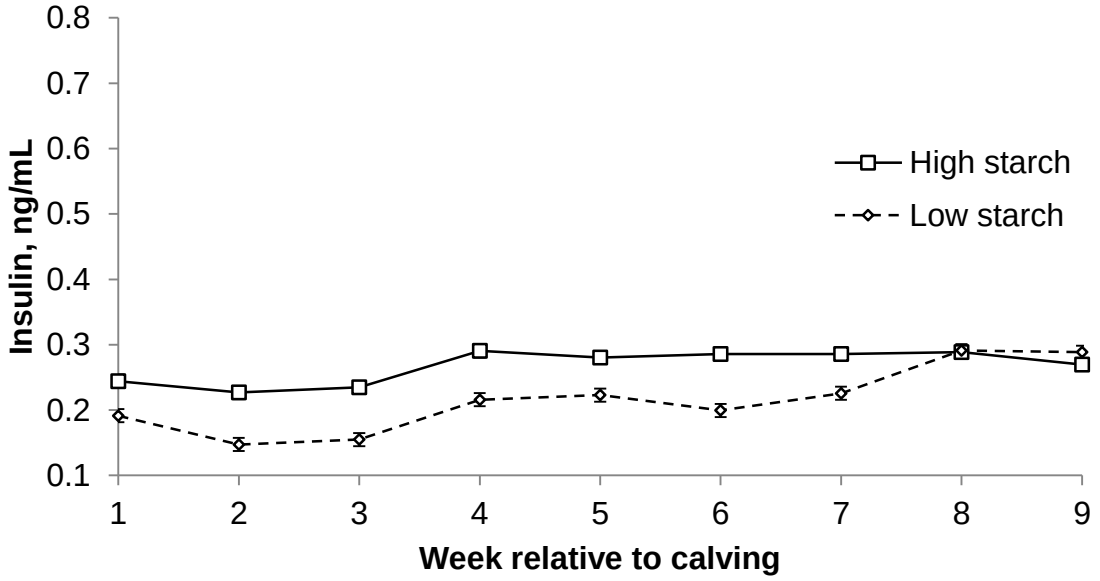


Figure 4-4. Least squares means for plasma insulin (ng/mL) for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data were analyzed separately as d 1 to 21 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs. low (21.5%) starch fed during wk 1 to 9. The *P* values for the overall effects of starch content were 0.008 during d 1 to 21 and 0.004 during wk 1 to 9. The *P* values for the interaction of starch content × time were 0.20 for d 1 to 21 and 0.14 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). The *P* values for the overall effect of monensin were 0.99 prepartum, 0.88 for d 1 to 21, and 0.97 for wk 1 to 9. The *P* values for the interaction of monensin × time were 0.24 prepartum, 0.04 for d 1 to 21, and 0.12 for wk 1 to 9.



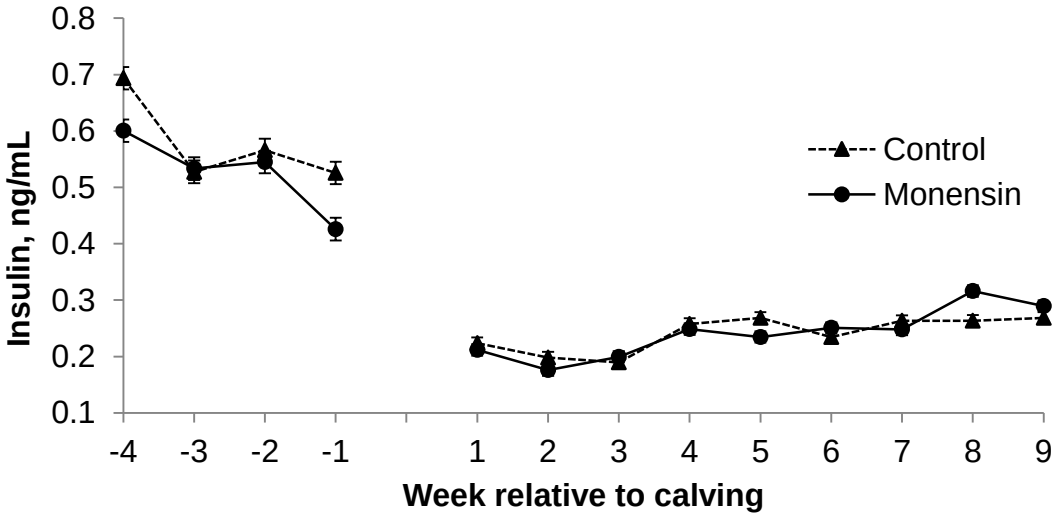
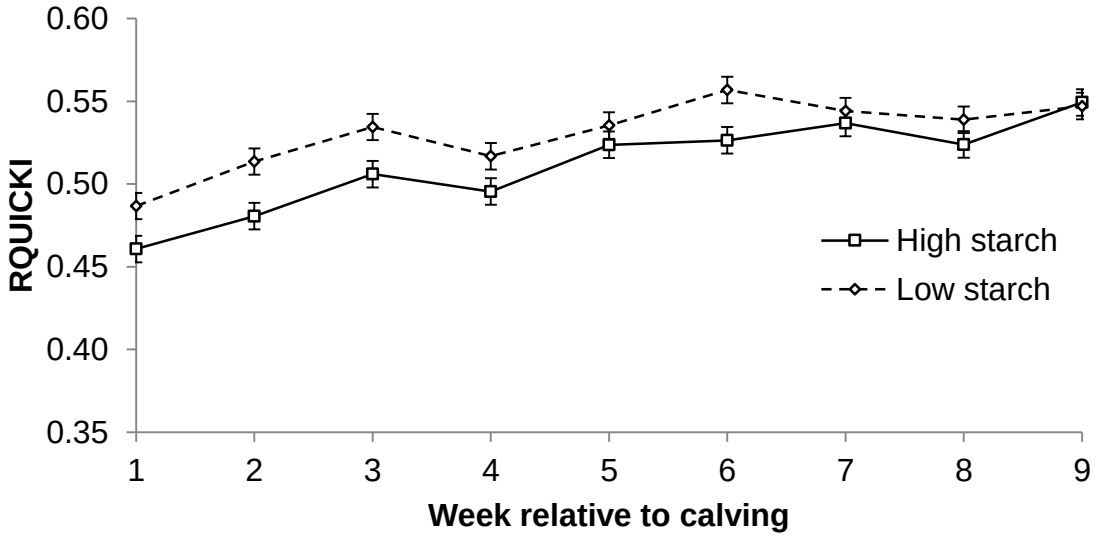


Figure 4-5. Least squares means for revised quantitative insulin sensitivity check index (RQUICKI) for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Postpartum data were analyzed separately as d 1 to 21 (dietary treatment period) and wk 1 to 9 (duration of experiment). Panel A. Effects of high (26.2%) vs. low (21.5%) starch fed during wk 1 to 9. The *P* values for the overall effects of starch content were 0.002 during d 1 to 21 and 0.09 during wk 1 to 9. The *P* values for the interaction of starch content × time were 0.95 for d 1 to 21 and 0.92 for wk 1 to 9. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). The *P* values for the overall effect of monensin were 0.94 prepartum, 0.69 for d 1 to 21, and 0.29 for wk 1 to 9. The *P* values for the interaction of monensin × time were 0.19 prepartum, 0.21 for d 1 to 21, and 0.38 for wk 1 to 9.



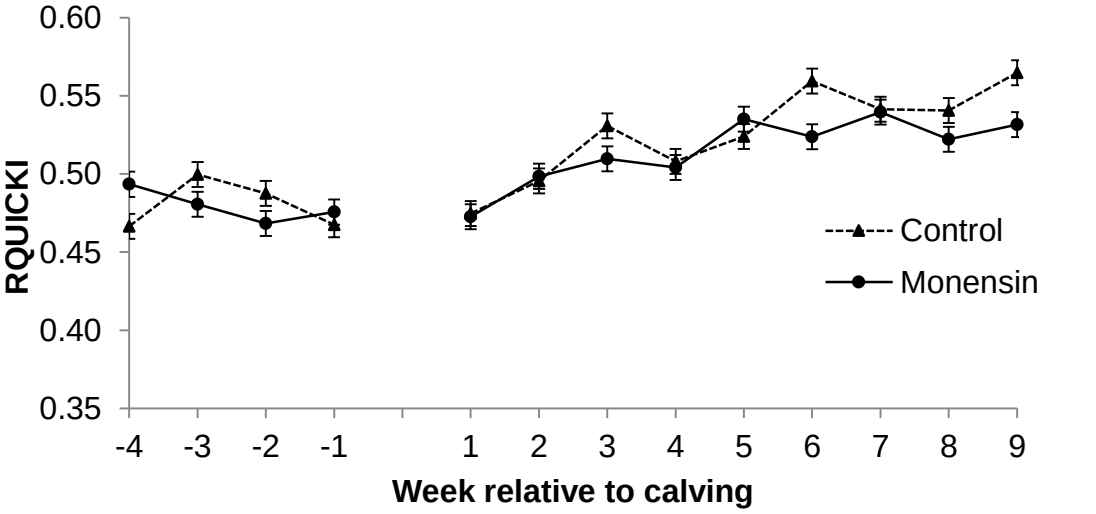
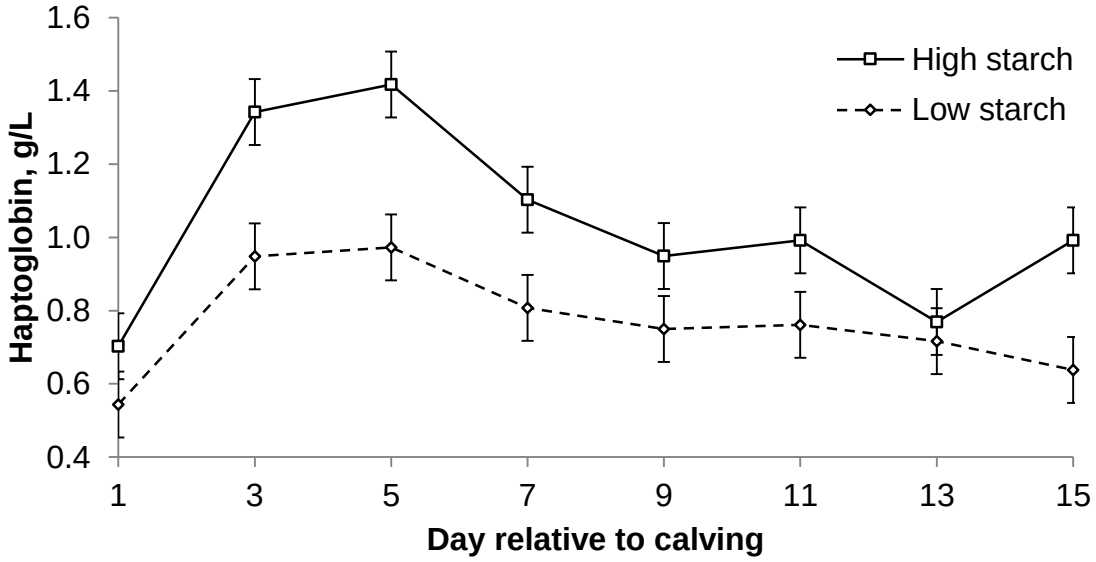
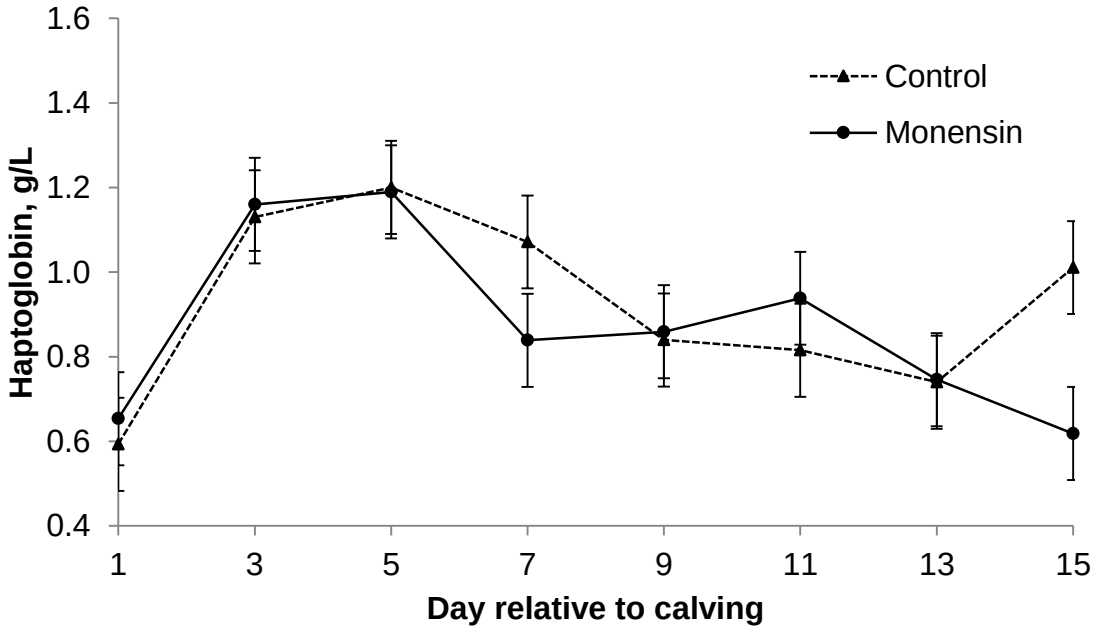


Figure 4-6. Least squares means for plasma haptoglobin (g/L) for cows fed different starch content in the postpartum diet and monensin throughout the periparturient period. Panel A. Effects of high (26.2%) vs. low (21.5%) starch fed during d 1 to 15. The *P* values for the overall effects of starch content were 0.04 and the *P* value for the interaction of starch content × d was 0.48. Panel B. Effects of monensin supplementation (0 vs. 400 mg/d prepartum and 450 mg/d postpartum). The *P* values for the overall effect of monensin were 0.70 and the *P* value for the interaction of monensin × d were 0.37.





DISCUSSION

We hypothesized that increasing starch content during the immediate postpartum period and feeding monensin throughout the periparturient period would increase hepatic gluconeogenesis and improve energy metabolism by increasing the amount of ruminal propionate available to the liver. During the weeks immediately following parturition, the increased glucose demands for milk production requires homeorhetic adaptations to support both the increased energy demands of the mammary gland as well as peripheral tissue metabolism (Bauman and Currie, 1980). In the current study, cows fed HS maintained greater plasma glucose concentrations during early lactation than cows fed the LS diet. In the study of Andersen et al. (2003) cows fed a higher energy propiogenic diet (26.7% starch) had higher plasma glucose concentrations in early lactation compared to cows fed a low energy diet (17.8% starch). Similarly, Rabelo et al. (2003) observed that cows fed a higher energy propiogenic postpartum diet (47.2% NFC) had increased plasma glucose concentrations compared to cows fed a lower energy diet postpartum (41.1% NFC). In the current study, because the cows fed Mon continued with their topdress treatment for the duration of the study, they maintained higher plasma glucose compared to Con cows for the duration of the study, likely a result of increased propionate supply available to the liver. In the meta-analysis of Duffield et al. (2008b), monensin treatment resulted in a 3.2% increase in plasma glucose concentration, whereas Arieli et al. (2008) similarly observed that cows treated with monensin had increased plasma glucose in the early postpartum period. Together these data suggest that feeding more propiogenic diets (with increased starch content or monensin inclusion) increased plasma glucose concentrations.

One homeorhetic adaptation that is employed to help meet the postpartum energy demand is increased lipolysis of adipose tissue and the mobilization of NEFA as an energy substrate (Vernon, 2005). The removal of NEFA by the liver is proportional to the concentration in the blood stream (Reynolds et al., 2003), and NEFA can be oxidized to generate reducing equivalents or ketone bodies, reesterified and exported as triglycerides in very low density lipoproteins (VLDL), or they can accumulate within the liver as stored triglycerides.

Cows that are consuming less energy in early lactation will need to mobilize more NEFA to meet energetic needs. In the current study, cows that were fed HS in early lactation had higher early lactation DMI, less negative EB (McCarthy et al., 2015), and lower plasma NEFA concentrations compared to cows fed LS. However, other studies have observed no effect of early lactation dietary energy content on NEFA mobilization (Andersen et al., 2004; Rabelo et al., 2005). The diets of Andersen et al. (2004) were not designed to be iso-energetic, however, the diets of Rabelo et al. (2003) were likely formulated to be comparable in energy as both diets have similar ether extracts, although diet formulation was not detailed further in their paper. In the current study, the concurrent observations of increased plasma glucose and insulin concentrations for cows fed HS, would indicate a decreased need for HS cows to mobilize adipose tissue compared to cows fed LS. Monensin treatment has also been shown to decrease circulating NEFA (Duffield et al., 2008b; Arieli et al., 2008), although, in the current study there was no effect of Mon treatment on plasma NEFA concentration. We did observe that cows fed Mon had lower BHBA, likely from increased DMI (McCarthy et al., 2015), increased gluconeogenic capacity, and

decreased ketogenesis with Mon treatment.

The partial oxidation of fatty acids to ketone bodies provides energetic substrate for the peripheral tissues, thereby sparing glucose for the mammary gland (Drackley et al., 2001). However, when there is an increased supply of propionate available to the liver, ketone synthesis is decreased (Drackley et al., 2001). This is likely because an increase in the concentration of succinyl CoA in the TCA cycle from increased propionate metabolism has inhibitory effects on 3-hydroxy-3-methylglutaryl CoA synthase (**HMGCS**) synthesis (Zammit, 1990), which is the rate limiting step in ketone body synthesis.

The removal of oxaloacetate from the TCA cycle to phosphoenolpyruvate for gluconeogenesis is a cataplerotic reaction (Owen et al., 2002). During gluconeogenesis additional oxaloacetate must be supplied to condense with acetyl CoA to maintain TCA cycle function and generate ATP. During the early postpartum period there is an increase in the activity of pyruvate carboxylase (Greenfield et al., 2000), which is a key anaplerotic enzyme in the TCA cycle (Owen et al., 2002). It is likely that the additional oxaloacetate entering the TCA cycle during early lactation is being supplied by amino acids and lactate. Although propionate likely supplies some oxaloacetate for oxidation, it is preferentially used for gluconeogenesis during this early lactation period (Drackley et al, 2001). Andersen et al. (2002) reported increased capacity for palmitate conversion to CO_2 in liver slices from early lactation cows fed more propiogenic diets compared to liver slices from cows fed less propiogenic diets, suggesting that feeding more propiogenic diets promotes more complete oxidation of acetyl CoA derived from mitochondrial β-oxidation of fatty acyl CoA. Accordingly, cows fed HS and cows fed

Mon in the current study had lower plasma BHBA concentrations in early lactation. Whereas both Andersen et al. (2004) and Rabelo et al. (2005) observed no difference between treatments for plasma NEFA, cows fed higher energy postpartum diets had lower plasma BHBA concentrations. Mullens et al. (2012) similarly observed that cows fed monensin had decreased plasma BHBA postpartum.

In the current study we observed greater increases in plasma glucose and reductions in plasma BHBA for primiparous cows fed Mon compared to multiparous cows fed Mon; however, increases in early lactation DMI and milk production were observed for all cows regardless of parity, as indicated by a lack of Mon × parity interactions for these measures (McCarthy et al., 2015). There have been relatively few other studies evaluating effects of parity with monensin treatment for comparison with these observations in the current study. Melendez et al. (2004) similarly observed greater reductions in plasma BHBA in primiparous cows administered a prepartum controlled-release capsule of monensin; however, milk and intake data were not reported. Arieli et al. (2008) utilized primiparous cows, and observed faster increases in early lactation milk in both monensin treated primi- and multiparous cows; however, they did not report monensin x parity interactions for plasma metabolites. Arieli et al. (2008) also observed that the rate of ketosis incidence was 60% lower in monensin-treated cows. In the current study, cows fed HS tended to have less subclinical ketosis than cows fed LS and cows fed Mon had less subclinical ketosis and also had less clinical cases of ketosis, likely from increased propionate supply to the liver. Interestingly, ketotic cows may also have a reduced ability for β-oxidation of NEFA as indicated by decreased mRNA levels of carnitine palmitoyl-transferase I and decreased

mRNA and protein levels of carnitine palmitoyltransferase II, acyl-CoA dehydrogenase long chain, HMGCS, and acetyl CoA carboxylase at increasing serum BHBA concentrations (Li et al., 2012), indicating further impairment in hepatic NEFA handling when ketone synthesis is elevated.

Propionate is a potent insulin secretogauge, likely because propionate production increases rapidly following meal consumption (Brockman, 1990). In the current study cows fed HS had increased plasma insulin concentrations. Andersen et al. (2004) and Rabelo et al. (2005) also observed similar increases in plasma insulin for cows fed higher energy propiogenic diets postpartum compared to cows fed lower energy diets. In early lactation the relatively low circulating insulin concentrations and peripheral tissue insulin resistance may help to attenuate the gluconeogenic contribution from other glucose precursors and increase liver glucose output (Drackley et al., 2001). Postpartum cows decrease whole body glucose oxidation to conserve glucose for milk lactose synthesis (Bell et al., 2000). In the current study cows fed HS had increased insulin concentrations and also exhibited increased insulin resistance as indicated by a lower RQUICKI. This increase in insulin resistance observed along with increases in early lactation milk yield (McCarthy et al., 2015) would suggest greater increase in glucose availability for the mammary gland in cows fed HS.

During the immediate postpartum period there is an increase in inflammation (Sordillo and Raphael, 2013). Cytokines that are produced by immune cells can elicit an inflammatory response and include the release of acute phase proteins from the liver, such as haptoglobin. Increased plasma haptoglobin is known to be a marker of postpartum inflammation in dairy cows (Humblet et al., 2006). In the current study cows

that were fed HS had elevated haptoglobin during the immediate postpartum period compared to LS cows; although, because haptoglobin is a nonspecific marker the exact cause of this inflammation is unknown. However, because the HS diet in the current study was more fermentable than the LS diet (21.5 vs. 16.8 starch fermentability as a % of DM; McCarthy et al., 2015), we speculate that it is possible that cows fed HS were experiencing a state of postpartum ruminal transition leading to increased inflammation and elevated haptoglobin concentrations. Penner et al. (2007) observed that primiparous cows experienced the occurrence of mild ruminal acidosis ($5.8 < pH > 5.5$) during d 1-5 postpartum that was greater than during the prepartum period, while Iqbal et al. (2010) observed increased haptoglobin concentrations in late lactation cows that were fed starch that was more rumen degradable. Although cows fed HS had increased haptoglobin concentrations in the current study, the overall effects of HS diet in the current study on production performance (McCarthy et al., 2015) and energy metabolism were positive.

The bovine liver has limited capacity for triglyceride export compared to monogastric animals (Chilliard, 1993), and the export of triglycerides is a rate limiting step in hepatic NEFA metabolism. Some lipid accumulation in the liver during the periparturient period seems to be a normal adaptation to lactation (Grum et al., 1996); however, excess liver triglyceride accumulation has been shown to decrease the ability of the hepatocyte to synthesize urea (Strang et al., 1998). The consequent increase in liver ammonia may decreases glucose synthesis from propionate (Overton et al., 1999). In the current study there was no effect of starch content on liver triglyceride content, although Andersen et al. (2004) observed that cows fed higher energy propiogenic diets

had decreased liver triglyceride content. Although elevated hepatic triglyceride accumulation in transition cows has been shown to have negative impact on gluconeogenesis in multiparous cows, the effect of triglyceride content in primiparous cows may not be as deleterious. In the current study primiparous cows fed Mon had increased liver triglyceride content compared to Con primiparous cows, although overall metabolism and energy balance would indicate positive effects of Mon treatment on primiparous cows. The observed increase in plasma glucose concentration with Mon treatment was mainly driven by a Mon × parity interaction, and we also observed a decrease plasma BHBA concentration with Mon, which was again mainly driven by decreases in primiparous cows. Mon treated primiparous cows likely had more glucose synthesis and decreased ketone body synthesis. However, we did not observe any treatment difference in plasma NEFA concentration, so it is likely that there was no difference in uptake of NEFA by the liver. Because there was likely less hepatic ketone synthesis, in Mon treated primiparous cows more of the NEFA taken up by the liver were likely reesterified into triglyceride. Because VLDL export is the rate limiting step in hepatic fatty acid export, Mon treated primiparous cows likely had increased triglyceride accumulation in the liver. Mullens et al. (2012) observed no difference in postpartum liver triglyceride content, although cows fed monensin had increased carnitine palmitolytransferase-1 (**CPT-1**) mRNA abundance immediately pre- and postpartum, indicating that perhaps cows fed monensin had increased capacity to oxidize fatty acids, as the entry of fatty acyl CoA into the mitochondria is regulated by CPT-1 and is a major control point in the mitochondrial β-oxidation of fatty acids (Drackley, 1999). This would be in accordance with the observations of Andersen et al.

