RELATIONSHIPS BETWEEEN CARBOHYDRATE NUTRITION AND METABOLISM, INFLAMMATION, AND PERFORMANCE OF DAIRY CATTLE DURING THE PERIPARTURIENT PERIOD

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The transition from pregnancy to lactation is a time of great metabolic adaptations for the dairy cow. Dry matter intake decreases, yet demand for nutrients increase vastly with the onset of milk production resulting in negative energy balance. Cows must mobilize body tissues to increase energy for the body while sparing glucose for the mammary gland. Due to this increased metabolic demand and negative energy balance, cows often end up in a state of metabolic dysfunction which can have disastrous consequences.

Nutritional strategies can help increase glucose precursors while decreasing body tissue mobilization that is linked to increased risk of disease. Previous research is mixed on the impacts of high starch to increase glucose precursors to the cow immediately postpartum, likely due to an increased risk for subacute ruminal acidosis that can result in metabolic dysfunction and inflammation. Feeding strategies which utilize high starch while also including higher fiber to promote rumen health have not been conducted.

The objectives of this dissertation were to: 1) investigate interplay of fiber fractions in high starch postpartum dairy cow diets on performance, hepatic

metabolism and energy balance, 2) examine interplay of inflammation, hepatic metabolism, energy metabolites, and metabolic hormones in the postpartum period, and 3) investigate opportunities to combine use of a higher digestibility corn silage with monensin to optimize production and health in the periparturient period.

Increased fiber in the postpartum diet can limit intake in early lactation, resulting in negatively altered metabolism and production. As intake restrictions were eased by feeding a more fermentable diet, cows were able to recover intake, production, and energy metabolites in a matter of days to match cows that were not limited in intake early postpartum. Increased fiber in diets that may pose a higher risk of subacute ruminal acidosis may still be warranted, though further research on fiber levels and fractions is needed. Correlations between improved hepatic metabolism and metabolic hormones indicative of energy balance were positive, though correlations between markers of improved hepatic metabolism and inflammation were negative. Cows fed corn silage with higher digestibility and monensin, which increases glucose precursors, showed a possible synergistic effect on milk production. Using either strategy alone increased energy status, improved metabolism and health status, however a combination of both strategies decreased milk components, indicating they might negatively alter the rumen environment and milk fat synthesis.

BIOGRAPHICAL SKETCH

Sarah LaCount was raised in Glen Burnie, Maryland and attended Virginia Polytechnic Institute and State University to study Dairy Science and Animal and Poultry Sciences where she graduated with a Bachelor's degree in 2013. Sarah completed a summer research internship at the Miner Institute in Chazy, NY while still a student at Virginia Tech. Upon graduation decided, she to continue her experience in agricultural research with a yearlong research position at the Miner Institute where she discovered an interest in research with transition cow nutrition. Sarah arrived at Cornell University in 2014 to begin graduate work with Dr. Tom Overton investigating the impacts of carbohydrate nutrition on metabolism, inflammation, and performance in the periparturient period. Sarah is defending her thesis in July 2019 and plans to work in the dairy nutrition industry.

Dedicated to my parents, Theresa & Dennis Williams, who fostered my love for learning and exploring from as early as I can remember.

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CHAPTER 1

INTRODUCTION

The transition from late gestation to early lactation is one of great metabolic adaptation for the dairy cow. Dry matter intake (DMI) decreases as cows approach parturition, and milk yield dramatically increases energy demands at the onset of lactation (Bell, 1995). Increases in DMI occur slowly after calving, and dietary glucose precursors are unable to meet the demand of the mammary gland. A number of physiological and hormonal shifts occur to spare glucose for milk production at the expense of catabolism of other tissues to provide fatty acids and amino acids for energy synthesis (Bauman and Currie, 1980). Increased mobilization of adipose tissue to produce non-esterified fatty acids (NEFA) for energy metabolism is necessary, but can be excessive with prolonged negative energy balance and increase the risk of postpartum metabolic disease (Ospina et al., 2010).

At the crux of this transition is the liver, tasked with increasing glucose production to support milk production, while utilizing an influx of fatty acids (FA) for energy production and storage, as ability to export FA by the ruminant liver is low. Hepatic gluconeogenesis is upregulated, though with a decrease of dietary glucose precursors accompanying decreased DMI, glucogenic amino acids such as alanine are utilized for gluconeogenesis through the action of pyruvate carboxylase which is upregulated in the early postpartum period (White et al., 2016). The liver takes up FA in proportion to their supply in the blood stream, thus excessive NEFA mobilization will lead to greatly increased quantity of FA uptake by the liver. Fatty acid infiltration of the liver can lead to fatty liver disease, which has been associated with increased

inflammation, decreases in gluconeogenesis, increased risk of disease, and poor performance (Bobe et al., 2004). Decreasing the energy deficit around the time of calving can help minimize excessive FA mobilization, ultimately improving liver metabolism and overall health status.

Nutritional strategies that aim to ease negative energy balance are necessary to help cows successfully navigate the transition period. Postpartum diets with high energy density have been shown to increase production and measures of energy balance postpartum, but others have shown exactly the opposite. Higher starch diets increase ruminal propionate production, the primary glucose precursor, and supply to the liver. Likely, this mix of responses are due to some of these high starch diets inducing subacute ruminal acidosis (SARA), due to the drastic change in diet from a bulky dry cow diet to a highly fermentable lactation diet (Nocek, 1997). Impacts of SARA can include inconsistent intakes, decreases in production, increases in adipose tissue mobilization and inflammation (Stone, 2004). One possible way to mitigate effects of SARA is to increase fiber content in the diet, though there have been no published studies assessing impacts of higher starch diets in conjunction with high fiber. Fiber is a major component of every ruminant diet, the digestibility of which can alter energy balance and intake dynamics. Highly digestible fiber can increase DMI and nutrient utilization in the total tract and the rumen (Oba and Allen, 1999). Indigestible aspects of fiber however, are also useful as physically effective neutral detergent fiber (peNDF) and undigested NDF at 240 h of in-vitro fermentation can impact rumination, rumen health, and milk components, but can also limit intake.

The optimal transition cow diet that increases DMI, optimizes hepatic metabolism, increases milk yield and protects against disease is far from being developed. The objective of this literature review is to examine the literature surrounding nutrition strategies in the transition period and their impacts on metabolism and production. Specific attention will be given to starch and fiber as sources of nutrients, and their impacts on hepatic metabolism, blood metabolites and energy balance.

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CHAPTER 2

LITERATURE REVIEW

METABOLIC CHANGES IN THE TRANSITION PERIOD

Prepartum Metabolic Shifts

Metabolic demands of pregnancy are relatively low until the third trimester of pregnancy, when the conceptus is growing rapidly, which coincides with the initiation of mammary development, placing greatly increased demands on the cow as parturition approaches (Bauman and Currie, 1980). Coordination of metabolic, hormonal, and endocrine signals from late pregnancy to early lactation are required to prepare for parturition and the initiation of lactation. As parturition approaches, over a 2-wk period gradual decreases in progesterone and increases in estradiol, prolactin, and growth hormone (GH) are observed. Days prior to calving, progesterone decreases dramatically, and glucocorticoids, prolactin, and GH concentrations increase rapidly (Bauman and Currie, 1980, Bell, 1995). Along with these changes, dry matter intake (DMI) begins to decrease gradually beginning 3 wk from parturition, ultimately decreasing ~30% in the week prior to parturition (Grummer, 1995). Coinciding with decreased DMI, insulin decreases gradually as parturition approaches, and adipose tissue is mobilized increasing blood non-esterified fatty acids (NEFA) to supply energy to the cow while glucose is being spared for the rapidly growing conceptus (Bell, 1995). Though increases in NEFA are likely related to DMI and decreases in insulin, force feeding cows to maintain intake as parturition approached resulted in a blunted NEFA increase, though the response was not completely eliminated

(Grummer, 1995). This would indicate metabolic changes are not necessarily related to DMI alone, but are likely related to coordination of hormones and endocrine factors. Several of these shifts prepartum (decreased insulin, increased GH and NEFA) are key to altering metabolism postpartum to spare glucose for the mammary gland to support milk synthesis.

Nutrient Partitioning and Negative Energy Balance

The onset of lactation increases demands for glucose rapidly; milk production can account for 85% of total glucose turnover, and lactose synthesis, the osmotic regulator of milk volume, alone utilizes 65-70% of total glucose (Baumgard et al., 2017). Nutrient requirements of the mammary gland by 4 days in milk (DIM) are greatly upregulated compared to the gravid uterus, specifically requirements increase: 2.7x for glucose, 2x for amino acids, and 4.5x for fatty acids (Bell, 1995). Blood glucose rapidly increases on the day of calving to support the initiation of milk production; however, this is likely due to the increases in glucocorticoids and glucagon at calving promoting the depletion of hepatic glycogen stores, which is unable to be sustained (Grummer, 1995). Ideally, postpartum DMI would increase immediately postpartum to meet demands of milk production; however, the time of calving represents the point of lowest DMI in the lactation cycle and DMI will not increase to a maximum until between 8-22 wk postpartum (Ingvartsen and Andersen, 2000). As requirements of glucose are high, and dietary supply is low, early lactation dairy cows undergo a period of negative energy balance (NEB) that will reach a nadir on wk 2 and on average will not be alleviated until between 6-8 wk postpartum (Butler, 2000, Grummer, 2012).

During NEB, metabolic and endocrine shifts allow the animal to spare glucose for the mammary gland, largely relying on mobilized fatty acids (FA) as fuel for extra mammary systems. The previously stated low levels of circulating insulin and high circulating GH at calving are sustained for several weeks after parturition (Bell, 1995). Increased circulating GH decreases insulin sensitivity, and together with the already low circulating insulin and IGF-I concentrations, this will decrease peripheral glucose utilization for lipogenesis or protein synthesis in adipose or muscle tissues, sparing glucose for use in the mammary gland, while promoting lipolysis and proteolysis for catabolism in the liver (Bell and Bauman, 1997). Negative protein balance is also apparent after calving as amino acids are utilized for gluconeogenesis, milk protein production, and elsewhere in the body. This negative protein balance will reach a nadir on d 7 and rebound to positive balance by 3-4 wk postpartum (Bell et al., 2000). It has been estimated that 25% of body protein can be mobilized and catabolized (mostly from skeletal muscle and skin) to help fill the requirement (Bell, 1995).

Mobilization of adipose tissue is critical in the transition period, complete oxidation of NEFA or partial oxidation resulting in ketone production (main ketone body discussed will be β-hydroxybutyrate; BHB) in the liver provides a source of energy for extra mammary tissues. However, hepatic uptake of NEFA is proportional to supply in the blood (Emery et al., 1992), thus excessive or prolonged adipose tissue mobilization can lead to increased triglyceride accumulation and fatty liver disease, which can negatively impact hepatic health and metabolism, milk production, and increase risk of other diseases (Herdt, 2000, Bobe et al., 2004). Aside from fatty liver, excessive increases of circulating NEFA and BHB in the periparturient period have

also been associated with decreased performance and reproductive parameters, and increased risk or odds of diseases such as displaced abomasum (DA), clinical ketosis (CK) retained placenta (RP), and metritis (Ospina et al., 2010b, a, Chapinal et al., 2011, McArt et al., 2013b).

Excessive NEB caused by maladaptation to metabolic shifts is unfortunately common, LeBlanc (2012) approximated that 75% of disease in dairy cows happens in the transition period. In a large field study in the Northeast US, incidence of clinical disease within 30 DIM (DA, CK, metritis or RP) ranged from 9.0% to 19.6% (Ospina et al., 2010b), while a large field study in Canada reported >30% of animals had one clinical disease within 90 DIM, 17.4% of which were energy related disorders (DA, CK, or RP). Incidence of subclinical disease, however, is even greater with reports of subclinical ketosis (BHB $\geq$ 1.2 mmol/L) incidence ranging from 43-59% on commercial dairy farms (McArt et al., 2013b). While disease incidence can be specific to animal, farm, or nutritional strategies, high rates of metabolic diseases are very common for animals in negative energy balance.

Negative energy balance plays an obvious role in metabolic disease, as disrupted adaptations in metabolism are generally caused by negative energy status, but NEB is also associated with immunosuppression. Macrophages and neutrophils rely primarily on glucose as a fuel source, and decreased glucose availability in NEB can decrease efficiency, while increases in NEFA can directly diminish antimicrobial capabilities of immune cells as well (Sordillo and Raphael, 2013). Kimura et al. (1999) monitored neutrophil myeloperoxidase activity of mastectomized cows in comparison with intact cows throughout the transition period to gauge the

immunosuppressive effects of parturition versus the effects of milk production and associated NEB. While myeloperoxidase activity decreased for both sets of cows around parturition, mastectomized cows were able to recover activity to prepartum values by one wk postpartum as compared to intact cows, in which myeloperoxidase activity remained depressed through 20 DIM. This would indicate while some level of immunosuppression is linked to parturition directly, sustained immunosuppression is more likely related to the NEB caused by the demands of lactation. Immunosuppression caused by NEB can increase susceptibility of cows in the early postpartum period to infectious as well as metabolic disorders, partially explaining the increased incidence of all health disorders around the transition period.

Negative energy balance impacts on reproduction

Fertility and reproductive performance can also be altered by NEB as shifts in metabolic hormones which are necessary to facilitate changes in hepatic metabolism and for glucose sparing can ultimately delay first ovulation (Butler, 2003). Under conditions of positive energy balance GH secretion is coupled with hepatic production of insulin like growth factor-I (IGF-I), which acts as negative feedback on GH (Lucy, 2008). During NEB, however, the GH-IGF-I axis is uncoupled. The liver becomes refractory to circulating GH due to a gradual decrease in hepatic GH receptor-1A mRNA (GHR) in the two wk leading up to calving, accompanied by a dramatic decline at the time of parturition (Radcliff et al., 2003). With few receptors for GH to act on the liver to increase IGF-I production, circulating IGF-I remains low and high levels of GH continues to promote catabolism of other tissues for fuel. As DMI increases, increasing glucose and insulin concentrations begin to increase, hepatic

GHR mRNA increases, number of hepatic GHR increase, the liver becomes responsive to GH, and IGF-I production increases (Butler et al., 2003, Radcliff et al., 2003). Increased IGF-I acts as feedback on circulating GH, which begins to decline approximately 2 wk postpartum, re-coupling the GH-IGF-I axis.

Minimizing NEB, or utilizing strategies to increase DMI postpartum to promote increases in insulin and IGF-I, can improve early reproductive performance. Circulating plasma insulin and IGF-I are critical to follicular development, increasing pulsatility of, and response to, luteinizing hormone (LH), which is necessary for successful ovulation (Beam and Butler, 1999). While all cows will undergo follicular wave development early postpartum, only 42% were able to ovulate a follicle successfully from that wave at 16-20 DIM, the remainder were nonovulatory or cystic (Beam and Butler, 1997). Of cows that successfully ovulated a dominant follicle, IGF-I was 40-50% higher in the first 2 wk postpartum than cows that had non-ovulatory follicles. Additionally, pregnancy rate of cows that ovulate successfully before 50 DIM is 30% higher at 200 DIM compared to cows that did not ovulate before 50 DIM, indicating how critical early metabolic status is, and that aiming to minimize NEB in early lactation can influence reproductive performance (Butler, 2003). Striving to minimize the impact of NEB in the transition period can improve animal health, metabolism, milk production and reproduction.

HEPATIC METABOLISM IN THE POSTPARTUM PERIOD

Gluconeogenesis

In ruminants, relatively small amounts of glucose are absorbed in the small intestine and enter into circulation. Instead, they must heavily on hepatic

gluconeogenesis, mainly volatile fatty acids produced in ruminal fermentation provide substrates for gluconeogenesis, though several other fuel sources can be used as well (Aschenbach et al., 2010). In a fed state, gluconeogenesis is tightly regulated through circulating concentrations of insulin and glucagon. Increases in insulin occur after a meal when blood glucose begins to rise, and promotes uptake of glucose and other nutrients (amino acids [AA], lactate, glycerol) by peripheral tissues, stimulating anabolism in adipose and muscle tissues. Insulin acts directly on the gluconeogenic pathway by decreasing cyclic-AMP (cAMP) which in turn downregulates gene expression of the rate limiting enzyme, phosphoenolpyruvate carboxykinase (PEPCK) and thus, decreases gluconeogenesis. Glucagon actions are completely opposite to those of insulin; glucagon increases when blood glucose levels are low to increase glycogenolysis and increase gluconeogenesis. Glucagon increases cAMP, which upregulates gene expression of PEPCK, decreases expression of pyruvate kinase, which promotes the breakdown of phosphoenolpyruvate (PEP), a necessary intermediate, ultimately increasing rates of gluconeogenesis.

Upon short term starvation or low glucose availability, glycogen stores are quickly depleted, insulin is low and glucagon increases which upregulates mRNA of PEPCK to increase gluconeogenesis (Pilkis et al., 1988). As DMI decreases in the days prior to calving and concentrations of circulating insulin are decreased, it is likely that these changes initiate the upregulation of gluconeogenesis prepartum (Drackley et al., 2001). Glucose demands increase approximately 2x from late pregnancy to early lactation, indicating rapid increases in gluconeogenesis are necessary to meet this demand (Bell, 1995, Reynolds et al., 2003). Rates of conversion of propionate to

glucose have been reported at 119 and 129% increases at 1 and 21 DIM, respectively, compared to prepartum values, demonstrating rates of gluconeogenesis are increased by 1 DIM (Drackley et al., 2001).

Glucogenic precursors

Propionate is the primary contributor to gluconeogenesis during all stages of lactation, however decreases in DMI around the time of calving decrease the ruminal availability of propionate, resulting in utilization of other fuels to meet glucose demands. Rates of *in-vitro* conversion of alanine to glucose have been markedly increased in instances decreased glucose availability, with increases in the rate of 198% at 1 DIM and 150% at 21 DIM as compared to prepartum rates, indicating the potential to increase utilization of amino acids for gluconeogenesis postpartum (Drackley et al., 2001). At 11 DIM gluconeogenic precursors other than propionate were measured to have maximal contribution to gluconeogenesis as compared to measurements taken at other points (21, 33, and 83 DIM) in lactation; contributions of propionate, lactate, alanine, and glycerol accounted for 69.2, 19.5, 7.5, and 4.5%, respectively, of glucose produced and secreted from the liver (Reynolds et al., 2003). As lactation progressed, contributions of lactate, alanine, and glycerol decreased while propionate contributions increased.

Propionate is mainly derived from dietary starch sources in the rumen and is quickly absorbed into the blood stream. Upon delivery to the liver approximately 90% of propionate is removed for metabolism, where it can either be utilized for oxidation or gluconeogenesis. Capacity of the liver to convert propionate to glucose is dependent on propionate supply, thus increased concentrations of propionate would increase

hepatic glucose output (Veenhuizen et al., 1988). *In-vitro* metabolism of liver slices with radiolabeled isotopes of [1-^{14}C] propionate suggests that propionate randomizes within the TCA cycle so that for every mole of labeled propionate that is directed towards oxaloacetate, 0.5 moles of CO_2 and 0.5 moles of glucose will be produced (Knapp et al., 1992). Increases in the ratio of labeled CO_2 to glucose, indicative of increased efficiency of glucose production, are reported for cows at 21 DIM compared to cows in the prepartum period or at 1 DIM (Drackley et al., 2001). This would indicate that as lactation progresses, DMI and thus propionate supply increases, and efficiency of glucose production from propionate also increases.

Propionate enters the mitochondria, being converted to methylmalonyl CoA prior to being converted to succinyl-CoA for entry into the TCA cycle. Once in the TCA cycle, succinyl-CoA can be completely oxidized or converted to oxaloacetate which can then be converted to phosphoenolpyruvate by the mitochondrial or cytosolic version of PEPCK (PEPCK-M or PEPCK-C, respectively), which is a rate-limiting step into the gluconeogenesis pathway. Gene expression and activity of PEPCK-C is regulated by hormones and is increased in times of fasting or starvation due to increases in glucagon and glucocorticoids, whereas PEPCK-M is not (Greenfield et al., 2000). Increases in PEPCK-C mRNA have been reported due to increased propionate supply, including in the postpartum period due to monensin supplementation prepartum (Karcher et al., 2007). However, PEPCK-C mRNA is not upregulated due to the onset of calving and was expressed similarly compared to prepartum levels in the immediate postpartum period; though gene expression of PEPCK-C did increase by 2.5 fold at 14 DIM, and reports of increases of between

50% and 3 fold by 28 DIM (Greenfield et al., 2000, White et al., 2016). This follows the idea of upregulation of PEPCK-C due to propionate supply, as DMI and propionate supply would be increasing in the weeks following parturition. This suggests immediate upregulation of gluconeogenesis to support lactation would not be reliant on PEPCK-C gene expression, and that PEPCK-C rather upregulates gluconeogenesis as lactation progresses.

Though other AA can be utilized for gluconeogenesis, alanine has been found to be the primary contributor to gluconeogenesis in the postpartum period, followed by glutamine (Aschenbach et al., 2010). As previously discussed, *in-vitro* rates of gluconeogenesis from alanine have been reported to increase drastically in the immediate postpartum period, and maximum alanine removal by the liver was achieved at 11 DIM, where alanine contributed to 7.5% of hepatic glucose synthesis. Amino acids used for gluconeogenesis are either absorbed from the diet or mobilized from body protein, namely skeletal muscle and skin (Bell et al., 2000). Ratios of urinary 3-methylhistidine to creatinine, which can be used to estimate rate of skeletal muscle degradation, increased to 3x prepartum values by 3 DIM, but decreased to only 2x prepartum values by 7 DIM, indicating AA from skeletal muscle are being mobilized from body protein for use as fuel in the immediate postpartum period (Drackley et al., 2001).

Lactate was measured to account for almost 20% of total glucose production at 11 DIM, being the second highest contributor to gluconeogenesis in the postpartum period (Reynolds et al., 2003). Contributions of lactate can occur from the diet, though little lactate is formed from common diets of transition dairy cows (Drackley et al.,

2001), or produced as a byproduct from glycolysis in peripheral tissues (Baird et al., 1980). Lactate dehydrogenase facilitates the conversion of pyruvate to lactate under conditions of anaerobic metabolism in peripheral tissues such as skeletal muscle. Conversion to lactate is critical as buildup of pyruvate would provide negative feedback, reducing rates of glycolysis as well as regenerating NAD+ from NADH for use in continued glycolysis. *In-vitro* rates of lactate conversion to glucose has been reported to increase ~3x in cows undergoing feed restriction compared to cows fed ad-libitum, increased lactate utilization for gluconeogenesis in times of glucose shortage (Velez and Donkin, 2005).

Amino acids and lactate are converted to pyruvate in the liver and can either be converted to acetyl-CoA for entry into the TCA cycle or are subsequently converted to oxaloacetate by the metabolic enzyme pyruvate carboxylase (PC), which can then be used for gluconeogenesis (dependent on PEPCK as previously described for propionate) or oxidation in the TCA cycle. Changes in gene expression and activity of PC in the periparturient period have been well documented, having been reported to increase 6-7.5x prepartum values at 1 DIM (Greenfield et al., 2000, White et al., 2016). This is a transient increase, however, as gene expression of PC decreases to only 4x at 14 DIM (White et al., 2016) and returned to postpartum values by 28 DIM (Greenfield et al., 2000), indicating the role of PC is likely critical in the immediate postpartum period.

Given increases in PC immediately postpartum without paired increases in PEPCK, the rate of gluconeogenesis may be unchanged, though increases in PC will supply a pool of oxaloacetate to the TCA cycle for oxidation (White et al., 2012).

Gene expression of PC is also regulated by acetyl-CoA concentrations in the early postpartum period (Aschenbach et al., 2010). This is critical as the catapleurotic pull of oxaloacetate away from the TCA cycle for gluconeogenesis is dependent on the ratio of available oxaloacetate to acetyl-CoA. The ratio of oxaloacetate to acetyl-CoA must be maintained at a 1:1 ratio for optimal TCA cycle function. If this ratio were to fall to <1 (less oxaloacetate available as compared to acetyl-CoA) the catapleurotic pull of PEP to extract oxaloacetate for gluconeogenesis would stop. This scenario is observed postpartum, as NEFA influx to the liver, transport across the mitochondria and conversion to acetyl-CoA are high, while propionate to supply to oxaloacetate is low. Increased PC expression increases providing anapleurotic supply oxaloacetate to the TCA in the immediate postpartum period, maintaining the 1:1 ratio for optimal function of the TCA cycle (White et al., 2012). That is not to say that increases in PC do not result in increases of AA and lactate use for gluconeogenesis. As propionate supply decreases immediately postpartum due to changes in DMI, it is likely that glucose precursors supplied through PC action contribute to gluconeogenesis to make up for the deficit of propionate, though does not actually increase the rate of gluconeogenesis. The availability of AA and lactate and activity of PC provide a critical increase in both glucose precursors and oxidative fuels for increased hepatic energy synthesis in the immediate postpartum period.

Fatty Acid Metabolism

Hepatic fatty acid metabolism is critical to provide energy to peripheral tissues and provide substrates for milk fat synthesis during NEB when glucose is being spared for the mammary gland. As previously described, low blood insulin and increased

concentrations of circulating GH stimulate lipolysis of adipose tissue, resulting in increased circulating NEFA concentrations. Uptake of NEFA by the liver is proportional to the supply in the blood stream, estimated to be approximately 25% (Emery et al., 1992, Drackley et al., 2001). Non esterified fatty acids in the liver have one of several metabolic fates; complete β-oxidation in the TCA cycle or peroxisome, incomplete oxidation and entry to the ketogenic pathway for synthesis and release of ketones, namely BHB, into circulation, re-esterification to triglyceride (TG) for either export as very low-density lipoprotein (VLDL) or storage in the liver (Drackley et al., 2001).

In the liver, NEFA are converted to acyl-CoA and at this point there are three options: conversion to malonyl CoA for TG synthesis, import into the mitochondria via metabolic enzyme carnitine palmitoyltransferase 1a (CPT1a), or peroxisomal oxidation which is thought of as a considerable avenue for oxidation when NEFA influx are very high. If converted to TG, the TG is then either stored in the liver, or exported as VLDL. If transported into the mitochondria for catabolism it is converted to acetyl CoA, where again there are two options. Acetyl-CoA can enter the TCA cycle for oxidation, or mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) can facilitate entry to the ketogenesis pathway (Hegardt, 1999). Oxidation of NEFA in the liver provide energy through catabolism in the TCA cycle, whereas ketone production provides a fuel source that can be utilized by peripheral tissues for energy and for milk fat synthesis.

Several factors can impact the metabolic fate of NEFA in the liver, uptake of NEFA into the mitochondria by CPT1a is the first regulated step. Activity of CPT1a is

governed by several factors, including malonyl CoA and methylmalonyl CoA concentrations, though these inhibitions are dependent on energy balance. Malonyl CoA production is regulated by the enzyme acetyl CoA carboxylase (Grummer, 1993). Under well-fed conditions and positive energy balance, increases in production of malonyl CoA would inhibit CPT1a to inhibit entry for catabolism in the mitochondria and instead favor storage as TG. During NEB however, when circulating insulin is low CPT1a is less sensitive to malonyl CoA inhibition (Drackley, 1999). Methylmalonyl CoA is a metabolic intermediate of propionate that is formed prior to entry into the TCA cycle once in the mitochondria. Increases in methylmalonyl CoA concentrations suggest that the TCA cycle is saturated, thus CPT1a is inhibited to avoid excess NEFA transportation to the mitochondria. Associations of decreased CPT1a activity with increased propionate have been reported previously, and this is likely one way propionate and fatty acid metabolism are linked (Drackley, 1999).

Similarly, succinyl CoA, the point of entry for propionate in the TCA cycle, is able to succinylate HMGCS2 when it is in excess inhibiting activity of HMGCS2 (Hegardt, 1999). Conversely, decreased propionate supply and thus decreased succinyl CoA would lift restrictions on HMGCS2. In NEB when insulin is low, NEFA load to the liver is high, and total propionate and associated metabolic intermediates would be low, both fatty acid oxidation and ketone production should be uninhibited to allow for maximum energy production.

Gene expression of CPT1a has been reported to increase immediately postpartum, and has been associated with increases in rates of ketogenesis, gluconeogenesis, and NEB (Drackley et al., 2001, Dann and Drackley, 2005). Gene

expression of HMGCS2 does not appear to change through the periparturient period (Graber et al., 2010, Chen et al., 2015, Mann et al., 2018). However, the changes in gene expression may not be representative of differences in rates of actual fatty acid metabolism observed through the transition period. Litherland et al. (2011) reported a small decrease in the rate of *in-vitro* palmitate metabolism to CO_2 , accompanied by slight increased rate of palmitate metabolism to ASP (representative of ketone production), and large increase in rates of palmitate esterification to TG as cows transitioned from pregnancy to lactation. Litherland et al. (2011) also reported a negative correlation between capacity for hepatic oxidation of fatty acids and capacity for esterification, indicating increases in hepatic TG content might be related to an alterations of oxidative capacity in the hepatocyte.

Re-esterification of NEFA is common postpartum due to the increased total NEFA load delivered to the liver, exceeding metabolic pathway capacity. Esterification rates have been reported as high as 188% of prepartum rates at 1 DIM, though this is highly influenced by factors of diet surrounding the transition period, as increases of only 7% were reported from the same study when feeding a high fat as opposed to high energy diet (Grum et al., 1996). Moderate positive correlations between rates of esterification and liver triglyceride content have been reported in the postpartum period (Grum et al., 2002, Litherland et al., 2011, McCarthy et al., 2015b). Export of esterified fat from the liver is possible, however, hepatic ability of ruminants to export VLDL is lower than other non-ruminant species, thus if supply of NEFA to the liver saturates pathways of catabolism, liver TG will begin to accumulate, causing fatty liver disease (Grummer, 1993). The incidence of moderate fatty liver (5-10% TG

as % wet weight) disease has been reported between 20-53% and severe fatty liver (>10% liver TG as % wet weight) disease as 11-20%, indicating how common increased liver triglyceride content is in the periparturient period (Bobe et al., 2004). Liver triglyceride content has been correlated negatively with DMI, plasma glucose, capacity to synthesize glucose from propionate, and energy balance (Grum et al., 2002, Piepenbrink and Overton, 2003, McCarthy et al., 2015b), and is associated with decreased production, increased risk of metabolic disorders, and associated with increases in inflammatory status (Bobe et al., 2004), demonstrating the multitude of metabolic shifts that coincide with increases in liver triglyceride content.

Though rates of VLDL synthesis are low in dairy cows, research would suggest supplementation of rumen protected choline may be able to increase liver TG export. Choline is a necessary precursor for phosphatidyl choline synthesis as a methyl donor, which is a required for VLDL packaging and secretion (Grummer, 1993). Though reports are mixed, choline has been shown to decrease liver TG in transition dairy cows and cows in feed restriction conditions (Grummer, 2008), and choline supplementation correlated negatively with rates of hepatic esterification (Piepenbrink and Overton, 2003). Rumen protection of choline is necessary as choline is metabolized in the rumen, and some variation in reports are thought to be due to adequacy of rumen protection (Grummer, 2008). Treatment with propylene glycol has also been reported to protect against hepatic triglyceride accumulation across the periparturient period due to related decreases in adipose tissue mobilization (Bobe et al., 2004, Grummer, 2008). This however is not likely a feasible prevention option for

cows under typical field conditions, as administration of propylene glycol to cows in the prepartum period multiple times would be laborious and time consuming.

INFLAMMATION IN THE TRANSITION PERIOD

Inflammation and health status

Inflammation is necessary to successfully undergo parturition, initiate lactation, and begin uterine involution in the postpartum period, several examples include macrophage and neutrophil influx that are necessary to promote dilation of the cervix prior to parturition, proliferation of mammary epithelial cells are promoted by nuclear factor κβ prior to lactation, and neutrophils are crucial for uterine involution, proper recovery from parturition, and first line of defense against infectious pathogens (Bradford et al., 2015). However these, and other inflammatory responses related to injury or illness, should be robust and rather short lived, eliminating the response following resolution of the infectious agent or cause, as excessive inflammation and/or prolonged inflammation can result in dysfunction and disease.

Inflammation generally begins with the signaling of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF), interleukin-1b and interleukin-6. Cytokines are produced by many cell types and activate leukocytes and endothelial cells, triggering the liver to start the acute phase response and ultimately increase inflammation further. During an inflammatory response, positive acute phase proteins (APP), such as haptoglobin, serum amyloid A, and bilirubin, that are produced in response to pro-inflammatory cytokine signaling increase, whereas negative acute phase proteins, such as albumin and cholesterol, which are produced in the liver under

normal conditions are decreased (Bertoni et al., 2008). While bilirubin and cholesterol are not actual APP, they are relatively easy to measure and the synthesis or clearance is related to an acute phase protein, thus the changes in blood concentrations are useful (Bertoni and Trevisi, 2013). Bertoni et al. (2008) calculated liver activity index of cows in early lactation based on several positive and negative APP, grouping cows into quartiles based on liver function. Cows in the lowest liver activity index group exhibited higher positive APP and lower negative APP, indicating more inflammation and less desirable liver function; these cows produced less milk and had longer time to pregnancy than the upper quartile group. It is important to note that cows in the highest liver activity index group still had increases in positive APP and decreases in negative APP, though the changes were less severe. Increases in specific acute phase proteins such as haptoglobin have also been associated with increased risk of periparturient disorders (Humblet et al., 2006, Huzzey et al., 2009), though given the non-specific nature of APP they may not be particularly useful in prediction of disease for individual cows.

Recently, it has been demonstrated that administration of non-steroidal anti-inflammatory drugs (NSAID) to cows after calving (delivered no sooner than 12 h after parturition) can increase milk yield from 7- 21%, though the mechanism of this increase remain unclear and the response is somewhat inconsistent (Farney et al., 2013, Carpenter et al., 2016, Carpenter et al., 2018). Administration of the NSAID sodium salicylate may influence insulin sensitivity, inducing a state of hypoglycemia, though the implications of this are not fully understood in terms of how it relates to the reported increased milk production. Timing of NSAID administration postpartum is

also vital. Newby et al. (2017) reported increases in stillbirths in animals given an NSAID before calving, and increased odds of having a retained placenta, increased temperature, and decreased milk production when the NSAID was delivered immediately postpartum. It would appear that decreasing inflammation in the postpartum period may increase milk production, though timing of delivery is critical, and further research is certainly warranted on this topic to investigate further.

Inflammation and hepatic metabolism

Inflammation and metabolism are linked in the transition period. As well as inflammation being associated with metabolic dysfunction, it was recently estimated that an activated immune response can utilize more than 2 kg/d of glucose (Kvidera et al., 2017), for a cow that is already in a state of NEB, this would add considerably to energy requirements and affect metabolism. Increases in NEFA and BHB that are related to immune suppression and increased risk of periparturient disorders such as DA, metritis, and CK were discussed previously (Ospina et al., 2010b, LeBlanc, 2012, Sordillo and Raphael, 2013).