(2002) that as the hepatic supply of propionate increased from feeding diets of greater propiogenic capacity this resulted in increased capacity for in vitro conversion of palmitate to CO_2.

In experiments using labeled isotopes, the [1-^{14}C]propionate label randomizes in the TCA cycle such that every mole of [1-^{14}C]propionate that is directed toward oxaloacetate would yield 0.5 moles of radiolabeled CO_2 and 0.5 moles of radiolabeled glucose (Knapp et al., 1992). Therefore, any increase in this ratio of labeled glucose to CO_2 would suggest an increase in the efficiency of utilization of propionate for gluconeogenesis. Cows treated with monensin had an increase in the ratio of glucose to CO_2, likely from an increase in propionate supply indicating an increased propensity to convert propionate to glucose with increased propionate supply. It appears that in the early lactation cow when there is an increase in propionate supply to the liver that there is an increased propensity to convert propionate to glucose rather than oxidize it (Drackley et al., 2001). The rates of gluconeogenesis from [1-^{14}C]propionate in early lactation liver slices are increased compared to rates in liver slices from the same cows once they have reached mid lactation (Aiello et al., 1989). Drackley et al. (2001) saw a positive correlation between carbohydrate intake in the immediate postpartum period with the efficiency of [1-^{14}C]propionate conversion to glucose in liver slices, which would suggest that the liver has the capacity to direct additional propionate toward glucose during this time. In the current study there was no effect of starch on the ratio of glucose to CO_2. However, because we biopsied cows at d 7 (± 1.6 SD) postpartum and increases in DMI in high starch cows were not observed until d 14 (McCarthy et al., 2015), likely starch intake had not increased sufficiently at the time of biopsy.

Although in vitro [1-^{14}C]palmitate metabolism was not measured in the current study, Andersen et al. (2002) observed that cows that were fed higher energy propiogenic diets in early lactation had increased oxidation of palmitate compared to cows fed lower energy diets. This increase in palmitate oxidation would suggest that increases in mitochondrial NEFA oxidation to acetyl CoA are providing oxidative carbon for the TCA cycle to accommodate the increased gluconeogenesis from propionate. Andersen et al. (2002) observed that cows fed the higher energy propiogenic diets had increased fatty acid oxidation and less liver triglyceride infiltration, perhaps because increased glucogenic capacity has been associated with a higher activity of carnitine palmitoyltransferase (Chow and Jesse, 1992) likely supplying additional acetyl CoA for the TCA cycle.

CONCLUSIONS

Overall effects of feeding a higher starch diet on metabolism appear to be positive, as observed by increased plasma glucose and insulin concentrations and decreased plasma NEFA and BHBA concentrations in early lactation. Cows fed monensin during the peripartal period had increased propensity to convert propionate to glucose in vitro as well as increased concentrations of plasma glucose and reduced concentrations of plasma BHBA, resulting in reduced subclinical ketosis. In conclusion, cows fed more propiogenic diets during the early lactation period either from increased starch content or inclusion of monensin had improved postpartum energy status.

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CHAPTER 5

GENE EXPRESSION OF HEPATIC GLUCONEOGENIC AND FATTY ACID METABOLISM IN EARLY LACTATION DAIRY COWS AS AFFECTED BY DIETARY STARCH AND MONENSIN SUPPLEMENTATION

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ABSTRACT

The objective of this study was to evaluate the impact of dietary starch level and monensin (Mon) on the gene expression of key enzymes related to hepatic gluconeogenesis and fatty acid metabolism in liver tissue obtained from dairy cows during early lactation. Prior to parturition primiparous (n = 21) and multiparous (n = 49) Holstein cows were fed a common controlled energy close up diet with a daily topdress of either 0 or 400 mg/d monensin. From d 1 to 21 postpartum, cows were fed a high starch (HS; 26.2% starch, 34.3% NDF, 22.7% ADF, 15.5% CP) or low starch (LS; 21.5% starch, 36.9% NDF, 25.2% ADF, 15.4% CP) total mixed ration (TMR) with a daily topdress of either 0 mg/d monensin (Con) or 450 mg/d monensin (Mon), continuing with prepartum topdress assignment. From d 22 through 63 postpartum all cows were fed HS and continued with assigned topdress treatment until d 63. Liver biopsies were obtained on d 7 postpartum. There was a tendency for an effect of starch on RNA content such that cows fed HS tended to have greater RNA content. There was a starch × monensin interaction for methylmalonyl CoA mutase (*MUT*) and cows fed HS + Mon and LS + Con had the lowest expression of *MUT*. There was also a tendency for a Mon × parity interaction such that primiparous cows fed Con had greater expression of *MUT* compared to primiparous cows fed Mon whereas cows fed Con had decreased expression of *MUT* compared to cows fed Mon. There was a tendency for cows fed Mon to have increased pyruvate carboxylase (*PC*) expression compared to Con cows. This appeared to be driven by a starch × monensin interaction such that cows fed HS + Mon had the greatest elevation in *PC* compared to the other treatments. There was a tendency for a starch × monensin interaction for cytosolic

phosphoenolpyruvate carboxykinase (*PCK1*) and cows that were fed LS + Con tended to have greater *PCK1* expression than cows that were fed HS + Con. There was also a tendency for a starch × monensin interaction for 3-hudroxy-3-methyl-glutaryl-CoA synthase (*HMGCS2*) such that cows fed LS + Con tended to have greater *HMGCS2* than LS + Mon cows. There was a starch × monensin interaction for acetyl-CoA carboxylase (*ACAC*) such that cows fed LS + Con had greater *ACAC* expression compared to cows fed LS + Mon. Overall, cows fed diets of different starch content in early lactation and monensin throughout the transition period had altered hepatic expression of genes related to glucose and fatty acid metabolism.

Keywords: early lactation, starch, monensin, hepatic gene expression

INTRODUCTION

Hepatic glucose production nearly doubles within 11 d of calving compared to prepartum hepatic glucose output (Reynolds et al., 2003), and propionate that is produced via fermentation of starch in the rumen is the main precursor for hepatic glucose production. Monensin supplementation also has been shown to increase ruminal propionate production (Armentano and Young, 1983). It has been observed that cows fed higher energy diets postpartum and monensin during the periparturient period have improvements in postpartum production (Andersen et al., 2003, Rabelo et al., 2003; McCarthy et al., 2015a), energy metabolism (Andersen et al., 2004, Arieli et al., 2008; McCarthy et al., 2015b), and less severe negative energy balance (McCarthy et al., 2015a).

The ability of the liver to convert propionate to glucose is responsive to propionate supply, and there is a positive linear relationship between increased

propionate supply and liver glucose output (Dijkstra et al., 2005). Therefore, cows that have an increase in propionate supply in early lactation (e.g. by feeding higher starch or monensin) should have the ability to increase hepatic gluconeogenesis. Because energy metabolism in the liver is regulated mainly by changes in activity of the enzymes that catalyze key steps in the gluconeogenic pathway, it is likely that any increases in hepatic gluconeogenic capacity will be reflected by changes in gene expression of these key enzymes. The expression of genes involved in gluconeogenesis also have been shown to be affected by diet and feed intake (Velez and Donkin, 2005, Loor et al., 2006). It was observed that increasing the propiogenic capacity of the early lactation diet by increasing starch fermentability (high moisture corn vs. dry ground corn) led to increased expression of pyruvate carboxylase, propionyl CoA synthase, and propionyl CoA carboxylase α (Ylioja et al., 2013). Karcher et al. (2007) similarly observed that cows fed monensin (300 mg/d) beginning at -28 d prior to calving until calving had an increase in cytosolic phosphoenolpyruvate carboxykinase mRNA expression on d -14 prepartum and d 1 postpartum compared to control cows. In addition when 400 mg/d monensin was fed to cows beginning 21 d prior to parturition through d 21 postpartum, monensin treated cows had increased carnatine palmitoyl-transferase 1A mRNA expression on d -7 and d 0 relative to parturition (Mullins et al., 2012), indicating potential for propiogenic diets to lead to increased capacity for hepatic fatty acid oxidation. Together these data suggest that increasing the gluconeogenic potential by feeding diets with greater propiogenic capacity during the early postpartum period may alter expression of enzymes involved in hepatic energy metabolism.

The objectives of this study were to examine the effect of dietary starch content during the immediate postpartum period and monensin inclusion throughout the periparturient period on early postpartum hepatic gene expression. We hypothesized that increasing starch level and feeding monensin during the immediate postpartum period would increase hepatic gluconeogenesis by increasing the expression of genes related to gluconeogenesis and fatty acid oxidation.

MATERIALS AND METHODS

Animals and dietary treatments

All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee and the experiment was conducted from March to October 2012. The experimental design and treatments were described more completely in a previous publication (McCarthy et al., 2015a). Briefly, the study was a completely randomized design with randomization restricted to balance for expected calving date of primiparous and multiparous cows and previous lactation 305-d mature-equivalent milk production for multiparous cows. A 2 × 2 factorial arrangement of postpartum treatments was utilized with early lactation period feeding strategy [high starch (**HS**) vs. low starch (**LS**) diet during the first 21 d postpartum] and monensin supplementation [0 mg monensin/d (**Con**) or 450 mg monensin/d (**Mon**); monensin; Elanco Animal Health, Greenfield, IN] as the variables of interest. In addition, cows that received Mon during the postpartum period were fed Mon (400 mg/d) initiated on 1 d between d 21-28 before expected parturition (average treatment of 25 d; minimum of 14 d on treatment before actual parturition was required for inclusion in the data set). The final dataset included 70 cows (primiparous n = 21, multiparous n = 49). Lactating cows were dried

off at least 45 d (average 53 d dry period length) prior to expected parturition, and moved to the experimental tie stall barn approximately 28 d prior to expected parturition where they began consuming the experimental close up dry cow diet.

Diet ingredients are presented in Table 5-1 and nutrient composition are presented in Table 5-2. Procedures and methods for feed sampling and analysis are detailed in McCarthy et al. (2015a). The topdress pellets were formulated to contain either 0 (Con) or 461 g/metric ton Mon and were fed as a daily topdress at rates of 0.85 kg/d prepartum and 0.95 kg/d postpartum. The Mon topdress was targeted to provide 400 mg/d prepartum and 450 mg/d postpartum. Cows continued to receive assigned topdress treatments through d 63 postpartum.

Liver biopsy and mRNA analysis

Liver tissue was sampled via percutaneous trocar biopsy (Veenhuizen et al., 1991) from cows under local anesthesia on d 7 (± 4 d range; ± 1.6 SD) relative to parturition. After blotting the liver tissue to remove excess blood and connective tissue, a portion of the liver sample was immersed in ice-cold PBS (0.015 M; 0.9% NaCl, pH 7.4) and transported to the laboratory within 45 min. of tissue collection (McCarthy et al., 2015b). The remaining portion of liver that was collected was snap-frozen in liquid nitrogen and stored at -80°C until analysis for DNA and RNA quantification, and gene expression.

DNA and RNA quantification

To quantify DNA and RNA, 150 μg of liver tissue was homogenized in 5 mL of DNA assay buffer (50 mMNa2PO4, 2 M NaCl, 2 mM Na2EDTA). Hoechst 33258 dye binding was used for DNA quantification (Labarca and Paigen, 1980). A standard

curve was prepared containing 0 to 2 μg of calf thymus DNA (Sigma Aldrich, Saint Louis, MO) per well. Triplicate 5 μL aliquots of sample homogenates were transferred to a 96-well microplate. Then 195 μL of DNA assay buffer containing Hoechst dye [99.8 mL of DNA assay buffer + 200 μL of dye solution (1 μg of Hoechst 33258 dye/μL of distilled water)] was added to each sample. Fluorescence was read with a SpectraMax M2 plate reader with 360/460 nm filter set (Molecular Devices, Sunnyvale, CA). A second 0.5 mL aliquot of the above liver tissue homogenate was placed in a 2 mL microcentrifuge tube and RNA was determined by ultraviolet absorbance using the method described by Schmidt and Thannhauser (1945). Absorbance was measured at 260 and 232 nm with a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA).

RNA extraction and real-time quantitative PCR

Total RNA was isolated and purified using miRNeasy minicolumns and on-column ribonuclease-free deoxyribonuclease treatment (QIAGEN Inc., Valencia, CA). Quantity and integrity of RNA was determined using the RNA Nano Lab chip kit and Bioanalyzer (Agilent, Palo Alto, CA). Reverse transcriptase reactions were performed with 2 μg of RNA in a 20-μL volume using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendation. Real-time PCR assays were performed in duplicate in a 20-μL volume using predesigned TaqMan primer probes for methylmalonyl CoA mutase (***MUT***), pyruvate carboxylase (***PC***), cytosolic phosphoenolpyruvate carboxykinase (***PCK1***),glucose 6 phosphatase (***G6PC***), 3-hudroxy-3-methyl-glutaryl-CoA synthase (***HMGCS2***), and acetyl-CoA carboxylase (***ACAC***), with the exception

of carnatine palmitoyl-transferase 1A (***CPT1A***) which was a custom designed primer probe (Applied Biosystems). All primer probes spanned exons. Reactions contained 500 nM of each primer (TaqMan probes) and diluted cDNA (20 ng). An arbitrary expression level was obtained by using the $\Delta\Delta C_t$ method. A calibrator sample was run for all genes and expression of β-2-microglobulin (***B2M***) was used as the invariant control. The C_t for the gene of interest in the test samples were adjusted in relation to expression of *B2M* ($\Delta C_{t\ sample}$), and the gene of interest C_t for calibrator samples were also adjusted in relation to expression of *B2M* ($\Delta C_{t\ calibrator}$). Any mRNA with a cycle number greater than 36 was declared undetectable.

Statistical analyses

Gene expression data was non-normally distributed and logarithmic transformation was conducted for all variables to reduce skewness prior to analysis. Statistical computations were performed using SAS software (version 9.2; SAS Institute Inc., Cary, NC). Data for DNA quantification, RNA quantification, and gene expression were subjected to ANOVA using the MIXED procedure of SAS. Fixed effects included starch level, monensin treatment, parity, and all 2-way interactions. The random effect was cow nested within starch and monensin treatment. Least squares means and standard error of the mean are reported. Statistical significance was declared at $P \leq 0.05$. Because a subset of cows were used for all liver analyses, trends were declared at $0.05 < P < 0.15$. Relationships between data for log transformed gene expression, liver composition (McCarthy et al. 2015b), and in vitro propionate metabolism (McCarthy et al. 2015b) on d 7 postpartum were analyzed using PROC CORR statement and the Pearson option in SAS.

RESULTS

DNA and RNA quantification

Results from hepatic DNA and RNA quantification are presented in Table 5-3. There was a tendency for a starch × parity interaction for DNA content ($P = 0.11$) such that primiparous cows fed LS tended to have greater DNA content compared to primiparous cows fed HS (8.77 vs. 7.34 mg DNA/g tissue) although DNA content between LS and HS multiparous cows was similar (7.67 vs. 7.79 mg DNA/g tissue). There was a tendency for an effect of starch on RNA content ($P = 0.13$) such that cows fed HS tended to have greater RNA content, and because of this tendency cows fed HS also had a numerically increased RNA:DNA ratio.

Gene expression

Hepatic gene expression results on d 7 postpartum are presented in Table 5-4. There was a starch × monensin interaction for *MUT* ($P = 0.005$; Figure 5-1A) such that cows fed HS + Mon and LS + Con had the lowest expression of *MUT*. There was also a tendency for a Mon × parity interaction ($P = 0.13$) such that primiparous cows fed Con had greater expression of *MUT* compared to primiparous cows fed Mon (1.62 vs. 1.38 $\log_2$ relative abundance) whereas cows fed Con had decreased expression of *MUT* compared to cows fed Mon (1.30 vs. 1.58 $\log_2$ relative abundance). There was a tendency ($P = 0.06$) for cows fed Mon to have increased *PC* expression compared to Con cows. This seemed to be driven by a starch × monensin interaction (Figure 5-1B; $P = 0.07$) such that cows fed HS + Mon had the greatest elevation in *PC* compared to the other treatments. There was a tendency for a starch × monensin interaction (Figure 5-1C; $P = 0.07$) for *PCK1* such that cows that were fed LS + Con tended to have

greater *PCK1* expression than cows that were fed HS + Con. There was also a tendency for a starch × monensin interaction (Figure 5-1F; $P = 0.08$) for *HMGCS2* such that cows fed LS + Con tended to have greater *HMGCS2* than LS + Mon cows. There was a starch × monensin interaction (Figure 5-1G; $P = 0.02$) for *ACAC* such that cows fed LS + Con had greater *ACAC* expression compared to cows fed LS + Mon. There was also a Mon × parity interaction ($P = 0.04$) and multiparous cows (**multi**) fed Con had greater *ACAC* compared to multiparous cows fed Mon and also primiparous cows (**primi**) fed Con (4.06 multi Con, 3.28 multi Mon, 3.36 primi Con, 3.53 primi Mon $\log_2$ relative abundance). There was no effect of treatment on *G6PC* (Figure 5-1D) or *CPT1A* (Figure 5-1E) expression at d 7 postpartum.

Correlations

Correlation analysis was performed for all gene expression variables with data for liver composition and in vitro [1-^{14}]C propionate metabolism presented in a previous publication (McCarthy et al., 2015 b). Measures of gene expression were well correlated with each other; however, there were poor relationships between measures of gene expression and liver composition and in vitro metabolism (data not shown). There was a strong negative relationship between *MUT* and *ACAC* ($r = -0.814$; $P = < 0.001$). Interestingly, there were similar negative relationships between *MUT* and *CPT1A* ($r = -0.614$; $P = < 0.001$), *PC* ($r = -0.748$; $P = < 0.001$), and *PCK1* ($r = -0.786$; $P = < 0.001$). However, relationships between *CPT1A* and *PCK1* were positive ($r = 0.521$; $P = < 0.001$), along with relationships between *PCK1* and *G6PC* ($r = 0.818$; $P = < 0.001$) and *PC* and *PCK1* ($r = 0.685$; $P = < 0.001$). For the majority of variables there was a similar directionality in the correlations between treatments

(data not shown). However, there were differences in the relationship between *PC* and *HMGCS2* gene expression (Figure 2A; Con $r = 0.806$, $P = 0.02$; Mon $r = 0.173$, $P = 0.41$) and also between *PC* and in conversion of [1-^{14}C]propionate to glucose (Figure 2 B; Con $r = 0.173$, $P = 0.97$; Mon $r = 0.436$, $P = 0.07$) for cows fed Mon and Con.

Table 5-1. Ingredient composition of the basal diets (% of DM)

	Prepartum	Postpartum[1]	
		HS	LS
Corn silage, processed	42.14	–	–
BMR corn silage[2]	–	38.50	38.50
Wheat straw	21.75	11.54	11.55
Legume silage	–	9.62	9.62
Shelled corn, finely ground	4.28	20.97	10.29
Citrus pulp	7.23	1.01	7.15
Corn germ meal	–	2.52	5.56
Soybean hulls	7.08	–	3.58
Soybean meal	5.27	5.87	3.86
Canola meal	4.63	2.73	2.08
Blood meal	1.05	1.94	1.93
Amino Plus[3]	1.78	1.70	2.34
Energy Booster 100[4]	–	0.77	0.96
Calcium carbonate	1.53	1.12	0.82
Sodium bicarbonate	–	0.86	0.85
Soy Chlor[5]	1.33	–	–
Salt	0.16	0.42	0.41
Calcium sulfate	0.73	0.17	0.17
Magnesium oxide	0.20	0.15	0.15
Magnesium sulfate, 9.9%	0.61	–	–
Selenium 0.06%	0.04	0.05	0.05
Mono dicalcium phosphate	–	0.02	0.07
Trace mineral premix[6]	0.02	0.03	0.03
Vitamin A, D, E premix[7]	0.05	0.02	0.02
Vitamin E premix[8]	0.005	–	–
Zinc sulfate	0.002	–	–
Copper sulfate	0.0004	0.001	0.001

[1]HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]BMR= brown mid-rib corn silage.
[3]AGP Inc., Omaha, NE.
[4]Milk Specialties Global; Carpentersville, IL.
[5]West Central, Ralston, IA.
[6]Contained 30,317 mg/kg of Cu, 136,466 mg/kg of Mn, 3,393 mg/kg of Co, 3,040 mg/kg of I, and 153,916 mg/kg of Zn.
[7]Contained 30,464 IU/kg of Vitamin A, 5,862 IU/kg of Vitamin D, and 93,784 IU/kg of Vitamin E.
[8]Contained 510,750 IU/kg of Vitamin E.

Table 5-2. Chemical composition of experimental diets (± SD[1])

	Prepartum diet	Postpartum diet[2]		Topdress pellet[3]	
Item		HS	LS	Con[4]	Mon[5]
DM, %	50.7 ± 2.4	48.3 ± 2.7	48.0 ± 3.2	93.2 ± 1.0	93.7 ± 1.2
CP, %	13.0 ± 0.8	15.5 ± 1.2	15.4 ± 0.8	37.5	37.0
ADF, %	28.2 ± 1.2	22.7 ± 1.2	25.2 ± 1.2	11.1	12.9
NDF, %	42.9 ± 2.0	34.3 ± 1.5	36.9 ± 1.5	22.6	21.3
30 h NDFD[6], %	–	18.9 ± 1.2	20.7 ± 1.1	–	–
30 h NDFD[6], % of NDF	–	55.1 ± 2.0	56.1 ± 1.4	–	–
Sugar, %	4.9 ± 0.8	3.5 ± 0.6	4.5 ± 0.4	10.6	11.3
Starch, %	17.4 ± 1.2	26.2 ± 1.2	21.5 ± 1.0	13.1	13.8
Fat, %	2.6 ± 0.2	4.0 ± 0.2	2.2 ± 0.6	2.4	2.5
Ca, %	1.28 ± 0.16	0.94 ± 0.09	1.01 ± 0.04	0.51	0.6
P, %	0.30 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.97	0.99
Mg, %	0.41 ± 0.04	0.28 ± 0.02	0.3 ± 0.03	0.48	0.48
K, %	1.12 ± 0.13	1.12 ± 0.09	1.18 ± 0.08	1.67	1.70
S, %	0.37 ± 0.04	0.21 ± 0.09	0.22 ±0.01	0.46	0.44
Na, %	0.12 ± 0.02	0.47 ±0.08	0.46 ± 0.05	0.07	0.07
Cl, %	0.37 ± 0.01	0.44 ± 0.04	0.44 ± 0.03	0.07	0.09
NE_L, Mcal/kg	1.48 ± 0.03	1.64 ± 0.02	1.56 ± 0.03	1.72	1.74

[1]Chemcial composition was analyzed on 6 composite samples of the prepartum diet, 7 composite samples of the high starch postpartum diet, and 6 composite samples of the low starch postpartum diet.

[2]HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).

[3]The composition of the topdress was 33.6% soybean meal, 33.2% wheat middlings, and 33.2% canola meal (DM basis).

[4]Con = control topdress, formulated to supplement 0 mg/d monensin.

[5] Mon = monensin topdress, formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.

[6]NDFD = NDF digestibility.

Table 5-3. Postpartum hepatic DNA and RNA content, and RNA:DNA ratio on d 7 (± 1.6 SD) postpartum for cows (n = 70) fed either high or low starch diets during the first 3 weeks postpartum and control or monensin treatments throughout the periparturient period and into early lactation

	Diet[1]			Topdress[2]			*P*-values[5]					
Item	HS	LS	SEM	Con[3]	Mon[4]	SEM	S	M	P	S×M	S×P	M×P
DNA	7.57	8.22	0.35	8.07	7.72	0.35	0.17	0.46	0.50	0.38	0.11	0.96
RNA	9.64	9.14	0.24	9.54	9.24	0.24	0.13	0.38	0.72	0.72	0.50	0.79
RNA:DNA ratio	1.32	1.16	0.08	1.25	1.23	0.08	0.17	0.86	0.51	0.76	0.22	0.94

[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, and P = parity.

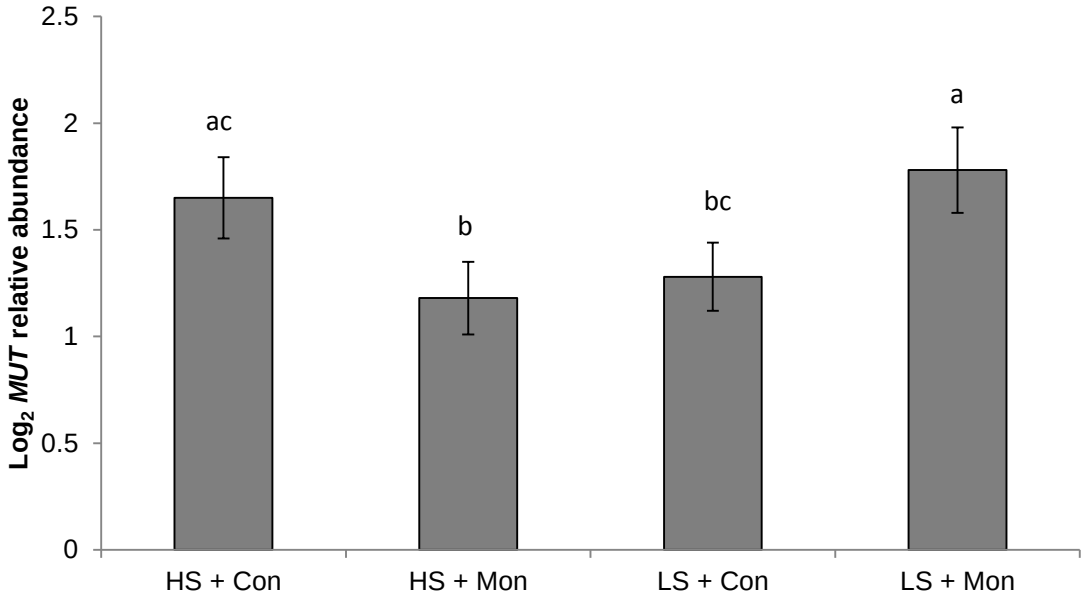
Table 5-4. Relative log_2 hepatic gene expression on d 7 (± 1.6 SD) postpartum for cows (n = 70) fed either high or low starch diets during the first 3 weeks postpartum and control or monensin treatments throughout the periparturient period and into early lactation

	Diet[1]			Topdress[2]			*P*-values[5]					
Item	HS	LS	SEM	Con[3]	Mon[4]	SEM	S	M	P	S×M	S×P	M×P
Gluconeogenesis related gene[6]												
MUT	1.42	1.53	0.13	1.46	1.48	0.14	0.53	0.93	0.76	0.005	0.92	0.13
PC	1.68	1.62	0.26	1.29	2.00	0.27	0.88	0.06	0.44	0.07	0.72	0.26
PCK1	3.22	3.43	0.18	3.38	3.27	0.19	0.41	0.66	0.97	0.07	0.75	0.28
G6PC	4.47	4.49	0.26	4.50	4.46	0.27	0.96	0.91	0.62	0.48	0.74	0.53
FA oxidation related gene[7]												
CPT1A	3.48	3.62	0.38	3.46	3.64	0.41	0.50	0.48	0.19	0.59	0.91	0.41
Ketogenesis related gene[8]												
HMGCS2	2.13	1.95	0.23	2.20	1.89	0.23	0.50	0.23	0.90	0.08	0.69	0.74
FA esterification related gene[9]												
ACAC	3.63	3.59	0.19	3.71	3.51	0.19	0.89	0.44	0.63	0.02	0.84	0.04

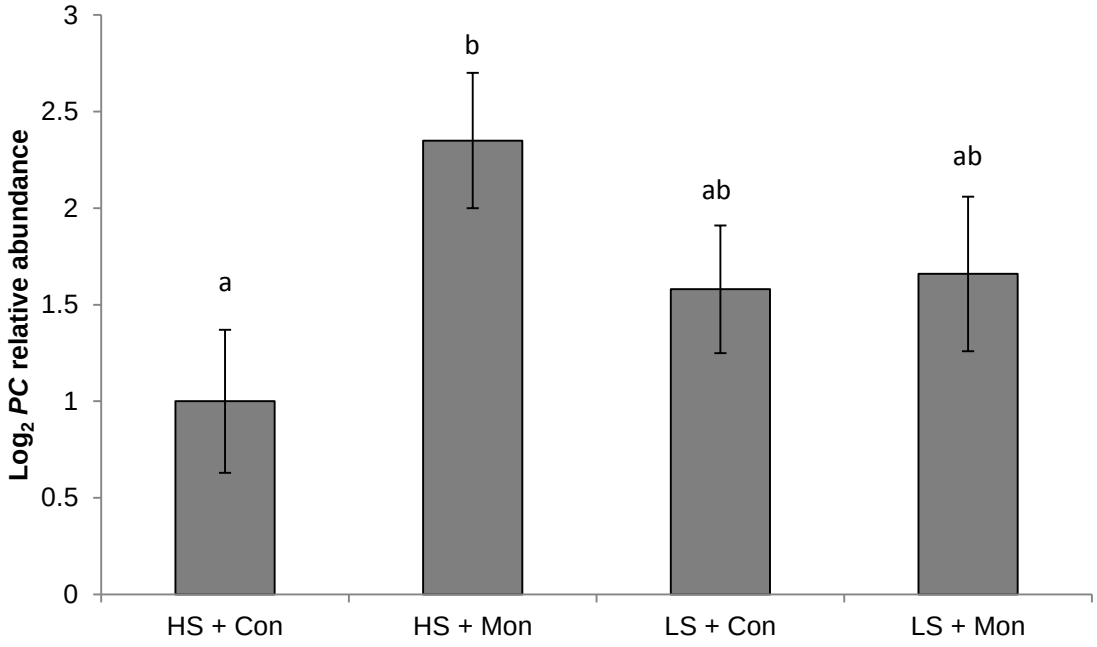
[1]Postpartum diets HS = high starch diet (26.2% starch) and LS = low starch diet (21.5% starch).
[2]Con = control topdress and Mon = monensin topdress.
[3]Formulated to supplement 0 mg/d monensin.
[4]Formulated to supplement 400 mg/d prepartum and 450 mg/d postpartum.
[5]S = starch, M = monensin, and P = parity.
[6]*MUT* = methylmalonyl CoA mutase; *PC* = pyruvate carboxylase; *PCK1* = cytosolic phosphoenolpyruvate carboxykinase; *G6PC* = glucose 6 phosphatase.
[7]*CPT1A* = carnatine palmitoyl-transferase 1A.
[8]*HMGCS2* = 3-hudroxy-3-methyl-glutaryl-CoA synthase.
[9]*ACAC* = acetyl-CoA carboxylase.

Figure 5-1. Starch × Mon interactions for hepatic gene expression of (A) methylmalonyl CoA mutase (*MUT* ; $P = 0.005$), (B) pyruvate carboxylase (*PC*; $P = 0.07$), (C) cytosolic phosphoenolpyruvate carboxykinase (*PCK1*; $P = 0.07$), (D) glucose 6 phosphatase (*G6PC*; $P = 0.48$), (E) carnatine palmitoyl-transferase 1A (*CPT1A*; $P = 0.59$), (F) 3-hudroxy-3-methyl-glutaryl-CoA synthase (*HMGCS2*; $P = 0.08$), (G) acetyl-CoA carboxylase (*ACAC*; $P = 0.02$).

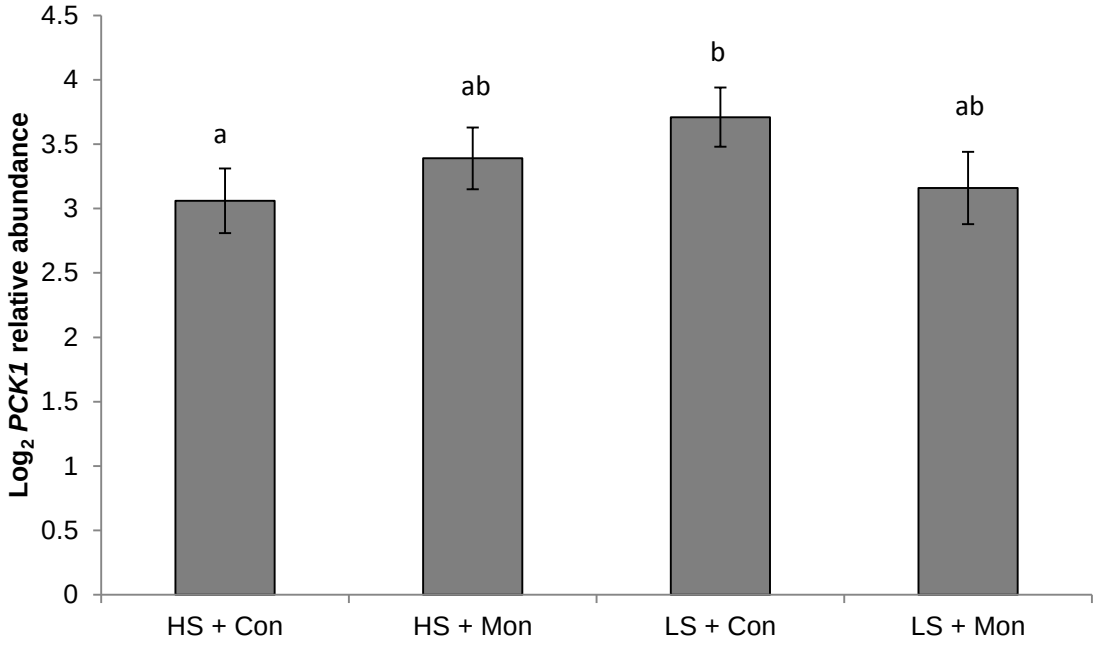
Panel 1A.



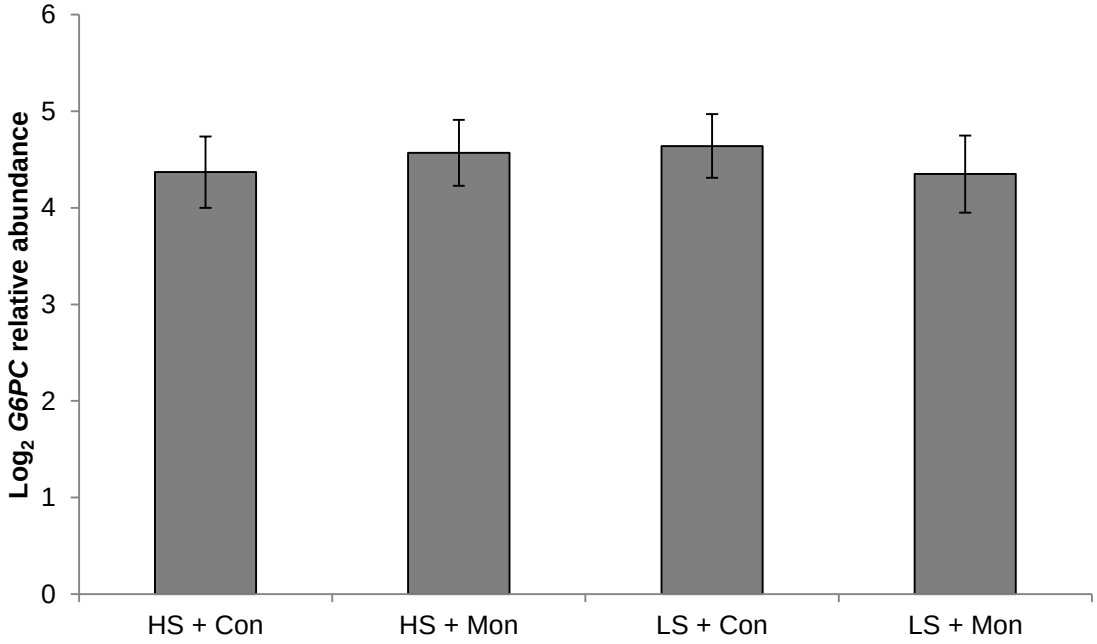
Panel 1B.



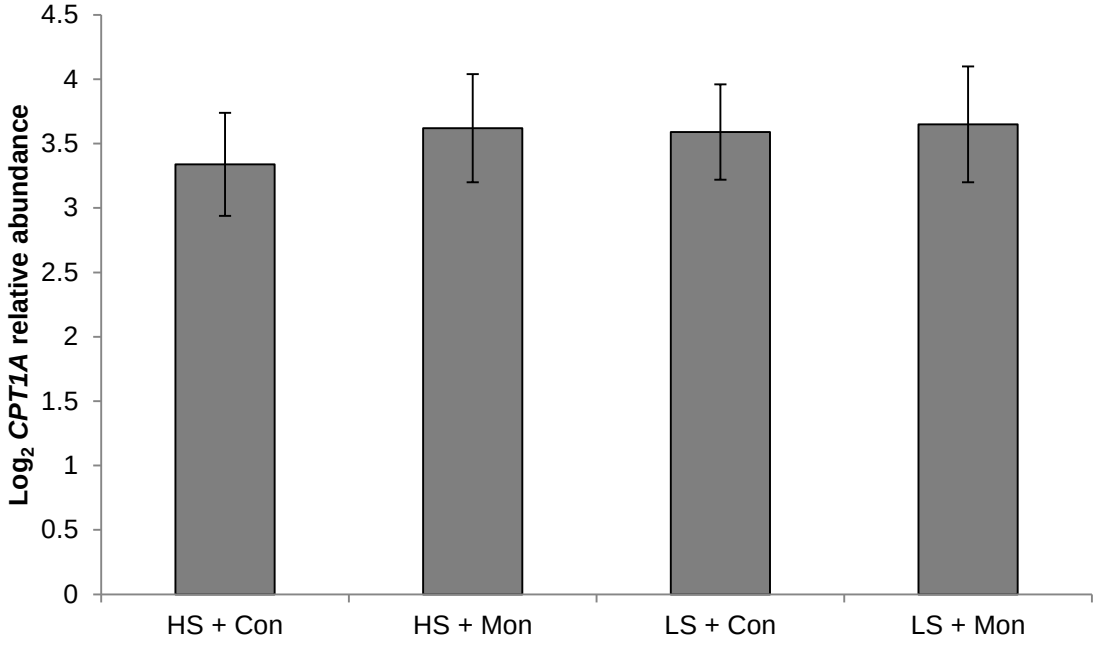
Panel 1C.



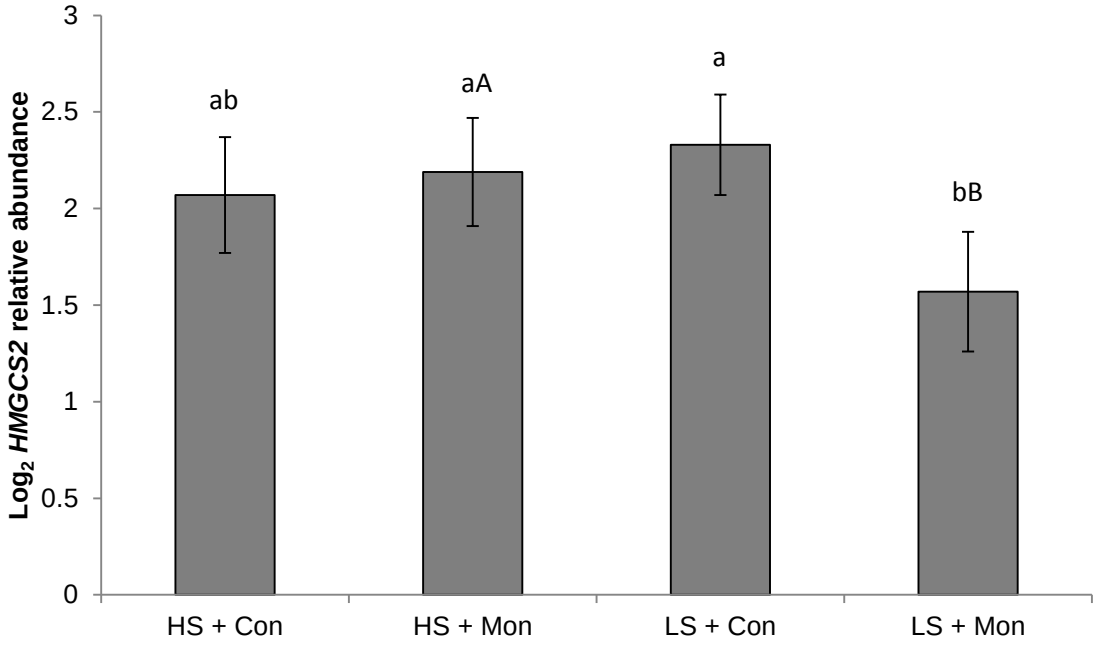
Panel 1D.



Panel 1E.



Panel 1F.



Panel 1G.

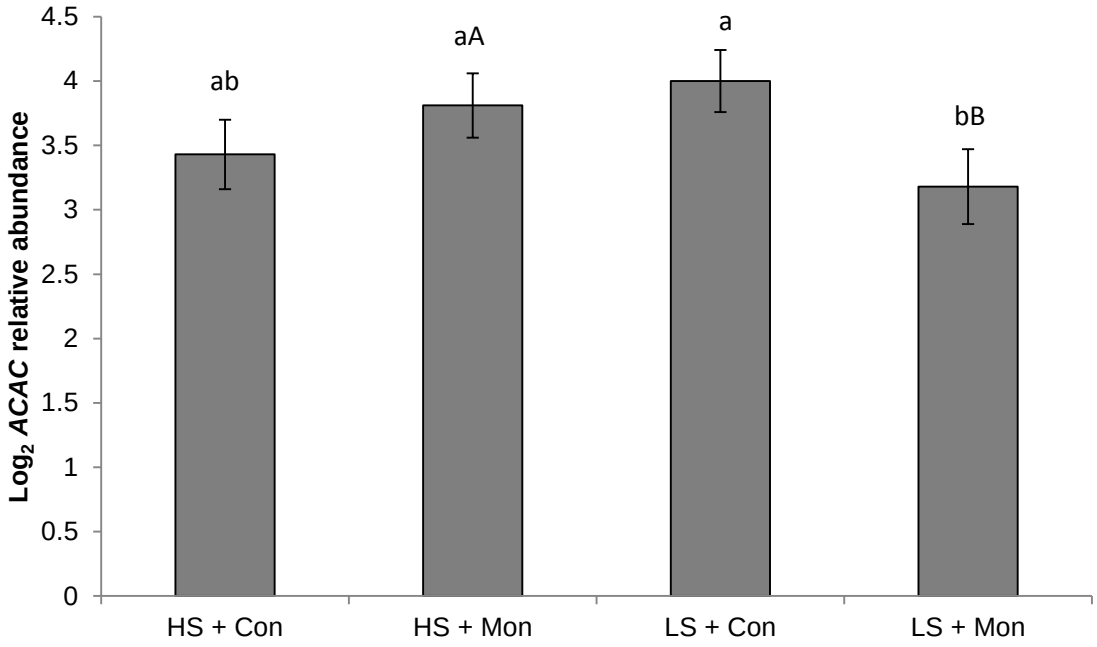
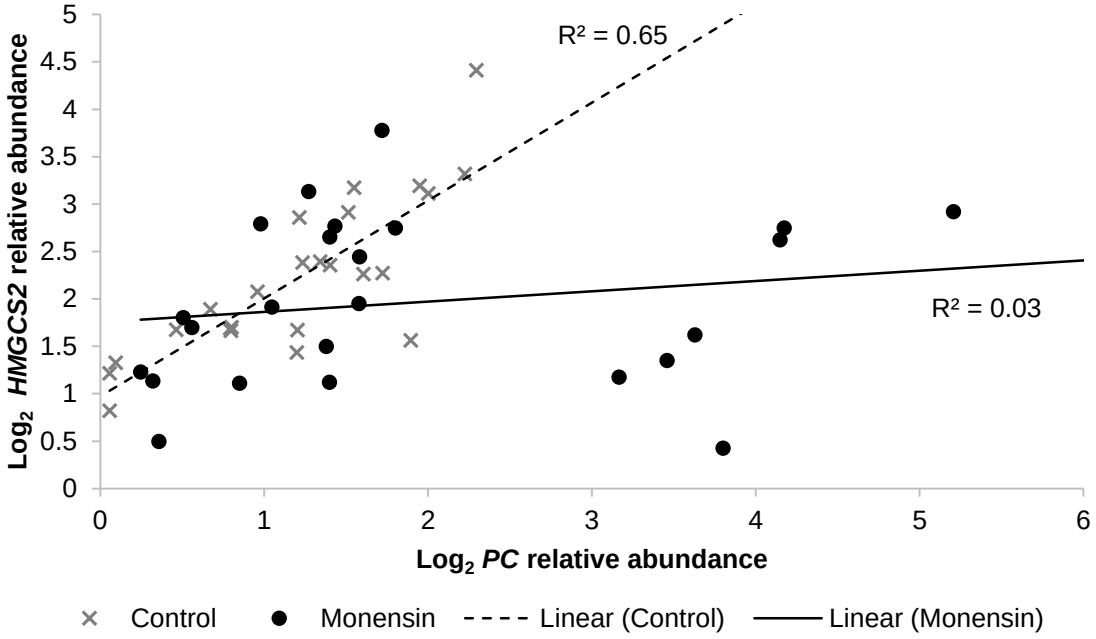
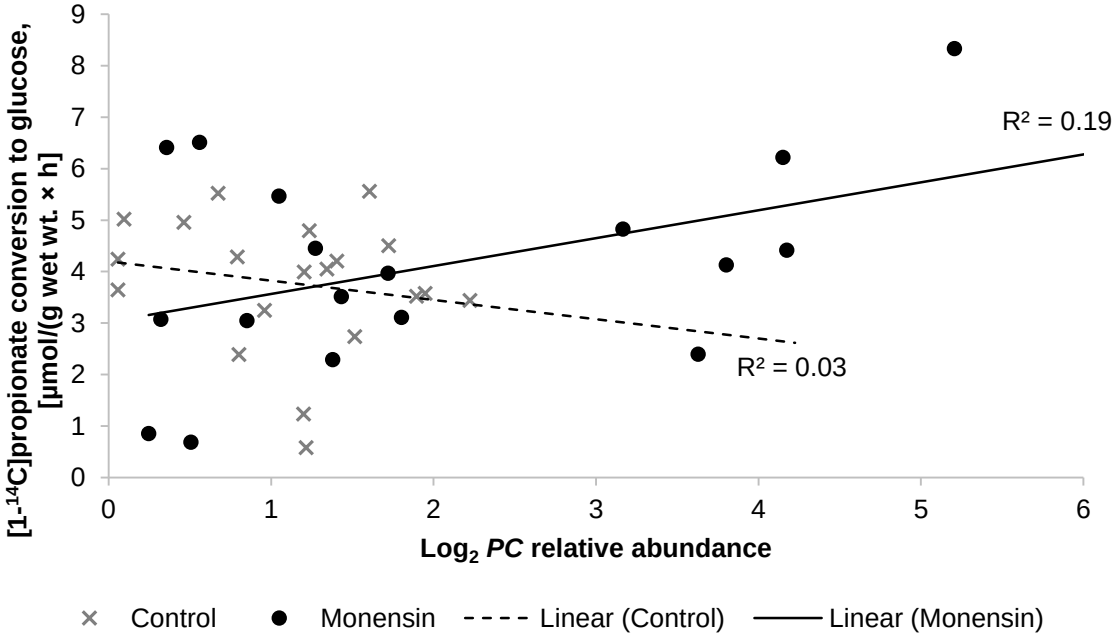

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Log2 ACAC relative abundance
ab
aA
a
bB
HS + Con
HS + Mon
LS + Con
LS + Mon

Figure 5-2. Pearson correlations for relationships for cows fed either control (Con; 0 mg monensin/d) or monensin (400 mg monensin/d prepartum and 450 mg monensin/d postpartum;) between (A) $\log_2$ relative abundance of *PC* and *HMGCS2* on d 7 postpartum (Con $r = 0.806$, $P = 0.02$; Mon $r = 0.173$, $P = 0.41$) and (B) $\log_2$ relative abundance of *PC* and in vitro [1-^{14}C]propionate conversion to glucose on d 7 postpartum (Con $r = 0.173$, $P = 0.97$; Mon $r = 0.436$, $P = 0.07$).

Panel 2A.



Panel 2B.



DISCUSSION

We hypothesized that increasing starch level and feeding monensin during the immediate postpartum period would increase hepatic gluconeogenesis by increasing the expression of gluconeogenic and fatty acid oxidation enzymes. In this study, there was an interesting lack of interactions of starch × Mon on production (McCarthy et al., 2015a) and metabolism (McCarthy et al., 2015b), including in vitro hepatic conversion of [1-^{14}C]propionate to CO_2 and glucose (McCarthy et al., 2015b). However, the current gene expression data from the same cows suggest multiple starch × Mon interactions, indicating differences in hepatic responses to treatments at the molecular level.

An increase in hepatic DNA content should indicate an increase in cell number and potential increase in liver mass, whereas an increase in RNA content would indicate a potential increase in protein synthesis capacity. The RNA:DNA ratio provides an index of the capacity of protein synthesis per cell as the DNA content per cell likely does not vary (Wagner et al., 1998). Baldwin et al. (2004) reported a quadratic increase in hepatic DNA content with increasing DIM, although there was no effect of time on RNA content in the same study. In the current study primiparous cows fed LS had greater hepatic DNA content compared to primiparous cows fed HS. However, there was a tendency for cows fed HS to have a greater RNA content and subsequent numerical increase in RNA:DNA ratio, indicating that cows fed HS may have had an increased ability for protein synthesis during the early postpartum period.