Excessive NEFA mobilization and delivery to the liver may also increase rates of peroxisomal oxidation (Drackley, 1999), which will increase oxidation and decreases liver lipid storage in the cell; however, it may also increase reactive oxygen species accumulation, as hydrogen peroxide is produced during peroxisomal oxidation (Sordillo, 2009). Cows with ketosis have been reported to have increased concentrations of APP, namely haptoglobin and serum amyloid A, when compared to non-ketotic control cows (Abuajamieh et al., 2016). Fatty liver disease and liver triglycerides have also been related to inflammation through increased haptoglobin,

(Katoh, 2002), TNFα and serum amyloid A (Ametaj et al., 2005), and infectious diseases such as mastitis and metritis (Bobe et al., 2004). Fatty liver is not just associated with increased inflammatory markers, but changes in immune function as well. In a study of endotoxin clearance, all clinically healthy (n = 6) cows were able to clear endotoxin from the plasma within 30 min, while cows with fatty liver disease were not able to clear the toxin for the entire study duration, with one of the 4 fatty liver cows involved dying prior to the end of the experiment (Andersen et al., 1996). This would indicate cow's fatty acids were not able to mount a proper immune response to clear the endotoxin from plasma, though the mechanism is not clear.

While there are several studies showing associations of alterations in hepatic metabolism and inflammation, there is a lack of research with a clear consensus on whether the disease and dysfunction causes the inflammation and immune status changes, or the inflammation and immunity changes are causing the disease. One example of this is for increases in inflammation associated with liver triglyceride accumulation. Evidence for exogenous TNFα administration increasing liver triglycerides exists for mid-lactation cows (Bradford et al., 2009); however, a similar study in transition cows found that exogenous TNFα administration decreased DMI, milk production, and impaired health status, but had no effect on liver triglyceride content or blood metabolites (Yuan et al., 2013). Given the close associations between altered metabolism, metabolic disease, and inflammation it may be difficult to elucidate the true cause and effect within these relationships.

Inflammation and subacute ruminal acidosis

Subacute ruminal acidosis is characterized by repeated bouts or a significant time period of low ruminal pH [threshold value of <5.8 pH; Penner (2006)] which leads to altered metabolism and potential damage to the ruminal epithelium. Although inconsistent and ambiguous, signs of SARA include decreased efficiency of milk production, fluctuating intake, loose manure, poor BCS, higher cull rates, milk fat depression, and lameness (Nocek, 1997). During SARA, low pH increases death and lysis of gram negative bacteria in the rumen causes release of the endotoxin lipopolysaccharide (LPS), as well as increases in circulating acute phase proteins such as haptoglobin and serum amyloid A (Gozho et al., 2005, Gozho et al., 2006, Emmanuel et al., 2008). One proposed mechanism is that long bouts of low pH in the rumen can cause damage to the rumen epithelium, resulting in tight junctions between cells to become leaky, allowing LPS into the blood stream (Zebeli and Metzler-Zebeli, 2012). Alternatively, others have proposed LPS might translate to the blood stream through leaky tight junctions in the hindgut rather than the rumen, as diets with high starch content may result in an increase in starch that escapes ruminal digestion, and is thus digested post-ruminally. Recent research however, has shown no differences in circulating APP or LPS binding protein after abomasal infusions of 1 kg corn starch delivered every 6 h to cows previously acclimated to a low starch diet. This would indicate that even with direct supplementation of starch to the hindgut, inflammation was not induced by post-ruminal starch digestion. Regardless of where LPS enters the bloodstream, translocation of ruminal LPS causes inflammation, activating pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6), acute phase proteins and systemic

inflammation (Baumann and Gauldie, 1994). Although inflammation in response to SARA is seen as a protective reaction against the free endotoxin in the blood (Zebeli and Metzler-Zebeli, 2012), prolonged systemic inflammation especially in this early lactation period, is detrimental to animal health and performance as discussed previously.

RUMEN FERMENATION

Fiber digestion

Fiber digestion is completed by microbes in the rumen, providing volatile fatty acids (VFA) to the animal for metabolism. Digestion of fiber is influenced by many factors including: animal characteristics, physiological state, microbial population, digestibility of feedstuff, chemical composition of feedstuff, and particle size. Rumen digestibility is determined by two rates, rate of digestion and rate of passage. The rate of digestion can be influenced by chemical and physical structure of the feedstuff, including fiber matrix and crosslinking (Raffrenato et al., 2017), all of which will influence microbial attachment, and enzyme production and activity. Rate of passage is influenced by particle size, rumen motility, DMI, and specific gravity of particles (Allen and Mertens, 1988, Dado and Allen, 1995). Rate of passage and rate of digestion are inversely related, as passage rate increases rate of digestion will decrease and vice versa (Oba and Allen, 2000c).

Neutral detergent fiber of feedstuffs is divided into different pools related to intrinsic factors affecting rate digestibility: a fast pool, a slow pool, and an indigestible pool (Raffrenato et al., 2019). Indigestible NDF is comprised of the portion of a feed

that is completely inaccessible to rumen microbes and would not be digested even if left in the rumen indefinitely (Allen and Mertens, 1988). Measuring true indigestible NDF is not possible, thus it is approximated by $uNDF_{240}$, or the amount of feedstuff left undigested after 240-h of *in-vitro* fermentation (Raffrenato et al., 2019). Feeding forage with increased digestibility has been linked to increases in DMI and milk production in several instances, as faster disappearance of NDF from the rumen reduces rumen fill and thus allows for greater feed intake (Oba and Allen, 2000a, c, Grant and Ferraretto, 2018). Oba and Allen (1999b) reported that a one unit difference in NDF digestibility (measured between 24 and 48 h of fermentation *in vitro*) was associated with a 0.12 kg increase in DMI and 0.25 kg increase in 4% FCM, calculated from an analysis of 13 different forage comparisons reported in the literature.

Microbial interaction with fiber can also impact rate of digestion. Rumen microbes can only attach to forage particles at sites of fracture due to chopping or mastication as forage particles are covered with cutin, thus surface area available for microbe attachment can influence digestibility. Fiber digesting bacteria utilize cell wall polysaccharides as a primary energy source, however adequate nitrogen must be available in the rumen as well to facilitate microbial proliferation (Allen and Mertens, 1988). These bacteria are also very sensitive to pH, and rumen pH of <6.0 can inhibit microbe attachment to feed particles, decreasing digestive ability and rates of growth. In diets with high fermentable carbohydrates which promote decreases in rumen pH, fiber digestibility can be altered (Zebeli and Metzler-Zebeli, 2012).

Passage rate through the rumen is regulated by digestibility, particle size, and animal factors such as physiological state and DMI. Cows fed forages with higher digestibility may have increased passage rate due to increased fragility that enhances particle size reduction and increases NDF turnover in the rumen, which could lessen physical fill restraints and allow for further increases in DMI (Oba and Allen, 2000c). Decreased digestibility and increased particle size has been reported to increase time required for rumen NDF turnover, ultimately slowing passage rate and decreasing DMI (Grant et al., 2018). Physiological state of the animal impacts passage rate due to differences in requirements and nutrient utilization. Dry cows have slower digestion and passage rate as compared with lactating cows, as energy demands and metabolic rates are lower during the dry period (Drackley et al., 2001, Reynolds et al., 2003).

Fiber impacts in transition cow diets

Fiber is often utilized in diets to promote chewing and rumination, ultimately fostering a more stable rumen environment, though diets high in NDF also have the capacity to limit intake via gut fill. This limiting effect of gut fill is due to signaling of satiety centers by stretch receptors in the rumen muscle layer (Van Soest, 1965, Allen, 1996, Mertens, 1997). Gut fill limitations are primarily noted during mid lactation rather than early lactation (Allen and Piantoni, 2013), though limitations due to inert mass in the rumen have been noted in times of negative energy balance as well (Allen, 2000), indicating these limitations are also possible in the transition period.

Physical size of fiber is capable of altering DMI, feeding behavior, and the rumen environment. Physically effective NDF (peNDF) is an estimate of physically

effective fiber, or fiber that is greater than 1.18 or 4 mm in length, depending on the study. The Penn State Particle Separator (PSPS) is a tool available to measure particle size on farm or in a lab. Originally peNDF was suggested as NDF content of all particles above the screen with 1.18 mm holes of the PSPS, however this has since been altered to a larger, 4mm screen (Kononoff et al., 2003a, Kmicikewycz et al., 2015). Previous research investigating effects of particle size on DMI provide mixed results; several studies have found increased DMI with decreased particle size (Kononoff and Heinrichs, 2003, Coon et al., 2018), whereas others have reported no differences in DMI (Beauchemin et al., 2003, Fernandez et al., 2004). Chewing activity is increased with diets higher in peNDF (Beauchemin et al., 2003, Kononoff et al., 2003b, Grant et al., 2018), however this is generally observed for time spent masticating at the feed bunk, rather than increases in time spent ruminating, but not in all cases (Krause et al., 2002). Increases in chewing or rumination are associated with increased salivary flow, which can help buffer the rumen, as evidenced by higher rumen pH for cows fed a diet containing longer particle size (Allen, 1997, Krause et al., 2002).

In addition to the physical aspects of fiber, fiber digestibility impacts DMI and nutrient utilization which are both critically important in the transition period. Stone et al. (2012) reported ~1 kg/d increase in prepartum DMI and 2 kg/d increase in postpartum DMI when feeding forage with higher digestibility, indicating capacity for intake responses in the transition period due to forage fiber digestibility. Investigation of dietary levels of $uNDF_{240}$ have not been widely reported in the literature, especially in the transition period. Fustini et al. (2017) reported that cows fed high $uNDF_{240}$ diets

exhibited no differences in DMI, though had less time of rumen pH <5.8 compared to cows fed lower $uNDF_{240}$ diets, likely due to the longer ruminal retention time and therefore stabilization. A case study presented by McCarthy et al. (2015a) reported $uNDF_{240}$ intake related to diets in the immediate postpartum period, indicating low $uNDF_{240}$ may play a role in metabolic function and health status. Diets were formulated for high starch or low starch prior to the start of a study, however a new bunk of corn silage was opened that had much higher digestibility and starch, and lower $uNDF_{240}$ than was used to formulate the diets. Upon feeding the diet that contained $uNDF_{240}$ at approximately 8% DM with 28% DM starch, the cows exhibited signs of severe metabolic dysfunction through increased prevalence of metabolic disorders. Of 7 cows that were fed this diet, 9 total cases of CK, DA, and RP were observed. Once diets were evaluated and adjusted through the removal of corn silage and addition of chopped straw, the new diet contained $uNDF_{240}$ at 10.5% DM and 26% DM starch. Throughout the rest of the study metabolic disorders were largely absent (of 38 cows that were fed the changed diet, only 6 total cases of CK, DA, and RP) and cows were highly productive. This would indicate some level of $uNDF_{240}$ is necessary in the diet for adequate rumen function.

Although $uNDF_{240}$ and peNDF are characterized differently (chemical vs. physical), they are related. Grant et al. (2018) evaluated effects of diets containing varying $uNDF_{240}$ and peNDF content in mid lactation cows, finding that cows fed a high $uNDF_{240}$/high peNDF diet had decreased milk yield and DMI, but increased daily rumen pH mean compared to the low $uNDF_{240}$/low peNDF, while all other combinations of $uNDF_{240}$ and peNDF levels were similar in all parameters. Both of

these feed fractions may play a role in intake and rumen dynamics, which are vital during the transition period.

Starch metabolism

Starch digestion in the rumen is accomplished by bacteria, fungi, and protozoa, though the roles of the latter two categories are not clearly defined (Tricario et al., 2008). Rumen bacteria that are primarily responsible for starch digestion produce α-amylase and isoamylase that are necessary for cleavage of internal linkages in starch. Starch is rapidly digested in the rumen to VFA, with the primary product being propionate (Allen, 2000). As discussed previously, propionate is the primary glucose precursor of hepatic gluconeogenesis, and it is quickly absorbed through the rumen wall after a meal. Though absorption rate is high for propionate, rapid digestion of starch can lead to an increase in concentration of total VFA in the rumen that can lower rumen pH, increasing risk for SARA (Zebeli and Metzler-Zebeli, 2012).

As for fiber digestion, feed characteristics such as total surface area and particle size can impact digestibility in the rumen (Ferraretto et al., 2013). Starch that has been stored or processed with higher moisture content (e.g. high moisture corn or steam flaked corn), or has a greater available surface area (ground corn > cracked corn > whole corn) will have a higher rate and likely more complete digestion in the rumen (Allen et al., 2009). Otherwise, as starch particle size is generally lower than that of forage, starch can pass through the rumen undigested and be degraded in the small intestine. Starch digested post-ruminally can be degraded and absorbed as glucose or VFA, whereas microbial digestion in the rumen will only yield VFA. If starch is digested and absorbed as glucose, it can be more energetically efficient than

gluconeogenesis from VFA provided by ruminally digested starch, however only a small portion of starch digested post-ruminally will be absorbed as glucose, as capacity for starch digestion and absorption post-ruminally may be limited in ruminants (Oba and Allen, 2000c).

NUTRITIONAL STRATEGIES IN THE PERIPARTURIENT PERIOD

Prepartum nutrition strategies

Prepartum nutritional strategies in the literature have evolved to meet different goals over time. One such previous goal included providing increased dietary energy in order to increase nutrient supply and also to develop the rumen epithelium in preparation for the switch to a highly fermentable diet, known as "steam up" diets (Grummer, 1995, Rabelo et al., 2003). Though rumen epithelial growth was reported in one study, data from cows fed diets more typical of in the transition period reported no changes in the rumen epithelium, indicating this strategy might not accurately reflect the needs of cows in this period and can lead to metabolic dysfunction in the postpartum period (Overton and Waldron, 2004). Increases in DMI in the postpartum period have been associated with increased DMI from d 21 to 1 d prepartum, (r^2 = 0.53), thus strategies have evolved to increase intake in the prepartum period (Grummer et al., 2004). However without qualification of nutrient profile, maximizing intake of one diet may not be the same as maximizing intake of a diet with a different nutrient profile. Prepartum increases in DMI of high energy diets may increase metabolic dysfunction after parturition, with increases in NEFA and BHB and risk of subsequent diseases, whereas maximizing DMI of a lower energy diet may improve energy metabolites and health status (Dann et al., 2006, Mann et al., 2015). High

energy diets (150% of NRC requirements) fed from dry off through calving which promoted high intake were also reported to alter rates of fatty acid metabolism at 1 DIM such that cows fed high energy had lower palmitate oxidation to CO_2, less conversion to ASP, and a higher proportion of palmitate metabolism to esterified products as compared to cows fed at 80% of NRC requirements. Decreases in CO_2 and ketone production would limit energy availability in the postpartum period, and increased conversion of palmitate to esterified products would increase chance of developing fatty liver disease.

Additionally, diets with high energy density that might encourage body weight or body condition score (BCS) gain in the dry period would be detrimental. It is well documented that cows with a high BCS (≥ 4) often undergo a period of more excessive NEB through the transition period compared to cows with BCS < 4. This is characterized by an 11% greater drop in DMI as calving approaches, higher rates of adipose tissue mobilization and circulating NEFA, and higher risk of metabolic disorders such as hyperketonemia (BHB ≥ 1.2 mmol/L) and fatty liver disease (Bobe et al., 2004, Grummer et al., 2004, McArt et al., 2013a).

More recently, decreasing the magnitude of DMI change from the dry period through parturition to protect against excessive NEB (Grummer et al., 2004) has been examined through both restriction of intake and controlled energy diets. Studies restricting intake to 80% or 100% of predicted requirements have reported more stable DMI as calving approached, decreased change in energy balance, increased postpartum DMI, improved energy metabolite status, and decreased incidence of health disorders, though milk yield was lower for cows fed the restricted treatments as

compared to an overfed (150% of requirements) group (Janovick and Drackley, 2010, Janovick et al., 2011). Though not all studies have reported an effect of restricting energy density on postpartum DMI or milk yield, the effects of improved postpartum energy metabolites and health status are consistent (Mann et al., 2015).

It would appear that in regards to metabolism and health, lower energy diets in the dry period may prove to be the best strategy, however milk yield may suffer. As restricting intake in a field setting would likely promote altered feeding behavior, controlled energy diets (formulated to provide 100% of requirements) are able to be provided ad libitum, with dilution of nutrient dense ingredients with addition of chopped straw.

Postpartum nutrition strategies

A major goal of the postpartum period is to promote increased DMI of an energy dense diet immediately postpartum to help meet demands of lactation and ease NEB. There are numerous ways to achieve this, though the three strategies discussed in this section are: high starch diets, increasing forage digestibility, and supplementation of monensin.

Higher starch. Increasing starch concentration in the immediate postpartum period provides additional dietary propionate, the primary glucose precursor, to help support the glucose demand by the mammary gland. Feeding higher starch diets in the immediate postpartum period have been shown to increase energy balance and decrease circulating NEFA and BHB (Rabelo et al., 2003, McCarthy et al., 2015c, McCarthy et al., 2015d). In the companion papers of McCarthy et al. (2015c) and McCarthy et al. (2015d), diets of differing starch content (26.2 vs. 21.5% DM) were

fed in the postpartum period, a treatment by time interaction revealed an increase in both DMI and milk yield were observed in wk 2 and 3 compared to cows fed lower starch. This would indicate that, while cows fed high starch did not maintain a difference throughout the rest of the study, they were able to increase DMI and milk yield faster that cows fed lower starch. Similar results for faster increases in milk yield have been reported in other studies feeding more propiogenic and higher energy density diets, indicating an increase in gluconeogenic precursors can support a faster increase in milk yield (Andersen et al., 2003, Rabelo et al., 2003). Cows fed high starch also exhibited higher plasma glucose and insulin, and lower NEFA concentrations as compared to cows fed lower starch diets, implying cows were in an improved state of metabolism and likely a more positive energy balance.

Despite improved performance and energy metabolites exhibited by cows fed high starch in McCarthy et al. (2015d), these cows also exhibited higher plasma haptoglobin concentrations, indicating they were experiencing a state of inflammation. Higher starch diets can lead to SARA due to the large changes in diet composition in the transition period and increased fermentable carbohydrate load provided by high starch (Nocek, 1997, Penner et al., 2007). Consequences of SARA have been described previously, though briefly SARA can cause inconsistent feed intake, decreased milk production, increased inflammation, and laminitis (Plaizier et al., 2008). There is some inconsistency in the literature surrounding higher starch fresh diets which is likely due to this increased risk of SARA and subsequent negative effects.

Recently, a comparison between studies that fed diets of similar fermentable carbohydrate concentrations in the postpartum period but reported opposite effects on measures of production and metabolism was conducted (McCarthy et al., 2015a). One glaring difference was found between the two trials: starch content of prepartum diet. Cows that transitioned from a low starch prepartum (13.5% DM) diet to a high starch lactating diet (25.5% DM) likely experienced SARA, which influenced the negative impacts on performance and metabolism, whereas cows that were fed a moderate starch diet prepartum (17.5% DM) transitioning on to a high starch lactation diet (26.2% DM) experienced only mild inflammation, and exhibited positive changes in production and energy metabolism.

Increases in dietary starch postpartum are likely to improve energy balance and allow an overall quicker adaptation to lactation, though the starch level in the prepartum diet needs to be considered, as too large of an increase in starch content can lead to SARA and subsequent negative effects, likely impacting performance and metabolism.

Increasing forage digestibility. Increasing forage digestibility in diets in the periparturient period can increase energy density of the diet without the high risk of SARA and altered rumen fermentation that is associated with increased dietary starch. Brown midrib corn silage (BMR) is a corn silage (CS) hybrid that has been shown to have higher NDF digestibility due to lower lignin, and likely lower $uNDF_{240}$ content (Oba and Allen, 1999a, Ferraretto and Shaver, 2015, Raffrenato et al., 2017). Impacts of feeding BMR CS compared to conventional CS can vary due to physiological state and milk production levels; however, in a recent meta-analysis across multiple trials

and stages of lactation feeding BMR is reported to increase total tract NDF digestibility, increase DMI, increase milk production by 1.5 kg/d, accompanied by a decrease (0.09%) in milk fat percentage, and an increase (0.05 kg/d) in milk protein yield (Ferraretto and Shaver, 2015).

Evidence of increased *in-vivo* NDF digestibility for BMR corn silage is somewhat mixed. In studies that report increased DMI with feeding BMR CS, passage rate is generally also increased, which can decrease overall NDF digestibility as it has less time to be digested in the rumen (Oba and Allen, 2000c, Holt et al., 2010). In other studies, however, increases of *in-vivo* NDF digestibility are increased, indicating increased energy availability due to increased ruminal and total tract digestibility (Oba and Allen, 1999a, Greenfield et al., 2001, Ferraretto and Shaver, 2015, Lim et al., 2015). Accompanying this increased digestion, several studies report decreases in the ruminal acetate:propionate ratio, or increases in ruminal propionate concentration alone, indicating the greater digestibility is responsible for a direct increase in propionate that can be utilized for glucose synthesis (Greenfield et al., 2001, Mutsvangwa et al., 2002, Taylor and Allen, 2005). This increase in rumen VFA concentration can result in a lower rumen pH, and altered rumen fermentation which presents with lower rumen pH is likely to alter biohydrogenation, causing the observed decrease in milk fat concentrations (Oba and Allen, 2000a, Lim et al., 2015).

Impacts of BMR on nutrient utilization, feeding behavior, production, and efficiency are well documented (Oba and Allen, 2000a, b, c, Ferraretto and Shaver, 2015); however, effects on energy metabolites are not often reported. Stone et al. (2012) observed no difference in plasma NEFA or BHB of cows fed BMR prepartum

or postpartum despite a 1.6 Mcal/d increase in energy balance for cows fed BMR prepartum. Blood samples were only collected 1x per wk in this study, from wk -3 to wk 3 relative to parturition, which may not have been frequent enough to pick up subtle and transient differences that can occur in the transition period.

Specifically in the transition period, feeding BMR for 3 wk prepartum and 3 wk postpartum has been reported to increase DMI, milk production and yield of components, with a significant carryover effect on milk yield that was apparent even after cows were switched to conventional corn silage, though there were no change in energy metabolites (Stone et al., 2012). However, Holt et al. (2013) fed BMR from parturition through 180 DIM and observed no effect of DMI or milk yield from 1-60 DIM, however post peak (>60 DIM) milk production increased and DMI tended to increase for cows fed BMR. These data would suggest that feeding BMR to cows in the transition period would likely increased performance, though impact on energy metabolites is unknown.

Monensin supplementation. Monensin is an ionophore that shifts rumen microbial population to increase production of propionate, ultimately increasing the glucose precursors supplied from rumen fermentation (Armentano and Young, 1983). This is achieved through disruption of the ion gradient of gram positive bacteria in the rumen, eventually causing death and lysis of those bacteria. This shift in rumen microbial population increases the proportion of gram negative bacteria which are the primary producers of propionate, increasing dietary glucose precursor supply (Bergen and Bates, 1984).

Effects of monensin are very well documented in the literature. A series of meta-analyses (Duffield et al., 2008a, b, c) that incorporated all stages of production, different management systems, and different methods of monensin delivery investigated effects of monensin on production, metabolism, and health parameters. Monensin was found to decrease DMI by 0.3 kg/d, but increase milk production by 0.7 kg/d, resulting in a milk production efficiency increase of 2.5%. Body weight and BCS increase, and milk composition is altered, with decreases in milk fatty acid and protein percent (0.13% and 0.03%, respectively). Monensin consistently decreases blood concentrations of BHB and NEFA, and increases blood glucose. These metabolic shifts are accompanied by decreased risk of ketosis, DA, and mastitis for cows fed monensin (75%, 75%, 91%, respectively).

Production effects of monensin are largely positive; however, the decrease in milk fat percentage could be detrimental. Monensin supplementation has been found to impact the rumen environment and disrupt biohydrogenation, potentially increasing absorption of biohydrogenation intermediates that can alter *de novo* milk fat synthesis (Fellner et al., 1997). Recently, McCarthy et al. (2018) conducted a large field trial investigating impacts of dietary factors, including monensin, on bulk tank milk fat. Though no single dietary characteristic accounted for >11% of variation in herd level milk fat, several factors including particle size, monensin dose, starch, and TMR PUFA and MUFA together accounted for 32% of the variation in milk fat depression. Duffield et al. (2008b) reported associations between monensin and diet on the reported decrease in milk fat. Nutrition modeling software was utilized to further elucidate the association with different dietary factors, and an association was found

such that milk fat decreased with increasing dietary C18:1 concentration. This fits well with findings of McCarthy et al. (2018), indicating that monensin alone may not meaningfully influence biohydrogenation in the rumen on its own, but combined with other dietary factors that pose a risk to milk fat there is a potential to cause milk fat depression.

Several studies have investigated use of monensin throughout the transition period on production and parameters of metabolism. Changes in DMI are not common, though trends for decreased intake over the periparturient period have been observed (Zahra et al., 2006, Petersson-Wolfe et al., 2007, Mullins et al., 2012). Blood BHB has been reported to decrease prepartum and postpartum, and increases of blood glucose in the postpartum period have also been reported in some, but not all studies (Zahra et al., 2006, Arieli et al., 2008, Mullins et al., 2012). Effects of monensin supplementation on milk production in the transition period however, is mixed. Reports of no change in milk yield or components, increases in milk yield, and increased or decreased component yields have all been reported (Arieli et al., 2008, Mullins et al., 2012, McCarthy et al., 2015c).

Impacts of monensin on hepatic fatty acid and glucose metabolism have also been reported in the transition period. Monensin supplementation prepartum reportedly increases gene expression of PEPCK-C and CPT1a mRNA, decreases rate of TG accumulation in the 2 wk around parturition, and increases hepatic glycogen at 3 wk of lactation (Zahra et al., 2006, Karcher et al., 2007, Mullins et al., 2012). Increases in supply of propionate prior to upregulation of gene expression of would fit

the feed forward model suggested for regulation of PEPCK-C, in that increased propionate availability due to monensin supplementation would influence increased mRNA of PEPCK-C by the time of calving. As gluconeogenesis is upregulated at a the cellular level through PEPCK-C and glucose precursors are increased via supplementation of monensin, this helps to explain increases in blood glucose concentration and increases in liver glycogen, as the cow is in a more positive energy balance. Upregulation of CPT1a mRNA at calving due to monensin is slightly surprising, as propionate supply can inhibit CPT1a activity. Perhaps with the increase in propionate thus contributions to oxaloacetate, upregulation of CPT1a would increase availability of acetyl-CoA to ensure maximum function of both gluconeogenesis and the TCA cycle. Monensin impacts on gene expression related to pathways of hepatic metabolism in the transition period likely warrant more investigation.

Taken together, it is clear that feeding monensin throughout the transition period results in improved metabolic status during the early postpartum period. Providing increased dietary glucose precursors without requiring large changes in DMI fits well with intake dynamics in the transition period, resulting in optimal hepatic metabolism and more positive energy balance.

RESEARCH OBJECTIVES

The transition period is a critical time, as changes in energy balance, vast metabolic shifts necessary to account for nutrient partitioning, and changes in immune function present great challenges for the dairy cow to overcome in order to have a

successful and healthy lactation. Gaining further understanding of energy demands, rumen function, and hepatic metabolic pathways will serve to increase our ability to evaluate nutritional strategies that can help the dairy cow navigate this critical period.

The objectives of this dissertation were to: 1) investigate interplay of fiber fractions in high starch postpartum dairy cow diets on performance, hepatic metabolism and energy balance, 2) examine interplay of inflammation, hepatic metabolism, energy metabolites, and metabolic hormones in the postpartum period, and 3) investigate opportunities to combine use of a higher digestibility corn silage with monensin to optimize production and health in the periparturient period.

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CHAPTER 3

THE EFFECT OF VARYING UNDIGESTED NDF AND PHYSICALLY EFFECTIVE NDF CONTENT OF POSTPARTUM DIETS ON PERFORMANCE AND ENERGY METABOLISM

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ABSTRACT

Our objective was to evaluate the effects of increased undigested NDF ($uNDF_{240}$; NDF undigested at 240-h of fermentation *in vitro*) and physically effective NDF (peNDF) in fresh cow diets containing high levels of starch on dry matter intake (DMI), rumination, milk yield, and plasma metabolites. Multiparous Holstein cows (n=56) were assigned to one of two higher starch postpartum diets with differing fiber levels with respect to $uNDF_{240}$ and peNDF fractions. The two treatment diets, high fiber (HF, n = 27) or low fiber (LF, n = 29), were formulated to differ in $uNDF_{240}$ (12.2% DM vs. 9.5% DM, respectively) and peNDF (23% DM vs. 21% DM, respectively) content. Higher $uNDF_{240}$ levels were achieved in the HF diet through the addition of chopped straw; treatment diets were similar in metabolizable protein (MP) and starch content. Cows were enrolled 28 d prior to expected parturition and individually fed a common dry cow diet in tie-stalls. Upon parturition cows were individually fed their respective treatment diets. At 29 d in milk (DIM), cows fed HF were switched to the LF diet and all cows were fed the LF diet through 42 DIM. Cows fed the HF diet had lower DMI during wk 2 through 5 postpartum compared with cows fed the LF diet. Dietary $uNDF_{240}$ intake as a percentage of BW was higher for cows fed the HF diet during wk 1 to 4 postpartum compared to cows fed the LF diet (0.32% vs 0.27% of BW). Despite differences in DMI, no differences in rumination were observed. Cows fed the HF diet lost more BW from wk 1 to 4 compared to cows fed LF. Energy balance of cows fed HF was more negative during wk 3, and tended to be lower during wk 2 and 4, than cows fed LF. A treatment by time interaction existed for milk yield such that milk yield was lower for cows fed HF during wk 3 and 4

postpartum compared with cows fed LF, though when expressed as fat- or energy-corrected milk, only trends for differences were observed. Cows fed the HF diet had higher plasma β-hydroxybutyrate from 12 to 31 DIM, and lower plasma glucose from 9 to 27 DIM compared to cows fed the LF diet. After switching diets, cows initially fed the HF diet quickly matched cows fed the LF diet in most performance and blood metabolites by wk 5 and in all outcomes by wk 6 postpartum. These effects suggest that additional $uNDF_{240}$ and peNDF during the fresh period can inhibit optimal performance and metabolism if maintained for several weeks postpartum.

INTRODUCTION

The transition from pregnancy to lactation is a period of great metabolic adaptation for the dairy cow. Early lactation demands for glucose, AA, and fatty acids increase dramatically compared to late gestation (Bell, 1995) and the DMI increase is insufficient to meet these demands in many cows resulting in negative energy balance, which reaches the lowest point about 2 wk postpartum (Butler, 2000). To satisfy overall energy demands, body reserves are mobilized as nonesterified fatty acids (NEFA), though high circulating concentrations of NEFA and BHB, another analyte related to energy metabolism, during the periparturient period are associated with increased risk of many diseases such as displaced abomasum, ketosis, and metritis, and can also result in decreased milk production in multiparous cows (Ospina et al., 2010a, b, McArt et al., 2013).

Feeding diets higher in energy density during the immediate postpartum period have been shown to increase energy balance and decrease circulating NEFA and BHB (Rabelo et al., 2003, McCarthy et al., 2015b, McCarthy et al., 2015c). However, these

higher energy diets often contain higher levels of starch, and large changes in diet composition in the transition period can lead to subacute ruminal acidosis (SARA; Nocek, 1997, Penner et al., 2007), which can cause decreased feed intake and milk production, increased inflammation, and laminitis (Plaizier et al., 2008). Postpartum diets that promote higher energy intakes, while ensuring a healthy rumen environment, would likely be ideal.

Fiber is often utilized in diets to promote chewing and rumination, ultimately fostering a more stable rumen environment (Mertens, 1997). Specifically, physically effective NDF (peNDF), which refers to the physical size of the fiber, has been found to reduce risk of SARA through increased chewing activity (Beauchemin et al., 2003). Cows at higher risk of acidosis have been reported to sort diets in favor of longer particles during acidosis challenge, likely to help buffer the rumen environment (DeVries et al., 2008). In the immediate postpartum period, however, Coon et al. (2019) reported that cows, regardless of SARA risk, sorted against long feed particles when fed a diet with longer chopped hay, thereby demonstrating that the severity of SARA challenges and the role of peNDF in this immediate postpartum period warrants further investigation.

In addition to the physical aspect of fiber, undigested NDF ($uNDF_{240}$, NDF undigested at 240 h of fermentation), a proxy for indigestible NDF, also plays a role in rumen dynamics. As the $uNDF_{240}$ of a feedstuff increases, it can accumulate in the rumen due to lack of degradation, which can decrease DMI (Harper and McNeill, 2015). However, Fustini et al. (2017) reported that cows fed high $uNDF_{240}$ diets had a

lower time of rumen pH <5.8 compared to cows fed lower $uNDF_{240}$ diets, which is likely due to the longer ruminal retention time and therefore stabilization.

Although $uNDF_{240}$ and peNDF are characterized differently (chemical vs. physical) they are related. Grant et al. (2018) evaluated effects of diets containing varying $uNDF_{240}$ and peNDF content in mid lactation cows, finding that cows fed a high $uNDF_{240}$/high peNDF diet had decreased milk yield and DMI, and increased daily rumen pH mean compared to the low $uNDF_{240}$/low peNDF, while all other combinations of $uNDF_{240}$ and peNDF levels were not different. Both of these feed fractions might play a role in intake and rumen dynamics, which are vital during the transition period.

During the early postpartum period, there is a lack of published information on the effects of increasing peNDF or $uNDF_{240}$ supply in the diet and their effect on production and metabolism. Our objective was to evaluate the effects of additional $uNDF_{240}$ in a higher starch fresh cow diet on DMI, rumination, milk yield, and plasma metabolites. We hypothesized that providing additional $uNDF_{240}$ in the early postpartum period would increase DMI and productive performance and potentially decrease circulating concentrations of NEFA and BHB during the postpartum period.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

All animal protocols were approved by the Cornell University Institutional Animal Care and Use Committee (protocol #2015-0024). Animals were enrolled in the experiment between July and September 2015. Multiparous Holstein cows (n=60) were enrolled in a randomized design study with randomization of treatment

assignment blocked to control for lactation number and previous 305-d mature equivalent milk production. A sample size calculation determined 25 cows per treatment would detect a difference in milk yield of 4 kg per day with 95% confidence and 80% power based on data from previous studies in our lab (McCarthy et al., 2015b).

Cows were moved to tie-stalls 28 d prior to expected parturition and fed a common close-up dry cow diet (Table 3-1) through parturition. After parturition, cows were fed their assigned treatment diets (Table 3-1), high fiber (HF) or low fiber (LF), which were formulated to differ in $uNDF_{240}$ (12.2% of DM vs. 9.5% of DM, respectively) and peNDF (23% of DM vs. 21% of DM, respectively) content. A total of 60 cows were enrolled in the experiment; four cows were removed from the data set prior to analysis for reasons unrelated to treatment by a researcher blinded to treatment assignment (three cows from HF and one cow from LF treatment: one suffered an injury in the calving pen and did not recover, two cows diagnosed with clinical hypocalcemia that did not recover, one cow with severe chronic mastitis). In addition, three cows were partially removed from the data set due to reasons unrelated to treatment. Two cows from the HF treatment were removed (d 11 and d 19 postpartum) after development of an infection post liver biopsy. One cow from LF treatment was removed after development of traumatic reticuloperitonitis (d 26 postpartum) and subsequent death. The final data set included 56 cows, 27 fed the HF diet and 29 fed the LF diet. Average lactation number of cows fed HF was 2.64, with 14 cows in their 2^{nd} lactation and 13 cows in their 3^{rd} and greater lactation. Average lactation number

of cows fed LF was 2.74, with 17 cows in their 2nd lactation and 12 cows in their 3rd and greater lactation.