In the liver propionate is metabolized through mitochondrial propionyl-CoA carboxylase and *MUT*. Methylmalonyl-CoA mutase is a key control point for

gluconeogenesis from propionate, although little is known about the regulation of *MUT* (Aschenbach et al., 2010). In the current study cows fed HS + Mon and LS + Con had the lowest expression of *MUT*. There was also a tendency for a Mon × parity interaction for *MUT* and primiparous cows fed Con had greater expression of *MUT* compared to primiparous cows fed Mon whereas cows fed Con had decreased expression of *MUT* compared to cows fed Mon. Interestingly, there were negative relationships between *MUT* and *CPT1A*, *PC*, and *PCK1* in the current study, indicating that an increase in *MUT* expression doesn't necessarily relate to increases in key gluconeogenic enzymes.

After the propionate carbon enters the tricarboxylic acid (**TCA**) cycle at succinyl-CoA, it is eventually converted to oxaloacetate and can then be metabolized by phosphoenolpyruvate carboxykinase to phosphoenolpyruvate and subsequently glucose (Aschenbach et al., 2010). The removal of oxaloacetate from the TCA cycle to phosphoenolpyruvate for gluconeogenesis is a cataplerotic reaction (Owen et al., 2002). During gluconeogenesis additional sources of oxaloacetate must be supplied to condense with acetyl CoA to maintain TCA cycle function and generate ATP. The activity of *PC*, which is a key anaplerotic enzyme in the TCA cycle (Owen et al., 2002), is critical in providing a pool of oxaloacetate for gluconeogenesis and TCA cycle activity (Aiello and Armentano, 1987). During early lactation it is likely that amino acids and lactate are supplying the additional sources of oxaloacetate to maintain this TCA cycle activity (Drackley et al., 2001). The mRNA expression of *PC* has been shown to increase during feed restriction in dairy cows (Velez and Donkin,

2005). Greenfield et al. (2000) observed an increase in *PC* mRNA abundance at calving, when cows exhibit a similar decrease in DMI (Ingvartsen and Andersen, 2000). This increase in mRNA abundance for *PC* was also strongly associated with an increase in *PC* activity (Greenfield et al., 2000). In the current study, there was a tendency for cows fed Mon to have increased *PC* expression compared to Con cows. This seemed to be driven by a starch × monensin interaction such that cows fed HS + Mon had the greatest elevation in *PC* compared to the other treatments. In this study we observed that cows fed Mon had an increased propensity to convert [1-^{14}C]propionate to glucose in vitro (McCarthy et al., 2015b), indicating a greater removal of TCA cycle intermediates. This increased removal of oxaloacetate from the TCA cycle would increase the need for additional oxaloacetate to condense with acetyl CoA and generate ATP to maintain TCA cycle function. The observed increase in *PC* abundance for HS + Mon cows likely reflects this increase in gluconeogenesis. In a study by Ylioja et al. (2013), cows fed a diet containing high moisture corn postpartum had increased *PC*, propionyl CoA synthase, and propionyl CoA carboxylase α expression during early lactation compared to cows fed postpartum diets containing dry corn. Similar to results from the current study (McCarthy et al., 2015a), Ylioja et al. (2013) also observed increased early lactation milk yield for cows fed more propiogenic diets with high moisture corn compared to cows fed dry corn during the early postpartum period. High moisture corn in the same study also tended to increase expression of citrate synthase compared to dry corn (Ylioja et al., 2013), also indicating an increased condensation of oxaloacetate and acetyl CoA to generate

citrate with increased gluconeogenic capacity.

One of the key regulatory enzymes in the gluconeogenic pathway is phosphoenolpyruvate carboxykinase (Croniger et al., 2002), and the phosphoenolpyruvate carboxykinase gene promoter is positively regulated by propionate (Hazelton et al., 2008). The transition to lactation does not appear to alter the mitochondrial form of phosphoenolpyruvate carboxykinase expression; however, expression of *PCK1* mRNA has been shown to increase during early lactation (Agca et al., 2002). Karcher et al. (2007) observed that cows fed monensin (300 mg/d) beginning -28 d prior to calving until calving had an increase in *PCK1* expression on d -14 prepartum and d 1 postpartum compared to control cows. This increase in expression of *PCK1* mRNA is matched by an increased activity of the enzyme (Agca et al., 2002). In the current study there was a tendency for a starch × monensin interaction for *PCK1* at d 7 postpartum; however, cows that were fed LS + Con tended to have greater *PCK1* expression than cows that were fed HS + Con. This is in contrast to observations of Karcher et al. (2007) for increased *PCK1* expression in cows fed more propiogenic diets in early lactation; however, by time of biopsy (d 7) in the current study, Karcher et al. (2007) observed no difference in *PCK1* expression between groups. In the current study correlation relationships between *PCK1* and *CPT1A* were positive, along with relationships between *PCK1* and *G6PC* and *PCK1* and *PC*, indicating increased gluconeogenic activity with increased *PCK1* expression. However, there have also been reports of no effect of propiogenic diet on *PCK1* expression (Ylioja et al., 2013) and a similar lack of effect of early lactation

propionate infusion on *PCK1* expression (Stocks and Allen, 2013), indicating variability in the response of *PCK1* expression to increased propiogenic substrate supply at the liver. Other reports suggest that *PCK1* expression is not upregulated until d 28 postpartum (Greenfield et al., 2000), indicating that perhaps other gluconeogenic enzymes are of greater importance during very early lactation.

Liver ketogenesis is regulated by the amount of substrate to the liver, the activity of *CPT1A* to promote the entry of NEFA into the mitochondria, as well as the intramitochondrial activity of *HMGCS2*, that is the rate limiting step in converting acetyl-CoA to ketone bodies (Drackley et al., 2001). The conversion of succinyl-CoA to succinate leads to inhibition of *HMGCS2* (Zammit, 1990). When there is a large supply of propionate, the pool of succinyl-CoA increases and leads to the inhibition of *HMGCS2* (Drackley et al., 2001) and decreased ketone body synthesis. Cows fed LS + Con tended to have greater *HMGCS2* than LS + Mon cows. In the current study, cows fed HS and Mon had reductions in plasma BHBA concentrations and greater plasma glucose concentrations compared to cows fed LS and Con (McCarthy et al., 2015b). This increase in plasma glucose is likely a result of increased hepatic gluconeogenesis from propionate (increasing the hepatic pool of succinyl-CoA) leading to an inhibition of *HMGCS2* and the observed reductions in BHBA in those cows. Interestingly, when we evaluated Pearson correlations there were differences in the relationship between *PC* and *HMGCS2* gene expression (Figure 2A; Con $r = 0.806$, $P = 0.02$; Mon $r = 0.173$, $P = 0.41$) and also between *PC* and in conversion of [1-^{14}C]propionate to glucose (Figure 2 B; Con $r = 0.173$, $P = 0.97$; Mon $r = 0.436$, $P = 0.07$) for cows fed

Mon and Con. Although in vitro [1-^{14}C]palmitate metabolism was not measured in the current study, Andersen et al. (2002) observed that cows that were fed higher propiogenic diets in early lactation had increased oxidation of palmitate compared to cows fed lower energy diets. This increase in palmitate oxidation would suggest that increases in mitochondrial NEFA oxidation to acetyl CoA are providing oxidative carbon for the TCA cycle to accommodate the increased gluconeogenesis from propionate. Andersen et al. (2002) observed that cows fed the higher energy propiogenic diets had increased fatty acid oxidation and less liver triglyceride infiltration, perhaps because increased gluconeogenic capacity has been associated with a higher activity of *CPT1A* (Chow and Jesse, 1992) likely supplying additional acetyl CoA for the TCA cycle.

One of the key enzymes in de novo fatty acid synthesis in the liver is *ACAC* (Vernon, 2005). The product of *ACAC* is malonyl-CoA and has been shown to modulate fatty acid oxidation by inhibiting *CPT1A* activity (Brindle et al., 1985, Guzman and Geelen, 1993). This inhibition of *CPT1A* is likely so that there is not a futile cycling of substrate between fatty acid synthesis and oxidation at the same time, although this relationship may be modulated during early lactation (Zammit, 1996), as will be discussed further. In the current study there was a starch × monensin interaction for *ACAC* such that cows fed LS + Con had greater *ACAC* expression compared to cows fed LS + Mon. Cows fed LS had greater NEFA mobilization than cows fed HS (McCarthy et al., 2015b) and the increase ACAC expression in LS + Con cows may be a reflection of exceeding the fatty acid oxidative pools for those cows, although there

was no effect of starch treatment on liver triglyceride content (McCarthy et al., 2015b). In contrast, Murondoti et al. (2004) observed that cows that were overfed during the dry period had elevated NEFA mobilization postpartum, and subsequently had reduced *ACAC* and fatty acid synthase activity, with no alterations *HMGCS2* or citrate synthase activity postpartum (Murondoti et al., 2004), leading to a reduction in TCA cycle capacity for fatty acid oxidation and increased triglyceride accumulation postpartum. Cows with high liver fat postpartum had greater PC mRNA abundance and tended to have greater *G6PC* as well (Hammon et al., 2009). Hammon et al. (2009) observed a positive relationship between *PC* abundance and liver triglyceride content (r = 0.48) however in the current study there was not a strong relationship between these variables (r = 0.21). In contrast to observations of Hammon et al., (2009), Li et al. (2012b) observed that bovine hepatocytes cultured with increasing NEFA concentration had a decrease in *PC* and *PCK1* mRNA expression, indicating that cows with excessive NEFA mobilization may have impairments in hepatic gluconeogenesis.

The β-oxidation of NEFA produces acetyl-CoA, which is an activator of *PC*, and can serve to help maintain oxaloacetate and TCA cycle intermediates in the mitochondria (Chow and Jesse, 1992). Both malonyl-CoA formation and methylmalonyl-CoA are inhibitory to *CPT1A* activity (Brindle et al., 1985, Drackley et al., 2001). However, during early lactation these inhibitory mechanisms may be decreased. In rodent models the inhibition of *CPT1A* by malonyl-CoA is diminished when insulin concentrations decrease, such as during early lactation in the dairy cow, and may increase NEFA transport into the mitochondria as well as oxidation (Zammit,

1996). Carnitine sensitivity to inhibition of malonyl-CoA has also been shown to decrease during starvation (Guzman and Geelen, 1993), and may be applied to the negative energy balance of early lactation as well. When 400 mg/d monensin was fed to cows beginning 21 d prior to parturition through d 21 postpartum, monensin treated cows had increased *CPT1A* mRNA expression on d -7 and d 0 relative to parturition; however, there was no difference from control cows by d 7 postpartum (Mullins et al., 2012). Similarly in the current study we observed no effect of treatment on *CPT1A* expression at the time of biopsy on d 7 postpartum. Recent work with early lactation animals has shown propionate infusion to be more hypophagic in animals with higher liver acetyl CoA concentrations, which is indicative of greater NEFA mobilization (Stocks and Allen, 2012, 2013). Interestingly, ketotic cows may also have a reduced ability for β-oxidation of NEFA as indicated by decreased mRNA levels of *CPT1A* and decreased mRNA and protein levels of carnitine palmitoyltransferase II, acyl-CoA dehydrogenase long chain, *HMGCS2*, and acetyl CoA carboxylase at increasing serum BHBA concentrations (Li et al., 2012a), indicating further impairment in hepatic NEFA handling when ketone synthesis is elevated.

CONCLUSIONS

Cows fed high starch diets during the early postpartum period feeding and diets containing monensin throughout the transition period had clear improvements in postpartum production and metabolism. However results of gene expression are less clear, although they do indicate alterations in gene expression for cows fed diets of different starch content either with or without monensin. Because cows were biopsied

at d 7 postpartum and treatment differences in DMI were not observed until later, it is likely that alterations in hepatic gene expression would be more apparent with increasing DIM. Overall, cows fed diets of different starch content in early lactation and monensin throughout the transition period had alterations in in hepatic gene expression of enzymes related to energy metabolism.

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CHAPTER 6

ASSOCIATIONS BETWEEN HEPATIC METABOLISM OF PROPIONATE AND PALMITATE IN LIVER SLICES FROM TRANSITION DAIRY COWS

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ABSTRACT

Multiparous Holstein cows (n = 95) were used to evaluate changes in hepatic propionate and palmitate metabolism and liver composition over time during the transition period, along with the relationships of these variables with the cumulative increases in NEFA and BHBA during the periparturient period. Data from three previous experiments were utilized to address the study objectives, accounting for a total of 95 multiparous Holstein cows. Liver slices from biopsies on d −21, 1, and 21 relative to parturition were used to determine conversion of [1-^{14}C]palmitate to CO_2 and esterified products (EP) and the conversion of [1-^{14}C]propionate to CO_2 and glucose. Hepatic glycogen content was highest on d -21 prepartum and was 26.9% and 36.5% of prepartum values on d 1 and 21 postpartum, respectively. Liver triglyceride content was lowest at d -21 prepartum and was 271% and 446% of prepartum values on d 1 and 21 postpartum, respectively. There was no difference in the capacity for the liver to oxidize [1-^{14}C]palmitate to CO_2 between d -21 prepartum and d 1 postpartum; however, on d 21 postpartum oxidation was 84% of prepartum values. The capacity of the liver to convert [1-^{14}C]palmitate to EP was 148% and 139% of prepartum values on d 1 and 21 postpartum, respectively. The capacity of liver to convert [1-^{14}C]propionate to CO_2 was 127% and 83% of prepartum values on d 1 and 21 postpartum, and the capacity of liver to convert [1-^{14}C]propionate to glucose was 126% and 85% of prepartum values on d 1 and 21 postpartum. Correlation relationships suggest that overall, cows with elevated prepartum liver triglyceride content had elevated triglycerides throughout the transition period along with

increased [1-^{14}C]palmitate oxidation and conversion to EP and a decreased propensity to convert [1-^{14}C]propionate to glucose. Cows with increased [1-^{14}C]propionate oxidation had increased conversion of [1-^{14}C]propionate to glucose throughout the transition period. Overall, conditions that lead to impairments in fatty acid metabolism during the transition period appear to be associated with impaired postpartum hepatic propionate metabolism.

Keywords: Early lactation, hepatic metabolism, propionate, palmitate

INTRODUCTION

The metabolic demand of lactation during the immediate postpartum period nearly doubles energy requirements compared to prepartum requirements and results in a state of negative energy balance (Drackley et al., 2001). One of the increases in this metabolic demand of lactation is the need to support milk lactose synthesis in the mammary gland (Bell and Bauman, 1997). At 4 d postpartum, glucose utilization by the mammary gland is estimated to be 2 times greater than the glucose utilization of the gravid uterus during late gestation (Bell, 1995). This rapid increase in glucose demand requires careful orchestration of the metabolic utilization of nutrients for other tissues (Bauman and Currie, 1980).

To accommodate this increase in glucose demand, the liver increases its metabolic activity (Reynolds et al., 2003; 2004). Propionate that is produced from ruminal fermentation is quantitatively the greatest contributor to gluconeogenesis during the periparturient period (Reynolds et al., 2003). However, during the

postpartum period there is a large increase in hepatic utilization of other gluconeogenic precursors (amino acids, lactate, and glycerol) to help meet glucose needs during early lactation (Reynolds et al., 2003). Postpartum endocrine changes and alterations in responses of insulin-dependent tissues to insulin stimulation leads to the sparing of glucose for mammary gland lactose synthesis (Bell, 1995). Because of these glucose sparing mechanisms, there is decreased lipogenesis and increased lipolysis in adipose tissue (Bell and Bauman, 1997). During the periparturient period this increase in net lipid mobilization increases the circulating pool of NEFA in the blood stream that can be incorporated into milk fat by the mammary gland, but also increases the proportional uptake of NEFA by the liver (Reynolds et al., 2003). These NEFA can be oxidized completely to CO_2 or partially oxidized to ketone bodies in the liver, including BHBA, that can be exported into circulation for further de novo fatty acid synthesis in the mammary gland and to help meet the energetic needs of the peripheral tissues, thereby sparing glucose for mammary lactose synthesis. Hepatic ketone synthesis depends on energy balance, availability of intermediates in the Krebs cycle, and may also be related to individual differences in hepatic gluconeogenic capacity. When there is a large supply of propionate, hepatic ketone body synthesis is reduced (Zammit, 1990; Drackley et al., 2001). When rates of lipid mobilization are high, uptake of NEFA by the liver commonly exceeds the rates of oxidation and export as very low density lipoproteins (**VLDL**), leading to liver triglyceride accumulation (Drackley, 1999). Excess liver triglyceride accumulation has been associated with a decreased ability for the hepatocyte to synthesize urea (Strang et al.,

1998). The consequent increase in liver ammonia may decrease glucose synthesis from propionate (Overton and Drackley, 1999). As such, there are many potential relationships between hepatic gluconeogenesis and fatty acid metabolism. The objective of this study was to further elucidate how these relationships of energy metabolism change during the transition to lactation. We were interested in evaluating changes in hepatic propionate and palmitate metabolism and liver composition over time during the transition period and the relationships between these variables, along with their relationships with the cumulative increases in circulating NEFA and BHBA concentrations during the periparturient period.

MATERIALS AND METHODS

Experimental animals and procedures

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee (Ithaca, NY) before the onset of the experiments. Data from 95 Holstein cows entering second lactation or greater from the Cornell University Teaching and Research Center Dairy were used for this data set from 3 separate experiments (Piepenbrink, 2003; Piepenbrink and Overton, 2003; Piepenbrink et al., 2004). Briefly, cows were housed in individual tie-stalls and fed the same prepartum and postpartum basal rations within each experiment. Cows received either no dietary treatment (Piepenbrink, 2003), varying amounts of rumen protected choline (Piepenbrink and Overton, 2003), or varying amounts of 2-hydroxy-4-(methylthio)-butanoic acid (Piepenbrink et al., 2004) beginning at 21 d before

expected calving and continuing through either d 63 (Piepenbrink, 2003; Piepenbrink et al., 2003) or 84 d (Piepenbrink et al., 2004) postpartum.

Plasma samples were obtained 3 times per week via venipuncture of the coccygeal vessels from d -21 prepartum through d 21 postpartum and analyzed for NEFA and BHBA. Plasma concentrations of NEFA and BHBA were analyzed by enzymatic analyses (NEFA-C; Wako Pure Chemical Industries, Osaka, Japan; and BHBA dehydrogenase; kit no. 310, Sigma Chemical, St. Louis, MO).

Liver tissue was sampled via percutaneous trocar biopsy (Veenhuizen et al., 1991) from cows under local anesthesia on d -21, 1, and 21 relative to parturition. After blotting the liver sample to remove excess blood and connective tissue, a portion of the sample was immersed in ice-cold PBS (0.015 M; 0.9% NaCl, pH 7.4) and transported to the laboratory within 45 min of tissue collection for measurement of in vitro metabolism of [1-^{14}C]propionate and [1-^{14}C]palmitate as previously described (Piepenbrink, 2003; Piepenbrink and Overton, 2003; Piepenbrink et al., 2004). The remaining portion of liver tissue that was collected was snap-frozen in liquid nitrogen and stored at -80°C until analysis for triglyceride and glycogen content as previously described (Piepenbrink, 2003; Piepenbrink and Overton, 2003; Piepenbrink et al., 2004).

Statistical analysis

All statistical computations were performed using SAS software (version 9.3; SAS Institute Inc., Cary, NC). Data for hepatic glycogen and triglyceride content, in vitro conversion of [1-^{14}C]palmitate to CO_2 and EP, and conversion of [1-

^{14}C]propionate to CO_2 and glucose and the ratio of glucose to CO_2 were subjected to repeated-measures ANOVA using PROC MIXED of SAS and the REPEATED statement. The fixed effect was time and the random effect was cow, and the PDIFF option was used to identify differences within time. Four covariance structures were tested: compound symmetry, heterogeneous compound symmetry, first-order autoregressive, and heterogeneous first-order autoregressive, and the covariance structure that resulted in the smallest Akaike information criterion was used. The degrees of freedom for PROC MIXED were estimated using the Kenward-Roger option in the model statement. Least squares means and standard error of the mean were reported. Statistical significance was declared at $P < 0.05$ and trends were discussed at $0.05 < P < 0.10$.

Eleven variables (Table 6-1) were chosen to test in Pearson correlation by SAS (SAS Users Guide, 2000). The area under the curve (**AUC**) was calculated for both NEFA and BHBA concentrations using the trapezoidal rule as described by Cardoso et al. (2011). If an individual cow was missing more than one-third of the variables, data for that cow were eliminated from the dataset prior to analysis. This resulted in the use of 95 cows; however, some variables only included 80 observations due to missing values.

RESULTS AND DISCUSSION

Hepatic glycogen and triglyceride content

Hepatic glycogen content was highest at d -21 prepartum and was 26.9% and 36.5% of prepartum values on d 1 and 21 postpartum, respectively (Figure 6-1). After

parturition, hepatic glycogen stores are released into circulation as another source of glucose during the immediate postpartum period. By d 21 postpartum hepatic gluconeogenesis has been sufficiently upregulated (Greenfield et al., 2000) such that hepatic glycogen stores begin to be replenished. Interestingly, prepartum concentrations of liver glycogen were not well correlated to glycogen concentrations at d 1 or 21 postpartum (Table 6-2), suggesting that prepartum glycogen stores are not predictive of their postpartum utilization, likely because to a large extent all cows mobilize glycogen during the immediate postpartum period (Hammon et al., 2009; Weber et al., 2013b).

Liver triglyceride content was lowest at d -21 prepartum and was 271% and 446% of prepartum values by d 1 and 21 postpartum, respectively (Figure 6-1). Liver triglyceride content at d -21 prepartum was correlated positively with concentrations of triglyceride in the liver on both d 1 and 21 postpartum (Table 6-2), suggesting that cows with higher prepartum liver triglyceride content also had greater accumulation of triglyceride postpartum. Some lipid accumulation in the liver during the periparturient period seems to be a normal adaptation to lactation (Grum et al., 1996b); however, excess liver triglyceride accumulation has been shown to impair hepatic metabolism (Strang et al., 1998).

Concentrations of liver glycogen and triglyceride were correlated positively at d -21 prepartum (Table 6-2), and cows with higher prepartum glycogen content also had higher triglyceride content prepartum. However, liver sampled on d 1 and 21 postpartum had weak negative correlations for concentrations of glycogen and

triglyceride, again suggesting that all cows mobilize glycogen postpartum regardless of liver triglyceride content, although cows with lower postpartum glycogen content may have greater depletion of glucose stores (Weber et al., 2013b). Glycogen on d 1 and 21 postpartum was correlated negatively with the capacity of liver to convert [1-^{14}C]palmitate to EP prepartum and on d 1 and 21 postpartum, respectively (Table 6-3), suggesting that cows with greater postpartum glycogen content may have decreased hepatic conversion of palmitate to triglycerides. Weber et al. (2013) observed that cows classified with low postpartum triglyceride content had greater postpartum glycogen content, higher postpartum DMI, and improved energy balance.

Prepartum concentrations of liver triglycerides had a positive correlation with liver capacity to convert [1-^{14}C]palmitate to CO_2 prepartum (Table 6-3). Concentrations of liver triglycerides on d 1 postpartum also were correlated positively with the capacity of liver to convert [1-^{14}C]palmitate to CO_2 on d 1 postpartum; however, relationships were less strong. There was a positive relationship between liver triglyceride content and the capacity of liver to convert [1-^{14}C]palmitate to EP on both d 1 and 21 postpartum. Litherland et al. (2011) observed that liver slices from overfed dry cows had decreased in vitro capacity to oxidize [1-^{14}C]palmitate and increase capacity for esterification on d 1 postpartum compared to energy restricted cows, leading to a greater likelihood for postpartum fatty liver accumulation.

Although there were no relationships between postpartum liver glycogen content and [1-^{14}C]propionate conversion to CO_2, liver triglyceride content on d 1 and 21 postpartum was negatively correlated with the capacity of liver to convert [1-

^{14}C]propionate to CO_2 on d 1 and 21 postpartum. Postpartum liver triglyceride content was also negatively correlated with the ratio of glucose to CO_2 at all postpartum time points, suggesting that cows with increased liver triglycerides had a reduced propensity to convert propionate to glucose. Similarly, Weber et al. (2013) observed that cows classified with greater postpartum triglyceride content had impaired mRNA expression of gluconeogenic enzymes.

Interestingly, there was a strong negative correlation between prepartum triglyceride content and prepartum NEFA AUC such that cows with elevated prepartum liver triglycerides had a lower prepartum NEFA AUC. However, there was a positive relationship between liver triglyceride content at d1 and 21 postpartum and NEFA AUC postpartum, indicating that as more NEFA are released into circulation during the postpartum period more triglycerides accumulate in the liver. Vazquez-Añon et al. (1994) similarly observed that elevated NEFA on d 1 postpartum was strongly related to hepatic triglyceride accumulation, and Strang et al (1998) observed a linear increase in triglyceride content in hepatocytes incubated in vitro with increasing levels of NEFA. This would lead to increased hepatic fatty acid metabolism and greater stored triglyceride in the liver, as Liu et al. (2014) observed decreased gene expression of key enzymes involved in VLDL export for hepatocytes that were incubated in vitro with increasing concentrations of NEFA. Postpartum liver triglyceride content on both d 1 and 21 were also positively correlated with postpartum BHBA AUC, indicating that perhaps as VLDL export is overwhelmed and stored triglycerides accumulate in the liver there is an increase in the partial oxidation

of fatty acids to ketone bodies.

Capacity of liver to metabolize palmitate

There was no difference in the capacity for the liver to oxidize [1-^{14}C]palmitate to CO_2 between d -21 prepartum and d 1 postpartum; however, on d 21 postpartum oxidation was 84% of prepartum values (Figure 6-2). The capacity of the liver to convert [1-^{14}C]palmitate to EP was 148% and 139% of prepartum values on d 1 and 21 postpartum, respectively (Figure 6-2).

The capacity for liver to oxidize [1-^{14}C]palmitate to CO_2 on d -21 prepartum was correlated with the capacity of the liver to oxidize [1-^{14}C]palmitate to CO_2 on both d 1 and 21 postpartum (Table 6-4), indicating that cows with a high oxidative capacity prepartum maintained a high oxidative capacity throughout the periparturient period. There was a negative relationship between prepartum liver capacity to oxidize [1-^{14}C]palmitate to CO_2 and the prepartum capacity for liver to convert [1-^{14}C]palmitate to EP; however, there was no relationship on d 1 postpartum, and the relationships between palmitate oxidation and conversion to EP was positive on d 21 postpartum. This alteration in the relationship postpartum between [1-^{14}C]palmitate oxidation to CO_2 and EP could suggest that these processes are responding to the postpartum negative energy balance and the increases in NEFA mobilization and ketone body synthesis. During the postpartum period there is a greater increase in partial oxidation to ketone bodies during early lactation (Zammit, 1990). Unfortunately in this data set ketone body synthesis, using the in vitro conversion of [1-^{14}C]palmitate to acid soluble products as a proxy, was not measured. However, we

could speculate that the lack of relationship between [1-^{14}C]palmitate oxidation and conversion to EP on d 1 postpartum may be because there is an increase in ketone synthesis during that period. Although the capacity of liver to convert [1-^{14}C]palmitate to CO_2 and EP at -21 d prepartum was negatively correlated with prepartum NEFA AUC, Litherland et al. (2011) observed that NEFA on d 1 postpartum was strongly correlated with [1-^{14}C]palmitate conversion to EP on d 1 postpartum. Although our data show a poor relationship between conversion to EP on d 1 postpartum with postpartum NEFA AUC, the capacity of liver to convert [1-^{14}C]palmitate to EP on d 21 was positively correlated with postpartum NEFA AUC and also postpartum BHBA AUC, indicating that as postpartum NEFA mobilization increases there is an increase in conversion of fatty acids to stored EP.

The capacity of liver slices to convert [1-^{14}C]palmitate to CO_2 at all time points was correlated negatively with the capacity to convert [1-^{14}C]propionate to glucose at all time points. Because prepartum [1-^{14}C]palmitate oxidation was correlated positively with liver triglyceride content, and liver triglycerides are associated with impaired gluconeogenesis (Drackley et al., 2001) it is possible that this negative correlation between fatty acid oxidation and gluconeogenesis is rather a reflection of triglyceride impaired liver function. On d 21 postpartum the capacity of liver slices to convert [1-^{14}C]palmitate to CO_2 was correlated negatively with the capacity of liver to oxidize [1-^{14}C]propionate to CO_2 on d 21 postpartum (Table 6-5), indicating that cows with increased [1-^{14}C]palmitate oxidation have decreased [1-^{14}C]propionate oxidation. The oxidation of NEFA in the liver provides ATP that is

needed for gluconeogenesis, and Andersen et al. (2002) reported increased capacity for palmitate conversion to CO_2 in liver slices from early lactation cows fed more propiogenic diets compared to liver slices from cows fed less propiogenic diets. Drackley et al. (2001) reported that liver slices from early postpartum cows have an increased propensity to convert propionate to glucose during early lactation. Together this may suggest that during early lactation cows have increased ability to use propionate for glucose synthesis and that feeding more propiogenic diets promotes more complete oxidation of acetyl CoA derived from mitochondrial β-oxidation of fatty acyl CoA.

Hepatic propionate metabolism

The capacity of liver to convert [1-^{14}C]propionate to CO_2 was 127% and 83% of prepartum values on d 1 and 21 postpartum, and the capacity of liver to convert [1-^{14}C]propionate to glucose was 126% and 85% of prepartum values on d 1 and 21 postpartum (Figure 6-3). In experiments using labeled isotopes, the [1-^{14}C]propionate label randomizes in the TCA cycle such that every mole of [1-^{14}C]propionate that is directed toward oxaloacetate would yield 0.5 moles of radiolabeled CO_2 and 0.5 moles of radiolabeled glucose (Knapp et al., 1992). Therefore an increase in this ratio of labeled glucose to CO_2 would suggest an increase in the efficiency of utilization of propionate for gluconeogenesis. However, because there were similar increases in both propionate oxidation and the capacity to convert propionate to glucose over time there was no effect of time on the ratio of glucose to CO_2 ($P = 0.91$).

There was a strong relationship between liver capacity for [1-^{14}C]propionate

oxidation to CO_2 and conversion to glucose that increased with time (Table 6-6). There was a strong positive correlation between [1-^{14}C]propionate conversion to glucose with the ratio of glucose to CO_2 for all time points. This would be expected as the [1-^{14}C]propionate conversion to glucose is included as part of the calculated ratio; however, interestingly there was no relationship between [1-^{14}C]propionate oxidation to CO_2 and the calculated ratio. The capacity for gluconeogenesis from [1-^{14}C]propionate on d 21 postpartum was correlated negatively with both postpartum NEFA and BHBA AUC (Table 6-7), indicating that as hepatic propionate utilization increases there is decreased ketogenesis. This is likely because succinyl CoA that is produced when propionate enters the citric acid cycle negatively inhibits 3-hydroxy-3-methylglutaryl-CoA synthase, the rate limiting step in ketone body synthesis (Zammit, 1990; Drackley et al., 2001).

Table 6-1. Variables used in Pearson correlation analysis.

Hepatic glycogen content on d - 21 prepartum and d 1 and 21 postpartum
Hepatic triglyceride content on d - 21 prepartum and d 1 and 21 postpartum
Capacity of liver to convert [1-^{14}C]palmitate to CO_2 on d - 21 prepartum and d 1 and 21 postpartum
Capacity of liver to convert [1-^{14}C]palmitate to EP on d - 21 prepartum and d 1 and 21 postpartum
Capacity of liver to convert [1-^{14}C]propionate to CO_2 on d - 21 prepartum and d 1 and 21 postpartum
Capacity of liver to convert [1-^{14}C]propionate to glucose on d - 21 prepartum and d 1 and 21 postpartum
Ratio of converted [1-^{14}C]propionate to glucose: CO_2 on d - 21 prepartum and d 1 and 21 postpartum
Calculated area under the curve for concentrations NEFA in plasma from d -21 to -1 prepartum
Calculated area under the curve for concentrations of NEFA in plasma from d 1 to 21 postpartum
Calculated area under the curve for concentrations of BHBA in plasma from d -21 to -1 prepartum
Calculated area under the curve for concentrations of BHBA in plasma from d 1 to 21 postpartum

Table 6-2. Pearson correlation among hepatic glycogen and triglyceride (n = 95) content across all other measured variables

	Glycogen			Triglyceride		
Variable[1]	d -21	d +1	d +21	d -21	d +1	d +21
Glycogen d -21	1.00	0.172	0.152	0.490	0.172	0.24
		0.10	0.14	<0.0001	0.10	0.02
Glycogen d +1		1.00	0.232	0.143	-0.243	-0.066
			0.03	0.17	0.02	0.53
Glycogen d +21			1.00	-0.064	-0.240	-0.285
				0.54	0.02	0.005
Triacylglycerol d -21				1.00	0.437	0.362
					<0.0001	0.0004
Triacylglycerol d +1					1.00	0.601
						<0.0001
Triacylglycerol d +21						1.00

[1]Correlation coefficient (*r*) is the top number, with corresponding *P* value beneath.

Table 6-3. Pearson correlation among hepatic glycogen and triglyceride content (n = 95) across all other measured variables

	Glycogen			Triglyceride		
Variable[1]	d -21	d +1	d +21	d -21	d +1	d +21
Palmitate to CO_2 d –21	0.274	0.150	-0.086	0.687	0.255	0.251
	0.008	0.15	0.41	<0.0001	0.01	0.02
Palmitate to CO_2 d +1	0.275	0.135	-0.019	0.682	0.328	0.248
	0.007	0.19	0.86	<0.0001	0.001	0.02
Palmitate to CO_2 d +21	0.323	0.113	-0.094	0.760	0.262	0.173
	0.002	0.28	0.37	<0.0001	<0.0001	0.09
Palmitate to EP d –21	-0.446	-0.147	-0.010	-0.651	-0.246	-0.293
	<0.0001	0.16	0.92	<0.0001	0.02	0.004
Palmitate to EP d +1	-0.104	-0.409	-0.076	-0.132	0.399	0.128
	0.32	<0.0001	0.47	0.21	<0.0001	0.22
Palmitate to EP d +21	-0.053	-0.087	-0.257	0.153	0.314	0.311
	0.61	0.41	0.01	0.14	0.002	0.002
Propionate to CO_2 d -21	-0.053	-0.087	-0.080	0.153	0.314	0.311
	0.61	0.41	0.46	0.14	0.002	0.002
Propionate to CO_2 d +1	-0.136	-0.163	-0.073	-0.210	-0.288	-0.145
	0.21	0.13	0.50	0.05	0.007	0.18
Propionate to CO_2 d +21	-0.296	0.068	0.087	-0.275	-0.405	-0.450
	0.005	0.53	0.42	0.01	<0.0001	<0.0001
Propionate to glucose d -21	-0.119	-0.116	-0.018	-0.400	-0.241	-0.137
	0.29	0.31	0.87	0.0002	0.03	0.23
Propionate to glucose d +1	-0.357	-0.141	-0.149	-0.408	-0.390	-0.278
	0.0007	0.20	0.17	<0.0001	0.0002	0.009
Propionate to glucose d +21	-0.333	-0.043	0.106	-0.444	-0.448	-0.477
	0.002	0.70	0.33	<0.0001	<0.0001	<0.0001
Ratio of glucose to CO2 d -21	-0.283	-0.198	-0.033	-0.556	-0.317	-0.235
	0.01	0.08	0.77	<0.0001	0.004	0.04
Ratio of glucose to CO2 d 1	-0.421	-0.018	-0.138	-0.392	-0.349	-0.303
	<0.0001	0.87	0.20	0.0002	0.001	0.005
Ratio of glucose to CO2 d +21	-0.149	-0.087	0.203	-0.450	-0.332	-0.323
	0.17	0.43	0.06	<0.0001	0.002	0.003
NEFA AUC prepartum	-0.105	0.031	-0.019	-0.454	-0.105	-0.155
	0.32	0.77	0.86	<0.0001	0.33	0.14
NEFA AUC postpartum	-0.025	-0.195	-0.285	-0.0526	0.290	0.356
	0.81	0.07	0.007	0.62	0.006	0.0006
BHBA AUC prepartum	0.055	0.030	-0.114	-0.016	0.007	-0.048
	0.61	0.87	0.29	0.88	0.95	0.65
BHBA AUC postpartum	0.041	-0.044	-0.220	0.246	0.361	0.310
	0.70	0.68	0.04	0.02	0.0005	0.003

[1]Correlation coefficient (*r*) is the top number, with corresponding *P* value beneath.

Table 6-4. Pearson correlation (n = 95) among in vitro conversion of [1-^{14}C]palmitate to CO_2 and intracellular stored esterified products (EP) across all other measured variables

	Palmitate to CO_2			Palmitate to EP		
Variable[1]	d -21	d +1	d +21	d -21	d +1	d +21
Palmitate to CO_2 d –21	1.00	0.671	0.771	-0.485	-0.106	0.226
		<0.0001	<0.0001	<0.0001	0.31	0.03
Palmitate to CO_2 d +1		1.00	0.735	-0.536	-0.091	0.07
			<0.0001	<0.0001	0.38	0.50
Palmitate to CO_2 d +21			1.00	-0.536	-0.241	0.247
				<0.0001	0.02	0.02
Palmitate to EP d –21				1.00	0.218	0.061
					0.03	0.56
Palmitate to EP d +1					1.00	0.289
						0.005
Palmitate to EP d +21						1.00

[1]Correlation coefficient (*r*) is the top number, with corresponding *P* value beneath.

Table 6-5. Pearson correlation (n = 95) among in vitro conversion of [1-^{14}C]palmitate to CO_2 and intracellular stored esterified products (EP) across all other measured variables

	Palmitate to CO_2			Palmitate to EP		
Variable[1]	d -21	d +1	d +21	d -21	d +1	d +21
Propionate to CO_2 d -21	-0.025	-0.056	-0.044	0.106	-0.119	-0.168
	0.82	0.61	0.69	0.33	0.27	0.12
Propionate to CO_2 d +1	-0.168	-0.078	-0.085	0.080	0.087	-0.056
	0.12	0.47	0.44	0.46	0.43	0.60
Propionate to CO_2 d +21	-0.254	-0.266	-0.264	0.393	-0.023	-0.142
	0.02	0.01	0.01	0.0002	0.83	0.19
Propionate to glucose d -21	-0.298	-0.351	-0.369	0.427	-0.043	-0.100
	0.007	0.001	0.0008	<0.0001	0.71	0.39
Propionate to glucose d +1	-0.315	-0.283	-0.272	0.234	0.092	-0.071
	0.003	0.008	0.01	0.03	0.40	0.52
Propionate to glucose d +21	-0.440	-0.388	-0.395	0.511	0.031	-0.144
	<0.0001	0.0003	0.0002	<0.0001	0.78	0.19
Ratio of glucose to CO2 d -21	-0.404	-0.445	-0.434	0.479	0.036	-0.037
	0.0002	<0.0001	<0.0001	<0.0001	0.75	0.74
Ratio of glucose to CO2 d 1	-0.314	-0.329	-0.308	0.263	-0.010	-0.040
	0.003	0.002	0.004	0.01	0.93	0.71
Ratio of glucose to CO2 d +21	-0.358	-0.315	-0.361	0.359	0.020	-0.139
	0.0008	0.003	0.0007	0.0008	0.72	0.20
NEFA AUC prepartum	-0.309	-0.307	-0.370	0.343	0.005	-0.012
	0.003	0.003	0.0003	0.0009	0.96	0.91
NEFA AUC postpartum	0.146	0.065	0.113	-0.007	0.081	0.258
	0.17	0.54	0.29	0.94	0.45	0.01
BHBA AUC prepartum	0.066	0.056	0.059	-0.032	0.005	0.062
	0.54	0.60	0.58	0.76	0.96	0.56
BHBA AUC postpartum	0.307	0.289	0.359	-0.190	0.070	0.376
	0.003	0.006	0.0005	0.07	0.52	0.0003

[1]Correlation coefficient (*r*) is the top number, with corresponding *P* value beneath.

Table 6-6. Pearson correlation (n = 95) among in vitro conversion of [1-^{14}C]propionate to CO_2 and glucose and the ratio of conversion to glucose and CO2 and all other measured variables

	Propionate to CO_2			Propionate to glucose			Ratio of glucose to CO2		
Variable[1]	d -21	d +1	d +21	d -21	d +1	d +21	d -21	d +1	d +21
Propionate to CO_2 d -21	1.00	0.210	0.156	0.701	0.049	-0.002	0.169	-0.106	-0.089
		0.05	0.15	<0.0001	0.65	0.98	0.13	0.33	0.42
Propionate to CO_2 d +1		1.00	0.216	0.260	0.725	0.142	0.251	0.207	0.023
			0.05	0.02	<0.0001	0.20	0.02	0.06	0.84
Propionate to CO_2 d +21			1.00	0.206	0.315	0.814	0.197	0.290	0.151
				0.07	0.003	<0.0001	0.08	0.007	0.17
Propionate to glucose d -21				1.00	0.174	0.162	0.795	-0.001	0.176
					0.12	0.16	<0.0001	0.99	0.12
Propionate to glucose d +1					1.00	0.385	0.297	0.800	0.158
						0.0003	0.008	<0.0001	0.15
Propionate to glucose d +21						1.00	0.263	0.455	0.573
							0.02	<0.0001	<0.0001
Ratio of glucose to CO2 d -21							1.00	0.177	0.320
								0.12	0.004
Ratio of glucose to CO2 d 1								1.00	0.240
									0.03
Ratio of glucose to CO2 d +21									1.00

[1]Correlation coefficient (*r*) is the top number, with corresponding *P* value beneath.

Table 6-7. Pearson correlation (n = 95) among in vitro conversion of [1-^{14}C]propionate to CO_2 and glucose and the ratio of conversion to glucose and CO2 and all other measured variables.

	Propionate to CO_2			Propionate to glucose			Ratio of glucose to CO2		
Variable[1]	d -21	d +1	d +21	d -21	d +1	d +21	d -21	d +1	d +21
NEFA AUC prepartum	-0.106	0.022	0.039	0.025	0.164	0.217	0.147	0.200	0.218
	0.34	0.84	0.72	0.83	0.14	0.05	0.21	0.07	0.05
NEFA AUC postpartum	-0.102	-0.031	-0.320	-0.081	-0.029	-0.219	-0.010	-0.055	-0.102
	0.36	0.78	0.003	0.49	0.80	0.05	0.93	0.62	0.36
BHBA AUC prepartum	-0.065	-0.049	-0.071	-0.162	-0.010	-0.045	-0.147	0.005	-0.048
	0.56	0.66	0.52	0.16	0.93	0.69	0.21	0.97	0.67
BHBA AUC postpartum	-0.142	-0.066	-0.374	-0.264	-0.164	-0.338	-0.207	-0.203	-0.22
	0.20	0.55	0.0005	0.02	0.14	0.002	0.07	0.07	0.05

[1]Correlation coefficient (*r*) is the top number, with corresponding *P* value beneath.

Figure 6-1. Hepatic glycogen (white bars) and triglyceride (gray bars) content as a percentage of tissue wet weight in transition dairy cows on d -21, 1, and 21 relative to calving. The effect of d relative to calving was significant for both glycogen ($P < 0.0001$) and triglyceride ($P < 0.0001$). Means with different letters are significantly different ($P = 0.05$).

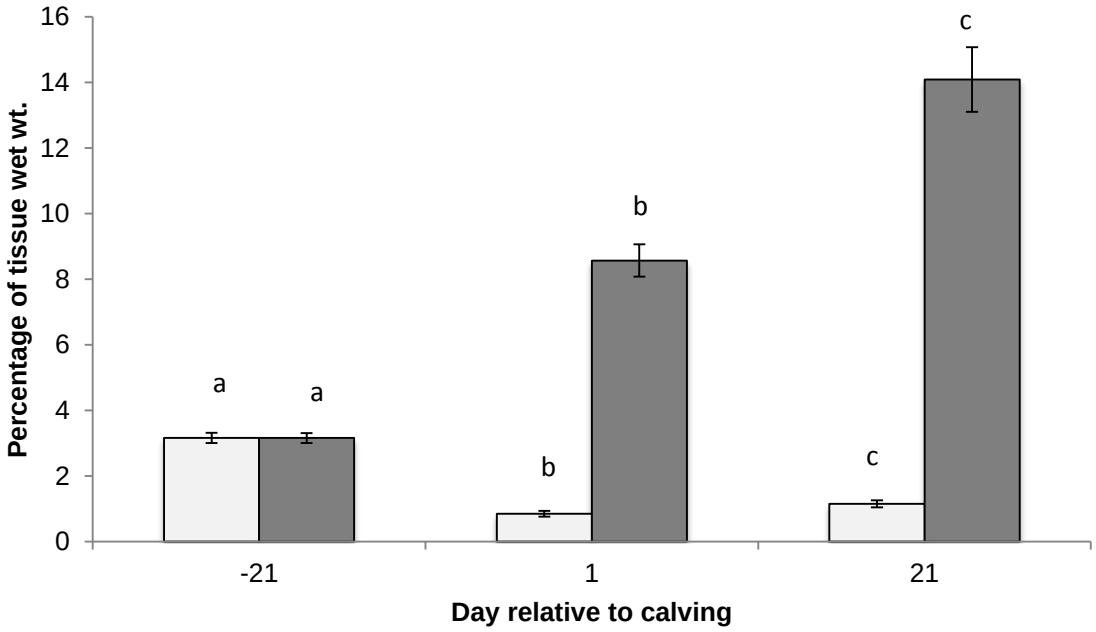


Figure 6-2. Rates of in vitro conversion of [1-^{14}C]palmitate to CO_2 (white bars) and stored esterified products (gray bars) in liver slices from transition dairy cows on d -21, 1, and 21 relative to calving (n = 95). The effect of d relative to calving was significant for both CO_2 ($P < 0.0001$) and stored esterified products ($P < 0.0001$). Means with different letters are significantly different ($P = 0.05$).

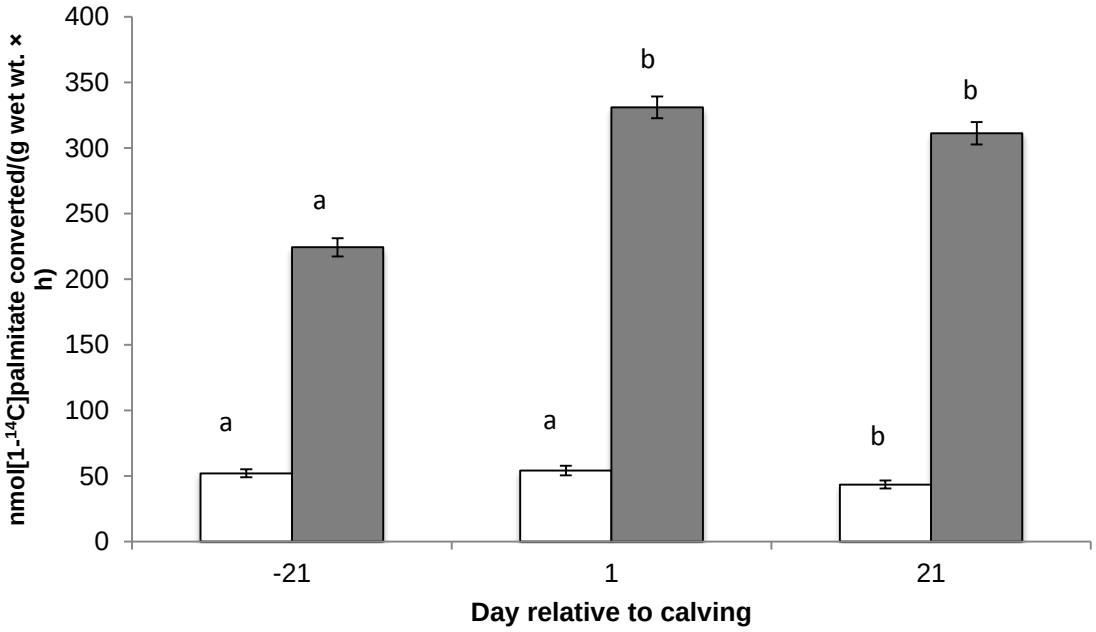
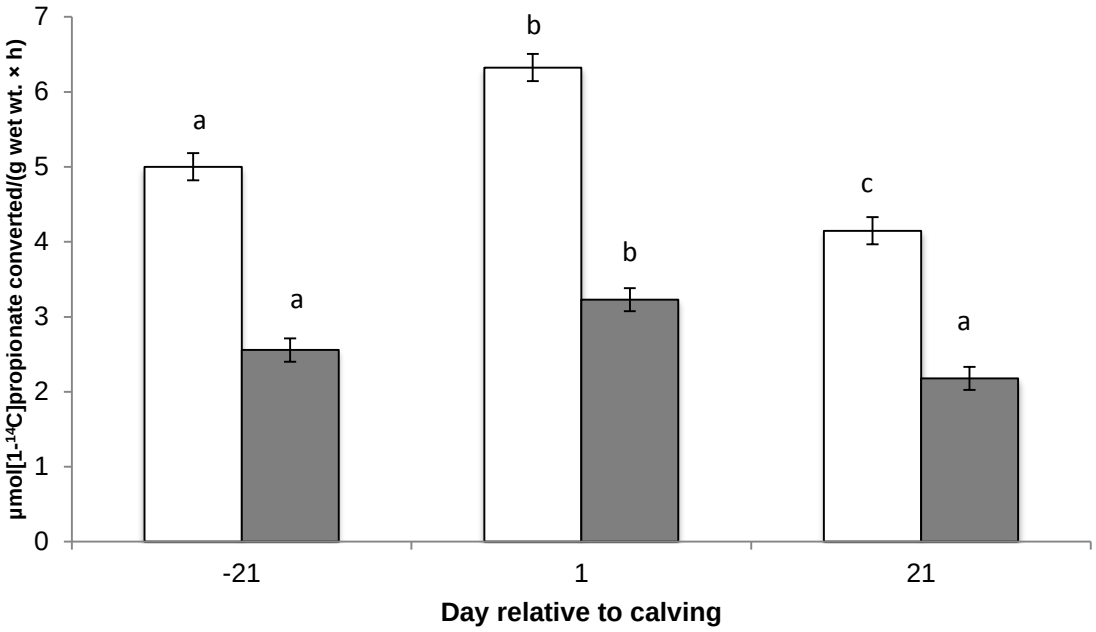


Figure 6-3. Rates of in vitro conversion of [1-^{14}C]propionate to CO_2 (white bars) and glucose (gray bars) in liver slices from transition dairy cows on d -21, 1, and 21 relative to calving (n = 95). The effect of d relative to calving was significant for both CO_2 ($P <$ 0.0001) and glucose ($P < 0.0001$). Means with different letters are significantly different ($P = 0.05$).