Diet Formulation, Feeding Management, and Analysis

Diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS, version 6.5, Cornell University, Ithaca, NY; Van Amburgh et al., 2015). The HF and LF treatment diets were fed from parturition until 28 DIM, after which all cows received the LF diet until the end of the study at 42 DIM. All diets were formulated to be similar to diets regularly fed in the Northeastern United States. The prepartum diet consisted mainly of corn silage and wheat straw with a grain mixture. Postpartum diets were formulated to contain corn silage, chopped alfalfa hay, wheat straw, and a grain mixture. Undigested fiber and peNDF content of the HF diet was increased by replacing a portion of the corn silage and grain mixtures with chopped wheat straw. Approximately one month after the first cow on study calved (20% of all postpartum days cows were fed), the diets were reformulated to better reach our target for starch in the diets by increasing the amount of corn meal and canola meal and decreasing the amount of grain mixture in both fresh diets. All forage amounts remained as originally formulated throughout the study. A weighted average of formulated diet ingredient composition (weighted to account for diet reformulation), forage analysis, and nutrient analysis of diets can be found in Table 3-1, Table 3-2, and Table 3-3, respectively.

Cows were housed in tie-stalls and fed once daily, with lactating cows fed between 0700 and 0900 h and dry cows between 0930 and 1100 h each day. Refusals were removed and weight was recorded each day prior to feeding. Weight of feed

delivered and refused along with weekly DM determinations were used to calculate daily individual DMI for each cow, and a refusal rate of 10% was targeted daily to ensure ad libitum intake.

Samples of all individual forages and grains and TMR were collected weekly. A duplicate of each weekly TMR sample collected was sent to a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) for DM determination at 135ºC (method 930.15, AOAC International, 2000) and NIR analysis of carbohydrate fractions (e.g., NDF, starch) to ensure diet targets were being achieved throughout the study. All other samples were dried at 40ºC for 96 h in a forced-air oven for on-farm DM determination and a portion of the sample was ground to 2-mm using a Wiley mill and retained. At the end of the experiment, all retained feed samples were composited by 4-wk intervals over the duration of the study and sent to a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) for wet chemistry determination of DM at 135ºC (method 930.15, AOAC International, 2000), ADF (method 973.18, AOAC International, 2000), CP (method 990.03, AOAC International, 2000), aNDFom (Van Soest et al., 1991, Mertens, 2002), starch (Hall, 2009), sugar (Dubois et al., 1952), ash (method 942.05, AOAC International, 2000), minerals (method 985.01, AOAC International, 2000), NE_L (NRC 2001) and *in vitro* NDF digestibility analysis at 30, 120, and 240 h (Goering and Van Soest, 1970, Raffrenato et al., 2019). Analysis of all composited ingredients were inputted into diets in CNCPS using chemical analysis of ingredients and actual average DMI for each diet and dietary MP supply was predicted. For determination of peNDF, two samples were taken throughout the study and assessed for particle size by

using the three sieve (19.0 mm, 8.0 mm, and 4.0 mm) Penn State Particle Separator (Nasco Inc., Fort Watkinson, WI) as described by Kononoff et al. (2003a) and modified to include a 4-mm sieve as reported by Kmicikewycz et al. (2015). Each sample was assessed for particle size twice and the values were averaged. To obtain diet peNDF, the material on the top three sieves were added together and multiplied by the NDF content of the diet, as reported by Kmicikewycz et al. (2015).

Data Collection, Sampling Procedures, and Analysis

Body weight was measured and BCS was assessed once weekly throughout the experiment beginning 28 d prior to expected parturition and continuing through 42 DIM. Body condition score was assigned by two scorers weekly according to Edmonson et al. (1989) and scores averaged prior to analysis. Rumination time was recorded in 2-h intervals starting 28 d prior to expected calving through 42 DIM using rumination collars (HR tags; SCR Dairy, Netanya, Israel). Daily rumination time was calculated by adding all 2-h intervals in a 24-h period and daily rumination was averaged by week prior to statistical analysis.

After calving, all cows were milked 3x daily at 0600, 1400, and 2200 h and individual milk weights were recorded. Daily milk yield was determined as the sum of the three daily milkings and weekly means of daily production were calculated prior to analysis. Once weekly, milk samples were collected from three consecutive milkings, mixed with a bronopol preservative immediately after collection, and stored at 4ºC prior to being sent to a commercial laboratory (DairyOne, Ithaca, NY) within 48 h of collection for analysis of milk protein, fat, lactose, total solids, and MUN using mid-infrared techniques (method 972.16 AOAC International, 2006), and SCC determined

using optical fluorescence (method 978.26, AOAC International, 2006); SCS was then calculated as SCS = $\log_2$(SCC / 100) + 3 (Shook, 1993). Milk fat and milk protein yield were calculated using the corresponding milk weight of each sample and used to calculate weekly 3.5% FCM, as 3.5% FCM = [0.432 × milk (kg)] + [16.216 × fat (kg)] (Gaines, 1928, Erdman, 2011), and ECM, as ECM= [0.327 × milk (kg)] + [12.95 × fat (kg)] + [7.65 true protein (kg)] (Tyrrell and Reid, 1965).

Prepartum and postpartum weekly energy balance (EBAL) was calculated according to NRC (2001) equations 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)], and

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 of NE_L/d) = weekly average DMI (kg/d) × diet NE_L (Mcal/kg of DM), maintenance requirement (Mcal of NE_L/d) = week metabolic BW ($kg^{0.75}$) × 0.08 (Mcal/ $kg^{0.75}$ per d); pregnancy requirement (Mcal) = (0.00318 × d of gestation - 0.0352) × (1/0.218); and lactation requirement (Mcal/d) = week average milk yield (kg/d) × [(0.0929 × fat percentage) + (0.0563 × true protein percentage) + (0.0395 × lactose percentage)].

Blood samples were collected via coccygeal vessels prior to fresh feed delivery between 0530 and 0730 h 2x/wk from d 28 prior to expected parturition through parturition, daily from 1 through 7 DIM, 3x/wk from 7 through 21 DIM, and 2x/wk from 21 through 42 DIM. Samples were collected using 10-mL sodium heparin evacuated tubes (158 USP, Becton Dickinson and Company, Franklin Lakes NJ),

using 20 ga vacutainer needles (Becton Dickinson and Company). Whole blood was harvested, placed on ice immediately after collection, and centrifuged for 20 min at 2,000 × *g* at 4ºC. Plasma was aliquoted into 1.7mL microfuge tubes, snap frozen in liquid N_2, and stored at -20ºC until analysis. Samples were analyzed for BHB using a commercially available kit (Catachem Inc., Oxford, CT) adapted as previously described by Leno et al. (2017), NEFA using a commercial enzymatic kit [HR series NEFA HG(2) Wako Pure Chemical Industries, Osaka, Japan], and glucose by enzymatic analysis (glucose oxidase; protocol from kit 510-A Sigma Aldrich, St. Louis, MO) using commercial products (PGO Enzyme Preparation and *o*-dianisidine dihydrochloride, Sigma Aldrich). Spectrophotometric measurements were collected using a tunable microplate reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA). Plasma insulin concentration was determined using a commercially available radioimmunoassay with a reported specificity for bovine insulin of 90% (Porcine Insulin RIA Kit, cat. no. PI-12K, EMD Millipore Corp, St. Louis, Missouri). Inter- and intra-assay coefficients of variation were maintained at <10% for all assays. The revised quantitative insulin sensitivity check index (RQUICKI), an indirect method to estimate insulin resistance using measured blood glucose, insulin, and NEFA, was calculated as described by Holtenius and Holtenius (2007).

Statistical Analysis

Daily means for DMI, milk yield, and rumination were averaged by week prior to analysis. To standardize blood metabolite data, day of blood samples collected 2x/wk or 3x/wk were averaged to reference days prior to analysis. Body weight change was calculated as the difference in BW from wk 1 to 4 and from wk 1 to 6. All

statistical analyses were conducted using SAS (version 9.4, SAS Institute Inc., Cary, NC). Prepartum data were analyzed separately to ensure no differences due to postpartum treatment assignment and overall means are reported. Postpartum data were analyzed as wk 1 to 4, representing the period of dietary treatment application, and wk 1 to 6 to analyze differences throughout the entire study period. All data collected over time were analyzed as repeated measures ANOVA using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). Fixed effects of treatment, time, parity group (2^{nd} vs. 3^{rd} and greater), and all 2-way interactions were included in the model. A variable to account for the diet change that occurred part way through the study was also included in all models. Cow nested within treatment was the random effect. Measurements collected during the week of enrollment were included in all models when available as covariates. Previous lactation 305-d mature-equivalent milk yield was used as a covariate for milk production. Denominator degrees of freedom were estimated using the Kenward Rogers method. Five covariance structures were tested for each model: first-order autoregressive, heterogeneous first-order autoregressive, compound symmetry, heterogeneous compound symmetry, and unstructured; the covariance structure with the lowest Akaike's information criterion was used (Littell et al., 1996). In instances of a 2-way interaction with time where $P \leq 0.10$, the slice option was added to the model to perform a partitioned analysis of the means of the interaction, producing an F-test for each level to determine at which levels treatments differed. In instances of a 2-way interaction without time where $P \leq 0.10$, the Tukey adjustment was utilized to correct for multiple comparisons. Residual analysis was performed to examine

normality and homogeneity of residuals, when non-normality of residual variance was evident, data were transformed (either log-transformed or square root transformation) and analysis repeated. Body weight and BCS change data, the only measurements not repeated over time, were analyzed using ANOVA in the MIXED procedure of SAS. All data presented are least squares means and standard error (SE), except in the case of transformed data (NEFA, BHB, Insulin, and RQUICKI) where geometric means with back-transformed 95% confidence intervals (CI) are reported. Statistical significance was declared at $P \leq 0.05$, and trends at $0.05 < P \leq 0.10$.

RESULTS

Dry Matter Intake, uNDF Intake, Rumination, BW, BCS, and Energy Balance

Prepartum DMI (15.5 ± 0.35 kg/d; mean ± standard error), $uNDF_{240}$ intake as percent of BW (0.24. ± 0.01 % of BW), BW (806 ± 3.25 kg), and BCS (3.4 ± 0.02) were not different ($P > 0.10$) due to postpartum treatment assignment; therefore, overall means are reported.

Postpartum DMI, intake of nutrients, rumination, BW, BW change, BCS, BCS change, and calculated energy balance are reported in table 3-4. Postpartum DMI was lower for cows fed HF compared to cows fed LF (19.4 vs. 21.1 kg/d in wk 1 to 4, $P < 0.01$, 21.0 vs. 22.3 kg/d in wk 1 to 6 $P = 0.03$; Table 3-4). Treatment by time interactions existed ($P < 0.01$) for postpartum DMI when analyzed as wk 1 to 4 and wk 1 to 6 such that cows fed HF had lower DMI compared to cows fed LF in wk 2 through 5 ($P < 0.05$; Figure 3-1). Postpartum DMI as percentage of BW also was lower for cows fed HF compared to cows fed LF depending on week relative to

calving, as there was a treatment by time interaction ($P < 0.01$) when analyzed as wk 1 to 6, though only a trend ($P = 0.08$) was present in wk 1 to 4. Cows fed HF had lower DMI, expressed as percentage of BW during wk 3 and 4 postpartum, and tended to have lower intake during wk 5 postpartum when compared to cows fed LF. Postpartum DMI (kg per day and percentage of BW) of all cows was similar during wk 6 ($P > 0.10$).

Intake of starch was higher for cows fed LF compared to cows fed HF (5.56 vs. 5.20 kg/d; $P < 0.01$), and differed by time such that cows fed LF had higher intake of starch in wk 2 through 5 postpartum as compared with cows fed HF. A treatment by time interaction was also present for intake of NDF fractions, shown in Figure 3-1. Cows fed HF tended to have higher intake (kg/d) of aNDFom in wk 1 and lower intake of aNDFom intake in wk 4, with statistically lower intake of aNDFom in wk 5 postpartum compared to cows fed LF. Intake of $uNDF_{240}$ (kg/d) was higher for cows fed HF in wk 1 to 4, and lower in wk 5 compared to cows fed LF. Dietary $uNDF_{240}$ intake as percentage of BW was also higher for cows fed HF compared to cows fed LF (0.32 vs. 0.27 % BW; $P = 0.01$) during wk 1 to 4. Intake of $uNDF_{240}$ as % of BW was higher ($P < 0.01$) for cows fed HF compared to cows fed LF during wk 1 to 4, representing the period of dietary difference; however, after cows fed HF were switched to the LF diet in wk 5 and 6, $uNDF_{240}$ intake as % BW was not different ($P > 0.10$). Despite these differences in $uNDF_{240}$ intake, no differences due to treatment diet were observed in rumination (overall mean 544 ± 8.2 min/d; $P < 0.56$).

Postpartum BCS was not different (overall mean 3.13 ± 0.03; $P > 0.10$) due to treatment. Postpartum BW tended to be lower for cows fed HF compared to those fed

LF (716 vs 723 kg; $P = 0.08$) when analyzed from wk 1 to 6. Postpartum BW change was more negative for cows fed HF compared to those fed LF (-46.2 vs. -24.5 kg, $P = 0.03$) when analyzed as wk 1 to 4; however, when analyzed as the change from wk 1 to 6 there was no longer an effect of treatment ($P > 0.10$).

Postpartum energy balance was lower for cows fed HF compared to cows fed LF during wk 1 to 4 (-10.2 vs -7.6 Mcal/d; $P = 0.05$). Over the entire study period (wk 1 to 6), there was a treatment by week interaction ($P = 0.03$) such that cows fed HF were in a more sever state of negative energy balance during wk 3 postpartum ($P = 0.005$), and tended ($P = 0.09$) to be more negative during wk 2 and 4 postpartum, as seen in Figure 3-2. During wk 5 and 6 postpartum, differences in energy balance related to previous dietary treatments were not statistically significant.

Milk Production and Composition

Milk yield, ECM, 3.5% FCM, and milk composition are presented in Table 3-5. A treatment by week interaction was present for milk yield during both wk 1 to 4 and wk 1 to 6 ($P = 0.04$, $P < 0.01$ respectively) such that milk yield was lower for cows fed HF than cows fed LF during wk 3 and 4 postpartum. During wk 5 and 6, however, there were no effects of treatment on milk yield. Milk yield over the duration of the study is shown in Figure 3-3. We observed a treatment by week interaction for ECM during wk 1 to 4 such that ECM tended ($P = 0.10$) to be lower for cows fed HF than those fed LF during wk 4. Over the entire study duration (wk 1 to 6), there were no effects of treatment on ECM. Yield of 3.5% FCM tended to be lower for cows fed HF compared to those fed LF during wk 4 both, when analyzed as wk 1 to 4 ($P= 0.06$) and wk 1 to 6 ($P= 0.08$).

We observed no differences in milk fat or total solids (percentage or yield) or somatic cell score (SCS) due to treatment. No differences in milk true protein percentage or yield due to treatment were observed during wk 1 to 4. When analyzed as wk 1 to 6, cows fed HF had lower milk true protein yield (1.32 vs. 1.41 kg/d; P= 0.03) and tended to have lower milk true protein percentage (3.02 vs. 3.09%; P= 0.10) compared to cows fed LF. No treatment or treatment by week interactions were observed for milk lactose (percentage or yield); however, there was an interaction for treatment by parity for milk lactose percentage (P= 0.04), when analyzed from wk 1 to 4 in which 3rd lactation and greater cows fed the HF diet had numerically lower lactose concentrations than 2nd lactation cows fed the HF diet, though in correcting for multiple comparisons the difference was not statistically significant (4.58 vs. 4.79%; P = 0.27). A treatment by time interaction was present for MUN such that cows fed the HF diet had lower MUN during wk 4 and tended to have higher MUN during wk 6 compared to cows fed LF.

Plasma Analytes

Prepartum and postpartum plasma concentrations of NEFA, BHB, glucose, insulin, and calculated RQUICKI are presented in Table 3-6. There were no differences in prepartum metabolites due to postpartum treatment assignment; therefore, overall means are reported. There were no differences in postpartum NEFA due to treatment or treatment by day interactions for wk 1 to 4, although there was a tendency for cows fed HF to have higher NEFA (472 vs. 430 μEq/L; P = 0.07) compared to cows fed LF from wk 1 to 6. There also was a trend for a treatment by parity interaction (P = 0.09) such that 2nd lactation cows fed LF tended to have

numerically lower NEFA than 3rd and greater lactation cows (418 vs. 589 μEq/L) fed LF, though in correction for multiple comparisions this difference was not statistically significant ($P = 0.14$). There was a treatment effect for BHB when analyzed from wk 1 to 4 such that cows fed HF had higher plasma BHB than cows fed the LF diet (8.28 vs. 6.21 mg/dL; $P = 0.01$). A treatment by week interaction was present when analyzed as wk 1 to 6, such that cows fed HF had higher ($P < 0.01$) plasma BHB from d 12 to d 31 postpartum compared to cows fed LF. During wk 1 to 4 postpartum, a trend was observed for plasma glucose such that cows fed HF had lower plasma glucose than cows fed LF (52.0 vs 55.3 mg/dL; $P = 0.09$). A treatment by week interaction was significant ($P < 0.01$) for plasma glucose during wk 1 to 6, such that plasma glucose was lower for cows fed HF compared to cows fed LF from d 9 to 27 postpartum. Plasma BHB and glucose are shown over time in Figure 3-4. No differences due to treatment were observed for plasma insulin concentration or insulin sensitivity (assessed using RQUICKI) from wk 1 to 4 or wk 1 to 6 postpartum.

Table 3-1. Formulated ingredient composition of the common prepartum diet and postpartum treatment diets formulated to differ in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF).

		Postpartum Diet[1]	
Ingredient, % of DM	Prepartum Diet	LF [1]	HF [1]
Conventional corn silage	45.19	42.31	38.46
Alfalfa hay	-	10.58	10.58
Wheat straw	20.84	1.15	8.65
Corn meal	2.43	17.64	20.51
Soybean meal	-	6.03	4.72
Canola meal	3.48	4.52	3.88
Wheat middlings	-	4.82	1.62
Amino Plus[2]	5.91	4.35	5.32
Corn gluten feed	1.74	1.60	0.47
Blood meal	2.43	0.95	1.09
Soybean hulls	6.95	2.42	-
Citrus pulp	4.52	-	0.79
Energy Booster[3]	-	1.29	1.57
Rumensin[4]	0.10	0.05	0.05
Salt	-	0.48	0.47
Ca carbonate	2.26	1.13	1.18
Mg oxide	-	0.28	0.28
Animate[5]	3.13	-	-
Alimet[6]	0.07	-	-
Vitamin and mineral mix[7]	0.94	0.40	0.39

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM). Treatment diets were fed from wk 1 to 4 after which all cows were switched to the LF diet.
[2] Heat-treated soybean meal, Ag Processing Inc., Omaha, NE.
[3] Commercial fat source, Milk Specialties Global, Eden Prairie, MN.
[4] Premix contained 26,400 g/t of monensin, Elanco Animal Health, Greenfield, IN.
[5] Anionic mineral supplement, Phibro Animal Health Corporation, Teaneck, NJ.
[6] 2-Hydroxy-4-(methylthio)-butanoic acid, Novus International, Saint Charles, MO.
[7] Prepartum mix contained 3,754 mg/kg Zn, 993 mg/kg Cu, 4,658 mg/kg Mn, 28.9 mg/kg Se, 82.8 mg/kg Co, 82.4 mg/kg I, 1,177 KIU/kg Vitamin A, 253 KIU/kg Vitamin D, and 55,784 IU/kg Vitamin E. Postpartum mix contained 25,560 mg/kg Zn, 7,154 mg/kg Cu, 21,958 mg/kg Mn, 214 mg/kg Se, 507 mg/kg Co, 331 mg/kg I, 3,704 KIU/kg Vitamin A, 922 KIU/kg Vitamin D, and 12,496 IU/kg Vitamin E (Central New York Feeds, Jordan, NY).

Table 3-2. Forage composition (DM basis except where noted) as analyzed by wet chemistry and *in-vitro* analysis by a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) on one composite sample for each forage used in treatment diets formulated to differ in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF), which was representative of the entire study period.

Item	Corn silage	Alfalfa hay	Wheat straw
DM, % of as fed[1]	26.8 ± 1.4	86.0 ± 1.5	87.2 ± 2.1
CP, %	7.8	17.0	4.9
ADF, %	27.0	38.1	54.5
aNDFom, %	43.6	45.6	77.8
Starch, %	31.6	1.2	0.8
Sugar, %	1.0	7.1	2.5
Fat, %	2.2	1.2	0.9
In-vitro digestibility analysis			
30-h NDFD[2], %	26.7	16.5	27.5
30-h NDFD, % of NDF	57.9	37.6	34.2
$uNDF_{30}$[3], %	19.4	27.4	53.0
$uNDF_{30}$, % of NDF	42.1	62.4	65.8
$uNDF_{240}$, %	12.4	24.1	35.4
$uNDF_{240}$, % of NDF	27.0	54.9	44.0

[1]DM was analyzed (dried at 40º C in a forced air oven for 96 h) on fresh forages weekly throughout the study and further corrected for residual moisture using DM obtained from Cumberland Valley Analytical Services upon wet chemistry analysis of each ground composite sample.

[2] NDF digestibility, determined via *in vitro* fermentation

[3] Undigested NDF at 30-h of *in vitro* fermentation

Table 3-3. Nutrient profile of all diets (mean ± SD), obtained through wet chemistry analysis and in vitro fermentation by Cumberland Valley Analytical Services (Hagerstown, MD), predicted by Cornell Net Carbohydrate and Protein System (version 6.5, Cornell University, Ithaca, NY), and obtained with a Penn State Particle Separator (Nasco Inc., Fort Watkinson, WI).

	Diet		
Item	Prepartum	Low Fiber (LF)[1]	High Fiber (HF)[1]
DM, % as fed[2]	45.9 ± 1.2	44.4 ± 2.2	47.4 ± 2.0
ADF, % DM	29.0 ± 0.5	21.3 ± 1.1	22.9 ± 2.1
aNDFom, % DM	43.1 ± 0.3	32.8 ± 1.4	35.3 ± 2.3
30-h NDFD[3], % DM	24.2 ± 0.5	20.2 ± 0.5	19.1 ± 1.7
30-h NDFD, % NDF	54.4 ± 1.5	59.5 ± 1.0	52.6 ± 2.2
$uNDF_{240}$[4], % DM	12.8 ± 0.5	9.5 ± 0.4	12.2 ± 1.6
CP, %	14.1 ± 0.8	15.5 ± 0.5	13.8 ± 1.4
Starch, %	15.7 ± 0.3	24.9 ± 1.7	24.6 ± 2.3
Sugar, %	3.5 ± 0.4	5.1 ± 0.7	3.9 ± 0.1
Fat, %	2.3 ± 0.2	3.3 ± 0.2	3.2 ± 0.2
peNDF[5], %	33.3[6]	21.6[7]	23.2[7]
MP[6], g/kg DM	89.0	112.1	108.0
NE_L, Mcal/kg	1.45 ± 0.02	1.65 ± 0.03	1.61 ± 0.02
Ca, %	1.46 ± 0.07	0.97 ± 0.12	0.98 ± 0.19
P, %	0.32 ± 0.02	0.41 ± 0.01	0.37 ± 0.01
Mg, %	0.51 ± 0.02	0.40 ± 0.04	0.39 ± 0.04
K, %	1.16 ± 0.02	1.24 ± 0.03	1.21 ± 0.04
S, %	0.39 ± 0.02	0.30 ± 0.02	0.26 ± 0.02
Na, %	0.13 ± 0.01	0.26 ± 0.02	0.23 ± 0.03
Cl, %	0.67 ± 0.02	0.49 ± 0.02	0.44 ± 0.04

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27). Treatment diets were fed from wk 1 to 4 after which all cows were switched to the LF diet.
[2] Determined via wet chemistry from fresh weekly samples sent to Cumberland Valley Analytical Services for NIR analysis.
[3] NDF digestibility, determined via *in vitro* fermentation
[4] Undigested NDF at 240-h of *in vitro* fermentation
[5] Physically effective NDF
[6] Formulated value given by Cornell Net Carbohydrate and Protein System v. 6.5 using analyzed forage values and mean DMI for each period and treatment.
[7] Determined using a Penn State Particle Separator from two samples collected throughout the study. Value obtained by multiplying the percentage of sample retained on screens ≥ 4mm by the diet NDF content.

Table 3-4. Least squares means and standard errors postpartum DMI, uNDF intake, rumination time, BCS, BW, BW change, and energy balance [calculated using NRC (2001) equations] for cows fed diets differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) in the postpartum period.

	Postpartum Treatment[1]			*P*-Value[2]			
Item	LF	HF	SEM	Trt	W	Parity	Trt × W
DMI, kg/d							
wk 1 to 4	21.1	19.4	0.36	<0.01	<0.01	0.31	0.002
wk 1 to 6	22.3	21.0	0.33	0.02	<0.01	0.10	<0.001
Starch intake, kg/d							
wk 1 to 4	5.24	4.79	0.10	<0.01	<0.01	0.01	0.002
wk 1 to 6	5.56	5.20	0.08	0.01	<0.01	<0.01	<0.001
aNDFom intake, kg/d							
wk 1 to 4	6.90	6.88	0.13	0.44	<0.01	<0.01	0.01
wk 1 to 6	7.32	7.23	0.11	0.44	<0.01	<0.01	0.001
$uNDF_{240}$ intake, kg/d							
wk 1 to 4	2.0	2.37	0.04	<0.01	<0.01	<0.01	0.23
wk 1 to 6	2.12	2.34	0.04	<0.01	<0.01	<0.01	<0.001
DMI, % of BW							
wk 1 to 4	2.9	2.7	0.05	<0.01	<0.01	0.19	0.08
wk 1 to 6	3.1	3.0	0.05	0.05	<0.01	0.42	0.002
$uNDF_{240}$ intake, % of BW							
wk 1 to 4	0.27	0.32	0.01	<0.01	<0.01	0.43	0.06
wk 1 to 6	0.29	0.32	0.01	<0.01	<0.01	0.81	<0.001
Rumination, min/d	544	543	8.20	0.56	<0.01	0.83	0.14
BCS	3.14	3.12	0.03	0.23	<0.01	0.89	0.72
BCS change							
wk 1 to 4	-0.26	-0.30	0.03	0.38	-	0.02	-
wk 1 to 6	-0.29	-0.33	0.04	0.40	-	0.82	-
BW, kg	723	716	4.90	0.08	<0.01	0.38	0.13
BW change, kg							
wk 1 to 4	-24.5	-46.2	6.80	0.03	-	0.48	-
wk 1 to 6	-28.5	-42.9	6.70	0.14	-	0.53	-
Energy balance, Mcal/d							
wk 1 to 4	-7.6	-10.2	0.83	0.05	<0.01	0.05	0.50
wk 1 to 6	-6.3	-8.4	0.76	0.21	<0.01	0.15	0.03

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27). Treatment diets were fed from wk 1 to 4 after which all cows were switched to the LF diet.
[2] All two way interactions were analyzed, interaction terms not presented were non-significant ($P > 0.10$) for all variables Trt = treatment; W= week

Table 3-5. Least square means and standard errors for milk yield, ECM, 3.5% FCM, and milk composition for cows fed differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) in the postpartum period.

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27). Treatment diets were fed from wk 1 to 4 after which all cows were switched to the LF diet.
[2] All two way interactions were analyzed, interaction terms not specified were non-significant ($P > 0.10$) for all variables presented. Trt = treatment; W = week; Par = parity
[3] ECM = (0.327 × kg wk average milk yield) + (12.95 × kg of fat) + (7.95 × kg of true protein)
[4] 3.5% FCM = (0.432 × kg wk average milk yield) + (16.216 × kg of fat)

Item	Postpartum Treatment[1]			*P*-Value[2]				
	LF	HF	SEM	Trt	W	Parity	Trt × Par	Trt × W
Milk yield, kg/d								
wk 1 to 4	44.1	41.7	0.9	0.08	<0.01	<0.01	0.31	0.04
wk 1 to 6	46.2	44.7	1.0	0.11	<0.01	<0.01	0.31	<0.01
ECM [3], kg/d								
wk 1 to 4	47.2	46.0	1.1	0.55	<0.01	<0.01	0.97	0.10
wk 1 to 6	48.4	47.1	0.9	0.45	<0.01	<0.01	0.97	0.14
3.5% FCM [4], kg/d								
wk 1 to 4	46.6	45.9	1.2	0.79	<0.01	<0.01	0.94	0.06
wk 1 to 6	48.1	47.4	1.0	0.77	<0.01	<0.01	0.85	0.08
Fat, %								
wk 1 to 4	3.89	4.06	0.10	0.27	<0.01	0.30	0.80	0.22
wk 1 to 6	3.72	3.86	0.09	0.45	<0.01	0.09	0.77	0.12
Fat, kg/d								
wk 1 to 4	1.72	1.72	0.06	0.91	<0.01	<0.01	0.95	0.14
wk 1 to 6	1.73	1.73	0.05	0.65	<0.01	<0.01	0.48	0.28
True protein, %								
wk 1 to 4	3.27	3.20	0.06	0.31	<0.01	0.88	0.57	0.41
wk 1 to 6	3.09	3.02	0.05	0.10	<0.01	0.66	0.19	0.62
True protein, kg/d								
wk 1 to 4	1.40	1.31	0.03	0.11	<0.01	<0.01	0.80	0.07
wk 1 to 6	1.41	1.32	0.03	0.03	<0.01	<0.01	0.42	0.11
Lactose, %								
wk 1 to 4	4.73	4.69	0.04	0.49	<0.01	0.04	0.04	0.39
wk 1 to 6	4.78	4.79	0.03	0.55	<0.01	0.28	0.22	0.63
Lactose, kg/d								
wk 1 to 4	2.02	2.00	0.05	0.37	<0.01	0.01	0.63	0.19
wk 1 to 6	2.26	2.15	0.05	0.33	<0.01	<0.01	0.70	0.11
Total solids, %								
wk 1 to 4	12.9	13.0	0.15	0.50	<0.01	0.60	0.57	0.57
wk 1 to 6	12.6	12.6	0.13	0.77	<0.01	0.12	0.35	0.52
Total solids, kg/d								
wk 1 to 4	3.23	3.10	0.08	0.41	<0.01	<0.01	0.86	0.40
wk 1 to 6	3.41	3.25	0.06	0.27	<0.01	<0.01	0.67	0.24
Milk Urea N, mg/dl								
wk 1 to 4	9.54	9.12	0.34	0.48	0.04	0.55	0.96	0.13
wk 1 to 6	9.64	9.39	0.27	0.64	<0.01	0.85	0.95	0.02
SCS								
wk 1 to 4	2.67	2.45	0.28	0.58	<0.01	0.35	0.83	0.91
wk 1 to 6	2.34	2.23	0.29	0.72	<0.01	0.38	0.81	0.55

Table 3-6. Back transformed geometric means and 95% confidence intervals or least squares means and standard errors for prepartum and postpartum plasma nonesterified fatty acids (NEFA), BHB, glucose, insulin, and revised quantitative insulin sensitivity check index (RQUICKI) for cows fed differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) in the postpartum period.

	Postpartum Treatment[1]		*P*-Value[2]				
Item	LF	HF	Trt	W	Par	Trt × Par	Trt × W
Prepartum[3]							
NEFA μEq/L	155 (138 - 174)		0.89	<0.01	0.92	0.50	0.27
BHB, mg/dL	4.75 (4.48-5.04)		0.53	0.37	0.33	0.41	0.67
Glucose, mg/dL	62.9 ± 0.58		0.66	0.71	0.28	0.54	0.44
Insulin, ng/mL	0.71 (0.65-0.76)		0.55	0.01	0.12	0.86	0.38
RQUICKI[4]	0.48 (0.47-0.49)		0.86	<0.01	0.06	0.62	0.17
Postpartum[5]							
NEFA μEq/L							
Week 1 to 4	500 (443 - 561)	550 (489 - 615)	0.25	<0.01	0.02	0.09	0.63
Week 1 to 6	430 (381 - 483)	472 (418 - 529)	0.07	<0.01	<0.01	0.15	0.50
BHB, mg/dL							
Week 1 to 4	6.21 (5.57 - 6.93)	8.28 (7.39 - 9.27)	<0.01	0.11	0.25	0.62	0.12
Week 1 to 6	6.02 (5.41 - 6.70)	7.82 (7.01 - 8.72)	<0.01	<0.01	0.12	0.33	<0.01
Glucose, mg/dL							
Week 1 to 4	55.3 ± 0.80	52.0 ± 0.83	0.09	<0.01	<0.01	0.39	0.14
Week 1 to 6	55.7 ± 0.73	53.0 ± 0.76	0.11	<0.01	<0.01	0.41	<0.01
Insulin, ng/mL							
Week 1 to 4	0.28 (0.24 – 0.33)	0.26 (0.22 – 0.30)	0.33	<0.01	0.02	0.49	0.53
Week 1 to 6	0.29 (0.25 – 0.33)	0.27 (0.24 – 0.32)	0.53	<0.01	0.02	0.54	0.19
RQUICKI							
Week 1 to 4	0.45 (0.44 – 0.46)	0.45 (0.44 – 0.47)	0.46	<0.01	0.80	0.12	0.62
Week 1 to 6	0.45 (0.44 – 0.46)	0.46 (0.44 – 0.47)	0.53	<0.01	0.83	0.11	0.76

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27). Treatment diets were fed from wk 1 to 4 after which all cows were switched to the LF diet.

Table 3-6. Continued
[2] All two way interactions were analyzed, interaction terms not specified were non-significant ($P > 0.10$) for all variables presented. Trt = treatment; W = week; Par = parity
[3] No treatment was administered during the prepartum period, prepartum data was analyzed completely separately with no observed effects due to treatment, overall least squares means from 3 wk prior to calving are reported. Samples were collected 2x/ wk in the 3 weeks prior to calving.
[4] RQUICKI=1/[log(glucose)+ log (insulin) + log (NEFA)].
[5] Samples collected daily on d 1 to 7 postpartum, 3x/ wk until d 28 and 2x/ wk thereafter.