CONCLUSIONS

There were decreases in hepatic glycogen content and increases in hepatic triglyceride content as cows transitioned to lactation. Similarly there was an increase in the capacity of the liver to convert [1-^{14}C]palmitate to EP during the postpartum period. There was also an increase in the capacity of liver to convert [1-^{14}C]propionate to CO_2 and glucose during the early postpartum period. Hepatic gluconeogenesis from propionate was negatively correlated with both postpartum NEFA and BHBA AUC, suggesting that cows with greater NEFA mobilization and cows with greater circulating BHBA have reduced gluconeogenic capacity from propionate. Similarly, correlation relationships suggest that excess liver triglyceride content is related to decreased capacity of the liver to convert propionate into glucose. Overall, alterations in fatty acid metabolism that lead to increased triglyceride accumulation during the transition period appear to impair postpartum hepatic gluconeogenesis.

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CHAPTER 7

ASSOCIATIONS BETWEEN THE DEGREE OF EARLY LACTATION INFLAMMATION AND PEROFRMANCE, METABOLISM, AND IMMUNE FUNCTION IN DAIRY COWS

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ABSTRACT

The objective of the current study was to determine associations between the severity of systemic inflammation during the early postpartum period and performance, energy metabolism, and immune function in dairy cows. Cows were assigned to categorical quartiles (Q; Q1 = 0.18-0.59, Q2 = 0.60-1.14, Q3 = 1.15-2.05, and Q4 = 2.06-2.50 g haptoglobin/L) based on the highest plasma haptoglobin (Hp) concentration measured during wk 1 postpartum. Although cows were assigned to different categories of inflammation during the postpartum period, there was a quadratic relationship of inflammation on prepartum dry matter intake (DMI) and net energy for lactation (NE_L) intake such that cows in Q3 had lower prepartum NE_L intake compared to cows in the other Q. There was a quadratic association of inflammation with postpartum DMI and NE_L intake such that cows in Q3 and Q4 also had generally lower postpartum NE_L intakes compared to cows with less inflammation. Similar quadratic relationships existed with inflammation and yields of 3.5% fat-corrected milk and energy-corrected milk. Plasma glucose concentrations tended to increase linearly, and plasma NEFA concentrations tended to decrease linearly with increasing inflammation. There was a quadratic relationship of inflammation with liver triglyceride and glycogen content at d 7 postpartum such that cows in Q3 had the lowest liver triglyceride and highest glycogen content. There was limited relationship of early lactation inflammatory quartile on immune function at d 7 postpartum. Overall, cows with elevated haptoglobin in the first week after calving had generally decreased milk yields and alterations in metabolism.

Keywords: Early lactation, haptoglobin, energy metabolism, immune function

INTRODUCTION

During the immediate postpartum period, systemic inflammation can be identified by a marked increase in plasma acute phase protein concentrations (Bionaz et al., 2007; LeBlanc, 2012). Inflammation develops upon pathogen recognition and is an essential component of the initial immune response for recruitment of immune cells to fight off infection. Cytokines that are produced by immune cells [especially proinflammatory tumor necrosis factor-α (**TNF-α**) and IL-6] induce the production and release of acute phase proteins (e.g. haptoglobin, **Hp**) from the liver. Haptoglobin has been used as a marker of systemic inflammation in transition dairy cows, as it is elevated during the immediate postpartum period (Huzzey et al., 2009; Galvão et al., 2010; Huzzey et al., 2011). Elevated postpartum Hp has been associated with the occurrence of metritis (Huzzey et al., 2009; Galvão et al., 2010), greater risk for developing a metabolic disorder within 30 d postpartum (Huzzey et al., 2011), and decreased milk production (Huzzey et al., 2012).

In human patients with severe sepsis or major trauma, resting energy expenditure increases to 40% above normal and continues to be elevated for 3 wk after the onset of illness (Plank and Hill, 2000). These data suggest that an immune disturbance increases the energy maintenance requirement. Negative energy balance (**NEB**) has been negatively correlated with both energy status (Galvão et al., 2010) and neutrophil function (Hammon et al., 2006). However, the energetic cost of immune activation in dairy cows has not been well-studied, and further investigation is warranted.

Elevated TNF-α has been shown to decrease glucose production (Kettelhut et

al., 1987) and decrease fatty acid oxidation (Nachiappan et al., 1994), likely leading to increased triglyceride accumulation in the liver, with further impairment of energy metabolism (Strang et al., 1998). Oral administration of cytokine IFN-α in late gestation resulted in increased plasma BHBA concentrations during the first 2 wk after calving (Trevisi et al., 2009), and injections of exogenous TNF-α for 7 d doubled liver triglyceride content in late lactation dairy cows (Bradford et al., 2009). Together these data suggest that increased inflammation disrupts normal energy metabolism processes. In addition, delaying the postpartum inflammatory response with salicylate treatment altered the metabolic adaptations of early lactation dairy cows and led to increased glucose utilization by peripheral tissues after salicylate treatment ended (Farney et al., 2013), suggesting that inflammatory pathways are also involved in the homeorhetic adaptation to lactation.

Many of the studies that have utilized early postpartum Hp as a marker of inflammation in early lactation dairy cows have been large field studies with infrequent sampling points (Huzzey et al., 2011) or measured Hp association with specific disease outcomes (Huzzey et al., 2009; Galvão et al., 2010; Yasui et al., 2014). Although excessive inflammation has negative downstream consequences, it is unclear how much or what type of inflammation is necessary for postpartum adaptation (Huzzey et al., 2011). This current study further evaluates associations between the severity of systemic inflammation during the early postpartum period and performance, energy metabolism, or immune function.

MATERIALS AND METHODS

Animals and treatments

All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee prior to the onset of the experiment. Data from 70 Holstein cows (primiparous n = 21, multiparous n = 49) from the Cornell University Teaching and Research Center Dairy were used for this study. Cows were recruited to address the hypotheses from a separate experiment (McCarthy et al., 2015a,b, in press) evaluating responses to early lactation period feeding strategy [high starch (**HS**) vs. low starch (**LS**) diet during the first 21 d postpartum] and monensin supplementation [0 mg monensin/d (**Con**) or 400 mg/d of monensin prepartum and 450 mg/d of monensin postpartum (**Mon**); Rumensin; Elanco Animal Health, Greenfield, IN] as the variables of interest, and detailed diet descriptions are provided in McCarthy et al. (2015a, in press). Briefly, all cows were fed the same prepartum diet (17.4% starch, 42.9% NDF, 28.2% ADF, 13.0% CP) and either a high starch (HS; 26.2% starch, 34.3% NDF, 22.7% ADF, 15.5% CP) or low starch (LS; 21.5% starch, 36.9% NDF, 25.2% ADF, 15.4% CP) postpartum diet depending on treatment assignment for the first 21 d of lactation. On d 22 postpartum all cows were fed the HS diet until d 63 of lactation. Lactating cows were dried off at least 45 d prior to expected parturition and moved to the experimental tie-stall barn 28 d prior to expected parturition and data were collected through d 63 of lactation.

Data collection, sampling procedures, and analytical methods

All cows were milked 2 times daily for the 9-wk lactation phase of the trial and daily milk yield was measured electronically. Daily milk yield was the sum of the 2

milkings, and weekly means of daily production were calculated. Weekly milk samples were collected from 2 consecutive milkings obtained over a 24-h period. Individual milk samples were sent to a commercial laboratory for analysis of milk composition (Dairy One, Ithaca, NY) as described by McCarthy et al. (2015a, in press). Weekly yields of milk components were calculated, as well as yields of fat-corrected milk (3.5% FCM = (0.432×milk kg) + (16.216×fat kg)) and energy-corrected milk (ECM = (0.327×milk kg) + (12.95×fat kg) + (7.65×true protein kg)).

Cows were fed once daily for ad libitum intake at 0700 h in amounts targeted to provide 2 to 3 kg (wet weight) of refusal. Refusals were removed daily before feeding, weighed, and recorded. All ingredients were sampled weekly for determination of DM content to adjust ration formulation. Weekly means of daily DMI were calculated prior to statistical analysis.

All cows were weighed once weekly and BCS were assigned for all cows weekly by 2 scorers using a 5 point system (Wildman et al., 1982), and scores were averaged prior to statistical analysis. Daily observations and general health records were maintained throughout the study.

Prepartum and postpartum energy balance calculations were determined according to NRC (2001) equations. Weekly values for prepartum calculated energy balance were determined as follows:

Prepartum NE_L (Mcal/d) balance = energy intake (Mcal of NE_L/d) – [maintenance requirement (Mcal of NE_L/d) + pregnancy requirement (Mcal of NE_L/d)], where energy intake (Mcal/d) = weekly DMI average (kg/d) × diet NE_L (Mcal/kg of DM); maintenance requirement (Mcal/d) = week metabolic BW (MBW; $kg^{0.75}$) × 0.08

(Mcal/$kg^{0.75}$); and pregnancy requirement (Mcal/d) = (0.00318 × d of gestation – 0.0352) × (1/0.218).

Weekly values for postpartum calculated energy balance were determined as follows:

Postpartum NE_L (Mcal/d) balance = energy intake (Mcal of NE_L/d) – [maintenance requirement (Mcal of NE_L/d) + lactation requirement (Mcal of NE_L/d)], where energy intake (Mcal/d) = weekly DMI average (kg/d) × diet NE_L (Mcal/kg of DM); maintenance requirement (Mcal/d) = week MBW ($kg^{0.75}$) × 0.08 (Mcal/ $kg^{0.75}$); and lactation requirement (Mcal/d) = Milk, kg × [(0.0929 × fat percentage) + (0.0563 × true protein percentage) + (0.0395 × lactose percentage)].

Plasma sampling and analyses

Blood samples were collected via venipuncture of the coccygeal vessels using heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) 1 h prior to feeding. Blood samples were collected 1×/wk prepartum beginning during the week prior to commencement of prepartum treatment assignment (covariate), 3×/wk from calving through 21 d postpartum, and 1×/wk from d 22 to 63 postpartum. Blood samples were placed on ice immediately following collection and plasma was harvested after centrifugation of the blood at 1300 × g for 15 min at 4°C. Plasma was stored at −20°C until subsequent analysis for Hp, glucose, insulin, NEFA, and BHBA.

Plasma concentrations of Hp were analyzed by enzymatic analysis using a commercial kit that measured haptoglobin-hemoglobin complex by estimated differences in peroxidase activity (Haptoglobin Assay, kit no. TP801, Tridelta Diagnostics Ltd., Morris Plains, NJ). Plasma concentrations of glucose were

determined by an enzymatic analysis (glucose oxidase; protocol from Sigma-Aldrich kit 510-A; St. Louis, MO) using commercial products (PGO Enzyme Preparation and o-dianisidine dihydrochloride, Sigma-Aldrich, St. Louis, MO). Plasma concentrations of NEFA also were analyzed by enzymatic analysis (HR Series NEFA HR (2), Wako Pure Chemical Industries, Osaka, Japan). Plasma concentrations of BHBA were analyzed by enzymatic analysis (BHBA dehydrogenase) using commercial products (Nicotinamide adenine dinucleotide and TRIS (hydroymethyl)aminomethane, Sigma-Aldrich; 3-hydroxybutyrate dehydrogenase, Roche Diagnostics Co., Indianapolis, IN). All spectrophotometric measurements were conducted using a Versamax tunable microplate reader (Molecular Devices, Sunnyvale, CA). Inter- and intra-assay variation was maintained at < 10% for all enzymatic analyses. Plasma concentrations of insulin were determined by double-antibody RIA (Porcine Insulin RIA cat. no. PI-12K, Linco Research, Millipore, St. Charles, MO), with a reported specificity to bovine insulin of 90%. Inter- and intra-assay coefficients of variation were 13.3 and 13.5%, respectively.

Liver biopsy, liver metabolic incubations, and liver composition

Liver tissue was sampled via percutaneous trocar biopsy (Veenhuizen et al., 1991) from cows under local anesthesia on d 7 (± 4 range; 1.6 SD) relative to parturition. Biopsies were obtained from 37 multiparous cows and 17 primiparous cows. After blotting the liver tissue to remove excess blood and connective tissue, a portion of the sample was immersed in ice-cold PBS (0.015 *M*; 0.9% NaCl, pH 7.4) and transported to the laboratory within 45 min of tissue collection for the in vitro experiment. The remaining portion of the liver sample was snap-frozen in liquid nitrogen and stored at -

80°C until analysis for triglyceride and glycogen content.

Hepatic capacities for conversion of [1-^{14}C]propionate (1 μCi per flask) in Krebs-Ringer bicarbonate media (final substrate concentration of 10 m*M*) to CO_2 and glucose were measured in triplicate flasks using tissue slices (60 to 80 mg) according to procedures described by McCarthy et al. (2015b, in press). Liver triglyceride content was determined using the Folch extraction method (Folch et al., 1957) followed by a colorimetric method based upon the Hantzsch condensation for estimating serum triglyceride (Fletcher, 1968) with modifications described by Foster and Dunn (1973). Glycogen content of liver was determined according to the procedures described by Bernal-Santos et al. (2003).

Phagocytosis, Oxidative Burst, and Flow Cytometric Analyses

Blood samples were obtained from each cow via coccygeal blood vessel puncture at 7 d (± 5 range; 2.1 SD) postpartum. Phagocytosis was analyzed using a commercial kit (Phagotest, Glycotope Biotechnology, Heidelberg, Germany). Briefly, heparinized and mixed whole blood (100 μL per test) was pipetted into the bottom of two 12 × 75 mm test tubes (Becton Dickinson Biosciences, San Jose, CA). Opsonized fluorescein isothiocyanate-labeled *Escherichia coli* (20 μL per test) was added to the whole blood samples. After gently vortexing, a control sample was left on ice while a test sample was incubated for 10 min at 37.0°C in a water bath for 15 min. Precisely at the end of the incubation time, all samples were taken out of the water bath and placed on ice simultaneously to stop phagocytosis. Quenching solution (100 μL) was then added to quench the fluorescence of bacteria that were attached but not internalized into phagocytes. The samples were washed twice with 3 mL of wash solution, centrifuged

at 250 × g for 10 minutes at 4°C after each washing, and the supernatant discarded. Erythrocytes in the samples were lysed and leukocytes fixed with the addition of 2 ml of the kit's lysing solution for 20 min at room temperature. After 2 wash steps (centrifugation 5 min, 250 × g, 4°C), the supernatant was discarded and DNA staining solution (200 µL) was added, followed by incubation for 10 min on ice (light protected). Flow cytometry was performed within 60 min after the incubation.

Oxidative burst activity was analyzed using a commercial kit (Phagoburst, Glycotope Biotechnology, Heidelberg, Germany). Briefly, heparinized and mixed whole blood (100 µL per test) was pipetted into the bottom of four 12 × 75 mm test tubes (Becton Dickinson Biosciences, San Jose, CA). For activation, the following solutions (20 µL per tube) were added to whole blood: wash solution as a negative control (tube #1); opsonized (non-labeled) *E. coli* (tube #2); chemotactic peptide N-formyl-MetLuePhe solution as a low physiological stimulus (tube #3); and phorbol 12-myristate 13-acetate (**PMA**) solution as a strong stimulus (tube #4). All tubes were mixed and incubated for 10 min at 37.0 °C in a water bath. After incubation, the fluorogenic substrate solution (20 µL) was added. The samples were vortexed thoroughly and incubated again for 10 min at 37.0 °C in the water bath. Erythrocytes in the samples were lysed and leukocytes fixed with the addition of 2 ml of the kit's lysing solution for 20 min at room temperature. After 2 wash steps (centrifugation 5 min, 250 × g, 4°C), the DNA staining solution (200 µL) was added, followed by incubation for 10 min on ice (light protected in the ice bath). Flow cytometry was performed within 30 min after the incubation.

Flow cytometric analyses were conducted as previously described (Flaminio et

al., 2002) using a BD FACScalibur (Becton Dickinson Biosciences, San Jose, CA) equipped with a 488 μm argon laser, 7600 Power Macintosh computer, and Cell Quest Analysis software (Becton Dickinson Biosciences, San Jose, CA). Leukocyte subpopulation (PMN and monocytes) were displayed in a dot plot and gated according to size (forward light scatter) and granularity (90-degree side light scatter). Ten thousand events in the gated area were collected following the guidelines described in the commercial kits. The percent fluorescence-positive events (% positive) and mean fluorescence intensity (**MFI**, which correlates with the mean number of bacteria ingested by a single phagocyte or the mean oxidative burst activity of a single phagocyte; Moya et al., 2008) were recorded for *E. coli* and PMA activation in each gated area (PMN or monocyte). An index of overall phagocytic or oxidative burst activity was also calculated by multiplying the percentage of responding cells by the corresponding MFI: [index = (positive %) × (MFI)]. Higher values for the index reflected higher phagocytic or oxidative burst activities (Canning et al., 1991). The control sample in each test was used to set the analyses threshold that indicated the positive and negative cell populations.

Blood Neutrophil Glycogen

Blood samples were obtained from each cow via coccygeal blood vessel puncture at 8 d postcalving. The PMNs were isolated using Ficoll 1083 density centrifugation as previously described (Galvão et al., 2010) and frozen for later glycogen determination. Glycogen in PMN was determined as previously described by (Galvão et al., 2010). Briefly, glycogen was hydrolyzed to glucose using amyloglucosidase; available glucose was determined by reacting 50 μL of supernatant

with a 1-mL mixture of 1 mM ATP, 0.9 m*M* NADP, 5 μg of glucose-6-phosphate dehydrogenase, 0.3 *M* triethanolamine, and 4 m*M* MgSO4 and recording the appearance of NADPH after the addition of 5 μL of hexokinase (2 mg/mL) as change in optical density at 340 nm on a spectrophotometer. This change in optical density was compared with a standard curve of glycogen, and results were expressed as micrograms of glycogen/10^6 PMN. Spectrophotometric measurements were conducted using a Versamax tunable microplate reader (Molecular Devices, Sunnyvale, CA).

Statistical Analyses

We were interested in evaluating the relationship between severity of systemic inflammation (as measured by plasma Hp concentration) during the postpartum period on production performance, metabolism, and immune function. Statistical computations were performed using SAS software (version 9.2; SAS Institute Inc., Cary, NC). Cows were assigned to the categorical quartile (**Q**; Q1 = 0.18-0.59, Q2 = 0.60–1.14, Q3 = 1.15–2.05, and Q4 = 2.06-2.50 g Hp/L) based on the highest Hp concentration during wk 1 postpartum. There was no effect of starch or Mon treatment on the distribution of cows to the quartiles, so dietary treatment was not included in the model. There were a low number of primiparous cows in each of the 4 quartiles, and parity was not included in the model. Because quartiles were not equally spaced, PROC IML was used to obtain linear and quadratic contrast coefficients for mean quartile values. Linear and quadratic contrasts were tested for all measurements. Data analyzed over time were subjected to repeated-measures ANOVA using PROC MIXED of SAS and the REPEATED statement. Fixed effects included inflammation quartile, time, and all 2-way interactions. The random effect was cow nested within inflammation quartile. Post hoc

comparisons between groups at each time point were obtained. Four covariance structures were tested: compound symmetry heterogeneous compound symmetry, first-order autoregressive, and heterogeneous first-order autoregressive and the covariance structure that resulted in the smallest Akaike information criterion was used. Data for liver composition, in vitro metabolism, and immune function were subjected to ANOVA using the PROC MIXED procedure. The degrees of freedom for all PROC MIXED were estimated using the Kenward-Roger option in the model statement. The fixed effect was inflammation quartile. Statistical significance was declared at $P < 0.05$ and trends were discussed at $0.10 < P < 0.05$.

RESULTS

Quartile distribution

All cows were categorized into quartiles (Q1 = 0.18 - 0.59, Q2 = 0.60 – 1.14, Q3 = 1.15 – 2.05, and Q4 = 2.06 - 2.50 g Hp/L) based on the highest Hp concentration during wk 1 postpartum (Table 7-1). Although cows were classified into inflammatory quartiles based on very early postpartum Hp concentration, this early classification resulted in distinctive Hp inflammatory responses through the first 9 wk of lactation (Figure 7-1). Cows in Q1 had very little inflammation during wk 1 postpartum, although they exhibited an increase in Hp during wk 2 with a peak in Hp concentration in wk 3. Cows in Q2 also had similar delays in Hp elevation until wk 3 postpartum. Cows in Q3 had an elevation of Hp in wk 1, but maintained similar Hp levels to other cows through d 63 postpartum, whereas cows in Q4 had an acute early lactation inflammatory response with a return to levels similar to the other quartiles by wk 3 postpartum.

Periparturient DMI, BW, BCS, NEB, milk production, and milk production efficiency

There was a quadratic relationship of postpartum inflammatory status with prepartum DMI ($P = 0.01$; Table 7-2). There was also a similar quadratic relationship ($P = 0.01$) and a tendency for a quadratic relationship ($P = 0.06$) of inflammatory quartile on prepartum NE_L intake and energy balance (Mcal/d) such that cows in Q3 had numerically lower prepartum DMI, NE_L intake, and energy balance compared to cows in the other quartiles (Table 7-2). There was a Q × wk interaction for both postpartum DMI ($P < 0.001$; Figure 7-2A) and postpartum NE_L intake ($P = 0.003$; Figure 7-2B) such that cows in Q3 had sustained decreases in DMI and NE_L intake through the end of the experiment. There was a quadratic association of inflammation with postpartum DMI ($P = 0.03$) and NE_L intake ($P = 0.02$), such that cows in Q3 and Q4 had generally lower NE_L intake than cows with less inflammation. There was a linear relationship ($P = 0.02$) of inflammatory quartile on postpartum BW such that cows with increasing inflammation had lower postpartum BW.

There was a Q × wk interaction on milk yield ($P = 0.009$; Figure 7-3A) such that differences between inflammatory groups were more pronounced earlier in lactation. Overall, increased inflammation was associated ($P = 0.05$) with quadratically decreased milk yield (Table 7-2). There were similar quadratic relationships with 3.5% FCM ($P = 0.02$) and ECM ($P = 0.03$). Although there was no association of inflammatory quartile with milk energy content (Mcal/kg) there was a Q × wk interaction for total milk energy output (Mcal/d; $P = 0.004$; Figure 7-3B) and a quadratic relationship ($P = 0.05$) such that cows in Q3 had numerically lower daily milk energy output. There was a Q × wk

interaction for energy difference (P = 0.04), measured as postpartum NE_L intake minus total daily milk energy output, and cows in Q3 tended to have lower energy available for maintenance in wk 6 and wk 8 compared to cows in other quartiles (Figure 7-4). There tended to be a Q × wk interaction for SCS (P = 0.09; data not shown) and cows in Q4 tended to have higher SCS in wk 1 postpartum compared to cows in the other quartiles.

Periparturient plasma metabolites

Periparturient plasma metabolites are presented in Table 7-3 and Figure 7-5. There was a Q × time interaction for plasma glucose concentration (P = 0.03; Figure 7-5A) and a tendency for a linear relationship of inflammation with plasma glucose (P = 0.10) such that cows with increasing inflammation had higher early lactation plasma glucose concentrations. There was a similar Q × time interaction for plasma insulin (P < 0.001; Figure 7-5B), and cows with increasing inflammation had higher early lactation plasma insulin concentrations. There was a Q × time interaction (P = 0.02; Figure 7-5C) for plasma NEFA, and a tendency for a linear relationship with plasma NEFA (P = 0.08) such that cows with increasing inflammation had lower plasma NEFA concentrations. Although there was no association of inflammation or interaction of Q × time on plasma BHBA concentration in the current study, there were treatment differences for individual sample days in which cows in Q1 and Q2 had higher peaks in BHBA compared to Q3 and Q4 cows (Figure 7-5D).