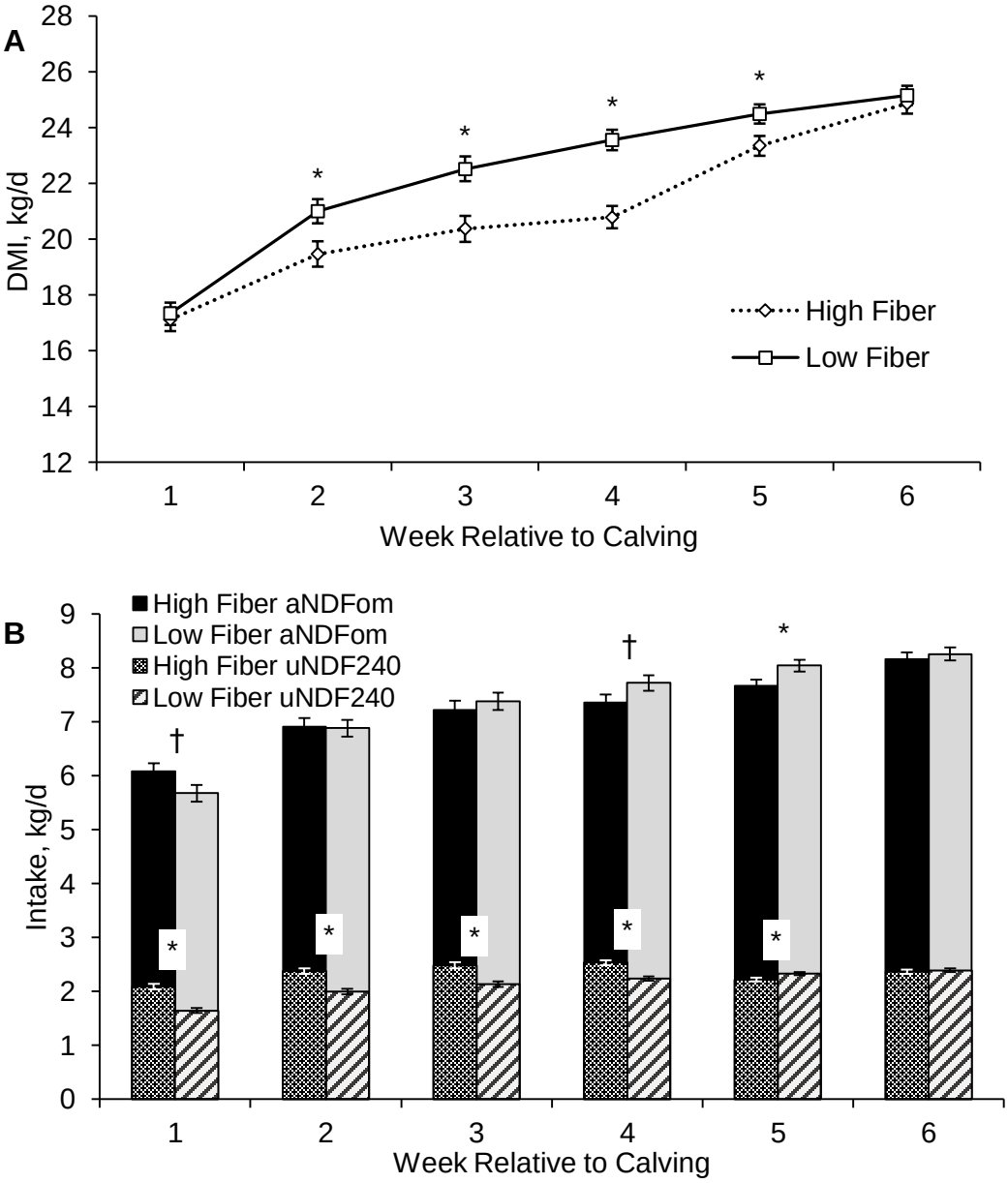

Figure 3-1. Least squares means (± SEM) of postpartum DMI (A) and intake of aNDFom and $uNDF_{240}$ (B) for cows fed diets differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) in wk 1 to 4 postpartum. Treatments were low fiber ($uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29) and high fiber ($uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27), at wk 5 all cows were fed the LF diet. Postpartum data were analyzed separately as wk 1 to 4 and 1 to 6. (A) A treatment by week interaction ($P < 0.01$) for DMI was detected for wk 1 to 4 and 1 to 6. (B) There was a treatment by week interaction for both aNDFom and $uNDF_{240}$ intake ($P < 0.01$), and an overall treatment difference for $uNDF_{240}$ intake ($P < 0.01$). For outcomes with treatment by week interaction, time points at which treatment means differ ($P \leq 0.05$) are indicated with an asterisk (*) and trends with a cross (†).

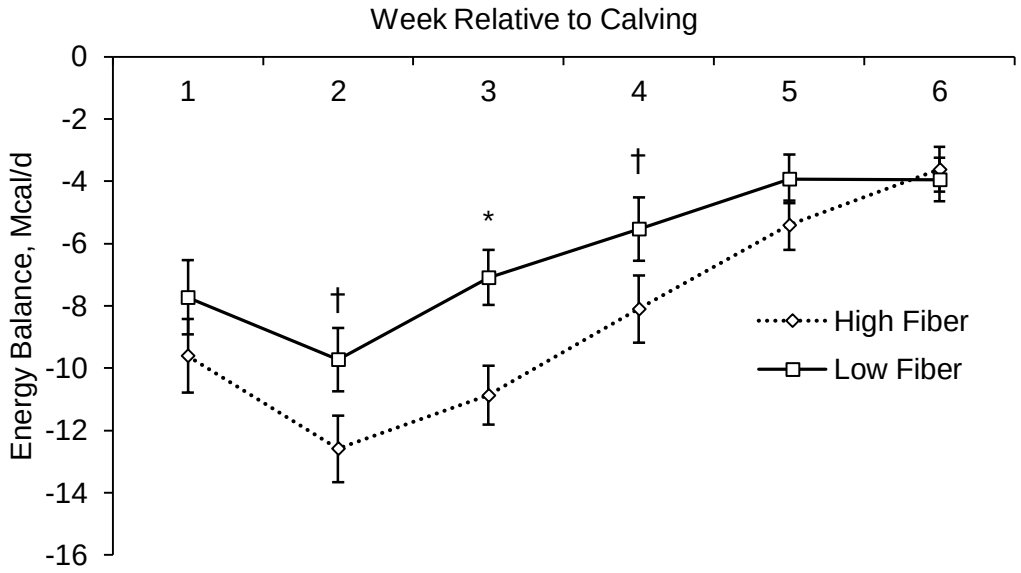

Figure 3-2. Least squares means (± SEM) of energy balance (Mcal/d) for cows fed diets differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) in wk 1 to 4 postpartum. Treatments were low fiber ($uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29) and high fiber ($uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27), at wk 5 postpartum all cows were fed the LF diet. Postpartum data were analyzed separately as wk 1 to 4 and 1 to 6. An overall treatment difference ($P = 0.05$) was observed when analyzed as wk 1 to 4, while for wk 1 to 6 there was an interaction ($P < 0.03$) of treatment by week. For outcomes with treatment by week interaction, time points at which treatment means differ ($P \leq 0.05$) are indicated with an asterisk (*), and trends ($P \leq 0.10$) with a cross (†).

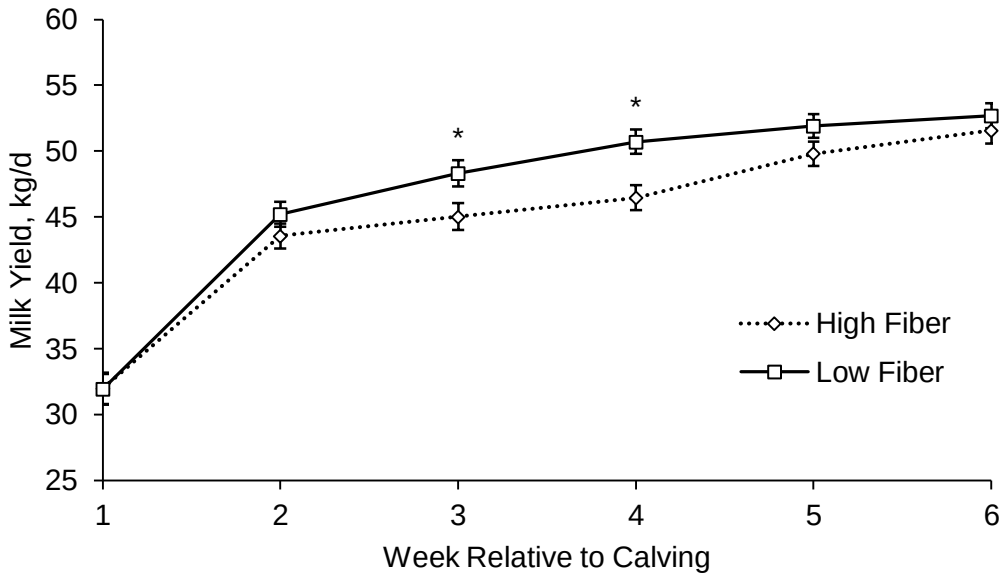

Figure 3-3. Least squares means (± SEM) of milk yield for cows fed diets differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) in wk 1 to 4 postpartum. Treatments were low fiber ($uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29) and high fiber ($uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM n = 27), at wk 5 all cows were fed the LF diet. Postpartum data were analyzed separately as wk 1 to 4 and 1 to 6. A treatment by week interaction for milk yield was detected for wk 1 to 4 ($P < 0.04$) and 1 to 6 ($P < 0.01$). For outcomes with treatment by week interaction, time points at which treatment means differ ($P \leq 0.05$) are indicated with an asterisk (*).

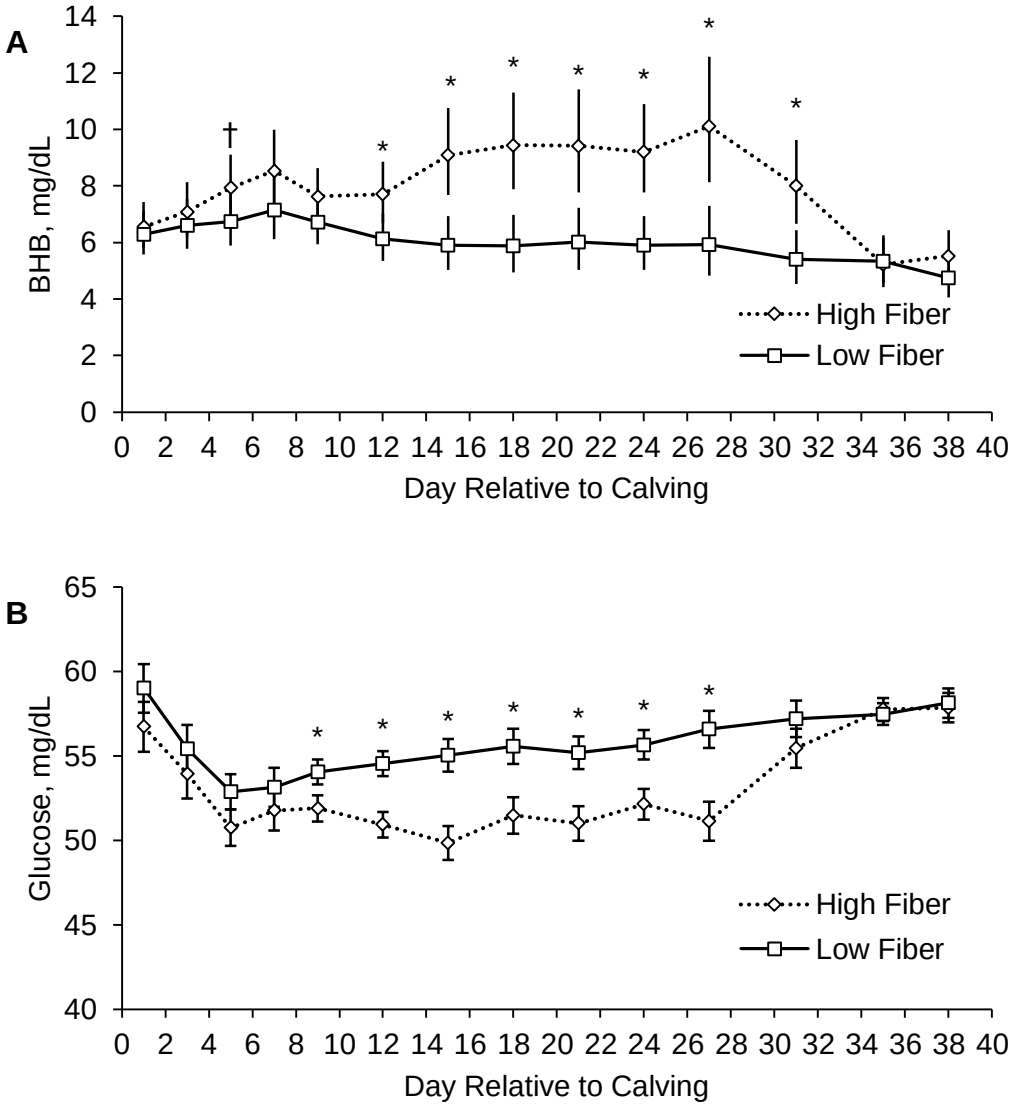

Figure 3-4. Geometric mean and back transformed 95% confidence intervals of (A) plasma BHB and least squares means (± SEM) of glucose (B) for cows fed diets differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) in wk 1 to 4 postpartum. Treatments were low fiber ($uNDF_{240}$ = 9.5% DM, peNDF = 21% DM, n = 29) and high fiber ($uNDF_{240}$ = 12.2% DM, peNDF = 23% DM, n = 27), treatment diets were fed from d 1 to d 28 postpartum, after which all cows were fed the LF diet. Postpartum data were analyzed separately as wk 1 to 4 and 1 to 6. A treatment by day interaction for plasma BHB was detected for 1 to 6 ($P < 0.01$), whereas a treatment difference ($P < 0.01$) was detected for wk 1 to 4. A treatment by day interaction for plasma glucose was detected for 1 to 6 ($P < 0.01$), while a treatment difference ($P = 0.01$) was detected for wk 1 to 4. For outcomes with treatment by week interaction, time points at which treatment means differ ($P \leq 0.05$) are indicated with an asterisk (*), and trends ($P \leq 0.10$) with a cross (†).

DISCUSSION

McCarthy et al. (2015a) suggested metabolic dysfunction (excessive metabolic disorders) occurred when diets containing $uNDF_{240}$ at approximately 8% DM with 28% DM starch were fed during the immediate postpartum period; however, after a diet correction in which diets fed containing $uNDF_{240}$ at 10.5% DM and 26% starch (DM basis) were fed, cows were highly productive and metabolic disorders were largely absent. Originally, postpartum treatment diets for the current study were formulated to contain 10.7% or 8.3% $uNDF_{240}$ as % DM (HF vs LF, respectively), to investigate this lower to adequate range of $uNDF_{240}$ in postpartum diets; however, due to higher than anticipated $uNDF_{240}$ levels in the alfalfa hay procured, overall dietary $uNDF_{240}$ levels were higher than anticipated. Therefore, our study ultimately provided data investigating the adequate to upper range of $uNDF_{240}$ (12.2% vs 9.5% $uNDF_{240}$, HF and LF respectively) in diets fed during the postpartum period. The LF diet appeared to provide adequate levels of $uNDF_{240}$, and was intermediate to the $uNDF_{240}$ levels of McCarthy et al. (2015a), whereas the HF diet was likely representative of the upper bound of $uNDF_{240}$ for this period, as discovered through limitations of DMI and production discussed subsequently. Limited previous research of effects of $uNDF_{240}$ levels on DMI is unclear, with diets containing high $uNDF_{240}$ (10.9% and 11.5% of diet DM) not limiting DMI of mid-lactation cows (Fustini et al., 2017, Grant et al., 2018), unless also combined with high levels of peNDF (21.9% DM; (Grant et al., 2018), which limited DMI and milk production. Grant et al. (2018) also fed diets containing 8.9% $uNDF_{240}$ (as % DM) as the low $uNDF_{240}$ diet, and achieved high production and intake in mid lactation cows. To the authors' knowledge there are no

published studies exploring $uNDF_{240}$ in diets fed during the immediate postpartum period; research exploring the lower bounds of $uNDF_{240}$ in the postpartum diet is still of interest, though investigation of the upper bounds of $uNDF_{240}$ also fills a necessary gap in the literature.

Cows fed the HF diet had lower DMI in wk 2 through 5 postpartum, though DMI expressed as a percentage of BW was lower only for these cows during wk 3 and 4 postpartum compared to cows fed the LF diet, and tended to be lower during wk 5 postpartum, the first week after switching to the LF diet. In the second week after cows fed HF were switched to the LF diet (wk 6), DMI was not different between groups. This decrease in DMI starting in wk 2 postpartum and continuing through to the switch to a lower fiber diet is likely due to the HF diet containing higher NDF, specifically $uNDF_{240}$ and peNDF. Previous research suggests that diets high in NDF have the capacity to limit intake via gut fill, due to signaling of satiety centers by stretch receptors in the rumen muscle layer (Van Soest, 1965, Allen, 1996, Mertens, 1997). Limitation of DMI via gut fill is primarily noted in times of peak milk production rather than early lactation (Allen and Piantoni, 2013), though DMI reductions due to inert rumen mass have been documented specifically in times of low or negative energy balance (e.g. early lactation), whereas DMI of cows in positive energy balance was not affected (Allen, 2000), suggesting cows in early lactation are susceptible to decreases in intake due to gut fill.

In the immediate postpartum period vast changes related to reproductive status and lactation lead to vast metabolic changes, many of which have also been implicated to play a role in the decrease of DMI in the time leading up to parturition, as well as

the lag of DMI increase after calving (Ingvartsen and Andersen, 2000). High energy diets have been linked to decreased intake as well, due to increased hepatic oxidation of fuels (namely starch) in the liver and the subsequent effects on satiety centers in the brain (Allen et al., 2009, Allen and Piantoni, 2013). This was likely not the cause for decreased intake in cows fed the HF diet in this study; however, as after switching to a diet with lower fiber and fill characteristics but similar starch content DMI increased quickly, indicating the limitation of intake was likely related to the fiber characteristics rather than energy density. Additionally, cows fed the LF diet consumed higher amounts of starch in wk 2 through 5 postpartum which accompanied increased DMI compared to cows fed HF, indicating increased starch intake was likely not inhibiting DMI in early lactation.

Oba and Allen (1999) reported a one unit difference in NDF digestibility (mostly measured between 24 and 48 h of fermentation *in vitro*) was associated with increased DMI and 4% FCM; however, NDF digestibility determined at 30 h (19.1 vs. 20.2% of DM) of the HF vs. LF diet were similar. The major differences between the two diets were in the $uNDF_{240}$ (12.2 vs 9.5% of DM) and peNDF (23.2 vs 21.6% of DM) fractions as designed. Previous research investigating effects of particle size on DMI provide mixed results; several studies have found increased DMI with decreased particle size (Kononoff and Heinrichs, 2003, Coon et al., 2018), whereas others have reported no differences in DMI (Beauchemin et al., 2003, Fernandez et al., 2004). Interestingly, Schadt et al. (2012) reported increases in chewing activity due to the smallest size particles collected by the PSPS, indicating that chemical parameters of feed particles can alter eating dynamics as well, not just physical particle size.

Most published research evaluated peNDF, but not $uNDF_{240}$, effects on DMI. Recently Grant et al. (2018) evaluated feeding both high and low $uNDF_{240}$ diets, each with high and low peNDF. When feeding low $uNDF_{240}$ diets, peNDF had no effect on DMI, though when feeding a diet with high $uNDF_{240}$ and higher peNDF DMI was lower compared with the cows fed high $uNDF_{240}$ and lower peNDF diet, which was similar to DMI of cows fed low $uNDF_{240}$. This could be attributed to rumen turnover, as cows fed the high $uNDF_{240}$ with high peNDF diet tended to have the slowest rumen turnover of aNDFom. Shifting rumen dynamics and turnover time could explain the lag in increase of DMI after cows fed HF were switched to the LF diet. The rumen might still be adjusting to lower levels of $uNDF_{240}$ and peNDF in the diet, clearing the extra fiber from the previous diet before reaching the faster turnover rate likely associated with the lower $uNDF_{240}$ diet and therefore increasing DMI. Increased BW loss for cows fed HF during wk 1 to 4 and more negative energy balance in early lactation compared to cows fed LF predictably follows decreased DMI due to gut fill limitations.

Intake of $uNDF_{240}$ differed by treatment, with cows fed HF consuming higher amounts of $uNDF_{240}$ both as kg/d (2.37 vs 2.0 kg/d; $P < 0.01$) and as percentage of BW than cows fed the LF treatment (0.32 vs 0.27 % of BW) during the period of dietary difference. These $uNDF_{240}$ intakes were considerably lower than reported elsewhere for mid lactation cows, where $uNDF_{240}$ intake ranged from 0.35% to 0.48% of BW for diets varying in fiber makeup and digestibility (Fustini et al., 2017, Grant et al., 2018). Early lactation cows are likely limited in intake by size constraints of the reticulo-rumen after calving, leading to lower capacity for NDF ingestion (Allen,

1996), while also being coupled with several changing metabolic signals around calving which can also lead to decreased overall DMI (Ingvartsen and Andersen, 2000). The physical and metabolic factors contributing to decreased DMI in this postpartum period would therefore limit total aNDFom and $uNDF_{240}$ ingestion compared with cows in later stages of lactation. Overall aNDFom intake was not statistically different due to treatment (6.88 vs 6.90 kg/d; $P = 0.44$) during the period of dietary different, though was different in specific weeks (treatment by time $P = 0.01$). Cows fed HF had higher intake of aNDFom in wk 1 compared to cows fed LF, which could indicate that DMI during the first week postpartum might not be limited by gut fill of aNDFom content; however, as lactation progressed aNDFom was not different between the diets, indicating gut fill due to total aNDFom intake was likely limiting DMI. Accompanying increases in $uNDF_{240}$ intake for cows fed HF with no differences in total aNDFom intake would indicate there is less potentially digestible material available to meet energy requirements of the animal, fitting the more severe energy deficit and altered energy metabolites observed in the cows fed HF.

Despite differences in DMI and intake of NDF fractions, there were no differences in rumination between treatments. Many studies report increases in chewing and rumination activity with increased particle size (Beauchemin et al., 2003, Kononoff and Heinrichs, 2003, Kononoff et al., 2003b), however it appears that most of the increase might be in actual eating and chewing time rather than rumination. Schadt et al. (2012) and Grant et al. (2018) reported that particles are chewed to a uniform size for bolus formation prior to swallowing, regardless of initial particle size. As a result, cows fed diets with higher peNDF might be expected to spend a longer

amount of time chewing and eating, but not necessarily ruminating. Though little research characterizing $uNDF_{240}$ in diets is published, both Fustini et al. (2017) and Grant et al. (2018) found no differences in rumination due to $uNDF_{240}$ content, which fits with the lack of differences observed in this study.

Milk production was lower for cows fed the HF diet during wk 3 and 4 compared to cows fed the LF diet, but not during wk 5 and 6 after the change to the LF diet, which is consistent considering the limited intake of the HF cows during that period. Whereas we did not observe any effects of treatment diet on milk fat and actually measured decreased true protein yield (tendency for decreased true protein percentage), ECM tended to be lower only during wk 4 when analyzed as wk 1 to 4, and 3.5% FCM tended to be lower in wk 4 for cows fed HF. Diets higher in fiber and with increased particle size have been shown to increase or maintain milk fat compared to cows fed diets lower in fiber or with smaller particle size (Sutton, 1989, Grant et al., 1990, Kononoff et al., 2003b), likely due to altered rumen dynamics which can shift fermentation and passage rate in lower fiber, higher energy density diets (Bauman and Griinari, 2003). It is possible that cows fed the HF diet produced numerically higher milk fat than cows fed LF, which contributed to slight increases in ECM and 3.5% FCM, though differences were not large enough to lead to statistically different means. Grant et al. (2018) reported higher milk fat percent from cows fed higher $uNDF_{240}$ diets, while noting no differences in milk fat percent due to peNDF. Lower milk protein percent has also been reported previously with high $uNDF_{240}$ diets by Fustini et al. (2017), and Grant et al. (2018), though only in the diet with high $uNDF_{240}$ and high peNDF, the high $uNDF_{240}$ and low peNDF diet was not different

than cows fed low $uNDF_{240}$. It is unlikely the high $uNDF_{240}$ alone that causes decreased milk protein percent, and is likely due to decreased DMI, decreased intake of potentially digestible NDF, and lower energy availability, which could limit microbial protein synthesis in the rumen. Future research is warranted to determine the influence of particle size versus chemical makeup of fiber, impacting the size of the potentially digestible NDF pool, and the effects on milk components.

Plasma metabolites also responded as expected with the decreased DMI and more negative energy balance observed for cows fed the HF diet compared to cows fed the LF diet. Cows fed the HF diet had lower plasma glucose from d 9 to 27 postpartum, which pinpoints the timeframe in which these cows had lower DMI. Plasma glucose has been shown to increase in cows fed higher energy density diets (Andersen J. B., 2003, Rabelo et al., 2003, McCarthy et al., 2015c); however the HF diet and LF diet were not different in starch or energy density, rather the decreased DMI of cows fed HF likely influenced the lower plasma glucose concentration directly as highlighted by the decreased total intake of starch when compared with cows fed LF. Once cows fed HF switched to the LF diet on d 29 postpartum, easing the physical intake limitations, by 31 DIM plasma glucose concentration of cows fed the HF diet was similar to cows fed LF throughout the whole experiment, and remained so throughout wk 6 postpartum. Despite these differences in blood glucose, no differences in blood insulin concentration or calculated RQUICKI were observed. This lack of difference in insulin concentrations could be due to the overall dampened secretion of insulin in response to blood glucose exhibited in early lactation (Piccioli-Cappelli et al., 2014, Baumgard et al., 2017), along with the lack of energy density

difference, namely glucose precursors such as propionate, between the two diets. While cows fed LF did have increased intake of starch (as kg/d) due to increased DMI, it is possible the subsequent increase in blood glucose was not large enough in magnitude to cause a response in plasma insulin. Blood NEFA tended to be higher for cows fed HF than cows fed LF from wk 1 to 6, which represents the greater negative energy balance of the cows fed HF. Nonesterified fatty acids increase in plasma as a result of lipolysis triggered to combat negative energy balance initiated around parturition and the onset of lactation (Herdt, 2000). Excessive NEFA mobilization, however, can lead to metabolic dysfunction as increased postpartum NEFA has been associated with higher risk of DA, metritis, ketosis, and early lactation culling (Ospina et al., 2010c). Blood BHB was higher for cows fed HF from d 12 to 31 postpartum, likely also to combat the decreased glucose availability and more negative energy balance of this period. In times of low glucose availability, fatty acids will be partially oxidized to ketone bodies and used for fuel throughout the body to spare available glucose for milk production by the mammary gland (Drackley et al., 2001). The timing of BHB differences being slightly offset from the time of blood glucose differences might reflect the slightly longer adjustment period of the body to respond to changes in available energy. It is well-documented in the literature that increased postpartum BHB can increase risk of displaced abomasum and early lactation culling, while it can also negatively impact reproduction and milk yield, though those responses are more variable (McArt et al., 2013). Although high BHB and low blood glucose were only observed for a subset of time within the experiment and NEFA only tended to be higher for cows fed the HF diet, these metabolic shifts early in lactation

resulted in metabolic dysfunction which could lead to disease and decreased productivity later in lactation.

CONCLUSIONS

In this experiment, levels of $uNDF_{240}$ (9.5% of DM; 0.27% of BW) and peNDF (21.6% of DM) achieved in the LF diet were adequate to support optimal metabolism and milk production in early lactation, whereas the levels in the high fiber diet ($uNDF_{240}$ at 12.2% of DM, 0.32% of BW, and 23.2% peNDF) limited DMI and performance during early lactation. It is important to note that, as a result of variation in forage composition, actual $uNDF_{240}$ levels were higher than intended, and the LF diet in the current study was intermediate to the levels previously determined to cause metabolic dysfunction of highly productive cows. Though not a definitive limit, these data would suggest a maximum for $uNDF_{240}$ intake of 0.32% of BW in the postpartum period, with an optimum range of $uNDF_{240}$ intake ranging from 0.29 to 0.32% of BW or as a percentage of the diet 9.5% to 11% DM. However, all fiber pools must be appropriately characterized and further investigated to fully understand what is first limiting for rumen fill, as in wk 2 and 3 postpartum total aNDFom intake was similar between treatments. Overall, cows fed increased levels of $uNDF_{240}$ and peNDF were restricted in intake thus in a more severe negative energy balance. However, the detrimental effects of this restriction did not appear to carryover once cows switched to a lower fiber diet. It appears that $uNDF_{240}$, overall forage digestibility and fiber pool size likely plays an important role in regulating intake in the early fresh period; this area warrants further investigation.

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CHAPTER 4

THE EFFECT OF VARYING UNDIGESTED NDF AND PHYSICALLY EFFECTIVE NDF CONTENT OF POSTPARTUM DIETS ON HEPATIC METABOLISM AND GENE EXPRESSION IN MULTIPAROUS HOLSTEIN COWS

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ABSTRACT

Our objective was to evaluate the effects of increased undigested NDF ($uNDF_{240}$, NDF undigested at 240-h of fermentation *in vitro*) and physically effective NDF (peNDF) in fresh cow diets containing higher levels of starch on hepatic metabolism and gene expression in early lactation. Multiparous Holstein cows (n=56) were assigned randomly to one of two higher starch postpartum diets with differing content of $uNDF_{240}$ and peNDF. The two treatment diets, high fiber (HF, n = 27) or low fiber (LF, n = 29), were formulated to differ in $uNDF_{240}$ (12.2% DM vs. 9.5% DM, respectively) and peNDF (23% DM vs. 21% DM, respectively) content. Higher $uNDF_{240}$ levels were achieved in the HF diet through the addition of chopped straw; treatment diets were similar in MP and starch content. Cows were enrolled 28 d prior to expected parturition and individually fed a common dry cow diet in tie-stalls. Upon parturition cows were individually fed their respective treatment diets through 28 DIM. Liver biopsies were obtained from a subset of 40 cows (HF n = 19, LF n = 21) on d 7 ± 1.1 (mean ± SD) and 14 ± 1.0 postpartum to determine liver composition and used in-vitro metabolism to determine rates of palmitate and propionate metabolism. Cows fed HF had lower DMI in wk 2 through 4, energy balance was lower overall, and milk yield was lower in wk 3 and 4 postpartum compared to cows fed LF. Cows fed LF had lower plasma globulin and total protein than cows fed HF, though no other differences in markers representing liver damage or inflammation were observed. Liver metabolism was altered for cows fed HF such that they tended to have higher rates of palmitate conversion to esterified products, lower rates of palmitate oxidation, higher liver triglyceride, and lower liver glycogen content when compared to cows fed

LF. Gene expression of pyruvate carboxylase at d 14 and carnitine palmitoyltransferase 1A overall tended to be higher for cows fed HF compared to those fed LF. Hepatic liver triglyceride content correlated positively with hepatic gene expression related to lipid metabolism and tumor necrosis factor α, while liver glycogen correlated negatively with gene expression related to lipid metabolism, and positively to gene expression of insulin-like growth factor I and growth hormone receptor. These effects suggest supplemental $uNDF_{240}$ and peNDF in the fresh period can inhibit DMI, increase severity of negative energy balance, and alter hepatic metabolism and gene expression.

INTRODUCTION

The dairy cow undergoes vast metabolic changes in the transition period from late pregnancy to lactation. Glucose demands during the early postpartum period are increased over 2 fold compared to late gestation and dietary glucose precursors are unable to fully meet this demand (Bell, 1995). Demands for AA increase for use in gluconeogenesis and milk protein production (Bell, 1995, Reynolds et al., 2003), and fatty acids mobilized from body tissue are utilized for oxidation and ketogenesis (Drackley et al., 2001) to provide energy to tissues as the majority of glucose is spared for the mammary gland (Bauman and Currie, 1980).

Changes in hepatic metabolism, including increases in gluconeogenesis and fatty acid metabolism, are crucial to avoid metabolic dysfunction. Gluconeogenesis is upregulated postpartum, though propionate supply is unable to meet the glucose demand (Reynolds et al., 2003), thus capacity for AA utilization for glucose production is highly upregulated postpartum (Drackley et al., 2001). Activity and gene

expression of pyruvate carboxylase (PC), a key enzyme which catalyzes the conversion of pyruvate to oxaloacetate, also is upregulated postpartum to facilitate this increase in gluconeogenesis from glucogenic AA (Greenfield et al., 2000).

Non-esterified fatty acids (NEFA) are mobilized from body fat reserves to combat negative energy balance (Herdt, 2000) and are catabolized in the liver. Oxidation of fat and ketogenesis are governed in part by the activity of carnitine palmitoyltransferase 1-A (CPT1A), responsible for transport of fatty acids into the mitochondria (Drackley et al., 2001). Rates of hepatic esterification and ketogenesis, and expression of CPT1A are upregulated during the early postpartum period (Dann and Drackley, 2005, Litherland et al., 2011). Though rates of NEFA oxidation are lower postpartum, prepartum diet has been shown to influence NEFA oxidation (Litherland et al., 2011) as well as CPT1A gene expression (Dann and Drackley, 2005). Excessive NEFA mobilization postpartum has been associated with negative impacts on health and performance, such as increased risk for DA, culling, and longer time to pregnancy (Ospina et al., 2010a, b). Excessive NEFA mobilization with insufficient oxidation or export of lipid from the liver can cause fatty liver, which ultimately can negatively impact hepatic capacity for gluconeogenesis (Rukkwamsuk et al., 1999).

Cows fed more propiogenic diets have exhibited lower NEFA mobilization, higher blood glucose and insulin, and increased propensity to convert propionate to glucose (Rabelo et al., 2003, McCarthy et al., 2015c), indicating more desirable liver function. These diets, typically with increased concentrations of starch, can lead to subacute ruminal acidosis, which could cause negative consequences such as

decreased DMI and milk production, and increased inflammation (Nocek, 1997, Penner et al., 2007, Plaizier et al., 2008). Feeding high starch diets that provide substrates for liver gluconeogenesis, while also promoting rumen function and increased DMI would be ideal. Less digestible fiber fractions can be utilized in diets to promote a more full rumen, and increased chewing and rumination activity (Mertens, 1997). Both physically effective NDF (peNDF) and undigested NDF ($uNDF_{240}$, NDF undigested at 240 h of fermentation) have been reported to decrease time spent under rumen pH of 5.8, indicative of SARA (Beauchemin et al., 2003, Fustini et al., 2017).

During the early postpartum period there is a lack of published information on the effects of increasing dietary peNDF or $uNDF_{240}$ on hepatic metabolism. Our objective was to evaluate the effects of supplemental $uNDF_{240}$ in a higher starch fresh cow diet on production, hepatic metabolism and gene expression, inflammation, and liver health markers. A secondary objective of this experiment was to establish correlations between hepatic metabolism parameters and gene expression of metabolic enzymes, markers of energy balance, and inflammation. We hypothesized that providing supplemental $uNDF_{240}$ in the early postpartum period would optimize hepatic metabolism, increasing energy supply while limiting hepatic damage and inflammation, subsequently increasing performance parameters.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

All animal protocols were approved by the Cornell University Institutional Animal Care and Use Committee (protocol #2015-0024). Experimental design and treatments are described in more detail in the companion paper (Chapter 3), and

described briefly here. Multiparous Holstein cows (n=60) were enrolled in a randomized design study between July and September 2015 where treatment was blocked to control for lactation number and previous 305-d mature equivalent milk production. A sample size calculation determined 25 cows per treatment would detect a difference in milk yield of 4 kg per day with 95% confidence and 80% power based on data from previous studies in our lab (McCarthy et al., 2015b). Cows were moved to tie-stalls 28 d prior to expected parturition and fed a common close-up dry cow diet through parturition, after which cows were fed their assigned treatment diets (Table 4-1), high fiber (HF) or low fiber (LF), which were formulated to differ in $uNDF_{240}$ (12.2% of DM vs. 9.5% of DM, respectively) and peNDF (23% of DM vs. 21% of DM, respectively) content. Four cows were removed completely from analysis and 3 cows were partially removed from the dataset by researcher blinded to treatment for severe illness or death which were unrelated to treatment prior to data analysis, resulting in a final dataset including 56 cows; 27 cows fed HF and 29 cows fed LF. Average lactation number of cows fed HF was 2.64, with 14 cows in their 2nd lactation and 13 cows in their 3rd and greater lactation. Average lactation number of cows fed LF was 2.74, with 17 cows in their 2nd lactation and 12 cows in their 3rd and greater lactation. A subset of 40 cows were subjected to liver biopsy at 7 ± 1.1 (mean ± SD) and 14 ± 1.0 DIM and all subsequent analyses which are the focus of this paper. A sample size calculation determined 16 cows per treatment would detect a difference of 1 standard deviation from the mean for conversion of propionate to glucose with 95% confidence and 80% power based on data from previous studies in our lab (McCarthy et al., 2015b). Eligibility for liver biopsy was dependent on DIM at the time of biopsy,

and was balanced for treatment as biopsies were conducted. Average lactation number for biopsied cows fed LF (n = 21) was 2.71, with 9 cows in 2nd lactation and 12 cows in their 3rd and greater lactation. Average lactation number for biopsied cows fed HF (n = 19) was 2.58 with 10 cows in 2nd lactation and 9 cows in their 3rd and greater lactation.

Diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS, version 6.5, Cornell University, Ithaca, NY; Van Amburgh et al., 2015). The HF and LF treatment diets were fed from parturition until 28 DIM, after which all cows received the LF diet until the end of the study at 42 DIM. Approximately one month after the first cow on study calved (20% of all postpartum days cows were fed), the diets were reformulated to better reach our target for starch in the diets increasing the amount of corn meal and canola meal, decreasing the amount of grain mixture in both fresh diets. All forage amounts remained as originally formulated throughout the study. A weighted average of formulated diet ingredient composition (weighted to account for diet reformulation), forage analysis, and nutrient analysis of diets can be found in Table 4-1, Table 4-2, and Table 4-3 respectively. Detailed animal feeding procedures, feed sampling and analysis information are detailed in Chapter 3.

Plasma sampling and analysis

Blood samples were collected via coccygeal vessels prior to fresh feed delivery between 0530 and 0730 h 2x/wk from d 28 prior to expected parturition through parturition, daily from 1 through 7 DIM, 3x/wk from 7 through 21 DIM, and 2x/wk from 21 DIM through 42 DIM. Samples were collected using 10-mL sodium heparin evacuated tubes (158 USP, Becton Dickinson and Company, Franklin Lakes NJ),

using 20 ga vacutainer needles (Becton Dickinson and Company). Whole blood was harvested, placed on ice immediately after collection, and centrifuged for 20 min at 2,000 × *g* at 4ºC. Plasma was aliquoted into 1.7mL microfuge tubes, snap frozen in liquid N_2 and stored at -20ºC until analysis. Plasma samples taken on the day corresponding with liver biopsy were sent to a commercial diagnostic lab for analysis (University of Guelph Animal Health Laboratory, Guelph, ON, Canada) of haptoglobin based on peroxidase activity (Makimura and Suzuki, 1982, Skinner et al., 1991), and aspartate aminotransferase (AST), cholesterol, glutamate dehydrogenase (GLDH), gamma-glutamyltransferase (GGT), bilirubin, total protein, albumin, globulin, and albumin:globulin ratio on a Roche Cobas 6000 c501 biochemistry analyzer (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. For all assays intra-assay CV was maintained below 5%, for all assays except for haptoglobin, inter-assay CV was maintained below 5%, while haptoglobin inter-assay CV was 10.2%.

Liver Biopsy, Metabolic Composition and Incubations

Liver samples (3 to 5 g) were obtained via percutaneous trocar biopsy (Veenhuizen et al., 1991) from 40 cows on day 7 ± 1.1 and 14 ± 1.0 (mean ± SD) postpartum from cows under local anesthesia. After blotting a liver sample to remove excess blood, part of the sample was immediately snap-frozen in liquid N_2 and stored at -80º C for subsequent analysis of liver composition and RNA extraction for gene hepatic expression analysis. Liver glycogen was determined according to procedures described by Bernal-Santos et al. (2003). Liver triglyceride content was determined using the Folch extraction method (Folch et al., 1957) followed by a colorimetric

method to estimate serum triglycerides (Fletcher, 1968) with modifications as described by Foster and Dunn (1973). The remainder of the liver sample was placed in ice cold PBS (0.015 *M*; 9% NaCl, pH 7.4) and transported to the laboratory within 1 h of tissue collection.

Fresh tissue transported to the lab was used immediately to determine hepatic capacity for gluconeogenesis via incubation with radiolabeled [1-^{14}C] propionate (American Radiolabeled Chemicals Inc., St. Louis, MO) and fatty acid metabolism via incubation with radiolabeled [1-^{14}C] palmitate (American Radiolabeled Chemicals Inc., St. Louis, MO). Liver samples were sliced using a Krumdieck Tissue Slicer (Alabama Research and Development, Munford, AL) filled with ice-cold transport buffer (0.015 *M* PBS). Tissue slices were weighed (60 to 80 mg) and blotted prior to being placed in flasks in triplicate to measure conversion of [1-^{14}C] propionate (1 μCi per flask) to glucose or CO_2 according to procedures previously described (Piepenbrink and Overton, 2003, Piepenbrink et al., 2004) with modifications as described by McCarthy et al. (2015c), or conversion of [1-^{14}C] palmitate (0.5 μCi per flask) to CO_2 and esterified products were measured as described by Piepenbrink et al. (2004), conversion of [1-^{14}C] palmitate to acid soluble products (ASP) was measured via processed incubation medium, as previously described (Drackley et al., 1991, Litherland et al., 2011) with the exception of modification of incubation medium as described by Piepenbrink and Overton (2003).

Briefly, liver tissue was placed into flasks containing [1-^{14}C] propionate in Krebs-Ringer bicarbonate medium [modified as described by Litherland et al. (2011)] and were incubated in a shaking water bath at 37°C to measure metabolism for 0

(control) or 120 min prior to termination via injection of 0.75 M H_2SO_4 into the medium. After termination of metabolism, the filter paper in the hanging well in each flask was soaked with NaOH (0.1mL; 30% wt/vol) prior to a 1 h incubation in a shaking ice-water bath to collect CO_2. After collection of CO_2 filter paper was removed and placed into a scintillation vial to be dried overnight under moving air at 39°C. Ten milliliters 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 removal of hanging well, the contents of each flask were processed for glucose production. An internal standard ([^{3}H] l-glucose, 0.055 μCi per flask; American Radiolabeled Chemicals Inc.) was added to the medium prior to being deproteinized and neutralized by additions of saturated $Ba(OH)_2$ solution. Using an ion-exchange method as described by Piepenbrink et al. (2004) radioactive glucose from media supernatants were isolated and measured using dual-labeled liquid scintillation spectroscopy.

To measure palmitate metabolism, liver tissue was placed into flasks containing [1-^{14}C] palmitate in Krebs-Ringer bicarbonate medium [modified as described by Litherland et al. (2011)] and were incubated in a shaking water bath at 37°C to measure metabolism for 120 min. Blank flasks did not receive any liver tissue and were terminated immediately. After incubation liver tissue was removed from the flask and washed with 3% BSA in KRB and saline prior to being moved to an extraction tube containing 3:2 (wt/vol) hexane:isopropanol to measure conversion of [1-^{14}C] palmitate to esterified products as described by Piepenbrink et al. (2004).

Flasks were capped with a rubber septa containing a well with filter paper for collection of CO_2. Incubation medium metabolism was stopped via injection of 1 mL 30% $HClO_4$ and filter paper was soaked with 0.1 mL NaOH (30% wt/vol) prior to be placed in a shaking ice-water bath for 1 hour to collect CO_2. Filter paper was removed and processed for CO_2 as previously described above for [1-^{14}C] propionate metabolism flasks. Contents of flasks were centrifuged and 1mL of supernatant were added to tubes containing 1 mL fisher universal indicator. Flasks were neutralized with K_2CO_3 prior to transfer of 0.5 mL medium to a scintillation vial, 10 mL of scintillation cocktail (Scintisafe Econo 2; Fisher Scientific) was added to each vial and radioactivity was measured using liquid scintillation spectroscopy to determine ASP.

Gene Expression

Total RNA was isolated and purified using miRNeasy minicolumns and on-column ribonuclease-free deoxyribonuclease treatment (QIAGEN Inc., Hilden, Germany). Concentration and integrity of RNA was determined using the 2100 Bioanalyzer system and RNA 6000 Nano kit (Agilent, Santa Clara, CA). Concentration of RNA ranged from 365 to 1,855 ng/μL and all RIN values were greater than 6.0 (range 6.1 to 8.9). Reverse transcriptase reactions were performed with 2 μg of RNA in a 20-μL volume using the high capacity cDNA reverse transcription kit with RNase Inhibitor (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendation. For all genes, Taqman probes were selected to span exon junctions and not detect genomic DNA, all probes were purchased from Applied Biosystems (Foster City, CA): angiopoietin-like 4 (ANGPTL4; assay Bt03234241), carnitine palmitoyltransferase 1A (CPT1A; assay

Bt04945715), mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2, assay Bt03233810), and pyruvate carboxylase (PC, assay Bt03244510). Two reference genes were selected to run on each plate TATA box binding protein (TBP, assay Bt03241948) and large ribosomal protein P0 (RPLP0, assay Bt03218086). Quantitative PCR reactions were conducted in duplicate using Taqman Fast Advanced master mix with 10-fold diluted cDNA as 10% of the reaction volume on a Step One Plus instrument (Applied Biosystems, Foster City, CA) according to manufacturer's recommendations set to 95°C for 1 second (denaturation) and 20 seconds at 60°C (annealing and extension), with a single UNG incubation period of 2 min at 50°C and polymerase activation of 2 min at 95°C prior to cycling. Negative control reactions lacking template were included for every gene on every plate, and a single pool of RNA from a liver sample in this study was used as calibrator cDNA used on all plates for the control following the $2^{-\Delta\Delta Ct}$ method, and results were expressed as a relative quantity. All above methods were utilized to determine expression of three additional genes for use in correlation analysis: insulin-like growth factor one (IGF-I; assay Bt03252282), growth hormone receptor (GHR; assay Bt03216844), and tumor necrosis factor alpha (TNFα; assay Bt03259156).

Statistical Analysis

Daily means for DMI, milk yield, and rumination were averaged by week prior to analysis. To standardize blood metabolite data, day of blood samples collected 2x / wk or 3x / wk were averaged to reference days prior to analysis. Body weight and BCS change was calculated as the difference in BW from wk 1 to 4. All statistical analyses were conducted using SAS (version 9.4, SAS Institute Inc., Cary, NC). All

data collected over time were analyzed as repeated measures ANOVA using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). Fixed effects of treatment, time, parity group (2^{nd} vs. 3^{rd} and greater), and all 2-way interactions were included in the model. A variable to account for the diet change that occurred mid study was also included in all models. Cow nested within treatment was the random effect. Measurements collected during the week of enrollment were included in all models when available as covariates. Previous lactation 305-d mature-equivalent milk yield was used as a covariate for milk production. Denominator degrees of freedom were estimated using the Kenward Rogers method. Five covariance structures were tested for each model: first-order autoregressive, heterogeneous first-order autoregressive, compound symmetry, heterogeneous compound symmetry, and unstructured; and the covariance structure with the lowest Akaike's information criterion was used (Littell et al., 1996). In instances of 2-way interaction with time where $P \leq 0.10$, the slice option was added to the model to perform a partitioned analysis of the means of the interaction, producing an F-test for each level to determine at which levels treatments differed. In instances of a 2-way interaction without time where $P \leq 0.10$, the Tukey adjustment was utilized to correct for multiple comparisons. Residual analysis was performed to examine normality and homogeneity of residuals, when non-normality of residual variance was evident, data were transformed (either log-transformed or square root transformation) and analysis repeated. Body weight and BCS change data, the only measurements not repeated over time, were analyzed using ANOVA in the MIXED procedure of SAS. All data presented are least squares means and standard error (SE), except in the case of

transformed data (haptoglobin, GLDH, AST, liver triglycerides, and gene expression of; CPT1A, HMGCS2, ANGPTL4) where geometric means with back-transformed 95% confidence intervals (CI) are reported. Statistical significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.15$. Sixteen variables including hepatic gene expression and rates of liver metabolism (listed in Table 4-4) were chosen to test in Pearson correlation in SAS, statistical significance was declared at $P \leq 0.05$.

RESULTS

Performance results were described in detail in the companion paper, in chapter 3. Data presented in this paper are limited to the period during which dietary treatments were applied, to relate more closely with the timing of liver sampling, though in general, results were similar to those described previously. Results related to cow performance are found in Table 4-5. Cows fed the HF diet had lower DMI compared to cows fed LF in wk 2 through 4 postpartum (trt × time $P = 0.002$); however DMI as a percentage of BW tended (trt × time $P = 0.08$) to be lower for cows fed HF in wk 3 and 4 postpartum. Intake of starch was higher for cows fed LF compared to cows fed HF (5.24 vs. 4.79 kg/d; $P < 0.01$), and differed by time such that cows fed LF had higher intake of starch in wk 2 through 4 postpartum as compared with cows fed HF. A treatment by time interaction was also present for intake of aNDFom such that cows fed HF tended to have higher intake (kg/d) of aNDFom in wk 1 (6.07 vs 5.66 ± 0.16 kg/d; $P = 0.07$) and lower intake of aNDFom intake in wk 4 (7.35 vs 7.71 ± 0.14 kg/d; $P = 0.08$) compared to cows fed LF. Intake of $uNDF_{240}$ was higher for cows fed HF compared to cows fed LF expressed as both total intake (2.37 vs 2.00 kg/d; $P < 0.01$) and as percent of BW (0.32 vs. 0.27; $P <$

0.01). Despite differences in DMI, there were no differences observed for rumination or BCS, though BW tended (trt × time $P = 0.10$) to be lower for cows fed HF and cows fed HF lost more BW from wk 1 to wk 4 postpartum (-46.2 vs. -24.5 kg; $P = 0.03$) compared to cows fed LF. A treatment by week interaction was present for milk yield ($P = 0.04$) such that milk yield was lower for cows fed HF than cows fed LF during wk 3 and 4 postpartum. We observed a treatment by week interaction for ECM such that ECM tended ($P = 0.10$) to be lower for cows fed HF than those fed LF during wk 4. Postpartum energy balance was lower for cows fed HF compared to cows fed LF (-10.2 vs -7.6 Mcal/d; $P = 0.05$).

Plasma chemistry variables on days corresponding to liver biopsy are reported in Table 4-6. Overall effects of treatment on plasma haptoglobin were not statistically significant; however, a treatment by parity interaction was present ($P = 0.01$) such that 2nd lactation cows fed the LF diet tended to have higher haptoglobin compared to 2nd lactation cows fed the HF diet (0.23 vs 0.17 g/L; $P = 0.15$). Plasma GLDH, AST, cholesterol, GGT, bilirubin, and albumin were not affected by treatment. A treatment by time interaction was present for globulin such that cows fed the LF diet had higher globulin on d 14 postpartum (42.7 vs 39.9 g/L; $P = 0.02$) compared to cows fed HF. A treatment by parity interaction was also present for globulin, such that 2nd lactation cows fed LF tended to have higher globulin concentrations compared to cows fed HF (40.6 vs 35.0 g/L; $P = 0.08$). Likely driven by these differences in globulin, treatment by parity interactions ($P < 0.05$) were evident in evaluation of total protein and albumin to globulin ratio, and a treatment by day of biopsy interaction was significant

for total protein ($P = 0.03$), and appeared as a trend for the albumin to globulin ratio ($P = 0.10$) with the direction and magnitude of differences similar to those of globulin.

Results from *in vitro* studies of palmitate and propionate metabolism in liver tissue are shown in Table 4-7. Cows fed HF tended to have lower overall conversion of [1-^{14}C] palmitate to CO_2 ($P = 0.15$) and higher conversion of [1-^{14}C] palmitate to esterified products ($P = 0.13$) than cows fed LF. Conversion of [1-^{14}C] palmitate to ASP, and conversion of [1-^{14}C] propionate to CO_2 and glucose did not differ by treatment. Conversion of [1-^{14}C] palmitate to CO_2 increased [10.4 vs 12.7 nmol/(g wet wt × h), $P = 0.01$], whereas conversion of [1-^{14}C] palmitate to esterified products tended to decrease [243 vs 231 nmol/(g wet wt × h), $P = 0.08$] over time, from d 7 compared to d 14 regardless of treatment. For [1-^{14}C] propionate metabolism, the ratio of production of glucose to CO_2 also tended to increase over time (0.53 vs 0.56, $P = 0.14$) from d 7 compared to d 14 regardless of treatment.

Liver composition results are reported in Figure 4-1. Liver glycogen content tended to be lower for cows fed HF compared to cows fed LF (0.54 vs 0.65% of wet weight, $P = 0.15$). We observed a treatment by time interaction for liver triglycerides such that cows fed HF tended ($P = 0.08$) to have higher liver triglycerides at d 14 compared to cows fed LF. Regardless of treatment both liver triglycerides (1.85 vs 1.95% of wet weight, $P = 0.03$) and liver glycogen increased (0.54 vs 0.65% of wet weight, $P = 0.01$) from d 7 to d 14, respectively.

Relative hepatic gene expression data are shown in Figure 4-2. A treatment by time interaction trend ($P = 0.14$) for hepatic gene expression of PC was present where cows fed HF tended to have higher expression of PC on d 14 compared to cows fed

LF. Cows fed HF tended to have higher overall expression of CPT1A (0.98 vs 0.88 RQ, $P = 0.08$) than cows fed LF. Gene expression of HMGCS2 by treatment or time were not different ($P > 0.15$); however there was a treatment by parity interaction ($P = 0.12$) in which numerically 2nd lactation cows fed LF tended to have lower expression of HMGCS2 than cows fed HF, however through correction for multiple comparisons the difference was not statistically different (0.73 vs. 0.88 RQ, $P = 0.38$). Gene expression of ANGPTL4 was not different ($P > 0.15$) due to treatment, time or any interactions.

Pearson correlations between liver composition and metabolism, and hepatic gene expression of PC, CPT1A, HMGCS2, and ANGPTL4 by day relative to calving are found in Table 4-8. Liver triglyceride content on both d 7 and d 14 postpartum correlated positively with d 7 gene expression of CPT1A, HMGCS2, and ANGPTL4. Liver glycogen content correlated negatively with gene expression of PC on day 7, CPT1A expression between all timepoints, and ANGPTL4 on d 7 and d 14. Metabolism of [1-^{14}C] palmitate to ASP on d 14 correlated negatively with ANGPTL4 gene expression on d 7. Metabolism [1-^{14}C] palmitate to CO_2 on d 14 correlated negatively with HMGCS2 gene expression on d 7. Metabolism of [1-^{14}C] propionate to glucose on d 14 correlated negatively with ANGPTL4 gene expression on d 7, and the ratio of glucose: CO_2 from [1-^{14}C] propionate metabolism showed the same correlation.

Pearson correlations between liver metabolism and composition, and hepatic gene expression of IGF-I, GHR, and TNFa, and plasma haptoglobin by day relative to calving are shown in Table 4-9. Liver triglyceride content on d 7 correlated positively

with TNFa gene expression on d 14. Liver glycogen content on d 7 and d 14 correlated positively with IGF-I gene expression on d 7. Liver glycogen content on d 14 also correlated positively with gene expression of GHR on d 7 and 14. Metabolism of [1-^{14}C] palmitate to ASP on d 14 correlated positively with GHR gene expression on d 7 and 14, negatively with gene expression of TNFa on d 14, and negatively with plasma haptoglobin on d 7. Metabolism of [1-^{14}C] palmitate to esterified products d 14 correlated negatively with IGF-I gene expression on d 7, and positively with gene expression of TNFa on d 14. Metabolism [1-^{14}C] propionate to CO_2 on d 14 correlated negatively with gene expression of TNFa on d 14. Metabolism [1-^{14}C] palmitate to glucose on d 14 correlated negatively with gene expression of TNFa on d 14, and with plasma haptoglobin on d 7 and 14. The ratio of glucose: CO_2 from [1-^{14}C] propionate metabolism correlated negatively on d 7 and 14 with TNFa gene expression, and on d 14 correlated negatively with plasma haptoglobin concentration on d 14.

Table 4-1. Formulated ingredient composition of the common prepartum diet and postpartum treatment diets formulated to differ in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF).

		Postpartum Diet[1]	
Ingredient, % of DM	Prepartum Diet	LF [1]	HF [1]
Conventional corn silage	45.19	42.31	38.46
Alfalfa hay	-	10.58	10.58
Wheat straw	20.84	1.15	8.65
Corn meal	2.43	17.64	20.51
Soybean meal	-	6.03	4.72
Canola meal	3.48	4.52	3.88
Wheat middlings	-	4.82	1.62
Amino Plus[2]	5.91	4.35	5.32
Corn gluten feed	1.74	1.60	0.47
Blood meal	2.43	0.95	1.09
Soybean hulls	6.95	2.42	-
Citrus pulp	4.52	-	0.79
Energy Booster[3]	-	1.29	1.57
Rumensin[4]	0.10	0.05	0.05
Salt	-	0.48	0.47
Ca carbonate	2.26	1.13	1.18
Mg oxide	-	0.28	0.28
Animate[5]	3.13	-	-
Alimet[6]	0.07	-	-
Vitamin and mineral mix[7]	0.94	0.40	0.39

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM). Treatment diets were fed from wk 1 to 4 postpartum.

[2] Heat-treated soybean meal, Ag Processing Inc., Omaha, NE.

[3] Commercial fat source, Milk Specialties Global, Eden Prairie, MN.

[4] Premix contained 26,400 g/t of monensin, Elanco Animal Health, Greenfield, IN.

[5] Anionic mineral supplement, Phibro Animal Health Corporation, Teaneck, NJ.

[6] 2-Hydroxy-4-(methylthio)-butanoic acid, Novus International, Saint Charles, MO.

[7] Prepartum mix contained 3,754 mg/kg Zn, 993 mg/kg Cu, 4,658 mg/kg Mn, 28.9 mg/kg Se, 82.8 mg/kg Co, 82.4 mg/kg I, 1,177 KIU/kg Vitamin A, 253 KIU/kg Vitamin D, and 55,784 IU/kg Vitamin E. Postpartum mix contained 25,560 mg/kg Zn, 7,154 mg/kg Cu, 21,958 mg/kg Mn, 214 mg/kg Se, 507 mg/kg Co, 331 mg/kg I, 3,704 KIU/kg Vitamin A, 922 KIU/kg Vitamin D, and 12,496 IU/kg Vitamin E (Central New York Feeds, Jordan, NY).

Table 4-2. Forage composition (DM basis except where noted) as analyzed by wet chemistry and *in-vitro* analysis by a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) on one composite sample for each forage representative of the entire study period.

Item	Corn silage	Alfalfa hay	Wheat straw
DM, % of as fed[1]	26.8 ± 1.4	86.0 ± 1.5	87.2 ± 2.1
CP, %	7.8	17.0	4.9
ADF, %	27.0	38.1	54.5
aNDFom, %	43.6	45.6	77.8
Starch, %	31.6	1.2	0.8
Sugar, %	1.0	7.1	2.5
Fat, %	2.2	1.2	0.9
In-vitro digestibility analysis			
30-h NDFD[2], %	26.7	16.5	27.5
30-h NDFD, % of NDF	57.9	37.6	34.2
$uNDF_{30}$[3], %	19.4	27.4	53.0
$uNDF_{30}$, % of NDF	42.1	62.4	65.8
$uNDF_{240}$[4], %	12.4	24.1	35.4
$uNDF_{240}$, % of NDF	27.0	54.9	44.0

[1]DM was analyzed (dried at 40º C in a forced air oven for 96 h) on fresh forages weekly throughout the study and further corrected for residual moisture using DM obtained from Cumberland Valley Analytical Services upon wet chemistry analysis of each ground composite sample.

[2] NDF digestibility, determined via *in vitro* fermentation

[3] Undigested NDF at 30-h of *in vitro* fermentation

[4] Undigested NDF at 240-h of *in vitro* fermentation

Table 4-3. Nutrient profile of all diets (mean ± SD), obtained through wet chemistry analysis and in vitro fermentation by Cumberland Valley Analytical Services (Hagerstown, MD), predicted by Cornell Net Carbohydrate and Protein System (version 6.5, Cornell University, Ithaca, NY), and obtained with a Penn State Particle Separator (Nasco Inc., Fort Watkinson, WI).

	Diet		
Item	Prepartum	Low Fiber (LF)[1]	High Fiber (HF)[1]
DM, % as fed[2]	45.9 ± 1.2	44.4 ± 2.2	47.4 ± 2.0
ADF, % DM	29.0 ± 0.5	21.3 ± 1.1	22.9 ± 2.1
aNDFom, % DM	43.1 ± 0.3	32.8 ± 1.4	35.3 ± 2.3
30-h NDFD[3], % DM	24.2 ± 0.5	20.2 ± 0.5	19.1 ± 1.7
30-h NDFD, % NDF	54.4 ± 1.5	59.5 ± 1.0	52.6 ± 2.2
$uNDF_{240}$[4], % DM	12.8 ± 0.5	9.5 ± 0.4	12.2 ± 1.6
CP, %	14.1 ± 0.8	15.5 ± 0.5	13.8 ± 1.4
Starch, %	15.7 ± 0.3	24.9 ± 1.7	24.6 ± 2.3
Sugar, %	3.5 ± 0.4	5.1 ± 0.7	3.9 ± 0.1
Fat, %	2.3 ± 0.2	3.3 ± 0.2	3.2 ± 0.2
peNDF[5], %	33.3[6]	21.6[7]	23.2[7]
MP[6], g/kg DM	89.0	112.1	108.0
NE_L, Mcal/kg	1.45 ± 0.02	1.65 ± 0.03	1.61 ± 0.02
Ca, %	1.46 ± 0.07	0.97 ± 0.12	0.98 ± 0.19
P, %	0.32 ± 0.02	0.41 ± 0.01	0.37 ± 0.01
Mg, %	0.51 ± 0.02	0.40 ± 0.04	0.39 ± 0.04
K, %	1.16 ± 0.02	1.24 ± 0.03	1.21 ± 0.04
S, %	0.39 ± 0.02	0.30 ± 0.02	0.26 ± 0.02
Na, %	0.13 ± 0.01	0.26 ± 0.02	0.23 ± 0.03
Cl, %	0.67 ± 0.02	0.49 ± 0.02	0.44 ± 0.04

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM). Treatment diets were fed from wk 1 to 4 postpartum.

[2] Determined via wet chemistry from fresh weekly samples sent to Cumberland Valley Analytical Services for NIR analysis.

[3] NDF digestibility, determined via *in vitro* fermentation

[4] Undigested NDF at 240-h of *in vitro* fermentation

[5] Physically effective NDF

[6] Formulated value given by Cornell Net Carbohydrate and Protein System v. 6.5 using analyzed forage values and mean DMI for each period and treatment.

[7] Determined using a Penn State Particle Separator from two samples collected throughout the study. Value obtained by multiplying the percentage of sample retained on screens ≥ 4mm by the diet NDF content.

Table 4-4. Variables used in Pearson Correlation analysis, run in SAS (version 9.4, Cary, NC).

Variable
Hepatic triglyceride content on d 7 and 14 postpartum
Hepatic glycogen content on d 7 and 14 postpartum
Capacity of liver to convert [1-^{14}C] palmitate to acid soluble products on d 7 and 14 postpartum
Capacity of liver to convert [1-^{14}C] palmitate to CO_2 products on d 7 and 14 postpartum
Capacity of liver to convert [1-^{14}C] palmitate to esterified products on d 7 and 14 postpartum
Capacity of liver to convert [1-^{14}C] propionate to CO_2 products on d 7 and 14 postpartum
Capacity of liver to convert [1-^{14}C] palmitate to glucose on d 7 and 14 postpartum
Ratio of Glucose:CO_2 from metabolism of [1-^{14}C] propionate on d 7 and 14 postpartum
Hepatic gene expression of PC on d 7 and 14 postpartum
Hepatic gene expression of CPT1A on d 7 and 14 postpartum
Hepatic gene expression of HMGCS2 on d 7 and 14 postpartum
Hepatic gene expression of ANGPTL 4on d 7 and 14 postpartum
Hepatic gene expression of IGF1 on d 7 and 14 postpartum
Hepatic gene expression of GHR on d 7 and 14 postpartum
Hepatic gene expression of TNFα on d 7 and 14 postpartum
Plasma haptoglobin

Table 4-5. Least squares means and standard errors for DMI, uNDF intake, rumination, BCS, BW, BW change, and energy balance for cows fed diets differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) from wk 1 to wk 4 postpartum.

	Postpartum treatment[1]			*P*-Value[2]			
Item	LF	HF	SEM	Trt	W	Parity	Trt × W
DMI, kg/d	21.1	19.4	0.36	<0.01	<0.01	0.31	<0.01
Starch intake, kg/d	5.24	4.79	0.10	<0.01	<0.01	0.01	0.002
aNDFom intake, kg/d	6.90	6.88	0.13	0.44	<0.01	<0.01	0.01
$uNDF_{240}$ intake, kg/d	2.0	2.37	0.04	<0.01	<0.01	<0.01	0.23
DMI, % of BW	2.9	2.7	0.05	<0.01	<0.01	0.19	0.08
$uNDF_{240}$ intake, % of BW	0.27	0.32	0.01	<0.01	<0.01	0.43	0.06
Rumination, min/d	535	538	8.5	0.58	<0.01	0.61	0.35
BCS	3.19	3.16	0.03	0.23	<0.01	0.79	0.50
BCS change	-0.26	-0.30	0.3	0.38	-	0.02	-
BW, kg	726	719	5.1	0.16	<0.01	0.41	0.10
BW change, kg	-24.5	-46.2	6.8	0.03	-	0.48	-
Milk yield, kg/d	44.1	41.7	0.9	0.08	<0.01	<0.01	0.04
ECM [3], kg/d	47.2	46.0	1.1	0.55	<0.01	<0.01	0.10
Energy balance[4], Mcal/d	-7.58	-10.2	0.83	0.05	<0.01	0.05	0.50

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27). Treatment diets were fed from wk 1 to 4 postpartum.

[2] All two way interactions were analyzed, interaction terms not presented were non-significant ($P > 0.10$) for all variables Trt = treatment; W= week

[3] ECM = (0.327 × kg wk average milk yield) + (12.95 × kg of fat) + (7.95 × kg of true protein)

[4] Energy balance calculated using NRC (2001) equations

Table 4-6. Geometric means and back transformed 95% confidence intervals of plasma haptoglobin, glutamate dehydrogenase (GLDH), aspartate dehydrogenase (AST), and least squares means and standard errors of plasma cholesterol, gamma glutamyl tranferase (GGT), bilirubin, total protein (TP), albumin, and globulin from cows fed diets differing in amount of undigested NDF at 240-h of in vitro fermentation (uNDF240) and physically effective NDF (peNDF) in the postpartum period.

	Postpartum treatment[1]			*P*-Value[2]				
Item	LF	HF	SEM	Trt	D	P	Trt × P	Trt × D
Haptoglobin, g/L	0.19 (0.17-0.23)	0.19 (0.17-0.22)	-	0.83	0.07	0.59	0.01	0.83
GLDH, U/L	12.0 (9.56-15.1)	15.5 (12.2-19.7)	-	0.13	<0.01	0.72	0.91	0.72
AST, U/L	53.3 (48.4-58.7)	54.9 (49.6-60.8)	-	0.66	0.57	0.83	0.96	0.94
Cholesterol, mmol/L	2.56	2.66	0.11	0.52	<0.01	0.19	0.98	0.80
GGT, U/L	22.8	24.6	0.90	0.17	<0.01	0.19	0.75	0.26
Bilirubin, µmol/L	1.89	1.75	0.16	0.53	0.33	0.06	0.88	0.79
TP, g/L	76.3	76.0	1.05	0.84	<0.01	0.01	0.03	0.03
Albumin, g/L	36.2	37.0	0.46	0.18	<0.01	0.36	0.18	0.63
Globulin, g/L	40.5	39.0	1.24	0.40	<0.01	0.02	0.02	0.02
Albumin:Globulin	0.90	0.99	0.04	0.10	<0.01	0.07	0.02	0.10

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 21), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 19). Treatment diets were fed from wk 1 to 4 postpartum.

[2] All two way interactions were analyzed, interaction terms not presented were non-significant ($P > 0.15$) for all variables. Trt = treatment, P = parity (2^{nd} vs 3^{rd} and greater lactation), D = day of biopsy, d7 vs d14

Table 4-7. Least squares means and standard errors of capacities for metabolism of [1-^{14} C]palmitate to CO_2, esterified products, and acid soluble products and capacities for metabolism of [1-^{14}C]propionate to CO_2 and glucose of liver slices from cows fed diets differing in amount of undigested NDF at 240-h of in vitro fermentation (uNDF240) and physically effective NDF (peNDF) in the postpartum period.

	Postpartum treatment[1]			*P*-Value[2]			
Item	LF	HF	SEM	Trt	D	Parity	Trt × D
Conversion of [1-^{14}C] palmitate, nmol/(g wet wt × h)							
CO_2	12.2	10.9	0.65	0.15	0.01	0.41	0.35
Esterified Products	226.8	246.5	9.2	0.13	0.08	0.02	0.36
Acid Soluble Products	132.3	137.0	9.3	0.52	0.59	0.52	0.94
Conversion of [1-^{14}C] propionate, μmol/(g wet wt × h)							
CO_2	6.9	6.8	0.26	0.79	0.35	0.62	0.51
Glucose	3.9	3.7	0.24	0.46	0.57	0.68	0.82
Glucose:CO_2	0.56	0.53	0.02	0.48	0.14	0.44	0.92

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 21), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 19). Treatment diets were fed from wk 1 to 4 postpartum.

[2] All two way interactions were analyzed, interaction terms not presented were non-significant ($P > 0.15$) for all variables. Trt = treatment; D = day of biopsy, d7 vs d14

Table 4-8. Pearson correlation[1] among liver composition and metabolism variables and gene expression of pyruvate carboxylase (PC), carnitine palmitoyltransferase 1A (CPT1A), mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), and angiopoietin-like 4 (ANGPTL4) from liver biopsies on d 7 ± 1.1 and d 14 ± 1.0 (mean ± SD) postpartum.