Liver composition and in vitro liver metabolism

There was a quadratic association of inflammation with liver triglyceride content at d 7 postpartum, such that cows in Q1 and Q4 had the highest triglyceride content (P

= 0.02; Table 7-4), and cows in Q3 had elevated liver glycogen content on d 7 postpartum compared to cows in other quartiles ($P = 0.02$).

There was no association of inflammation on in vitro conversion of [1-^{14}C]propionate to either CO_2 or glucose (Table 7-4). In experiments using labeled isotopes the [1-^{14}C]propionate label randomizes in the TCA cycle such that every mole of [1-^{14}C]propionate that is directed toward oxaloacetate should yield 0.5 moles of radiolabeled CO_2 and 0.5 moles of radiolabeled glucose (Knapp et al., 1992). Any increase in this ratio of labeled glucose to CO_2 would suggest an increase in the efficiency of utilization of propionate for gluconeogenesis; however, there were no relationships of inflammation with the ratio of glucose to CO_2 in the current study (Table 7-4).

Immune function

Relationships of inflammatory quartile with measures of immune function at d 7 postpartum are presented in Table 7-5. Cows in all quartiles had similar neutrophil phagocytosis; however, there was a relationship of inflammatory quartile with monocyte phagocytosis ($P = 0.02$), but the linear and quadratic contrasts were not significant. There was a tendency for an effect of quartile on the monocytes phagocytosis index ($P = 0.06$); however; linear and quadratic contrasts were not significant. There was a quadratic relationship of inflammatory quartile with monocyte *E. coli* stimulated oxidative burst such that cows in Q2 and Q3 had lower monocyte *E. coli* stimulated oxidative burst MFI ($P = 0.02$) and index ($P = 0.04$). There was no association of inflammatory quartile with neutrophil glycogen content at d 7 postpartum.

Table 7-1. Distribution of cows based on highest haptoglobin concentration during d 1 to 7 postpartum (n = 70)

	Inflammation Quartile[1]			
	1	2	3	4
Primiparous				
n	2	3	10	6
percentage	9.5	14.3	47.6	28.6
Multiparous				
n	15	15	7	12
percentage	30.6	30.6	14.3	24.5
Total, n	17	18	17	18

[1]Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum. Q × parity interaction $P = 0.01$.

Table 7-2. Associations of early postpartum inflammation with pre- and postpartum intake, BW, BCS, energy balance, postpartum milk production, and feed efficiency (n = 70)

	Inflammation Quartile[1]					*P*-values[2]				
Item	1	2	3	4	SEM	Q	Wk	Q×Wk	Linear	Quadratic
Prepartum										
DMI, kg/d	14.21	12.99	11.25	13.18	0.70	0.03	<0.001	0.71	0.15	0.01
NEL intake, Mcal/d	21.10	19.19	16.64	19.55	1.02	0.03	<0.001	0.68	0.14	0.01
BW, kg	713	682	636	686	14	0.003	<0.001	0.60	0.06	0.002
BCS	3.55	3.43	3.59	3.48	0.07	0.47	0.005	0.49	0.92	0.93
Energy Balance, Mcal/d	7.84	6.28	4.56	6.56	1.04	0.17	<0.001	0.53	0.27	0.06
Postpartum										
DMI, kg/d	22.31	21.31	18.26	20.66	0.92	0.02	<0.001	<0.001	0.0.06	0.03
NEL intake, Mcal/d	36.28	34.62	29.79	33.74	1.38	0.01	<0.001	0.003	0.05	0.02
BW, kg	643	616	578	607	11	0.003	<0.001	0.01	0.008	0.008
BCS	3.02	2.91	3.09	2.97	0.08	0.45	<0.001	0.61	0.86	0.77
Energy Balance, Mcal/d	-2.99	-3.15	-3.02	-1.91	0.82	0.67	<0.001	0.08	0.33	0.46
Milk yield, kg/d	42.55	40.10	34.20	37.35	1.77	0.009	<0.001	<0.001	0.008	0.05
3.5% FCM, kg/d[3]	43.41	41.10	34.89	38.76	1.61	0.003	<0.001	0.03	0.007	0.02
ECM, kg/d[4]	43.10	41.06	34.84	38.35	1.58	0.003	0.009	0.008	0.005	0.03
MUN, mg/dL	12.02	11.96	11.67	11.21	0.42	0.49	0.01	0.75	0.14	0.71
SCS[5]	2.14	2.51	2.49	2.99	0.38	0.45	<0.001	0.09	0.14	0.89
Milk energy, Mcal/kg	0.70	0.72	0.72	0.71	0.02	0.82	<0.001	0.74	0.78	0.40
Milk energy, Mcal/d	29.09	27.98	23.76	25.93	1.04	0.003	<0.001	0.001	0.004	0.05
Energy difference, Mcal/d	7.17	6.66	6.14	7.81	0.88	0.57	<0.001	0.04	0.68	0.20
Milk/DMI	1.94	1.93	1.89	1.84	0.05	0.47	<0.001	0.55	0.13	0.75
ECM/DMI	2.00	2.03	1.97	1.93	0.06	0.67	<0.001	0.83	0.29	0.65

[1]Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum.
[2]Q = quartile.
[3]Fat corrected milk (FCM) calculated as FCM = 0.432 × milk kg + 16.216 × fat kg;
[4]Energy corrected milk (ECM) calculated as ECM = 0.327 × milk kg + 12.95 × fat kg + 7.65 × true protein kg;
[5]Somatic Cell Score calculated as SCS = log2(SCC/100) + 3.
[6]Energy difference (ED) calculated as ED = postpartum NEL intake (Mcal/d) - milk energy (Mcal/d).

Table 7-3. Association of early postpartum inflammation with periparturient plasma metabolites (n = 70)

	Inflammation Quartile[1]					*P*-values[2]				
Item	1	2	3	4	SEM	Q	Time	Q×Time	Linear	Quadratic
Glucose, mg/dL	56.9	55.5	60.8	59.4	1.8	0.15	<0.001	0.03	0.10	0.77
Insulin, ng/mL	0.27	0.26	0.29	0.32	0.03	0.52	<0.001	<0.001	0.15	0.72
NEFA μEq/L	490	452	449	403	33	0.32	<0.001	0.02	0.08	0.94
BHBA mg/dL	10.22	9.83	9.33	9.41	0.63	0.73	<0.001	0.62	0.30	0.64

[1]Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum.
[2]Q = quartile.

Table 7-4. Association of early postpartum inflammation with postpartum liver composition and in vitro [1-^{14}C]propionate metabolism (n = 70)

	Inflammation Quartile[1]					*P*-values[2]		
Item	1	2	3	4	SEM	Q	Linear	Quadratic
Liver composition								
Triglycerides, % of wet wt.	8.60	5.66	4.39	8.52	1.49	0.11	0.92	0.02
Glycogen, % of wet wt.	0.73	0.69	1.21	0.46	0.17	0.02	0.68	0.02
In vitro metabolism								
CO_2	6.36	6.34	5.59	5.92	0.54	0.63	0.34	0.60
Glucose	4.06	3.79	3.83	3.49	0.46	0.83	0.41	0.94
Glucose:CO_2 Ratio	0.70	0.57	0.68	0.59	0.07	0.37	0.50	0.83

[1]Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum.
[2]Q = quartile.

Table 7-5. Association of early postpartum inflammation with indices of immune function at d 7 postpartum (n = 70)

	Inflammation Quartile[1]					*P*-values[2]		
Item	1	2	3	4	SEM	Q	Linear	Quadratic
Phagocytosis								
Neutrophil, %*	85.90	88.68	83.72	83.77	1.04	0.62	0.36	0.79
Neutrophil, MFI	152	152	142	155	9	0.73	0.98	0.39
Neutrophil, Index*	12,684	13,259	11,531	12,490	1	0.71	0.59	0.71
Monocyte, %	40.42	45.97	38.89	43.32	1.71	0.02	0.96	0.95
Monocyte, MFI*	70.58	73.11	62.78	68.08	1.06	0.25	0.26	0.49
Monocyte, Index*	2,812	3,322	2,411	2,913	1	0.06	0.47	0.60
Oxidative burst								
E coli stimulation								
Neutrophil, %	88.69	82.77	82.59	83.35	2.50	0.24	0.18	0.17
Neutrophil, MFI	21.34	19.08	20.20	18.81	1.45	0.58	0.34	0.80
Neutrophil, Index	1,888	1,647	1,693	1,598	144	0.50	0.22	0.61
Monocyte, %*	8.57	5.78	6.52	8.60	1.29	0.60	0.83	0.21
Monocyte, MFI*	8.67	7.89	7.33	9.76	1.09	0.09	0.38	0.02
Monocyte, Index*	74.23	45.62	47.79	83.92	1.29	0.20	0.60	0.04
PMA stimulation								
Neutrophil, %*	90.85	81.00	85.31	80.89	1.05	0.14	0.17	0.55
Neutrophil, MFI*	43.65	41.58	43.55	36.65	1.13	0.70	0.37	0.59
Neutrophil, Index	4,533	4,042	4,353	3,307	534	0.39	0.17	0.59
Monocyte, %*	24.44	16.35	22.58	21.99	1.31	0.71	0.92	0.55
Monocyte, MFI	28.58	31.10	25.46	33.78	2.99	0.23	0.45	0.25
Monocyte, Index*	528	481	517	700	1	0.75	0.54	0.37
Neutrophil glycogen, μg/10^6 PMN								
7 d postpartum	7.58	6.96	8.52	8.02	1.17	0.81	0.57	0.93

[1]Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum.
[2]Q = quartile.
*Indicates that distribution was non-normal and values were log transformed prior to statistical analysis. Values presented in the table were back transformed after analysis.

Figure 7-1. Periparturient haptoglobin concentrations for cows (n = 70) assigned to an inflammatory quartile based on the highest haptoglobin concentration during wk 1 postpartum (Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum). The box indicates the window in which quartiles were defined. There was an interaction of Q × wk during the periparturient period ($P < 0.001$). *Indicates differences between inflammation quartiles ($P < 0.05$) within sampling day.

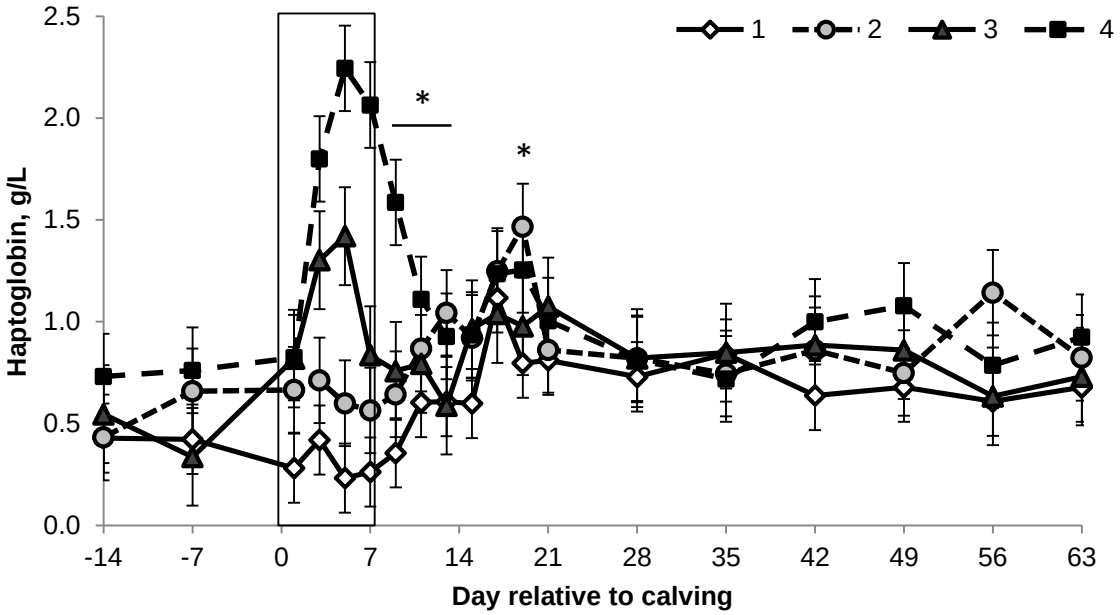
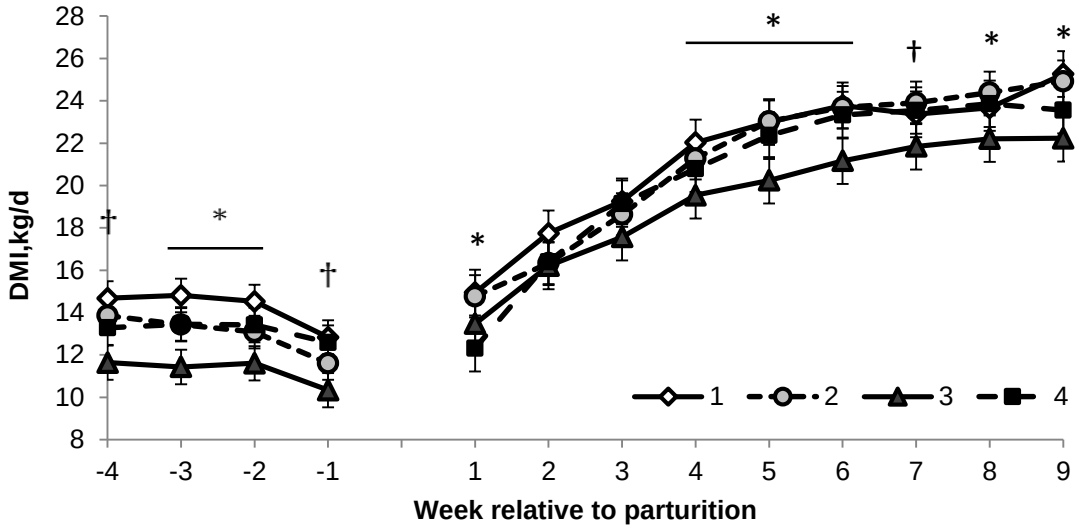


Figure 7-2. Least square means for periparturient DM and NE_L intake for cows (n = 70) assigned to an inflammatory quartile based on the highest haptoglobin concentration during wk 1 postpartum (Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum). Panel A. Periparturient dry matter intake. The *P* value for the main effect of inflammation was 0.03 during the prepartum period and 0.02 during the postpartum period. There was an interaction of Q × wk postpartum ($P < 0.001$). Panel B. Periparturient NE_L intake. The *P* value for the main effect of inflammation was 0.03 during the prepartum period and 0.01 during the postpartum period. There was an interaction of Q × wk during the postpartum period ($P = 0.003$). *Indicates a difference between inflammation quartiles ($P < 0.05$) and † indicates a tendency for differences between inflammation quartiles ($0.10 < P > 0.05$) within sampling week.



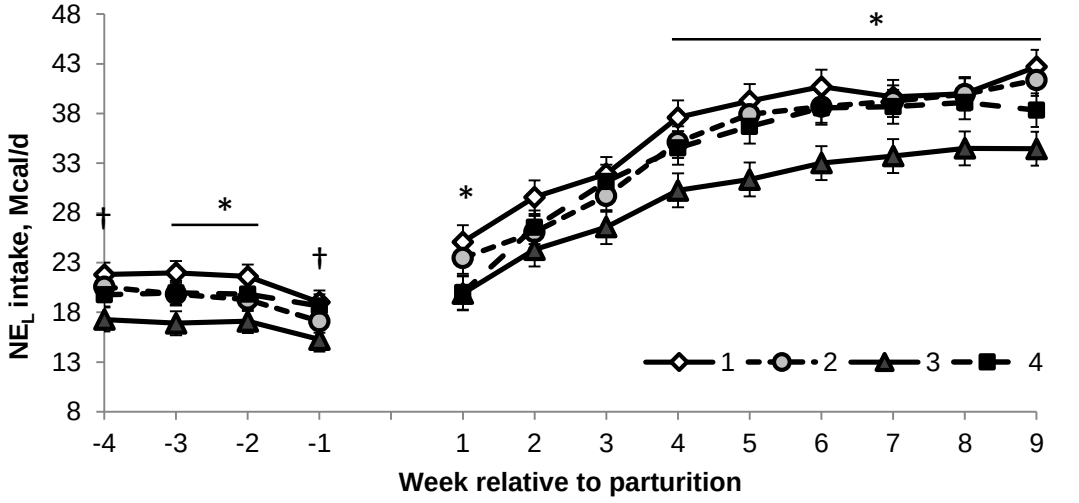
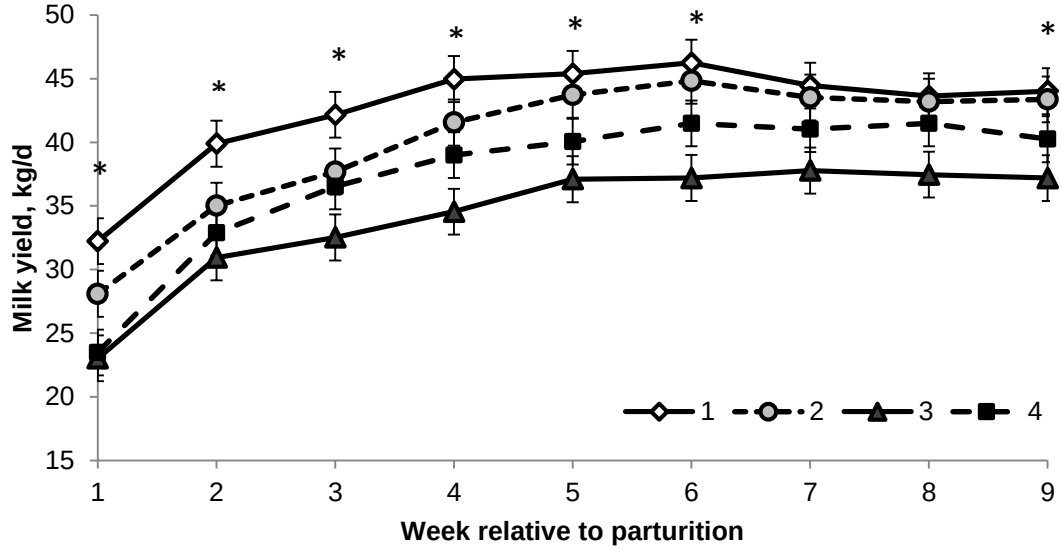


Figure 7-3. Least square means for postpartum milk yield (kg/d) and milk energy output (kg/d) for cows (n = 70) assigned to an inflammatory quartile based on the highest haptoglobin concentration during wk 1 postpartum (Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum). Panel A. Postpartum milk yield. The *P* value for the main effect of inflammation was 0.009 during the postpartum period. There was an interaction of Q × wk during the postpartum period ($P < 0.001$). Panel B. Postpartum milk energy output. The *P* value for the main effect of inflammation was 0.003 during the postpartum period. There was an interaction of Q × wk during the postpartum period ($P = 0.001$). *Indicates a difference between inflammation quartiles ($P < 0.05$) and † indicates a tendency for differences between inflammation quartiles ($0.10 < P > 0.05$) within sampling week.



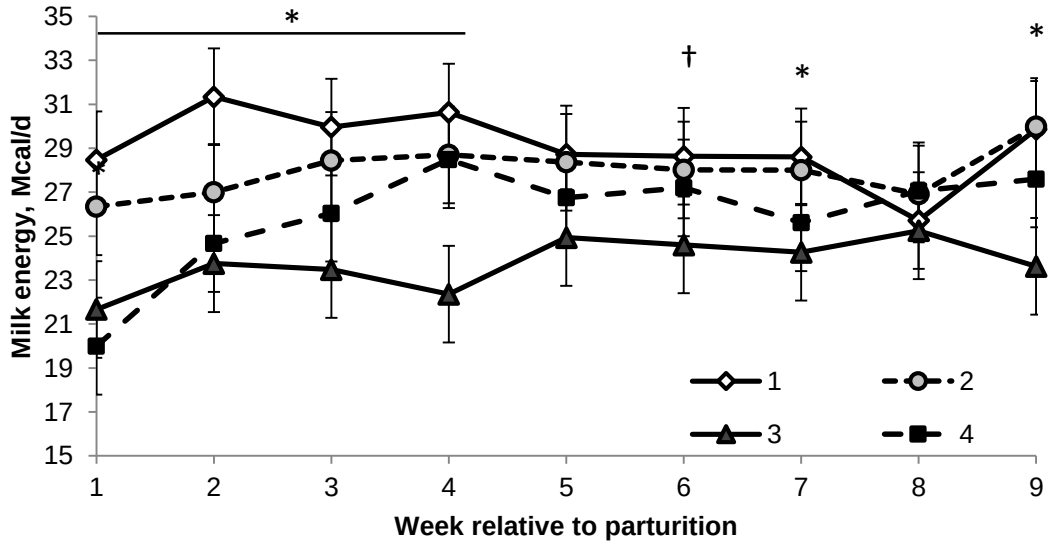


Figure 7-4. Least square means for postpartum energy difference calculated as the NE_L intake minus milk energy output for cows (n = 70) assigned to an inflammatory quartile based on the highest haptoglobin concentration during wk 1 postpartum (Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum). The *P* value for the main effect of inflammation was 0.57 during the postpartum period, however there was an interaction of Q × wk during the postpartum period ($P = 0.04$). †Indicates a tendency for differences between inflammation quartiles ($0.10 < P > 0.05$) within sampling week.