		Hepatic Gene Expression							
		PC		CPT1A		HMGCS2		ANGPTL4	
		d 7	d 14	d 7	d 14	d 7	d 14	d 7	d 14
Liver Triglycerides		0.188	-0.081	0.328	-0.019	0.381	0.203	0.413	0.024
	d 7	0.25	0.62	0.04	0.91	0.02	0.21	0.01	0.88
		0.124	-0.028	0.321	0.087	0.412	0.298	0.329	0.129
	d14	0.45	0.86	0.04	0.59	0.01	0.06	0.04	0.43
Liver Glycogen		-0.576	-0.294	-0.525	-0.370	-0.213	-0.268	-0.528	-0.163
	d 7	<0.01	0.07	<0.01	0.02	0.19	0.09	<0.01	0.31
		-0.279	-0.282	-0.346	-0.418	-0.111	-0.093	-0.431	-0.350
	d14	0.08	0.08	0.03	0.01	0.50	0.57	0.01	0.03
Palmitate to Acid Soluble Products		0.045	-0.095	0.021	-0.103	-0.270	-0.188	-0.130	0.038
	d 7	0.78	0.56	0.90	0.53	0.09	0.25	0.42	0.82
		-0.165	0.006	-0.179	0.076	-0.113	0.080	-0.343	-0.065
	d14	0.31	0.97	0.27	0.64	0.49	0.62	0.03	0.69
Palmitate to CO_2		0.280	0.004	0.171	-0.085	-0.269	0.001	0.040	-0.182
	d 7	0.08	0.98	0.29	0.60	0.09	0.99	0.80	0.26
		0.083	0.099	-0.057	0.174	-0.321	-0.157	-0.066	0.276
	d14	0.62	0.55	0.73	0.29	0.05	0.34	0.69	0.09
Palmitate to Esterified Products		0.023	-0.057	0.231	0.060	0.308	0.258	0.171	0.086
	d 7	0.89	0.73	0.16	0.72	0.06	0.11	0.30	0.60
		-0.016	0.054	0.188	-0.056	0.283	0.060	0.261	-0.011
	d14	0.92	0.74	0.25	0.73	0.08	0.71	0.10	0.94
Propionate to CO_2		0.045	-0.013	0.085	0.105	-0.278	-0.106	-0.022	-0.109
	d 7	0.78	0.94	0.61	0.53	0.09	0.52	0.89	0.51
		-0.074	0.080	-0.070	0.222	0.021	0.131	-0.257	0.270
	d14	0.66	0.63	0.67	0.18	0.90	0.43	0.12	0.10
Propionate to Glucose		0.183	0.026	0.058	0.044	-0.237	-0.224	-0.059	-0.074
	d 7	0.27	0.88	0.73	0.79	0.15	0.18	0.73	0.66
		-0.170	-0.065	-0.061	0.112	0.109	0.134	-0.366	0.078
	d14	0.31	0.70	0.72	0.50	0.51	0.42	0.02	0.64
Propionate Glucose:CO_2		0.163	0.066	-0.026	0.015	-0.151	-0.184	-0.082	0.021
	d 7	0.33	0.69	0.88	0.93	0.36	0.27	0.62	0.90
		-0.195	-0.155	-0.034	0.012	0.084	0.079	-0.373	-0.139
	d14	0.24	0.35	0.84	0.94	0.62	0.64	0.02	0.41

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

Table 4-9. Pearson correlation[1] among liver composition and metabolism variables and gene expression of insulin like growth factor-1 (IGF1), growth hormone receptor (GHR), and tumor necrosis factor-α (TNFa), and plasma haptoglobin from liver biopsies on d 7 ± 1.1 and d 14 ± 1.0 (mean ± SD) postpartum.

		Hepatic Gene Expression						Plasma Haptoglobin	
		IGF1		GHR		TNFa			
		d 7	d 14	d 7	d 14	d 7	d 14	d 7	d 14
Liver Triglycerides	d 7	-0.175	0.118	-0.054	0.093	-0.028	0.332	0.159	-0.160
		0.28	0.47	0.74	0.57	0.86	0.04	0.33	0.32
	d14	-0.142	0.085	0.073	0.155	-0.084	0.257	0.188	-0.193
		0.38	0.60	0.65	0.34	0.61	0.11	0.25	0.23
Liver Glycogen	d 7	0.514	0.069	0.190	-0.018	-0.002	-0.297	-0.282	-0.007
		0.01	0.67	0.24	0.91	0.99	0.06	0.08	0.97
	d14	0.410	0.297	0.366	0.353	0.056	-0.245	-0.059	-0.101
		0.01	0.06	0.02	0.03	0.73	0.13	0.72	0.53
Palmitate to ASP	d 7	0.370	0.190	-0.003	0.061	0.089	-0.099	-0.279	-0.013
		0.02	0.24	0.99	0.71	0.59	0.55	0.08	0.94
	d14	0.303	0.033	0.358	0.324	0.128	-0.335	-0.349	-0.180
		0.06	0.84	0.02	0.04	0.43	0.03	0.03	0.27
Palmitate to CO_2	d 7	0.140	0.214	-0.081	0.240	0.147	0.058	-0.208	-0.127
		0.39	0.19	0.62	0.14	0.37	0.72	0.20	0.43
	d14	0.034	-0.064	-0.058	0.052	0.208	-0.149	-0.130	0.125
		0.84	0.70	0.73	0.75	0.20	0.37	0.43	0.45
Palmitate to Esterified Products	d 7	-0.277	-0.073	-0.041	0.107	-0.129	0.291	0.106	-0.217
		0.09	0.66	0.81	0.52	0.43	0.07	0.52	0.18
	d14	-0.343	-0.276	-0.129	-0.167	-0.101	0.432	0.161	0.043
		0.03	0.08	0.43	0.30	0.53	0.01	0.32	0.79
Propionate to CO_2	d 7	0.021	0.010	-0.071	-0.037	0.255	0.068	-0.339	-0.022
		0.90	0.95	0.67	0.82	0.12	0.68	0.03	0.90
	d14	0.131	-0.172	0.286	0.107	0.180	-0.348	-0.204	-0.190
		0.43	0.30	0.08	0.52	0.28	0.03	0.22	0.25
Propionate to Glucose	d 7	0.081	0.173	-0.201	-0.002	-0.123	-0.081	-0.313	-0.079
		0.63	0.30	0.23	0.99	0.46	0.63	0.06	0.64
	d14	0.201	0.048	0.243	0.179	-0.084	-0.440	-0.316	-0.409
		0.23	0.78	0.14	0.28	0.62	0.01	0.05	0.01
Propionate Glucose:CO_2	d 7	0.097	0.194	-0.213	0.014	-0.343	-0.185	-0.189	-0.108
		0.56	0.24	0.20	0.93	0.03	0.27	0.26	0.52
	d14	0.218	0.254	0.087	0.161	-0.323	-0.415	-0.310	-0.502
		0.19	0.12	0.60	0.33	0.05	0.01	0.06	<0.01

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

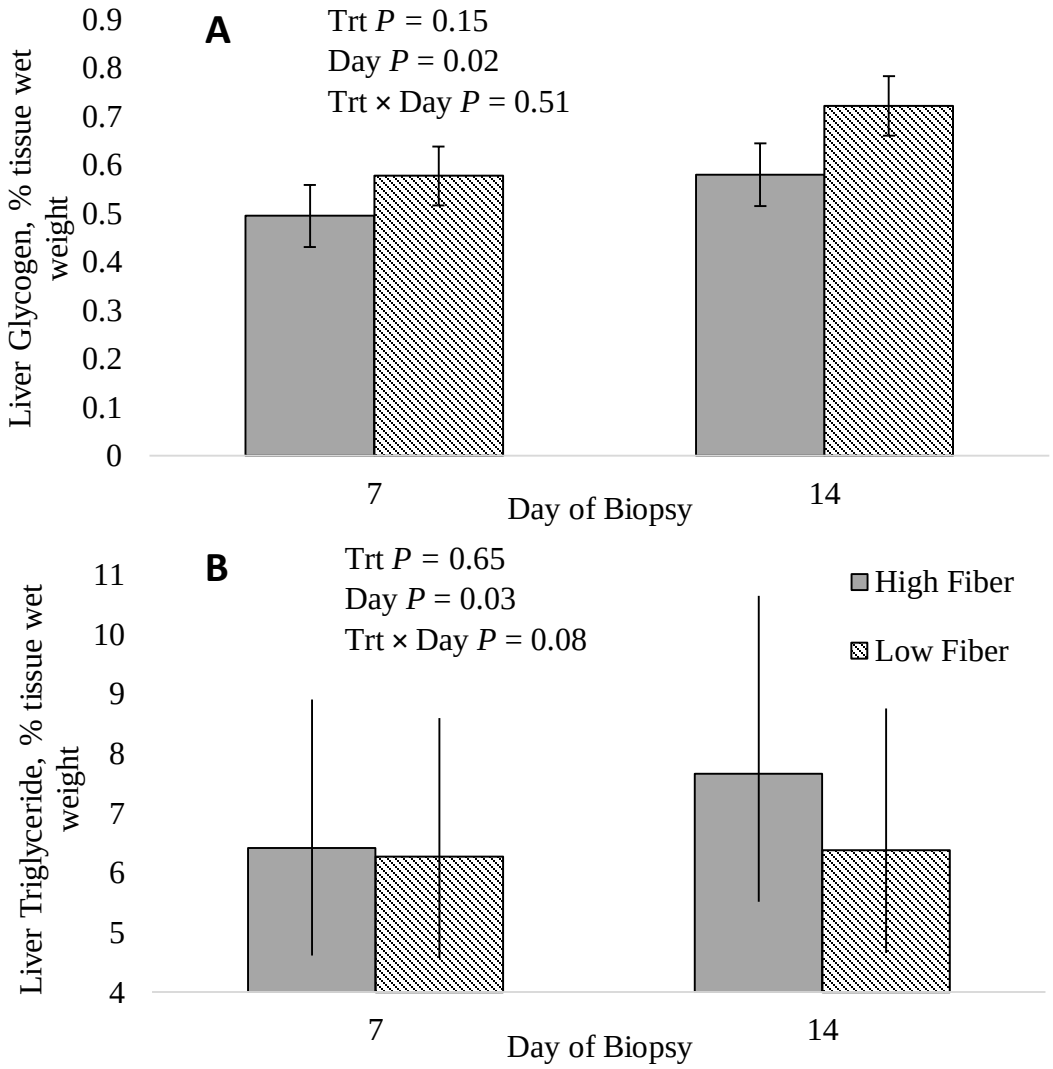

Figure 4-1. Least squares means and standard errors of liver glycogen (A) and geometric means and back transformed 95% confidence intervals of liver triglycerides (B) from liver biopsies on d 7 ± 1.1 and d 14 ± 1.0 (mean ± SD) postpartum of cows fed diets differing in amount of undigested NDF at 240-h of in vitro fermentation ($uNDF_{240}$) and physically effective NDF (peNDF). Treatments were low fiber ($uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 21) and high fiber ($uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 19).

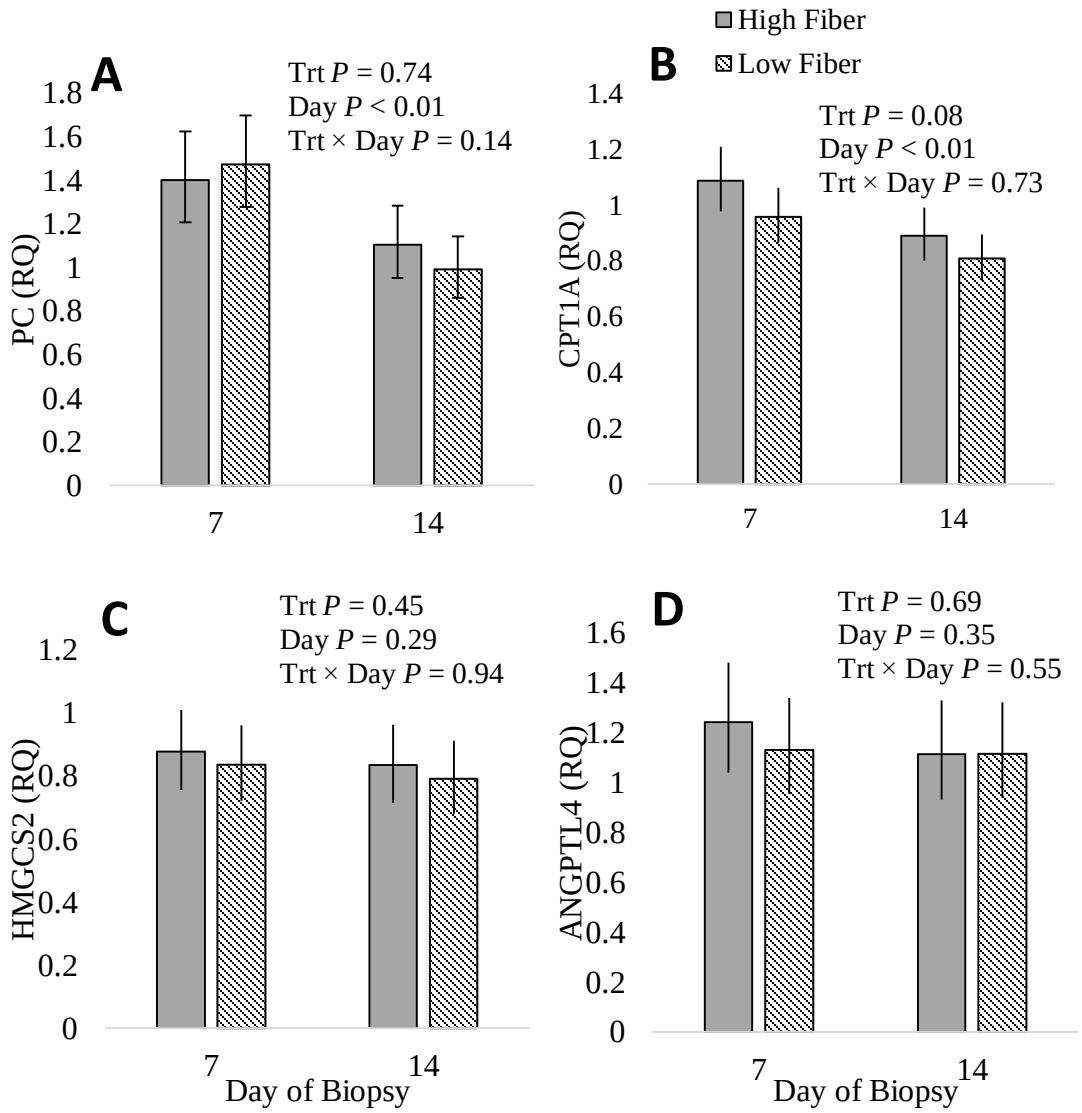

Figure 4-2. Least squares means (± SEM; A) or geometric means and back transformed 95% confidence intervals (B, C, D) for hepatic gene expression of pyruvate carboxylase (PC; A), carnitine palmitoyltransferase 1-A (CPT1A; B), 3-hydroxy-3methylglutaryl-CoA synthase-2 (HMGCS2; C), and angiopoietin like 4 (ANGPTL4; D) expressed as relative gene expression (RQ) from liver biopsies on d 7 ± 1.1 and d 14 ± 1.0 (mean ± SD) postpartum of cows fed diets differing in amount of undigested NDF at 240-h of in vitro fermentation ($uNDF_{240}$) and physically effective NDF (peNDF). Treatments were low fiber ($uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 21) and high fiber ($uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 19).

DISCUSSION

Fiber levels in the immediate postpartum period have not widely been explored, though case study data from McCarthy et al. (2015a) would suggest insufficient $uNDF_{240}$ in the immediate postpartum ($uNDF_{240}$ = 8% of DM) period can lead to metabolic dysfunction and high disease incidence, though cows were able to recover after switching to a higher $uNDF_{240}$ level (10.5% of DM). Originally, postpartum treatment diets for the current study were formulated to contain 10.7% or 8.3% DM $uNDF_{240}$ (HF vs LF, respectively), to investigate this lower to adequate range of $uNDF_{240}$ in postpartum diets; however, due to higher than anticipated $uNDF_{240}$ levels in the alfalfa hay procured, overall dietary $uNDF_{240}$ levels were higher than anticipated. Therefore, our study provided data investigating the adequate to upper range of $uNDF_{240}$ (12.2% vs 9.5% $uNDF_{240}$, DM basis HF and LF respectively) in diets fed during the postpartum period. The LF diet appeared to provide adequate levels of $uNDF_{240}$, and was intermediate to the $uNDF_{240}$ levels of McCarthy et al. (2015a), whereas the HF diet was likely representative of the upper bound of $uNDF_{240}$ for this period, as discovered through limitations of DMI, production and altered metabolism discussed subsequently. Few studies have focused on $uNDF_{240}$ levels, those that have were investigating those effects on mid lactation cows (Fustini et al., 2017, Grant et al., 2018) with mixed effects on intake and milk production. To the authors' knowledge there are no published studies exploring $uNDF_{240}$ in diets fed during the immediate postpartum period; research exploring the lower bounds of $uNDF_{240}$ in the postpartum diet is still of interest, though investigation of the upper bounds of $uNDF_{240}$ also fills a necessary gap in the literature. Performance results were described in detail in

companion paper, in chapter 3, whereas performance and DMI data presented in this paper is limited to the period of dietary difference only, to relate more closely with liver parameters, results were similar to those described previously.

Overall very few differences were seen in plasma chemistry parameters due to treatment. Several plasma chemistry parameters resulted in very small numerical differences, which we lacked the statistical power to detect given only samples from cows that were used for livery biopsy were analyzed. This would indicate the type II error is likely high. Higher plasma globulin and total protein concentrations were detected in cows fed LF on d 14 postpartum compared to cows fed HF, and within second lactation cows in the experiment, cows fed LF had higher haptoglobin as well. Globulin and haptoglobin are positive acute phase proteins (APP) produced by the liver, and which higher levels are deemed indicative of a state of inflammation (Bionaz et al., 2007). Some level of inflammation postpartum is expected and necessary to undergo calving, initiate lactation, and begin uterine involution, though high levels of inflammation, or sustained systemic inflammation postpartum can have negative consequences (Bradford et al., 2015).

Bertoni et al. (2008) calculated liver activity index of cows in early lactation based on several positive and negative APP, grouping cows into quartiles based on liver function. Cows in the lowest liver activity index group exhibited higher positive APP (including haptoglobin and globulin), and lower negative APP, indicating lower liver function; these cows produced less milk and had longer time to pregnancy than the upper quartile group. However, the cows fed LF in our experiment demonstrated higher DMI, milk production, and overall a more favorable metabolic status compared to cows

fed HF. While the globulin levels may have been higher on d 14 for cows fed LF compared to those fed HF, plasma haptoglobin levels and all other measured APP or liver enzymes were not different, indicating if there was inflammation, it was likely mild. In 2nd lactation cows fed LF, higher haptoglobin levels were evident, though the haptoglobin level (0.23 g/L) would be considered relatively low for postpartum cows, and is comparable to haptoglobin levels of cows in the upper quartile for liver activity index by Bertoni et al. (2008).

Markers of increased inflammation several weeks postpartum even in low quantities, could be indicative of subacute inflammation. Cows fed highly fermentable diets after calving are more susceptible to SARA (Nocek, 1997, Penner et al., 2007), which can result in increased inflammation (McCarthy et al., 2015c) and negative impacts on DMI and milk production (Plaizier et al., 2008). This could relate to the higher overall energy intake by cows fed LF, as cows fed LF exhibited higher DMI of a more fermentable diet and higher overall intake of starch. Though we observed no detrimental effects on production or energy metabolites (Chapter 3), cows fed LF could have been experiencing mild SARA to cause the slight increase in inflammatory markers observed.

Cows fed the HF diet had altered hepatic fatty acid metabolism, with tendencies for lower conversion of [1-^{14}C] palmitate to CO_2 and higher conversion of [1-^{14}C] palmitate to esterified products overall, and lower liver glycogen and higher liver triglycerides at d 14 than cows fed LF. Litherland et al. (2011) reported a negative correlation between capacity for hepatic oxidation of fatty acids and capacity for esterification, and several others have reported positive correlations between capacity

for esterification and liver triglyceride content (Grum et al., 2002, Litherland et al., 2011, McCarthy et al., 2015b), similar to the trends observed in this study. Fatty acids play a vital role in providing energy to the cow in the immediate postpartum period, as glucose is spared for the mammary gland for lactation (Bell, 1995). However, NEFA are taken up in proportion to their supply to the liver (Emery et al., 1992, Reynolds et al., 2003); therefore, excess NEFA mobilization and hepatic infiltration can lead to excessive triglyceride accumulation causing fatty liver disease and metabolic dysfunction (Grummer, 1993, Drackley, 1999, Bobe et al., 2004). Liver triglyceride content has been correlated negatively with DMI, plasma glucose, capacity to synthesize glucose from propionate, and energy balance (Grum et al., 2002, Piepenbrink and Overton, 2003, McCarthy et al., 2015b), demonstrating the multitude of metabolic shifts that coincide with increases in liver triglyceride content. Of cows that were biopsied, 34% of biopsies from cows fed HF had severe (>10% liver triglycerides; Bobe et al., 2004) fatty liver, compared with only 19% of biopsies from cows fed LF (data not shown), indicating cows fed HF had more severe fatty liver disease. Cows fed HF also exhibited lower DMI, more negative energy balance, and tended to have lower plasma glucose (Chapter 3) compared to cows fed LF, fitting well with previously reported associations and indicating these cows were in a state of altered hepatic metabolism.

The altered hepatic metabolism of cows fed HF was also evident through changes in gene expression; cows fed HF not only had increased liver triglycerides and lower liver glycogen but also trends for increased PC at d 14 and overall CPT-1A mRNA abundance compared to cows fed LF. Increased PC levels are common after calving (Greenfield et al., 2000, Aschenbach et al., 2010, Graber et al., 2010), as DMI is

low and glucose demand by the mammary gland is high, and AA and lactate are utilized in higher quantities for gluconeogenesis in the liver (Reynolds et al., 2003). Hepatic levels of PC mRNA were found to correlate negatively with hepatic glycogen content, which cows fed HF also demonstrated. The higher PC gene expression and lower glycogen content of cows fed HF, coupled with trends for lower plasma glucose (Chapter 3) compared to cows fed LF, indicates lower overall glucose availability in cows fed HF. The lack of difference in conversion of [1-^{14}C] propionate to glucose due to treatment would indicate there were no direct differences on rates hepatic glucose production from propionate. Considering the limitations of DMI and decreased starch intake starting in wk 2, this would indicate a direct lack of glucose precursors being provided by the diet, causing higher PC activity utilizing lactate and AA for gluconeogenesis, to compensate for lower dietary glucose levels. Velez and Donkin (2005) noted similar increases in PC mRNA after feed restriction of cows in mid-lactation and verifying that these increases in PC levels were accompanied by increased conversion of lactate to glucose. With no differences in conversion of propionate to glucose in the same cows, this confirms increases in PC levels under feed restriction were indicative of increased gluconeogenic capacity of lactate. Similarly, under conditions of increased glucose demand in wethers, Overton et al. (1999) demonstrated an increased capacity for conversion of alanine to glucose compared to capacity for conversion propionate to glucose (285% vs 166% of controls), indicating the increased gluconeogenic capacity of glucogenic amino acids during conditions of low glucose availability, such as that seen under feed restriction or negative energy balance.

Utilization of fatty acids for hepatic metabolism is governed by plasma supply of NEFA to the liver (Emery et al., 1992), but also by CPT-1A, which facilitates their transport into the mitochondria (McGarry and Brown, 1997). Expression of CPT-1A is upregulated postpartum, associated with higher rates of ketogenesis and gluconeogenesis, and generally with negative energy balance (Dann and Drackley, 2005). Hepatic expression of CPT-1A correlated positively with liver triglyceride content and negatively with liver glycogen content, which is indicative of severity of negative energy balance (Veenhuizen et al., 1991), indicating CPT-1A upregulation would coincide with altered hepatic lipid metabolism and negative energy balance. Higher expression levels of CPT-1A by cows fed HF would be in accordance with these findings, as cows fed HF had higher PC mRNA expression, liver triglycerides, plasma BHB, lower liver glycogen and were in more severe negative energy balance for a longer duration than cows fed LF (Chapter 3).

Despite higher expression of CPT-1A to bring NEFA into the mitochondria for metabolism, conversion of [1-^{14}C] palmitate to CO_2 was also lower for cows fed HF; therefore, the increased NEFA were not being utilized for higher rates of oxidation but perhaps were being converted to ketone bodies, as evidenced by higher plasma BHB (Chapter 3) compared to cows fed LF. In fact, conversion of [1-^{14}C] palmitate to CO_2 was negatively correlated with gene expression of HMGCS2, a key enzyme which catalyzes the rate limiting step of conversion of acetyl-CoA to ketone bodies (Hegardt, 1999), indicating cows with lower rates of fatty acid oxidation have higher expression of HMGCS2 and subsequently higher ketone production. Given this correlation and the increased ketone body production of cows fed HF, we expected to see a difference in

expression of HMGCS2 mRNA due to treatment, the absence of such could represent a lack of statistical power. While there were no main effect treatment differences for cows fed HF vs LF, there was a treatment by parity interaction such that cows in their 2nd lactation fed LF had numerically lower HMGCS2 gene expression compared to 2nd lactation cows fed HF. As 2nd lactation cows fed LF were able to consume more DMI and starch compared to those fed HF, it would follow that these cows had a larger supply of propionate that is converted to methylmalonyl-CoA, and ultimately enters the tricarboxylic acid cycle in the form of succinyl-CoA (Ingvartsen, 2006, Aschenbach et al., 2010), which is a potent inhibitor of HMGCS2 (Hegardt, 1999, Drackley et al., 2001). With cows fed LF having increased DMI and thus increased propionate supply for metabolism in the liver, it would also follow that these cows were in a more positive energy balance, produced less ketone bodies, and had less liver triglyceride content, as HMGCS2 also positively correlated with liver triglyceride content.

It is unclear why this effect was only for 2nd lactation cows as compared to all cows on treatment, but differences in overall DMI and milk production may be the cause. Cows in their 3rd and greater lactation, regardless of treatment, had statistically higher milk production ($P < 0.01$) but only tended to have higher DMI ($P = 0.10$) through wk 6 postpartum (Chapter 3). Higher milk production requires more glucose to be spared for the mammary gland, and metabolism of more fuels in the liver to support other metabolic processes (Baumgard et al., 2017). With a higher demand for glucose by the mammary gland, there would likely be less feedback of succinyl-CoA on HMGCS2, resulting in no difference between its expression in cows of 3rd lactation and greater. Overall, hepatic metabolism of cows fed HF was altered compared to cows fed

LF, with shifts of gene expression and metabolism to combat lower glucose availability and more severe negative energy balance.

Gene expression of ANGPTL4, an endocrine signal for change in energy status and a potent inhibitor of lipoprotein lipase (Kersten, 2005), showed no differences across treatments or time. Gene expression of ANGPTL4 has been shown to increase to different degrees across multiple instances of negative energy balance; in the transition from pregnancy to lactation, through feed restriction, and through administration of exogenous GH (Koltes and Spurlock, 2012). Recently, Silva et al. (2017) reported differences in ANGPTL4 expression prepartum in response to treatment with rBST; postpartum levels of ANGPTL4 expression were higher than prepartum but did not differ due to rBST treatment. Gene expression of ANGPTL4 appears to be altered due to vast changes in energy status and metabolism, it may be that the differences in energy balance between dietary treatments in this experiment, and changes in energy balance status between d 7 and 14 postpartum were not large enough to give rise to differences in ANGPTL4 expression.

While treatment did not impact gene expression of ANGPLT4, there were several significant correlations with hepatic composition and metabolism. Hepatic triglyceride content was positively correlated with ANGPTL4 gene expression. As ANGPTL4 is known to increase plasma triglycerides in mice (Kersten, 2005), and in dairy cows gene expression of ANGPTL was upregulated in coordination with increased NEFA (Koltes and Spurlock, 2012), it would follow that liver triglyceride content would increase in the dairy cow, given the relationship of NEFA uptake by the liver that is proportional to supply (Emery et al., 1992). Hepatic glycogen content,

capacity for conversion of palmitate to ASP, conversion of propionate to glucose, and the glucose:CO_2 ratio were all negatively correlated with ANGPTL4 expression. Given the relationship of ANGPTL4 expression increasing with a larger energy deficit, negative correlations with measures expected to increase in times of positive energy balance might be expected. The ratio of glucose to CO_2 tended to increase from d 7 to 14 postpartum indicating increased efficiency of utilization of propionate for gluconeogenesis (Knapp et al., 1992) as lactation progresses, while liver glycogen, which again is indicative of severity of negative energy balance (Veenhuizen et al., 1991), was also increased from d 7 to 14. These changes in hepatic metabolism occur as negative energy balance is reaching and beginning to rebound from a nadir, though energy balance did not reach a positive status in the 6 wk of this experiment (Chapter 3). Koltes and Spurlock (2012) demonstrated that ANGPTL4 gene expression was similar at d 5 and 21 postpartum, but had decreased by 150 DIM when energy balance was calculated to be positive.

Correlation analysis between variables related to hepatic metabolism and composition, and gene expression of IGF-I, GHR, TNFα, and plasma haptoglobin allow us to further investigate the changes in energy balance and inflammatory status related to these metabolic shifts. Growth hormone plays a crucial role in nutrient partitioning in the early postpartum period (Lucy, 2008). During negative energy balance the GH-IGF-I axis is uncoupled due to low circulating insulin and lack of GH receptors on the liver (Kobayashi et al., 1999), to recognize and produce IGF-I which acts as an insulin sensitizing factor, and as negative feedback on GH secretion (Butler, 2003). Increases in insulin have been known to overcome this uncoupling (Butler et al., 2003), natural

increases in insulin would occur as DMI increases, after the nadir of negative energy balance. Our study shows that both GHR and IGF-I gene expression correlated positively with liver glycogen, indicating that cows with higher glycogen were likely in more positive energy balance overall in agreement with Veenhuizen et al. (1991). Gene expression of IGF-I also negatively correlated with conversion of palmitate to esterified products. As palmitate conversion to esterified products has previously correlated negatively with plasma glucose (Grum et al., 2002), and with propionate conversion to glucose in our study (data not shown) it would follow that cows with higher conversion of palmitate to esterified products would be in more severe negative energy balance, and likely the GH-IGF-I axis would remain uncoupled resulting in lower IGF-I expression and production.

Interestingly, gene expression of TNFα correlated positively with conversion of palmitate to esterified products and liver triglyceride content on d 14. Other than the aforementioned relationship with negative energy balance, liver triglyceride accumulation and fatty liver disease have been associated with altered immune status, including increased TNFα and other APP (Ametaj et al., 2005), and infectious diseases such as mastitis and metritis (Bobe et al., 2004). It is unclear whether this increased inflammatory response is part of the etiology of fatty liver, or a consequence of the alterations in metabolic hormones and metabolites affected by triglyceride accumulation. While evidence for exogenous TNFα administration increasing liver triglycerides exists for mid-lactation cows (Bradford et al., 2009), a similar study in transition cows found that exogenous TNFα administration decreased DMI, milk production, and impaired health status, but had no effect on liver triglyceride content or

blood metabolites (Yuan et al., 2013). Study of endothelial cells in vitro suggests that increased NEFA concentrations can increase pro-inflammatory cytokine release (Contreras et al., 2012), indicating increased NEFA concentration itself could be responsible for increases in inflammation accompanying increased liver triglyceride accumulation. Though the cause is not clear, inflammation and liver triglyceride accumulation appear to be closely related, as demonstrated by the positive correlations observed.

Gene expression of TNFα correlated negatively with capacity to convert propionate to CO_2, and both plasma haptoglobin and TNFα gene expression were moderately negatively correlated with capacity of the liver to convert propionate to glucose and the glucose to CO_2 ratio. Evidence for a direct role for inflammation in alteration of hepatic glucose metabolism is also mixed. Waldron et al. (2006) reported increases in plasma glucose appearance rate following intramammary LPS injection in early lactation dairy cows, Bradford et al. (2009) reported downregulation of key gluconeogenic enzymes in response to TNFα administration in mid-lactation cows, while Yuan et al. (2013) found no differences in measures of glucose metabolism and gluconeogenic enzymes with TNFα administration in early lactation cows. There is, however, strong evidence that increased liver lipid content, fatty liver, and ketosis can downregulate hepatic gluconeogenesis, including downregulation of key enzymes and decreased capacity to convert propionate to glucose (Mills et al., 1986, Veenhuizen et al., 1991, Rukkwamsuk et al., 1999, Bobe et al., 2004). Given the positive relationship of TNFα gene expression and liver triglyceride content, it is likely that these effects could be attributed to the altered lipid metabolism its impacts on hepatic glucose

metabolism. Though haptoglobin was not correlated with any other measures of hepatic metabolism in this experiment, it has been associated with increased liver triglycerides and fatty liver (Katoh, 2002), and with decreased milk production previously (Huzzey et al., 2015). To our knowledge, there have been no previous associations of haptoglobin directly with glucose metabolism and gluconeogenic capacity of the liver, while it could be explained by associations with liver triglycerides, inflammatory status, and associated decreases in liver function overall, this potential link should be investigated further.

As mentioned previously, inflammation is a natural process and is necessary for the cow to undergo and recover from calving, however prolonged inflammation has been associated with many alterations in metabolism and health status (Bradford et al., 2015). It follows then, that the majority of correlations between inflammatory markers and altered metabolism occurred on d 14 rather than at d 7. Increases in these markers at d 7 might be associated with recovery from calving, however increases sustained to d 14 could be indicative of unresolved subacute inflammation, or inflammation linked to some other injury, or due to shifted metabolic status. Overall, gene expression of GHR, IGF-I, TNFα and plasma haptoglobin correlated with aspects of liver composition and hepatic metabolism that are closely related to energy balance and metabolic state.

CONCLUSIONS

In this experiment, levels of $uNDF_{240}$ (9.5% of DM; 0.27% of BW) and peNDF (21.6% of DM) achieved in the LF diet were adequate to promote optimal hepatic metabolism in early lactation cows whereas the levels in the high fiber diet ($uNDF_{240}$ at 12.2% of DM, 0.32% of BW, and 23.2% peNDF) limited DMI and performance during

early lactation. It is important to note that, as a result of variation in forage composition, actual $uNDF_{240}$ levels were higher than intended, and the LF diet in the current study was intermediate to the levels previously determined to cause metabolic dysfunction or highly productive cows. Overall, cows fed increased levels of $uNDF_{240}$ and peNDF resulted in restricted in intake and subsequently altered hepatic metabolism and gene expression. Alterations in hepatic metabolism and gene expression were mostly related to fatty acid metabolism, indicating increased hepatic fatty acid load and increased liver triglyceride accumulation. It appears that $uNDF_{240}$, likely plays an important role in regulating intake in the early fresh period, which can greatly impact hepatic metabolism, this area warrants further investigation.

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CHAPTER 5

INFLUENCE OF INFLAMMATION AND ACUTE PHASE PROTEINS ON REGULATION OF GROWTH HORMONE-IGF-I AXIS IN PERIPARTURIENT DAIRY COWS

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ABSTRACT

Our objective was to investigate impacts of inflammation and the regulation of the growth hormone (GH)-insulin like growth factor-I (IGF-I) axis and hepatic metabolism when feeding increased undigested NDF ($uNDF_{240}$, NDF undigested at 240-h of fermentation *in vitro*) and physically effective NDF (peNDF) in fresh cow diets containing higher levels of starch. Multiparous Holstein cows (n=56) were assigned randomly to one of two higher starch postpartum diets with differing content of $uNDF_{240}$ and peNDF. The two treatment diets, high fiber (HF, n = 27) or low fiber (LF, n = 29), were formulated to differ in $uNDF_{240}$ (12.2% DM vs. 9.5% DM, respectively) and peNDF (23% DM vs. 21% DM, respectively) content. Cows were enrolled 28 d prior to expected parturition and individually fed a common dry cow diet in tie-stalls. Upon parturition cows were individually fed their respective treatment diets through 28 DIM, at 29 DIM all cows were fed the LF diet through 42 DIM. Liver biopsies were obtained from a subset of 40 cows (HF n = 19, LF n = 21) on d 7 ± 1.1 (mean ± SD) and 14 ± 1.0 postpartum and used for gene expression analysis of key metabolic enzymes and inflammatory markers. Blood samples were used for DNA genotyping of polymorphisms in the genes of GH receptor (GHR), IGF-I, and tumor necrosis factor α (TNFα). Cows fed HF had lower DMI in wk 2 through 4, energy balance was lower overall, and milk yield was lower in wk 3 and 4 postpartum compared to cows fed LF. Despite this difference in energy balance, there were no effects of dietary treatment on gene expression of GHR, IGF-I, TNFα or TNF receptor (TNFRSF1a), and genotypes of GHR, IGF-I and TNFα did not influence production or DMI. Though dietary treatment did not alter gene expression, a treatment by time effect

was apparent for plasma IGF-I concentration. Correlation analysis revealed GHR and IGF-I correlate positively with measures of improved energy balance and liver health and negatively with metabolic enzymes and liver acute phase proteins which are associated with negative energy balance and inflammatory conditions. Gene expression of TNFα and TNFRSF1a correlated positively with metabolic enzymes that are upregulated during negative energy balance and positive acute phase proteins, while correlating negatively with blood glucose, negative acute phase proteins and milk production. Metabolism, energy balance and inflammation are linked in the early postpartum period.

INTRODUCTION

The transition from late pregnancy to early lactation is a time of great metabolic adaptation and change for the dairy cow. Energy requirements dramatically increase with the onset of milk production, accompanied by low dietary energy supply caused by low DMI, thus animals enter a state of negative energy balance (NEB; Bell, 1995, Grummer et al., 2004). Glucose demands increase over 2-fold compared to late pregnancy and the majority of glucose is spared for lactose synthesis, making the peripheral systems reliant on utilization of energy derived from adipose tissue mobilization and fatty acid catabolism (Bauman and Currie, 1980, Baumgard et al., 2017).

Growth hormone (GH) plays a crucial role in the nutrient partitioning of the transition period (Lucy, 2008). Concentrations of GH increase prepartum, reaching a peak around calving and remain elevated for several weeks postpartum (Bell, 1995). During this time, the liver is refractory to circulating GH, as the gene expression and

number of GH receptors (GHR) on the liver are greatly decreased, resulting in low plasma concentration of insulin like growth factor–I (IGF); this altered state is referred to as uncoupling the GH-IGF-I axis (Radcliff et al., 2003a). This uncoupling is necessary for nutrient partitioning during NEB, as high circulating GH promotes lipolysis and proteolysis that supply glucose precursors and fatty acids to the liver, which would be downregulated with subsequent increases in IGF-I (Bell and Bauman, 1997, Bell et al., 2000).

As energy balance begins to become more positive due to increased DMI, and therefore increased plasma glucose and insulin concentrations, gene expression and number of GHR on the liver increase, increasing plasma IGF-I concentrations in response to high circulating GH (Butler et al., 2003, Radcliff et al., 2003b). This recoupling of the GH-IGF-I axis is indicative of increases in energy balance which are critical for reproductive success. Circulating IGF-I is higher during the first 2 wk postpartum of cows that were able to ovulate a dominant follicle at 16-20 DIM (Beam and Butler, 1997), and this early ovulatory success can increase pregnancy rate, as cows that successfully ovulated a follicle by 50 DIM had 30% higher pregnancy rate at 200 DIM compared to cows that did not (Butler, 2003). Genotype of GHR has been found to positively impact both circulating IGF-I concentrations and calving to conception interval (Schneider et al., 2013), indicating that genetic differences could play a role in energy balance regulation and subsequent reproductive success.

Accompanying these metabolic shifts during the periparturient period is increased inflammation. Though inflammation is critical for tissue recovery and uterine involution following parturition, excessive and prolonged inflammation is associated

with decreased production and increases in disease, both infectious and metabolic (Bobe et al., 2004, Bertoni and Trevisi, 2013, Bradford et al., 2015). Increases in circulating concentrations of the acute phase protein haptoglobin has been associated with increases in periparturient disorders and decreased milk production (Humblet et al., 2006, Huzzey et al., 2009, Huzzey et al., 2015), and increased or prolonged uterine inflammation is associated with more severe NEB and decreased reproductive performance (LeBlanc, 2012).

Impacts of diet that affect DMI and subsequently energy balance likely alter metabolic changes and inflammation, as they are all invariably linked in the transition period. In postpartum diets with high levels of dietary starch, milk production and metabolism can be increased, though these diets can also increase in inflammation due to risk of subacute ruminal acidosis (Stone, 2004, McCarthy et al., 2015a, McCarthy et al., 2015b). Dietary levels of undigested NDF ($uNDF_{240}$, NDF undigested at 240-h of fermentation *in vitro*) have been recently investigated and may play a role in rumen stabilization and intake regulation (Fustini et al., 2017, Grant et al., 2018), though consensus in the research is not clear and research in transition cows is lacking. Thus, the objective of this study was to investigate impacts of inflammation and the acute phase response on regulation of GH-IGF-I axis and hepatic metabolism when feeding increased $uNDF_{240}$ and physically effective NDF (peNDF) in fresh cow diets containing higher levels of starch.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

All animal protocols were approved by the Cornell University Institutional Animal Care and Use Committee (protocol #2015-0024). Experimental design and treatments are described in more detail in the companion paper (Chapter 3), and described briefly here. Multiparous Holstein cows (n=60) were enrolled in a randomized design study between July and September 2015 with randomization of treatment assignment blocked to control for lactation number and previous 305-d mature equivalent milk production. Cows were moved to tie-stalls 28 d prior to expected parturition and fed a common close-up dry cow diet through parturition, after which cows were fed their assigned treatment diets (Table 5-1), high fiber (**HF**) or low fiber (**LF**), which were formulated to differ in $uNDF_{240}$ (12.2 vs. 9.5% of DM, respectively) and peNDF (23 vs. 21% of DM, respectively) content. Four cows were removed completely from analysis and 3 cows were partially removed by researcher blinded to treatment for severe illness or death which were unrelated to treatment prior to data analysis, resulting in a final dataset including 56 cows; 27 cows fed HF and 29 cows fed LF. Average lactation number of cows fed HF was 2.64, with 14 cows in their 2^{nd} lactation and 13 cows in their 3^{rd} and greater lactation. Average lactation number of cows fed LF was 2.74, with 17 cows in their 2^{nd} lactation and 12 cows in their 3^{rd} and greater lactation. A subset of 40 cows were subjected to liver biopsy at 7 ± 1.1 (mean ± SD) and 14 ± 1.0 DIM and all subsequent analyses which are the focus of this paper. A sample size calculation determined 16 cows per treatment would detect a difference of 1 standard deviation from the mean for conversion of propionate to glucose with 95% confidence and 80% power based on data from previous studies in our lab (McCarthy et

al., 2015b). Eligibility for liver biopsy was dependent on DIM at the time of biopsy, and was balanced for treatment as biopsies were conducted. Average lactation number for biopsied cows fed LF (n = 21) was 2.71, with 9 cows in 2nd lactation and 12 cows in their 3rd and greater lactation. Average lactation number for biopsied cows fed HF (n = 19) was 2.58 with 10 cows in 2nd lactation and 9 cows in their 3rd and greater lactation.

Diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS, version 6.5, Cornell University, Ithaca, NY; Van Amburgh et al., 2015). The HF and LF treatment diets were fed from parturition until 28 DIM, after which all cows received the LF diet until the end of the study at 42 DIM. Approximately one month after the first cow on study calved (20% of all postpartum days cows were fed), the diets were reformulated to better reach our target for starch in the diets increasing the amount of corn meal and canola meal, decreasing the amount of grain mixture in both fresh diets. All forage amounts remained as originally formulated throughout the study. A weighted average of formulated diet ingredient composition (weighted to account for diet reformulation), forage analysis, and nutrient analysis of diets can be found in Table 5-1, Table 5-2, and Table 5-3 respectively. Detailed animal feeding procedures, feed sampling and analysis information are detailed in Chapter 3.

Plasma Sampling and Analysis

Blood samples were collected via coccygeal vessels prior to fresh feed delivery between 0530 and 0730 h 2x/wk from d 28 prior to expected parturition through parturition, daily from 1 DIM through 7 DIM, 3x/wk from 7 through 21 DIM, and 2x/wk from 21 DIM through 42 DIM. Samples were collected using 10-mL sodium heparin evacuated tubes (158 USP, Becton Dickinson and Company, Franklin Lakes

NJ), using 20 ga vacutainer needles (Becton Dickinson and Company). Whole blood was harvested, placed on ice immediately after collection, and centrifuged for 20 min at 2,000 × *g* at 4ºC. Plasma was aliquoted into 1.7 mL microfuge tubes, snap frozen in liquid N_2 and stored at -20ºC until analysis. Plasma samples taken on the day corresponding with liver biopsy were sent to a commercial diagnostic lab for analysis (University of Guelph Animal Health Laboratory, Guelph, ON, Canada) of haptoglobin based on peroxidase activity (Makimura and Suzuki, 1982, Skinner et al., 1991), and aspartate aminotransferase (AST), cholesterol, glutamate dehydrogenase (GLDH), gamma-glutamyltransferase (GGT), bilirubin, total protein, albumin, globulin, and albumin:globulin ratio on a Roche Cobas 6000 c501 biochemistry analyzer (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. For all assays intra-assay CV was maintained below 5%, for all assays except for haptoglobin, inter-assay CV was maintained below 5%, while haptoglobin inter-assay CV was 10.2%.

Plasma IGF-I concentrations were assayed using an radioimmunoassay procedure and following the extraction procedure described previously (Butler et al., 2003, Butler et al., 2004). DNA genotyping was completed from blood samples for all IGF-I, GHR, and TNFa single nucleotide polymorphisms (SNP) using PCR-restriction fragment length methods, as described previously (Higuchi et al., 1999, Hax et al., 2017). Primers, annealing temperature, fragment lengths, and enzymes used can be found in Table 5-4. The genotype of each animal was determined by the fragment size reported as base pairs. The genotypes identified for IGF-I were: *Sna*BI (A/A) 249bp, *Sna*BI (A/B) 249bp, 226 bp, *Sna*BI (B/B) 226bp (Ge et al., 2001). The genotypes

identified for GHR were: AluI (A/A) 747 bp, 75 bp; AluI (A/B) 747 bp, 602 bp, 145 bp, 75 bp; *Alu*I (B/B) 602 bp, 145 bp, 75 bp (Aggrey et al., 1999). The genotypes identified for TNFa were: Allele A: 1233bp, Allele B: 928 bp, 305 bp (Higuchi et al., 1999).

Analysis of plasma NEFA concentrations [HR Series NEFA HR (2), Wako Pure Chemical Industries, Osaka, Japan], were conducted in triplicate using commercial enzymatic kits, as described in Leno et al. (2017). Plasma glucose concentrations were determined by enzymatic analysis (glucose oxidase; protocol from kit 510-A; Sigma-Aldrich, St. Louis, MO) using commercial products (PGO Enzyme Preparation and o-dianisidine dihydrochloride, Sigma-Aldrich) as described in McCarthy et al. (2015c). Coefficients of variation for all assays (both inter- and intra-assay) were maintained below 10%.

Liver Biopsy and Gene Expression

Liver samples (3-5 g) were obtained via percutaneous trocar biopsy (Veenhuizen et al., 1991) from 40 cows on day 7 ± 1.1 and 14 ± 1.0 (mean ± SD) postpartum from cows under local anesthesia. After blotting liver sample to remove excess blood, part of the sample was snap-frozen immediately in liquid N_2 and stored at -80º C for subsequent RNA extraction for hepatic gene expression analysis.

Total RNA was isolated and purified using miRNeasy minicolumns and on-column ribonuclease-free deoxyribonuclease treatment (QIAGEN Inc., Hilden, Germany). Concentration and integrity of RNA was determined using the 2100 Bioanalyzer system and RNA 6000 Nano kit (Agilent, Santa Clara, CA). Concentration of RNA ranged from 365 to 1,855 ng/µL and all RIN values were greater than 6.0 (range 6.1 to 8.9). Reverse transcriptase reactions were performed with 2 µg of RNA in

a 20-µL volume using the high capacity cDNA reverse transcription kit with RNase Inhibitor (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendation. For all genes, Taqman probes were selected to span exon junctions and not detect genomic DNA, all probes were purchased from Applied Biosystems (Foster City, CA): insulin-like growth factor one (IGF-I; assay Bt03252282), growth hormone receptor (GHR; assay Bt03216844), tumor necrosis factor alpha (TNFα; assay Bt03259156) and tumor necrosis factor alpha receptor super family one alpha (TNFSRF1a; assay Bt03215763). Two reference genes were selected to run on each plate; TATA box binding protein (TBP, assay Bt03241948) and large ribosomal protein P0 (RPLP0, assay Bt03218086). Quantitative PCR reactions were conducted in duplicate using Taqman Fast Advanced master mix with 10-fold diluted cDNA as 10% of the reaction volume on a Step One Plus instrument (Applied Biosystems, Foster City, CA) according to manufacturer's recommendations set to 95°C for 1 sec (denaturation) and 20 sec at 60°C (annealing and extension), with a single UNG incubation period of 2 min at 50°C and polymerase activation of 2 min at 95°C prior to cycling. Negative control reactions lacking template were included for every gene on every plate, and a single pool of RNA from a liver sample in this study was used as calibrator cDNA used on all plates for the control following the $2^{-\Delta\Delta Ct}$ method, and results were expressed as a relative quantity. All above methods were utilized to determine expression of four additional genes for use in correlation analysis: angiopoietin-like 4 (ANGPTL4; assay Bt03234241), carnitine palmitoyltransferase 1A (CPT1A; assay Bt04945715), mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2, assay Bt03233810), and pyruvate carboxylase (PC, assay Bt03244510).

Statistical Analysis

Daily means for DMI, milk yield, and rumination were averaged by week prior to analysis. To standardize blood metabolite data, day of blood samples collected 2x/wk or 3x/wk were averaged to reference days prior to analysis. Body weight change and BCS change were calculated as the difference from wk 1 to 4. All statistical analyses were conducted using SAS (version 9.4, SAS Institute Inc., Cary, NC). All data collected over time were analyzed as repeated measures ANOVA using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). Fixed effects of treatment, time, parity group (2nd vs. 3rd and greater), genotype for GHR, IGF, and TNFα, and all 2-way interactions were included in the model. A variable to account for the diet change that occurred part way through the study was also included in all models. Cow nested within treatment was the random effect. Measurements collected during the week of enrollment were included in all models when available as covariates. Previous lactation 305-d mature-equivalent milk yield was used as a covariate for milk production. Denominator degrees of freedom were estimated using the Kenward Rogers method. Five covariance structures were tested for each model: first-order autoregressive, heterogeneous first-order autoregressive, compound symmetry, heterogeneous compound symmetry, and unstructured; and the covariance structure with the lowest Akaike's information criterion was used (Littell et al., 1996). In instances of 2-way interaction with time where $P \leq 0.10$, the slice option was added to the model to perform a partitioned analysis of the means of the interaction, producing an F-test for each level to determine at which levels treatments differed. In instances of a 2-way interaction without time, the Tukey-Kramer adjustment was used to correct for

multiple comparisons. Residual analysis was performed to examine normality and homogeneity of residuals, when non-normality of residual variance was evident, data were transformed (either log-transformed or square root transformation) and analysis repeated. Body weight change data, the only measurement not repeated over time, were analyzed using ANOVA in the MIXED procedure of SAS. All data presented are least squares means and standard error (SE), except in the case of transformed data (haptoglobin, GLDH, AST, and gene expression of; IGF-I and TNFα) where geometric means with back-transformed 95% confidence intervals (CI) are reported. Significance was declared at $P \leq 0.05$, and trends discussed at $0.05 < P \leq 0.15$. Twenty-three variables (Table 5-5) were chosen to test in Pearson correlation in SAS, significance was declared at $P \leq 0.05$.

RESULTS

Table 5-6 contains the count of cows with each SNP of IGF-I, GHR, and TNFa for cows that underwent a liver biopsy and are included in gene expression data. Table 5-7 contains the SNP count within each genotype for all cows in the study that was utilized for plasma IGF-I analysis.

Performance results were described in detail in companion Chapters 3 and 4, but are reported here to help characterize the animals and effects of treatment. Results related to cow performance are found in Table 5-8, there were no differences ($P > 0.15$; data not shown) due to genotype of GHR, IGF-I, or TNFα on any cow performance parameters. Performance results due to treatment are the same as described in Chapter 4, but will be described here briefly. Cows fed the HF diet had lower DMI compared to cows fed LF in wk 2 through 4 postpartum (trt × time $P = 0.002$). Intake of starch was

higher for cows fed LF compared to cows fed HF (5.24 vs. 4.79 kg/d; $P < 0.01$), and differed by time such that cows fed LF had higher intake of starch in wk 2 through 4 postpartum as compared with cows fed HF. A treatment by time interaction was also present for intake of aNDFom such that cows fed HF tended to have higher intake (kg/d) of aNDFom in wk 1 (6.07 vs 5.66 ± 0.16 kg/d; $P = 0.07$) and lower intake of aNDFom intake in wk 4 (7.35 vs 7.71 ± 0.14 kg/d; $P = 0.08$) compared to cows fed LF. Intake of $uNDF_{240}$ was higher for cows fed HF compared to cows fed LF expressed as both total intake (2.37 vs 2.00 kg/d; $P < 0.01$) and as percent of BW (0.32 vs. 0.27; $P < 0.01$). Despite differences in DMI, there were no differences observed for rumination or BCS, though cows fed HF lost more BW from wk 1 to wk 4 postpartum (-46.2 vs. -24.5 kg; $P = 0.03$) compared to cows fed LF. A treatment by week interaction was present for milk yield ($P = 0.04$) such that milk yield was lower for cows fed HF than cows fed LF during wk 3 and 4 postpartum, and a trend for treatment by week interaction for ECM such that ECM tended ($P = 0.10$) to be lower for cows fed HF than those fed LF during wk 4. Postpartum energy balance was lower for cows fed HF compared to cows fed LF (-10.2 vs -7.6 Mcal/d; $P = 0.05$).

Plasma chemistry variables on days corresponding to liver biopsy, and plasma IGF-I concentrations throughout the experiment are reported in Table 5-8. There was a tendency for a treatment by time interaction for plasma IGF-I concentration (Figure 5-1; $P = 0.08$); cows fed LF tended to have higher IGF-I compared to cows fed HF during wk 3 postpartum (85.3 vs 64.6 ng/mL; $P = 0.11$). Differences in liver health and inflammatory markers due to treatment were described in Chapter 4. There were no interactions of treatment and genotype of any gene, but haptoglobin tended to differ

with GHR genotype and TNFα genotype influenced plasma bilirubin and cholesterol as reported in Figure 5-2. The cow (n = 1) with GHR SNP AB tended (P = 0.09) to have lower plasma haptoglobin than cows with GHR SNP AA. Cows with TNFα SNP of AA had higher concentrations of plasma bilirubin compared to cows with SNP BB (P = 0.03), and had lower plasma cholesterol than cows with SNP AB or BB (P = 0.03).

There were no differences of treatment (data not shown; $P > 0.15$) on IGF-I, GHR, TNFα, and TNFRSF1a hepatic gene expression. Genotype effects on gene expression of IGF-I, GHR, TNFα, and TNFRSF1a are reported in figure 5-3. Gene expression of IGF-I tended to be influenced by both GHR and TNFα genotypes (P = 0.06, P = 0.11 respectively). For GHR, genotype AB had higher gene expression of IGF-I as compared to those with AA or BB. For TNFα, genotype AB exhibited lower gene expression of IGF-I when compared with genotype BB. Gene expression of GHR was only influenced by GHR genotype such that the cow (n = 1) with genotype AB tended (P = 0.11) to have higher gene expression of GHR than genotypes AA or BB. Gene expression of TNFα did not differ due to any genotypes, but it did differ over time. As cows progressed from d 7 to d 14 in lactation, hepatic TNFα gene expression increased ($P < 0.01$). Gene expression of TNFRSF1a was similar to TNFα in that only time influenced gene expression. From d 7 to 14 in lactation TNFRSF1a gene expression tended to increase (P = 0.12).

Pearson correlations between hepatic gene expression of IGF-I, GHR, TNFα, and TNFRSF1a, with markers of liver health and inflammation are reported in Table 5-10. Gene expression of IGF-I and GHR correlated negatively with gene expression of PC, CPT1a, and ANGPTL4 on d 7 postpartum, and correlated positively with energy

balance on d 7 postpartum and gene expression of HMGCS2 on d 14 postpartum. Gene expression of TNFα positively correlated with gene expression of CPT1a on d 14, and expression of TNF on d 14 correlated positively with ANGPTL4 expression on d 7 postpartum. Gene expression of TNFα also correlated positively with plasma NEFA on d 14 postpartum, and negatively with plasma glucose at the same timepoint. Gene expression of TNFRSF1a correlated positively with gene expression of PC and ANGPTL4 on d 7 and negatively with plasma NEFA content on d 7, and gene expression of HMGCS2 on d 14. Gene expression of TNFRSF1a on d 7 also correlated negatively with average 14 d milk yield. Plasma IGF-I moderately correlated positively with plasma glucose on d 7 (data not shown, r = 0.48; $P < 0.01$), though no other correlations were observed for plasma IGF-I.

Pearson correlations between hepatic gene expression of IGF-I, GHR, TNFα, and TNFRSF1a, and hepatic gene expression of PC, CPT1A, HMGCS2, and ANGPTL4, and measures of energy balance and metabolism are reported in Table 5-11. Gene expression of IGF-I correlated positively with plasma albumin and cholesterol on d 7. Gene expression of IGF-I on d 7 and GHR on d 14 were correlated negatively with plasma globulin and bilirubin on d 7 and positively with albumin to globulin ratio. Gene expression of TNFα on d 14 correlated positively with bilirubin at d 7, though gene expression of TNFRSF1a on d 7 correlated negatively to bilirubin a. Gene expression of TNFRSF1a at d 14 was negatively correlated with plasma albumin at d 7 and a positively correlated with plasma haptoglobin at d 14. Gene expression of TNFRSF1a correlated negatively with GGT on d 7, and d 14 expression of TNFRSF1a negatively with AST and GLDH on d 7.

Table 5-1. Formulated ingredient composition of the common prepartum diet and postpartum treatment diets formulated to differ in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF).

		Postpartum Diet[1]	
Ingredient, % of DM	Prepartum Diet	LF [1]	HF [1]
Conventional corn silage	45.19	42.31	38.46
Alfalfa hay	-	10.58	10.58
Wheat straw	20.84	1.15	8.65
Corn meal	2.43	17.64	20.51
Soybean meal	-	6.03	4.72
Canola meal	3.48	4.52	3.88
Wheat middlings	-	4.82	1.62
Amino Plus[2]	5.91	4.35	5.32
Corn gluten feed	1.74	1.60	0.47
Blood meal	2.43	0.95	1.09
Soybean hulls	6.95	2.42	-
Citrus pulp	4.52	-	0.79
Energy Booster[3]	-	1.29	1.57
Rumensin[4]	0.10	0.05	0.05
Salt	-	0.48	0.47
Ca carbonate	2.26	1.13	1.18
Mg oxide	-	0.28	0.28
Animate[5]	3.13	-	-
Alimet[6]	0.07	-	-
Vitamin and mineral mix[7]	0.94	0.40	0.39

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM). Treatment diets were fed from wk 1 to 4 postpartum.

[2] Heat-treated soybean meal, Ag Processing Inc., Omaha, NE.

[3] Commercial fat source, Milk Specialties Global, Eden Prairie, MN.

[4] Premix contained 26,400 g/t of monensin, Elanco Animal Health, Greenfield, IN.

[5] Anionic mineral supplement, Phibro Animal Health Corporation, Teaneck, NJ.

[6] 2-Hydroxy-4-(methylthio)-butanoic acid, Novus International, Saint Charles, MO.

[7] Prepartum mix contained 3,754 mg/kg Zn, 993 mg/kg Cu, 4,658 mg/kg Mn, 28.9 mg/kg Se, 82.8 mg/kg Co, 82.4 mg/kg I, 1,177 KIU/kg Vitamin A, 253 KIU/kg Vitamin D, and 55,784 IU/kg Vitamin E. Postpartum mix contained 25,560 mg/kg Zn, 7,154 mg/kg Cu, 21,958 mg/kg Mn, 214 mg/kg Se, 507 mg/kg Co, 331 mg/kg I, 3,704 KIU/kg Vitamin A, 922 KIU/kg Vitamin D, and 12,496 IU/kg Vitamin E (Central New York Feeds, Jordan, NY).

Table 5-2. Forage composition (DM basis except where noted) as analyzed by wet chemistry and *in-vitro* analysis by a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) on one composite sample for each forage representative of the entire study period.

Item	Corn silage	Alfalfa hay	Wheat straw
DM, % of as fed[1]	26.8 ± 1.4	86.0 ± 1.5	87.2 ± 2.1
CP, %	7.8	17.0	4.9
ADF, %	27.0	38.1	54.5
aNDFom, %	43.6	45.6	77.8
Starch, %	31.6	1.2	0.8
Sugar, %	1.0	7.1	2.5
Fat, %	2.2	1.2	0.9
In-vitro digestibility analysis			
30-h NDFD[2], %	26.7	16.5	27.5
30-h NDFD, % of NDF	57.9	37.6	34.2
$uNDF_{30}$[3], %	19.4	27.4	53.0
$uNDF_{30}$, % of NDF	42.1	62.4	65.8
$uNDF_{240}$[4], %	12.4	24.1	35.4
$uNDF_{240}$, % of NDF	27.0	54.9	44.0

[1]DM was analyzed (dried at 40º C in a forced air oven for 96 h) on fresh forages weekly throughout the study and further corrected for residual moisture using DM obtained from Cumberland Valley Analytical Services upon wet chemistry analysis of each ground composite sample.

[2] NDF digestibility, determined via *in vitro* fermentation

[3] Undigested NDF at 30-h of *in vitro* fermentation

[4] Undigested NDF at 240-h of *in vitro* fermentation

Table 5-3. Nutrient profile of all diets (mean ± SD), obtained through wet chemistry analysis and in vitro fermentation by Cumberland Valley Analytical Services (Hagerstown, MD), predicted by Cornell Net Carbohydrate and Protein System (version 6.5, Cornell University, Ithaca, NY), and obtained with a Penn State Particle Separator (Nasco Inc., Fort Watkinson, WI).

	Diet		
Item	Prepartum	Low Fiber (LF)[1]	High Fiber (HF)[1]
DM, % as fed[2]	45.9 ± 1.2	44.4 ± 2.2	47.4 ± 2.0
ADF, % DM	29.0 ± 0.5	21.3 ± 1.1	22.9 ± 2.1
aNDFom, % DM	43.1 ± 0.3	32.8 ± 1.4	35.3 ± 2.3
30-h NDFD[3], % DM	24.2 ± 0.5	20.2 ± 0.5	19.1 ± 1.7
30-h NDFD, % NDF	54.4 ± 1.5	59.5 ± 1.0	52.6 ± 2.2
$uNDF_{240}$[4], % DM	12.8 ± 0.5	9.5 ± 0.4	12.2 ± 1.6
CP, %	14.1 ± 0.8	15.5 ± 0.5	13.8 ± 1.4
Starch, %	15.7 ± 0.3	24.9 ± 1.7	24.6 ± 2.3
Sugar, %	3.5 ± 0.4	5.1 ± 0.7	3.9 ± 0.1
Fat, %	2.3 ± 0.2	3.3 ± 0.2	3.2 ± 0.2
peNDF[5], %	33.3[6]	21.6[7]	23.2[7]
MP[6], g/kg DM	89.0	112.1	108.0
NE_L, Mcal/kg	1.45 ± 0.02	1.65 ± 0.03	1.61 ± 0.02
Ca, %	1.46 ± 0.07	0.97 ± 0.12	0.98 ± 0.19
P, %	0.32 ± 0.02	0.41 ± 0.01	0.37 ± 0.01
Mg, %	0.51 ± 0.02	0.40 ± 0.04	0.39 ± 0.04
K, %	1.16 ± 0.02	1.24 ± 0.03	1.21 ± 0.04
S, %	0.39 ± 0.02	0.30 ± 0.02	0.26 ± 0.02
Na, %	0.13 ± 0.01	0.26 ± 0.02	0.23 ± 0.03
Cl, %	0.67 ± 0.02	0.49 ± 0.02	0.44 ± 0.04

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM). Treatment diets were fed from wk 1 to 4 postpartum.

[2] Determined via wet chemistry from fresh weekly samples sent to Cumberland Valley Analytical Services for NIR analysis.

[3] NDF digestibility, determined via *in vitro* fermentation

[4] Undigested NDF at 240-h of *in vitro* fermentation

[5] Physically effective NDF

[6] Formulated value given by Cornell Net Carbohydrate and Protein System v. 6.5 using analyzed forage values and mean DMI for each period and treatment.

[7] Determined using a Penn State Particle Separator from two samples collected throughout the study. Value obtained by multiplying the percentage of sample retained on screens ≥ 4mm by the diet NDF content.

Table 5-4. Primers, annealing temperatures, and enzymes used to genotype insulin-like growth factor one (IGF-I), growth hormone receptor (GHR), and tumor necrosis factor alpha (TNFa) genes.

Gene	Anneal Temp[1]	Frag length[2]	Enzy[3]	Ref[4]
IGF-I primers FW: TTAAATAATTGGGTTGGAAGACTGC RV: ACCTTACCCGTATGAAAGGAATATACGT	58 °C	249 bp	*Sna*BI	Ge et al., 2001
GHR primers FW: TGCGTGCACAGCAGCTCAACC RV: AGCAACCCCACTGCTGGGCAT	66 °C	836 bp	*Alu*I	Aggrey et al., 1999
TNFa primers FW: GGGTGACTTGCTCTAACACTCATC RV: AGGCCTCACTTCCCTACATCCCTA	63 °C	1233 bp	*Rsa*I	Higuchi et al., 1999

[1]Annealing temperature, [2]Fragment Length, bp=base pair, [3] Enzyme, [4]Reference

Table 5-5. Variables used in Pearson Correlation analysis, run in SAS (version 9.4, Cary, NC).

Variable
Hepatic gene expression of IGF1 on d 7 and 14 postpartum
Hepatic gene expression of GHR on d 7 and 14 postpartum
Hepatic gene expression of TNFα on d 7 and 14 postpartum
Plasma IGF-I concentration on d 7 and 14 postpartum
Plasma Aspartate Aminotransferase on d 7 and 14 postpartum
Plasma Cholesterol on d 7 and 14 postpartum
Plasma Glutamate dehydrogenase on d 7 and 14 postpartum
Plasma Gamma-glutamyltransferase on d 7 and 14 postpartum
Plasma Haptoglobin on d 7 and 14 postpartum
Plasma Bilirubin on d 7 and 14 postpartum
Plasma Total Protein on d 7 and 14 postpartum
Plasma Albumin on d 7 and 14 postpartum
Plasma Globulin on d 7 and 14 postpartum
Albumin:Globulin ratio on d 7 and 14 postpartum
Hepatic gene expression of PC on d 7 and 14 postpartum
Hepatic gene expression of CPT1A on d 7 and 14 postpartum
Hepatic gene expression of HMGCS2 on d 7 and 14 postpartum
Hepatic gene expression of ANGPTL4 on d 7 and 14 postpartum
Calculated energy balance for wk 1 and 2 postpartum
Week average plasma NEFA for wk 1 and 2 postpartum
Weel average plasma glucose for wk 1 and 2 postpartum
Average DMI for the first 14 DIM
Average milk yield for the first 14 DIM

Table 5-6. Total number of cows determined with each single nuclear polymorphism genotype for insulin-like growth factor one (IGF-I), growth hormone receptor (GHR), and tumor necrosis factor alpha (TNFa) out of subset of cows that were liver biopsied (n = 40) for which data was utilized for gene expression and correlation analysis.

Allele Type	IGF-I Genotype	GHR Genotype	TNFα Genotype
AA	22	16	5
AB	11	1	13
BB	2	17	14

Table 5-7. Total number of cows determined with each single nuclear polymorphism genotype for insulin-like growth factor one (IGF-I) and growth hormone receptor (GHR) , and tumor necrosis factor alpha (TNFa) for use in blood IGF-I analysis.

Allele Type	IGF-I Genotype	GHR Genotype	TNFα Genotype
AA	33	21	6
AB	18	4	18
BB	3	28	22

Table 5-8. Least squares means and standard errors for DMI, uNDF intake, rumination time, BCS, BW, BW change, and energy balance for cows fed diets differing in amount of undigested NDF at 240-h of *in vitro* fermentation ($uNDF_{240}$) and physically effective NDF (peNDF) from wk 1 to wk 4 postpartum.

	Postpartum treatment[1]			*P*-Value[2]			
Item	LF	HF	SEM	Trt	W	Parity	Trt × W
DMI, kg/d	21.1	19.4	0.36	<0.01	<0.01	0.31	<0.01
Starch intake, kg/d	5.24	4.79	0.10	<0.01	<0.01	0.01	0.002
aNDFom intake, kg/d	6.90	6.88	0.13	0.44	<0.01	<0.01	0.01
$uNDF_{240}$ intake, kg/d	2.0	2.37	0.04	<0.01	<0.01	<0.01	0.23
DMI, % of BW	2.9	2.7	0.05	<0.01	<0.01	0.19	0.08
$uNDF_{240}$ intake, % of BW	0.27	0.32	0.01	<0.01	<0.01	0.43	0.06
Rumination, min/d	535	538	8.5	0.58	<0.01	0.61	0.35
BCS	3.19	3.16	0.03	0.23	<0.01	0.79	0.50
BCS change	-0.26	-0.30	0.3	0.38	-	0.02	-
BW, kg	726	719	5.1	0.16	<0.01	0.41	0.10
BW change, kg	-24.5	-46.2	6.8	0.03	-	0.48	-
Milk yield, kg/d	44.1	41.7	0.9	0.08	<0.01	<0.01	0.04
ECM [3], kg/d	47.2	46.0	1.1	0.55	<0.01	<0.01	0.10
Energy balance[4], Mcal/d	-7.58	-10.2	0.83	0.05	<0.01	0.05	0.50

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27). Treatment diets were fed from wk 1 to 4 postpartum.