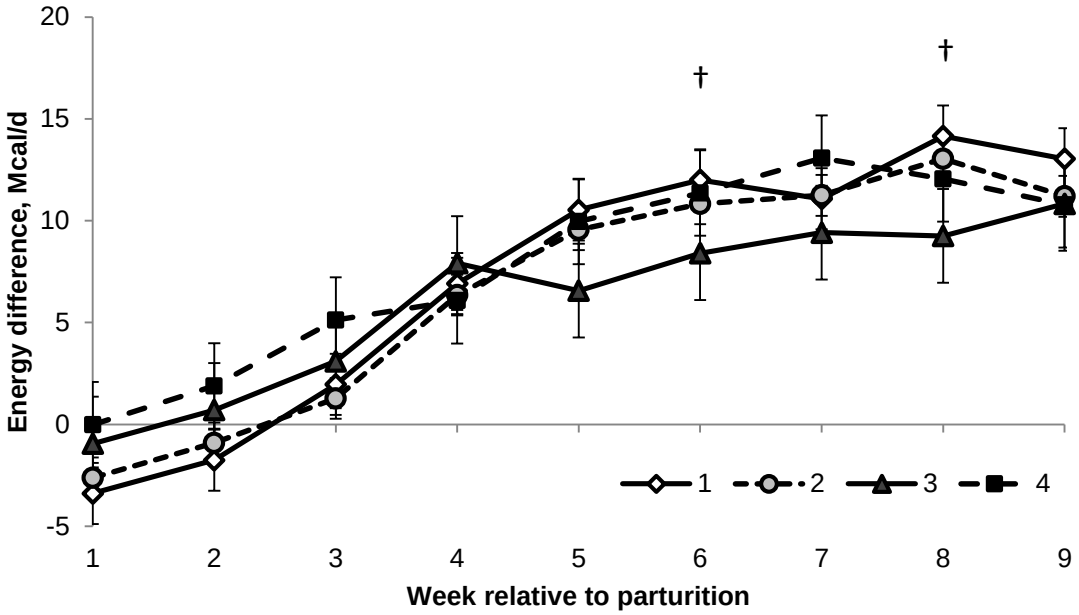
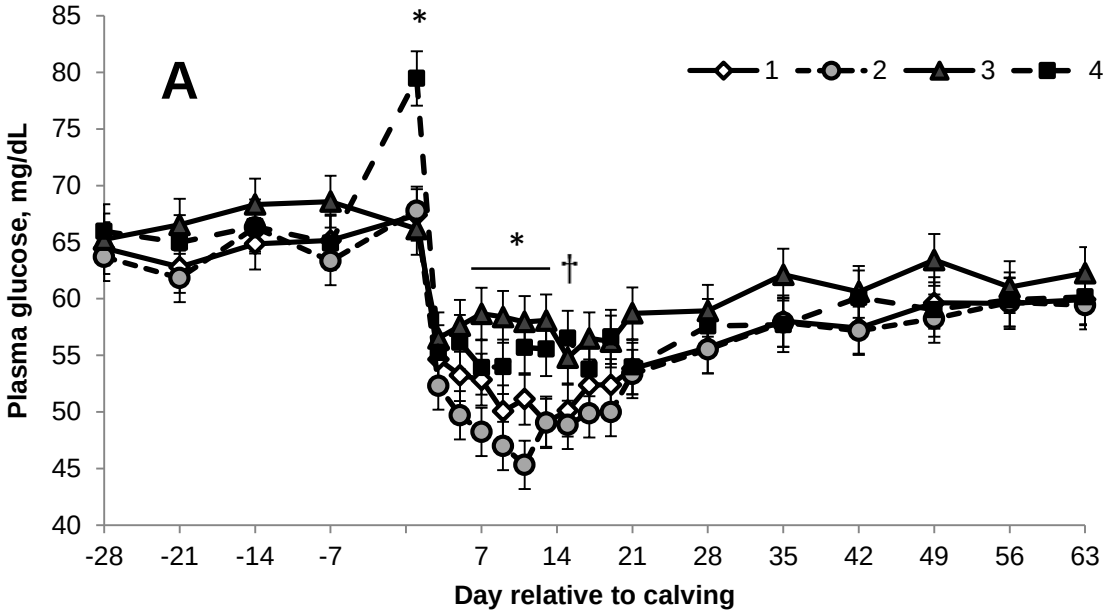
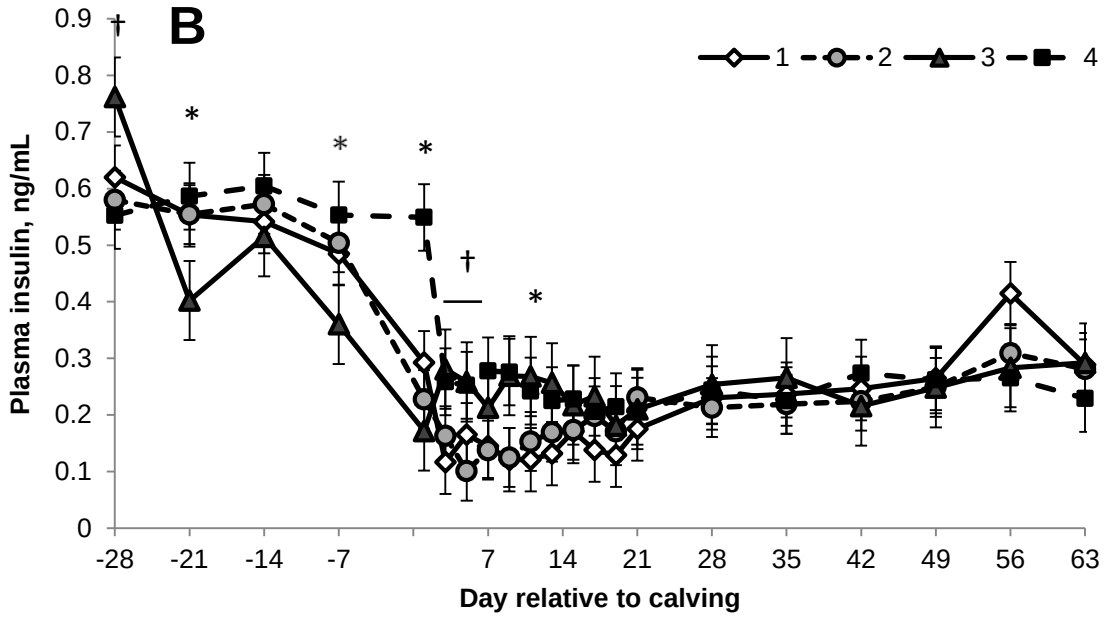
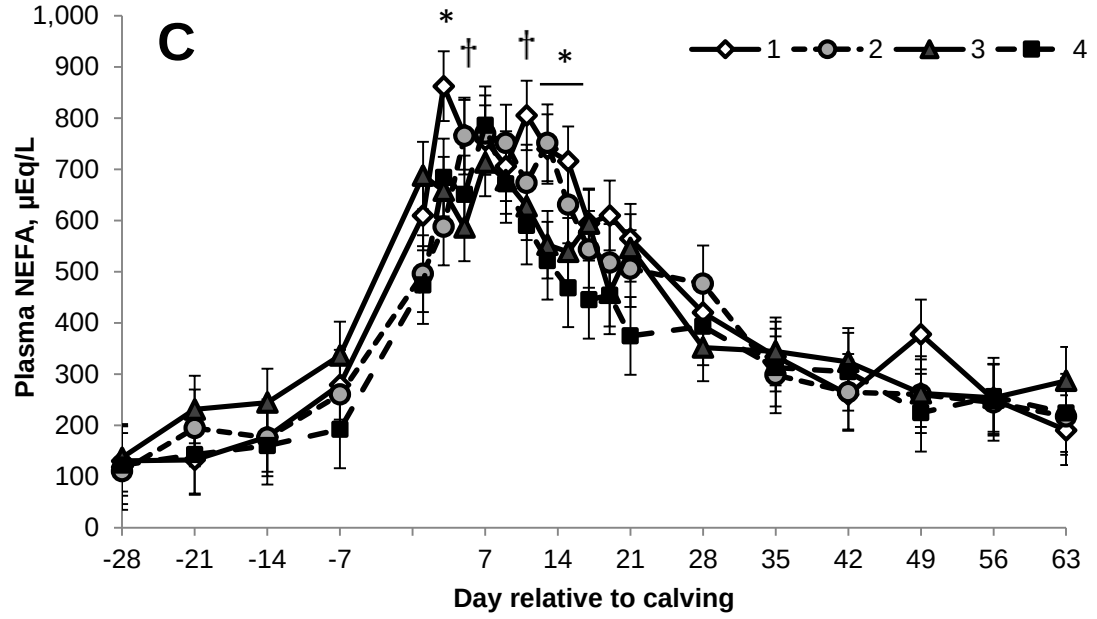
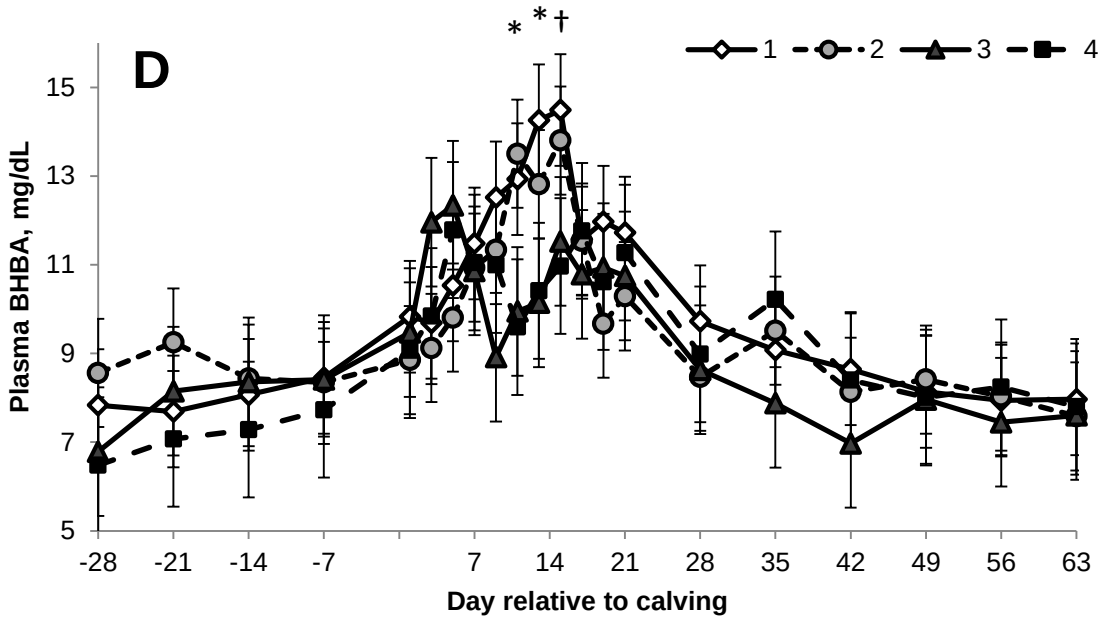


Figure 7-5. Least square means for periparturient plasma glucose, insulin, NEFA, and BHBA concentrations for cows (n = 70) assigned to an inflammatory quartile based on the highest haptoglobin concentration during wk 1 postpartum (Quartile 1 = 0.18 - 0.59, quartile 2 = 0.60 – 1.14, quartile 3 = 1.15 – 2.05, quartile 4 = 2.06 - 2.50 g haptoglobin/L during wk 1 postpartum). Panel A. Periparturient plasma glucose concentrations. The *P* value for the main effect of inflammation was 0.15 during the periparturient period, however, there was a Q × time interaction for plasma glucose concentrations during the periparturient period ($P = 0.03$). Panel B. Periparturient plasma insulin concentrations. The *P* value for the main effect of inflammation was 0.52 during the periparturient period, however, there was a Q × time interaction for plasma insulin concentrations during the periparturient period ($P < 0.001$). Panel C. Periparturient plasma NEFA concentrations. The *P* value for the main effect of inflammation was 0.32 during the periparturient period, however, there was a Q × time interaction for plasma NEFA concentrations during the periparturient period ($P = 0.02$). Panel D. Periparturient plasma BHBA concentrations. There was no main effect of inflammation on BHBA ($P = 0.65$) and no Q × time interaction for plasma BHBA concentrations during the periparturient period ($P = 0.64$), however, there were differences on individual sampling days. *Indicates a difference between inflammation quartiles ($P < 0.05$) and † indicates a tendency for differences between inflammation quartiles ($0.10 < P > 0.05$) within sampling day.


A
1
2
3
4
Plasma glucose, mg/dL
85
80
75
70
65
60
55
50
45
40
-28
-21
-14
-7
7
14
21
28
35
42
49
56
63
Day relative to calving
*
*
†


B
1
2
3
4
Plasma insulin, ng/mL
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-28
-21
-14
-7
7
14
21
28
35
42
49
56
63
Day relative to calving
†
*
*
*
†
*


C
1
2
3
4
Plasma NEFA, µEq/L
1,000
900
800
700
600
500
400
300
200
100
0
-28
-21
-14
-7
7
14
21
28
35
42
49
56
63
Day relative to calving


D
1
2
3
4
Plasma BHBA, mg/dL
15
13
11
9
7
5
-28
-21
-14
-7
7
14
21
28
35
42
49
56
63
Day relative to calving

DISCUSSION

The objective of this study was to evaluate relationships between severity of systemic inflammation during the early postpartum period, using haptoglobin as a marker of inflammation, and performance, energy metabolism, or immune function. Elevated early postpartum Hp has been associated with the incidence of metritis (Galvão et al., 2010) and reductions in milk yield and reproductive function (Huzzey et al., 2012). In the current study, cows in Q3 and Q4 had Hp concentrations > 1.15 g/L, which is similar to the high Hp cutoff point of 1.1 g/L during the first week of lactation established by Huzzey et al. (2011). Interestingly, there were very different responses in production performance for cows in Q3 and Q4 in the current study, even though they would both be above the high Hp cutoff point. Although the mobilization of active neutrophils is one of the key defenses against infectious disease in dairy cows (Burvenich et al., 2003), monocytes have been shown to be more responsive to inflammatory stimulation during the transition period than during other physiological states, resulting in greater cytokine production (Sordillo et al., 1995). Cows in Q3 had a lower percentage of monocytes involved in phagocytosis and tended to have a lower monocyte phagocytosis index at d 7 postpartum, perhaps indicating that Q3 cows had an impaired early lactation immune response compared to Q4 cows.

Cows in Q3 had lower prepartum DMI and NE_L intake compared to cows in other quartiles, which continued through the postpartum period. The decrease in prepartum DMI and NE_L intake for cows in Q3 indicates that perhaps they did not have enough energy to mount a sufficient immune response during the immediate postpartum period, leading to observed postpartum alterations in metabolism and decreased

productive performance. Although cows in Q4 had reduced DMI during the early postpartum period, they returned to similar intake compared to cows in the low Hp quartiles. However, cows in Q3 had greater sustained reductions in DMI compared to cows in the other quartiles. Weber et al. (2013) observed that cows with lower liver fat, and likely in a less inflammatory state, had faster increases in postpartum DMI compared to cows with medium and high liver fat content (greater inflammatory state). Together this would suggest that cows with postpartum inflammation experience reductions in DMI, perhaps because some of the pro-inflammatory cytokines that are secreted in response to an inflammatory event have been shown to be hypophagic (Klasing, 1988); however, the sustained reductions in milk and DMI for cows in Q3 compared to Q4 cows requires further investigation.

In the current study all cows had similar milk energy content (Mcal/kg), however, cows in Q3 and Q4 had decreased early postpartum milk yield compared to cows with less inflammation, and cows in Q3 maintained the lowest milk yields and energy output (Mcal/d) throughout the entire trial period. Huzzey et al. (2012) observed that high Hp in the early postpartum period was associated with reduced subsequent ME305-predicted milk yields. Bertoni et al. (2008) also observed that cows with a low liver activity index in early lactation (indicating a greater degree of inflammation) had a 6.5 kg/d reduction in milk yield compared to cows with very little early lactation inflammation, and numerical reductions in milk yield with each increase in inflammatory quartile. The lack of difference in milk energy content on a Mcal/kg basis suggests that cows do not alter milk composition in response to an inflammatory event but rather reduce overall milk yield.

Because calculated energy balance doesn't take into account the potential increases in maintenance requirements during immune activation and the reductions in milk yield that likely result from the inflammatory event, we calculated energy difference as postpartum energy intake minus milk energy output. This energy difference would indicate relative DMI energy that is available for other body functions. This of course does not take into account the mobilization of body tissues and the offset of energy provided from these energy stores, however, we can extrapolate relative contribution of these body energy stores from plasma metabolite data. During wk 6 and 8 postpartum cows in Q3 tended to have a lower energy difference indicating that the reductions in DMI and milk yield for Q3 cows provided less DMI energy available for maintenance requirements. This should suggest that Q3 cows would have increased mobilization of body tissue stores to make up the difference. However, this was not the case in the current dataset as indicated by similar NEFA mobilization in all cows during those time points. In human patients that suffered from major trauma, it was reported that resting energy expenditure increased to 40% above normal and continued to be elevated 3 wk after the onset of illness (Plank and Hill, 2000). In context of the current study this would perhaps indicate that cows in Q3, that had lower DM and NE_L intake, had less available energy to mount a sufficient immune response to the inflammatory event and experienced a state of prolonged immunosuppression because of the lack of sufficient energy.

In the current study the metabolic response to inflammation as indicated by plasma metabolites were similar between Q3 and Q4. Cows in Q3 and Q4 had elevated early lactation plasma glucose and insulin, perhaps indicating increased

insulin resistance in these cows as insulin resistance has been implicated in the inflammatory response (LeBlanc, 2012). Although TNF-α administration in early postpartum cows did not alter postpartum plasma metabolite response (Yuan et al., 2013), salicylate treatment during the immediate postpartum period altered the metabolic adaptations to lactation after salicylate treatment ended on d 7, leading to increases in BHBA and NEFA, indicating that inflammation associated pathways are involved in the adaptations to lactation (Farney et al., 2013). In contrast to results of the current study, Travesi et al. (2012) observed that cows with a low liver activity index (indicative of a greater degree of inflammation) had lower glucose at calving and elevated postpartum NEFA and BHBA compared to cows with a high liver activity index. Energy for phagocytosis of neutrophils is mostly dependent on intercellular glycogen (Naidu and Newbould, 1973) and glucose is one of the main oxidative fuels for macrophages (Newsholme et al., 1987). In the current study we observed the greatest relationships of early lactation inflammation on impairments in monocyte function. Because the main energetic source for monocytes is glucose, it is likely that cows in Q3 also had less energy available for monocyte function and activity. Insulin modulates immune cell response (Helderman, 1981), and may also have a direct effect on metabolism and function in neutrophils (Alba-Loureiro et al., 2006), although we did not observe any effects of inflammation on neutrophil glycogen content in the current study. During the early postpartum period it appears that cows in Q3 and Q4 had higher plasma glucose compared to cows in Q1 and Q2, with a similar pattern for higher plasma insulin concentrations in cows in Q3 and Q4. Macrophages and neutrophils have been shown to express GLUT1, GLUT 3 and

GLUT4 isoforms on the plasma membrane of both resting and activated cells (Maratou et al., 2007), and insulin stimulates both phagocytosis and the rate of glucose utilization (Newsholme et al., 1996). In vitro experiments utilizing activated macrophages and neutrophils incubated at increasing concentrations of insulin observed an increase in the GLUT4 MFI (Maratou et al., 2007). Glucose utilization rates in activated immune cells are high and a large proportion of the glucose is metabolized to lactate in both macrophages (Newsholme et al., 1986) and neutrophils (Curi et al., 1997).

Cytokines IL-1 and TNF-α have been shown to increase gluconeogenesis and glucose oxidation (Klasing, 1988). In the current study there was no difference between quartiles in utilization of [1-^{14}C]propionate for in vitro liver slice conversion to CO_2 or glucose at d 7 postpartum, however, differences in productive performance were not observed until later, perhaps indicating that alterations in hepatic glucose metabolism also did not occur until later postpartum. Karnovsky et al. (1975) observed a fourfold increase in conversion of [6-^{14}C]glucose to $^{14}CO_2$ during phagocytosis in activated macrophages compared with resting murine cells. In vitro leukocyte activation index was positively correlated with glucose status in blood from periparturient dairy cows (Schwarm et al., 2013), which would indicate that innate immune activation is modulated by energy availability. Because Hp is a non-specific marker of inflammation, we are not able to speculate on the cause of the early lactation elevation in Hp for cows in Q3 and Q4 in the current study. Although both quartiles mounted some degree of inflammatory response during the early postpartum period, it is likely that Q4 cows were able to mount a more sufficient anti-

inflammatory response to clear up the pathogen insult, whereas Q3 cows were not able to mount a sufficient response, resulting in performance losses for those cows. The decrease in prepartum DMI and NE_L intake for cows in Q3 indicated that perhaps this decrease in prepartum energy intake impaired their ability to mount a sufficient immune response during the immediate postpartum period, leading to postpartum impairments in immune function and sustained decreased productive performance.

Fatty liver has been reported to induce production of Hp (Murata et al., 2004; Guzelbektes et al., 2010), although it has also been suggested that fatty liver may suppress the secretion of acute phase proteins (Ingvartsen et al., 2003). Cytokine TNF-α has been shown to increase hepatic triglyceride synthesis (Klasing, 1988), although cytokines were not measured in the current study. Interestingly, at d 7 postpartum cows in Q4 had high triglyceride content while Q3 cows had the lowest triglyceride content, again indicating that there are differences in response to early lactation inflammation.

CONCLUSIONS

Although Q3 cows had similar alterations in metabolism as Q4 cows they exhibited more negative detriments in production, as indicated by decreased DMI and reduced milk yield through wk 9 of lactation compared to cows in Q4. It is likely that because Q3 cows had lower DM and NE_L intakes prepartum they had less available energy to mount a sufficient immune response to the early lactation inflammatory event and experienced a state of prolonged immunosuppression because of the lack of sufficient energy leading to greater reductions in milk yield. More in depth investigations of early lactation inflammation and the mechanistic response and role of

immune function are necessary to better understand the different adaptation strategies of individual cows to inflammation in early lactation. Cows that fell within the established high haptoglobin cut points (> 1.1 g/L) in this study had a diverse range of production responses. These cut points may not be specific enough to be useful as a managerial tool in their present state, and further investigation is warranted.

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CHAPTER 8

OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

After parturition the nutrients required for milk synthesis utilize a large portion of maternal nutrients, and this adaptation to lactation also requires homeorhetic changes of the immune system as well. Higher DMI postpartum generally results in lower circulating NEFA and has been associated with improved health, performance, and less severe postpartum negative EB. Optimizing DMI during this postpartum period is especially important to provide sufficient energy to support milk production. Because hepatic energy requirements increase dramatically at the onset of lactation and adipose mobilization is increased, As such there appear to be many complex interactions in the utilization and regulation of energy substrates postpartum.

Conclusions

The first objective of this research were to evaluate the effects of different energetic nutritional strategies on postpartum production and metabolism and to evaluate the impact of these nutritional strategies on liver metabolism. Cows that were fed more propiogenic diets in early lactation via increased starch content or monensin inclusion had increased milk yield and DMI during the immediate postpartum period. Cows fed high starch diets or monensin had less negative EB during the immediate postpartum period, and overall more propiogenic diets favorably affected postpartum production outcomes. These same propiogenic diets appeared have positive effects on metabolism as well, as indicated by increased plasma glucose and insulin concentrations and decreased plasma NEFA and BHBA concentrations in early lactation. Cows fed monensin during the peripartal period had increased propensity to convert propionate to glucose in vitro. However, results of gene expression were less clear, although they did indicate altered gene expression for cows fed diets of different

starch content either with or without monensin. Because cows were biopsied at d 7 postpartum and treatment differences in DMI were not observed until later, it is likely that treatment differences in hepatic gene expression would be more apparent with increasing DIM.

As cows transitioned to lactation, there were decreases in hepatic glycogen content and increases in hepatic triglyceride content. Similarly there was an increase in the capacity of the liver to convert [1-^{14}C]palmitate to EP during the postpartum period. There was also an increase in the capacity of liver to convert [1-^{14}C]propionate to CO_2 and glucose during the postpartum period. Hepatic gluconeogenesis from propionate was negatively correlated with both postpartum NEFA and BHBA AUC, suggesting that cows with greater NEFA mobilization and greater circulating BHBA have reduced gluconeogenic capacity from propionate. Similarly, correlation relationships suggest that excess liver triglyceride content is related to decreased capacity of the liver to convert propionate into glucose. Overall, alterations in fatty acid metabolism that lead to increased triglyceride accumulation during the transition period appear to impair postpartum hepatic gluconeogenesis.

The last objective of this research was to evaluate the association between the degree of early lactation inflammation and production and metabolism. Although cows with elevated plasma haptoglobin concentrations in the first week postpartum cows had similar alterations in metabolism, cows with a lower degree of elevated haptoglobin exhibited more negative detriments in production, as indicated by decreased DMI and reduced milk yield through wk 9 of lactation compared to cows with the highest inflammation. It is likely that because these cows with a lower degree

of elevated haptoglobin had lower DM and NE_L intakes prepartum they had less available energy to mount a sufficient immune response to the early lactation inflammatory event and experienced a state of prolonged immunosuppression because of the lack of sufficient energy leading to greater reductions in milk yield.

Future directions

As has been shown in the above chapters cows that were fed diets with greater propiogenic capacity during the postpartum period had improvements in production and metabolism and increased conversion of propionate to glucose in the liver. Alterations in fatty acid metabolism that lead to increased triglyceride accumulation during the transition period appear to impair postpartum hepatic gluconeogenesis, and cows that had elevated inflammation in the first week postpartum exhibited a diverse range of production responses, indicating that there is a large degree of variation in individual adaptation.

As part of the propiogenic diet study the first cows that calved onto either ration developed significant health problems. Upon investigating, we discovered a new bunk of corn silage was being used in the rations. The new bunk of corn silage had an increased starch content and lower NDF than when we had originally formulated the ration. While cows on both diets had significant health disorders, incidence in cows on the high starch diet was greater, likely because they had a more fermentable diet and less fiber in the diet, as cows on the low starch ration had greater inclusion of by-product non-forage fiber. Once the physically effective fiber content of the diet was corrected by adding more chopped straw, the metabolic disorder problem disappeared for cows fed both rations. As a result of these observations research will

be underway shortly to further investigate the importance of fiber content in early lactation diets. This is an important area of work as we tend to focus on energy and negative energy balance in early lactation, however fiber content remains a vital component of the early lactation ration and there is currently a lack of work in this area.

One of my interests that stemmed from my liver metabolism work has been on the interactions between hepatic glucose and fatty acid metabolism. The traditional focus has been an "either or" approach to studying macronutrient metabolism; however, especially during the early lactation period there are many interactions between the metabolism of these macronutrients to meet the needs of the cow. To address these interactions and the changes in the hepatic intermediary metabolism during early lactation I am currently looking at gene expression of some key hepatic enzymes in liver tissue obtained from early lactation cows, and evaluate the change in enzyme expression postpartum to better understand the interaction of these key regulatory steps in liver energy metabolism in the early lactation cow.

Another area of interest that arose from this work is in the large degree of variation in individual adaptation to lactation, and how individual animals handle this transition. From results of my haptoglobin study, I believe that more in depth investigations of early lactation inflammation and the mechanistic response and role of immune function are necessary to better understand the different adaptation strategies of individual cows to inflammation in early lactation. Cows that fell within the established high haptoglobin cut points in this study had a diverse range of production responses and further investigation is warranted.

I hope that all of my current and future research endeavors continue to help dairy producers and dairy cows by increasing our collective understanding of the underlying mechanisms that take place during the transition to lactation thereby allowing us to maximize their productive performance and also improve the quality of care we provide them.