[2] All two way interactions were analyzed, interaction terms not presented were non-significant ($P > 0.10$) for all variables Trt = treatment; W= week

[3] ECM = (0.327 × kg wk average milk yield) + (12.95 × kg of fat) + (7.95 × kg of true protein)

[4] Energy balance calculated using NRC (2001) equations

Table 5-9. Geometric means and back transformed 95% confidence intervals of plasma haptoglobin, glutamate dehydrogenase (GLDH), aspartate dehydrogenase (AST), and least squares means and standard errors of plasma cholesterol, gamma glutamyl tranferase (GGT), bilirubin, total protein (TP), albumin, globulin, and insulin like growth factor-I (IGF-I) from cows fed diets differing in amount of undigested NDF at 240-h of in vitro fermentation (uNDF240) and physically effective NDF (peNDF) in the postpartum period.

	Postpartum treatment[1]			*P*-Value[2]				
Item	LF	HF	SEM	Trt	D	P	Trt × P	Trt × D
Haptoglobin, g/L	0.19 (0.17-0.23)	0.19 (0.17-0.22)	-	0.83	0.07	0.59	0.01	0.83
GLDH, U/L	12.0 (9.56-15.1)	15.5 (12.2-19.7)	-	0.13	<0.01	0.72	0.91	0.72
AST, U/L	53.3 (48.4-58.7)	54.9 (49.6-60.8)	-	0.66	0.57	0.83	0.96	0.94
Cholesterol, mmol/L	2.56	2.66	0.11	0.52	<0.01	0.19	0.98	0.80
GGT, U/L	22.8	24.6	0.90	0.17	<0.01	0.19	0.75	0.26
Bilirubin, µmol/L	1.89	1.75	0.16	0.53	0.33	0.06	0.88	0.79
TP, g/L	76.3	76.0	1.05	0.84	<0.01	0.01	0.03	0.03
Albumin, g/L	36.2	37.0	0.46	0.18	<0.01	0.36	0.18	0.63
Globulin, g/L	40.5	39.0	1.24	0.40	<0.01	0.02	0.02	0.02
Albumin: Globulin	0.90	0.99	0.04	0.10	<0.01	0.07	0.02	0.10
Plasma IGF-I, ng/mL	80.1	69.7	11.2	0.30	<0.01	0.18	0.65	0.08

[1] Treatments: low fiber (**LF;** $uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 21), high fiber (**HF;** $uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 19). Treatment diets were fed from wk 1 to 4 postpartum.

[2] All two way interactions were analyzed, interaction terms not presented were non-significant ($P > 0.15$) for all variables. Trt = treatment, P = parity group (2^{nd} lactation vs 3^{rd} and greater lactation), D = day of biopsy (d7 vs d14 except for IGF-I, factor of time was for weekly samples through 1-42 DIM)

Table 5-10. Pearson correlations[1] between insulin-like growth factor one (IGF-I), growth hormone receptor (GHR), tumor necrosis factor alpha (TNFa), and TNFa receptor superfamily 1a (TNFRSF1a) hepatic expression and plasma variables related to liver health from liver biopsies on d 7 ± 1.1 and d 14 ± 1.0 (mean ± SD) postpartum.

		Hepatic Gene Expression							
		IGF1		GHR		TNFa		TNFRSF	
		d 7	d 14	d 7	d 14	d 7	d 14	d 7	d 14
		-0.090	-0.059	-0.002	0.031	-0.173	-0.131	-0.273	-0.354
	d 7	0.58	0.72	0.99	0.85	0.28	0.42	0.09	0.02
		-0.068	-0.040	-0.020	-0.013	-0.234	-0.171	-0.210	-0.296
AST[2]	d14	0.68	0.81	0.90	0.94	0.15	0.29	0.19	0.06
		0.431	0.356	0.199	0.171	-0.110	-0.033	0.006	0.070
	d 7	0.01	0.02	0.28	0.29	0.50	0.84	0.97	0.67
		0.312	0.243	0.061	0.117	-0.184	-0.081	0.063	-0.001
Cholesterol	d14	0.05	0.13	0.71	0.47	0.25	0.62	0.70	0.99
		-0.036	0.133	0.173	0.254	-0.218	-0.238	-0.254	-0.347
	d 7	0.82	0.41	0.29	0.11	0.18	0.14	0.11	0.03
		-0.110	-0.135	-0.071	-0.18	-0.175	-0.187	-0.270	-0.23
GLDH[3]	d14	0.50	0.41	0.66	0.27	0.28	0.25	0.09	0.15
		0.285	0.084	0.122	0.055	-0.199	-0.289	-0.360	-0.227
	d 7	0.07	0.61	0.45	0.73	0.22	0.07	0.02	0.16
		0.101	0.031	-0.057	-0.179	-0.255	-0.256	-0.274	-0.134
GGT[4]	d14	0.53	0.85	0.72	0.27	0.11	0.11	0.09	0.41
		-0.273	0.047	-0.035	-0.136	-0.011	0.001	0.326	0.007
	d 7	0.09	0.77	0.83	0.40	0.95	0.99	0.04	0.96
		-0.184	-0.047	-0.039	-0.234	0.069	0.140	0.220	0.373
Haptoglobin	d14	0.25	0.77	0.81	0.14	0.67	0.39	0.17	0.02
		-0.357	-0.166	-0.113	-0.015	0.258	0.400	-0.031	-0.186
	d 7	0.02	0.30	0.49	0.93	0.11	0.01	0.85	0.25
		-0.160	-0.243	-0.098	-0.195	-0.046	0.070	-0.383	-0.257
Bilirubin	d14	0.32	0.13	0.55	0.22	0.78	0.67	0.01	0.11
		-0.182	-0.273	-0.083	-0.282	-0.069	0.138	-0.254	-0.047
	d 7	0.26	0.09	0.61	0.08	0.67	0.39	0.11	0.77
		-0.249	-0.151	-0.065	-0.224	-0.067	0.043	0.209	0.088
Total protein	d14	0.12	0.35	0.69	0.16	0.68	0.79	0.20	0.59
		0.451	0.451	0.226	0.180	0.081	-0.155	-0.210	-0.321
	d 7	0.01	0.01	0.16	0.26	0.62	0.34	0.19	0.04
		0.223	0.223	0.150	0.231	-0.109	-0.159	0.032	-0.207
Albumin	d14	0.18	0.18	0.35	0.15	0.50	0.32	0.85	0.20
		-0.316	-0.241	-0.151	-0.315	-0.089	0.177	-0.158	0.065
	d 7	0.05	0.13	0.35	0.05	0.58	0.27	0.33	0.69
		-0.317	-0.163	-0.107	-0.293	-0.040	0.087	0.207	0.146
Globulin	d14	0.05	0.31	0.51	0.06	0.80	0.59	0.20	0.37
		0.379	0.176	0.199	0.309	0.141	-0.162	0.123	-0.12
	d 7	0.02	0.28	0.22	0.05	0.38	0.32	0.45	0.46
Albumin:		0.436	0.224	0.223	0.414	-0.002	-0.126	-0.096	-0.156
Globulin	d14	0.01	0.17	0.16	0.01	0.99	0.44	0.55	0.34

[1]Correlation coefficient (r) is the top number, corresponding *P* value is beneath. [2]Aspartate aminotransferase , [3]Glutamate dehydrogenase, [4]Gamma-glutamyltransferase

Table 5-11. Pearson correlations[1] between insulin-like growth factor one (IGF-I), growth hormone receptor (GHR), tumor necrosis factor alpha (TNFa), and TNFa receptor superfamily 1a (TNFRSF1a) hepatic gene expression and hepatic gene expression of pyruvate carboxylase (PC), carnitine palmitoyltransferase 1A (CPT1A), mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), and angiopoietin-like 4 (ANGPTL4), calculated weekly energy balance, plasma NEFA and glucose concentrations, and 14d average DMI and milk yield from liver biopsies on d 7 ± 1.1 and d 14 ± 1.0 (mean ± SD) postpartum.

		Hepatic Gene Expression							
		IGF1		GHR		TNFa		TNFRSF	
		d 7	d 14	d 7	d 14	d 7	d 14	d 7	d 14
PC Gene Expression	d 7	-0.448	-0.037	-0.447	-0.191	0.047	0.219	0.379	0.106
		<0.01	0.82	<0.01	0.24	0.77	0.17	0.02	0.52
	d14	-0.160	-0.109	-0.172	-0.070	-0.142	-0.039	0.029	-0.066
		0.32	0.50	0.29	0.67	0.38	0.81	0.86	0.69
CPT-1A Gene Expression	d 7	-0.370	0.024	-0.340	-0.115	0.070	0.306	0.159	0.081
		0.02	0.88	0.03	0.48	0.67	0.05	0.33	0.62
	d14	-0.062	0.118	-0.053	0.035	0.008	-0.195	0.222	-0.168
		0.70	0.47	0.75	0.83	0.96	0.23	0.17	0.30
HMGCS2 Gene Expression	d 7	0.093	0.182	0.170	0.211	-0.176	-0.015	-0.011	0.023
		0.57	0.26	0.29	0.19	0.28	0.93	0.95	0.89
	d14	0.200	0.397	0.191	0.378	-0.226	-0.288	-0.026	-0.337
		0.22	0.01	0.24	0.02	0.16	0.07	0.87	0.03
ANGPTL-4 Gene Expression	d 7	-0.459	0.038	-0.417	-0.112	0.126	0.341	0.313	0.157
		<0.01	0.82	0.01	0.49	0.44	0.03	0.05	0.33
	d14	-0.045	0.025	0.092	-0.048	-0.031	-0.097	0.149	0.135
		0.78	0.88	0.57	0.77	0.85	0.55	0.36	0.41
Weekly Energy Balance	d 7	0.409	0.280	0.324	0.152	0.058	-0.191	0.222	0.166
		0.01	0.10	0.05	0.38	0.74	0.26	0.19	0.33
	d14	0.325	0.141	0.213	0.132	0.218	-0.131	0.262	0.225
		0.06	0.43	0.23	0.46	0.22	0.47	0.14	0.21
Week Average NEFA	d 7	-0.299	-0.008	-0.122	0.042	-0.235	0.173	-0.404	-0.208
		0.06	0.96	0.46	0.80	0.15	0.29	0.01	0.20
	d14	-0.209	-0.139	-0.095	-0.049	-0.005	0.317	-0.124	-0.099
		0.20	0.40	0.56	0.77	0.98	0.05	0.45	0.55
Week Average Glucose	d 7	0.286	0.143	0.235	0.057	0.107	-0.239	0.105	-0.116
		0.08	0.39	0.15	0.73	0.52	0.14	0.53	0.48
	d14	0.300	0.262	0.234	0.185	0.056	-0.334	0.210	-0.030
		0.06	0.11	0.15	0.26	0.74	0.04	0.20	0.85
14d average DMI		0.115	0.021	0.151	0.189	-0.139	-0.169	-0.165	0.101
		0.49	0.90	0.36	0.25	0.40	0.30	0.31	0.54
14 d average Milk Yield		-0.266	-0.110	-0.219	0.065	-0.153	0.099	-0.447	-0.075
		0.10	0.50	0.18	0.69	0.35	0.55	<0.01	0.65

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

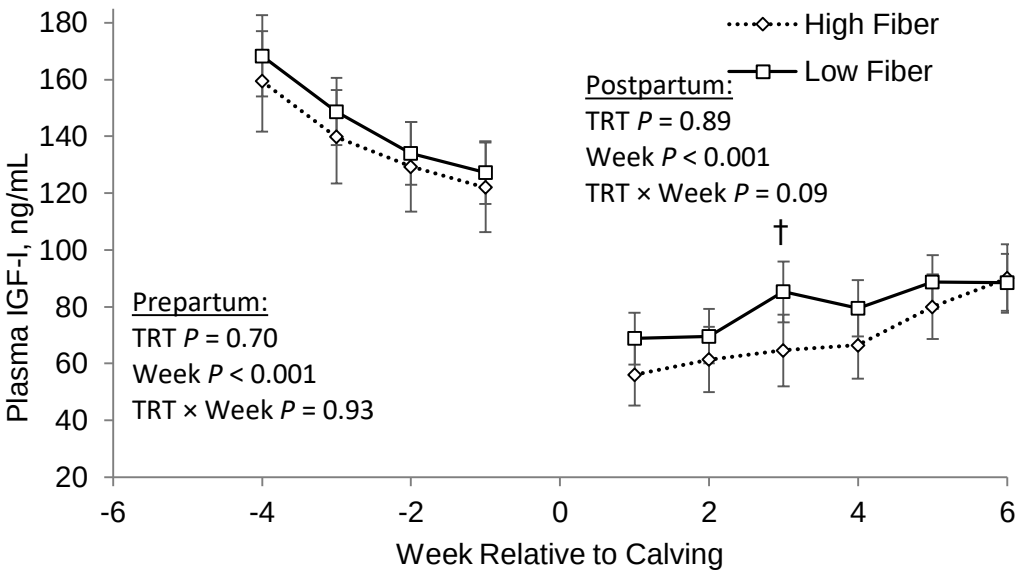


Figure 5-1. Least square means and standard errors for plasma IGF-I concentration by week relative to calving from cows fed diets differing in amount of undigested NDF at 240-h of in vitro fermentation ($uNDF_{240}$) and physically effective NDF (peNDF). Samples were collected on d 7 ± 1.1 and d 14 ± 1.0 (mean ± SD) postpartum. Treatments were low fiber ($uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 29) and high fiber ($uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 27). Cows fed HF were switched to a low fiber diet at 29 DIM. A cross (†) denotes a timepoint in which the means tended ($0.05 < P \leq 0.15$) to differ.

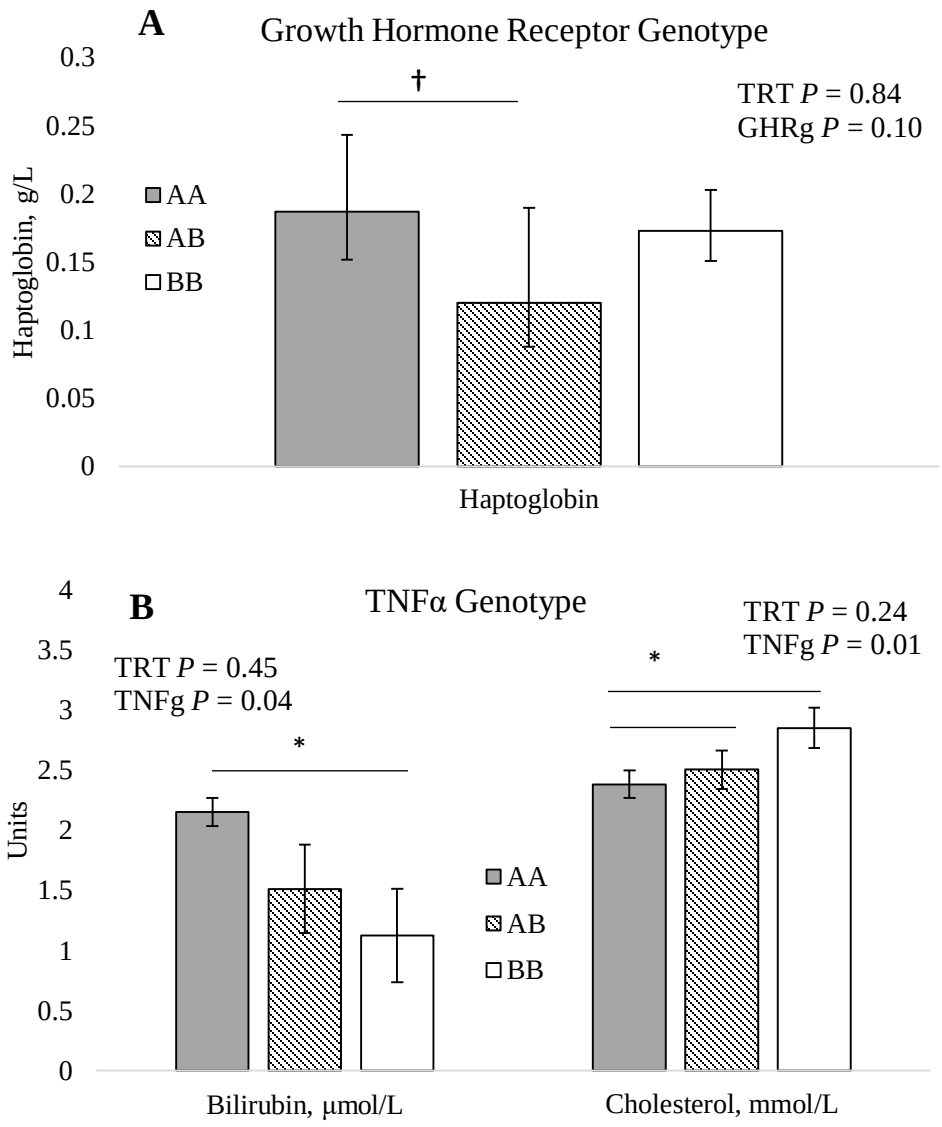
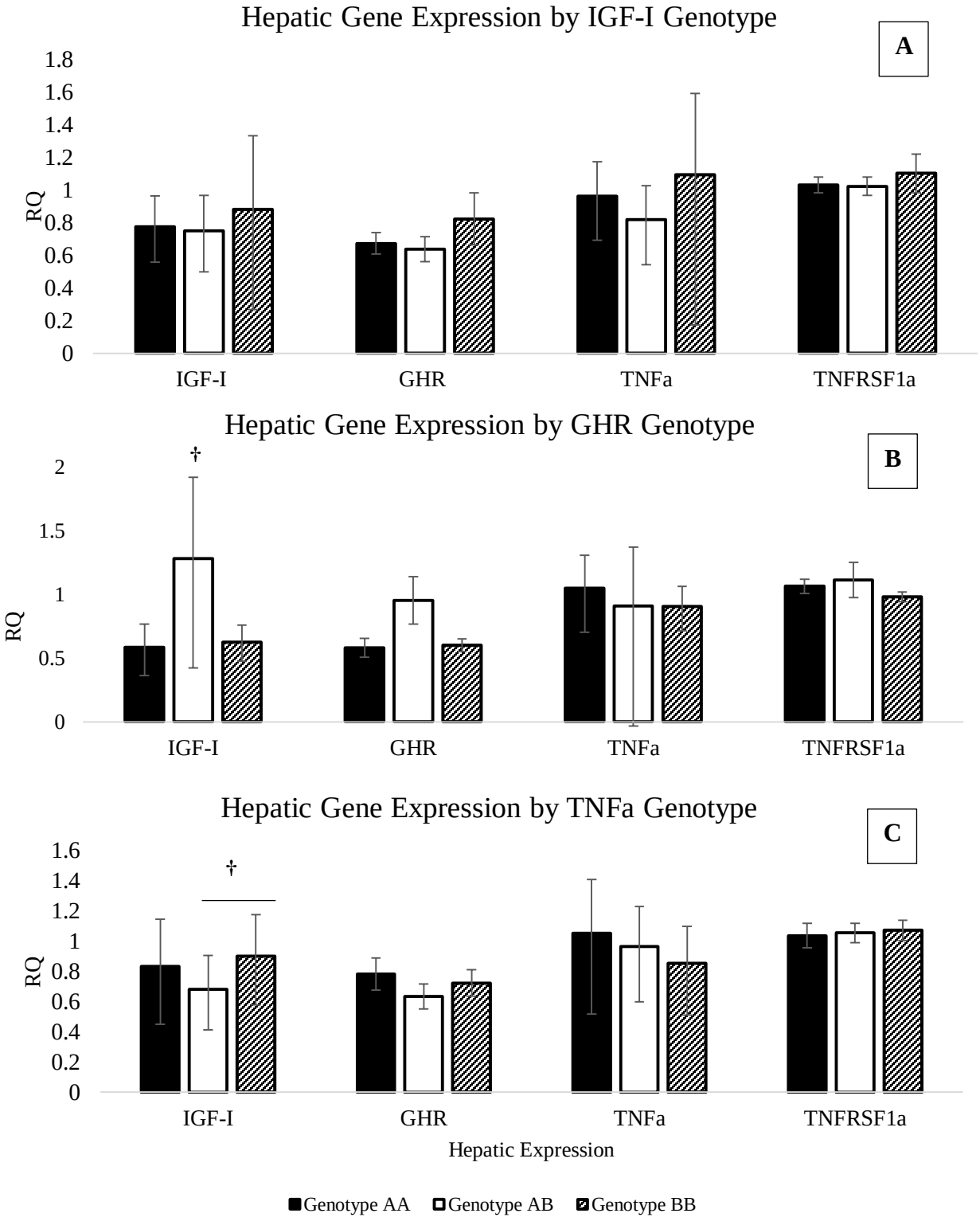


Figure 5-2. Geometric means and backtransformed 95% confidence intervals for haptoglobin concentration as affected by growth hormone receptor genotype (A) and least square means and standard errors of plasma bilirubin and cholesterol as affected by tumor necrosis factor-α (TNFα) genotype (B) from cows fed diets differing in amount of undigested NDF at 240-h of in vitro fermentation ($uNDF_{240}$) and physically effective NDF (peNDF). Samples were collected on d 7 ± 1.1 and d 14 ± 1.0 (mean ± SD) postpartum. Treatments were low fiber ($uNDF_{240}$ = 9.5% of DM, peNDF = 21% of DM, n = 21) and high fiber ($uNDF_{240}$ = 12.2% of DM, peNDF = 23% of DM, n = 19). Bars and asterisks (*) represent genotype means that were statistically different ($P \leq 0.05$), while crosses (†) represent statistical trends ($0.05 < P \leq 0.15$), with bars reflecting a difference between only the two specified genotype. *P* values not reported were not significantly different ($P > 0.15$).

Figure 5-3. The effect of IGF-I (A), GHR (B), and TNFa (C) genotype on overall mean of hepatic gene expression of insulin like growth factor one (IGF-I), growth hormone receptor (GHR), tumor necrosis factor alpha (TNFa), and TNFa receptor superfamily 1a (TNFRSF1a). IGF-I and TNFa gene expression are reported as geometric means and back transformed 95% confidence intervals. GHR and TNFRSF1a gene expression are reported as least square means and standard error. Cross (†) represents a tendency ($0.05 < P < 0.15$) for difference in gene expression due to genotype, with bars reflecting a difference between only the two specified genotype.


Hepatic Gene Expression by IGF-I Genotype
A
RQ
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IGF-I
GHR
TNFa
TNFRSF1a
Hepatic Gene Expression by GHR Genotype
B
†
RQ
2
1.5
1
0.5
0
IGF-I
GHR
TNFa
TNFRSF1a
Hepatic Gene Expression by TNFa Genotype
C
†
RQ
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IGF-I
GHR
TNFa
TNFRSF1a
Hepatic Expression
Genotype AA
Genotype AB
Genotype BB

DISCUSSION

Originally, postpartum treatment diets for the current study were formulated to contain 10.7% or 8.3% $uNDF_{240}$ (HF vs LF, respectively), to investigate the lower to adequate range of $uNDF_{240}$ in postpartum diets; however, due to higher than anticipated $uNDF_{240}$ levels in the alfalfa hay procured, overall dietary $uNDF_{240}$ levels were higher than anticipated. Therefore, the present study ultimately provided data investigating the adequate to upper range of $uNDF_{240}$ (12.2% vs 9.5% $uNDF_{240}$, HF and LF respectively) in diets fed during the postpartum period. The LF diet appeared to provide adequate levels of $uNDF_{240}$, whereas the HF diet was likely representative of the upper bound of $uNDF_{240}$ for this period, as discovered through limitations of DMI, production, more negative energy balance and altered metabolism discussed in Chapters 3 and 4.

As the main goal of this study was to investigate impacts of dietary treatment on performance and metabolism parameters, it is important to note that the number of cows within each genotype SNP were not balanced in this experiment. Table 5-6 and Table 5-7 report the frequency of which the different alleles were present in our study, where at least one genotype SNP for each gene is underrepresented due to the population studied. Further research is needed to fully investigate effects of genotype, and a more robust and balanced study should be performed to adequately examine any effects proposed here, which should be interpreted with caution.

Circulating concentrations of IGF-I are low after calving, as the animal is in negative energy balance and the GH-IGF-I axis is uncoupled (Lucy, 2008). As DMI

and subsequently energy balance begin to increase, increasing blood glucose and insulin concentrations, GHR expression increases, the liver becomes reactive to circulating GH and IGF-I production commences, increasing IGF-I in the blood stream (Butler, 2003). Thus, animals with higher plasma IGF-I concentrations are likely in more positive energy balance. In our study, this is confirmed by the moderately positive correlation between plasma IGF-I and glucose levels during the first week postpartum ($r = 0.48$; $P < 0.01$). Cows fed LF in wk 3 tended to have greater IGF-I concentrations compared to cows fed HF, which fits well with this relationship, and the understanding of IGF-I production in relation to energy balance status. Cows fed LF had increased DMI and increased blood glucose (Chapter 3), and were in less of an energy deficit in wk 3 postpartum compared to cows fed HF (-7.1 vs. -10.9 Mcal/D; $P = 0.005$).

Cows with GHR genotype AB had both higher IGF-I gene expression (n = 4) and higher GHR gene expression (n = 1), in agreement with the known relationship of GHR and circulating concentrations of IGF-I (Butler et al., 2003); however, it is inconsistent with previous literature. Schneider et al. (2013) reported GHR genotype AA cows to have increased plasma IGF-I expression and improved reproductive parameters in a larger study (n = 94) with more animals in each respective genotype group. Given the small and uneven distribution of genotypes for cows in this study, and that we observed no differences due to overall genotype in circulating IGF-I, more research is necessary to fully understand the impact of GHR genotype on IGF-I production and subsequent effects.

Haptoglobin, bilirubin, and cholesterol are all related to the acute phase response. During an inflammatory response, positive acute phase proteins (APP), such as haptoglobin and bilirubin, that are produced in response to pro-inflammatory cytokines, increase while negative acute phase proteins, such as albumin and cholesterol, that are produced in the liver under normal conditions are decreased (Bertoni et al., 2008). Although bilirubin and cholesterol are not actual APP, they are easy to measure and the synthesis or clearance is related to APP (Bertoni and Trevisi, 2013). Cows with higher positive APP and lower negative APP are likely in a state of inflammation or altered metabolism; animals such as these have been shown to produce less milk and have longer time to pregnancy compared to cows with lower positive APP and higher negative APP (Bertoni et al., 2008).

In our study, plasma cholesterol and bilirubin were influenced by TNFα genotype. Cows with TNFα SNP BB had higher plasma cholesterol, indicating a better state of liver metabolism. This group of cows also had the higher IGF-I gene expression than cows with SNP AB, indicating they were likely in a more positive energy balance, allowing for increased liver function. Cows with TNFα SNP AA had higher concentration of bilirubin as compared to those cows with SNP BB. This could be a negative reflection of cows with SNP AA, however it could also be a sign of the overall improved liver function of cows with SNP BB. Bilirubin is a marker of liver enzyme status, as the enzymes are necessary to clear bilirubin. As bilirubin concentrations increase, this would imply liver enzymes are not present in sufficient quantity and liver function is impaired (Bertoni and Trevisi, 2013). As cows with TNFα SNP BB had higher IGF-I gene expression and increased cholesterol

concentrations, it would follow that bilirubin would be low, as the liver is functioning optimally. Interestingly, hepatic IGF-I expression correlated positively with plasma cholesterol and albumin concentrations, and negatively with plasma bilirubin concentration, indicating that increases in energy balance likely would be associated with increased liver function. Both IGF-I and GHR gene expression negatively correlated with plasma globulin as well, another positive APP which can indicate inflammation, further linking energy balance and inflammatory status (Bionaz et al., 2007).

Cows with GHR SNP AA demonstrated a tendency for higher haptoglobin concentration compared to cows with SNP AB. The cow (n = 1) with SNP AB had the highest IGF-I gene expression, indicating a more positive energy status that would likely be associated with decreased disease incidence. As many periparturient diseases and altered metabolic status associated with negative energy balance can increase haptoglobin concentrations (Humblet et al., 2006, Huzzey et al., 2009, McCarthy et al., 2015b), it could be that lower haptoglobin for cows with SNP AB is indicative of the absence of disease or metabolic inflammation that could be associated with more negative energy balance of cows with SNP AA.

The positive correlation of TNFRSF1a with haptoglobin and negative correlation to albumin is expected, as APP production is in response to pro-inflammatory cytokines such as TNFα. As TNFα signals through TNFRSF1a, increases in TNFα that would increase its receptor (Jiang et al., 2008) would likely increase positive APP and haptoglobin production, and decrease negative APP such as albumin. Somewhat unexpected is the negative correlation of TNFRSF1a with

concentrations of GGT and GLDH. Liver enzymes GLDH and GGT are generally associated with liver damage (Mann et al., 2018), in which inflammation would likely play a role. Loor et al. (2007) reported a different member of the TNFRSF family (TNFRSF25) was upregulated during cell death, but also upregulated during cellular growth and proliferation. It is possible TNFRSF1a was similarly upregulated due to cellular growth and proliferation in the liver rather than increased inflammation that would induce liver damage and thus result in a positive correlation, though the mechanism is not clear and should be investigated further.

Hepatic metabolism is critical to ensuring adequate fuels are available to the mammary gland and all extra-mammary tissues. During negative energy balance to cope with the influx of NEFA delivered by the blood stream fatty acid metabolism must be upregulated along with increased gluconeogenesis to support the mammary gland (Bauman and Currie, 1980). Gene expression of several key enzymes change around parturition to accommodate these changes, and return to lower levels as energy balance becomes more positive. Gene expression of IGF-I and GHR correlated negatively with gene expression of PC, CPT-1A, and ANGPTL4 at day 7 postpartum, while correlating positively with energy balance at d 7 and HMGCS2 gene expression at d 14. Conversely, gene expression of TNFα and TNFRSF1a correlated positively with PC, CPT-1A, and ANGPLT4, and negatively with milk production, plasma glucose, and HNGCS2 expression. Activity of PC and CPT-1A and ANGPTL4 increase postpartum in order to supply precursors to gluconeogenesis, increase NEFA transport into the mitochondria for oxidation or ketogenesis, and increase mobilization of adipose tissue via inhibition of lipoprotein lipase (Drackley et al., 2001, Dann and

Drackley, 2005, Kersten, 2005, Koltes and Spurlock, 2012). All three enzymes play an important role in metabolism during negative energy balance, and thus would be negatively correlated with GHR and IGF-I expression as for the liver to be responsive to GH and produce IGF-I, the animal needs to be in a more positive energy balance (Radcliff et al., 2003b). As stated previously, inflammation can present with both infectious and metabolic diseases. Gene expression of TNFα correlated positively with CPT-1A and NEFA specifically at d 14 rather than d 7, this likely would indicate that inflammation present with prolonged adipose tissue mobilization associated with excessive negative energy balance (Bobe et al., 2004, Bradford et al., 2015). Overall, gene expression of GHR, IGF-I, TNFα and TNFRSF1a correlated with aspects of liver health, inflammation, and hepatic metabolism that are closely related to energy balance and metabolic state.

CONCLUSIONS

In this experiment, levels of uNDF240 (9.5% of DM; 0.27% of BW) and peNDF (21.6% of DM) achieved in the LF diet were adequate for optimal hepatic metabolism and performance in early lactation cows whereas the levels in the high fiber diet (uNDF240 at 12.2% of DM, 0.32% of BW, and 23.2% peNDF) limited DMI and performance during early lactation. Despite changes in calculated energy balance, no differences due to diet were apparent for IGF-I or GHR gene expression, though IGF-I tended to be higher for cows fed LF compared to cows fed HF in wk 3 postpartum. Associations between genotype of GHR and TNFα and expression of IGF-I and markers of liver health suggest genotype can impact energy balance and health status, though more robust studies are needed. Correlation analysis revealed

relationships between IGF-I and GHR and measures of improved energy balance and metabolism, while TNFα and TNFRSF1 were associated with markers of decreased liver health, more negative energy balance, and altered metabolism. Overall, metabolic parameters, energy balance, and inflammation within the transition period are all related, though more research is needed to fully investigate the mechanisms.

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CHAPTER 6

PERFORMANCE AND METABOLISM OF MULTIPARIOUS HOLSTEIN DAIRY COWS AS AFFECTED BY CORN SILAGE TYPE AND SUPPLEMENTATION WITH MONENSIN THROUGHOUT THE TRANSITION PERIOD

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ABSTRACT

Our objective was to evaluate the effects of corn silage (CS) type and monensin supplementation throughout the transition period on metabolism and performance. Multiparous Holstein cows (n= 85) were enrolled 28 d before expected parturition and randomly assigned to treatments in a 2 × 2 factorial arrangement with diets containing conventional (CON) or brown midrib (BMR) CS, with monensin (M; 330 mg/d prepartum and 450 mg/d postpartum) or without M (NO; 0 mg/d). Diets were formulated for high forage content and to be identical except for CS type and small inclusion mix delivering M or NO. Prepartum diets (75% forage, 25% concentrate) were fed from -21 d through calving, and postpartum (65% forage, 35% concentrate) diets formulated to follow the original treatment assignment were fed from calving through 42 d postpartum. Blood samples were taken 1x/wk prepartum, 2x/wk during wk 1 and 2, and 1x/wk through wk 6 postpartum and analyzed for β-hydroxy butyrate (BHB), non-esterified fatty acids (NEFA), and glucose. Prepartum dry matter intake (DMI) was higher for cows fed BMR than CON (14.7 vs. 14.0 kg/d), whereas cows fed M had lower DMI than cows fed NO (13.9 vs. 14.8 kg/d). Postpartum DMI was not affected by treatment. A three-way interaction of CS, M, and time (T) was observed for milk yield where BMR-M was highest and CON-NO lowest during wk 5 and 6 postpartum. Interactions of CS and M were present for percentages of fat, true protein, and total solids in milk, and milk FA composition. A CS × T interaction was observed such that NEFA in wk -2 and -1 and BHB at d 32 and 39 postpartum were lower for cows fed BMR than those fed CON. Postpartum, cows fed BMR had lower NEFA and higher blood glucose than cows fed CON. Cows fed M

had lower BHB than cows fed NO both prepartum and postpartum, and M × T interaction was present such that cows fed M had lower NEFA on d 11, 13, and 20 postpartum. Incidence of hyperketonemia was lower for cows fed M than those fed NO in cows fed CON, and for cows fed BMR compared to cows fed CON in cows not supplemented with M. Cows fed BMR and cows fed M throughout the transition period exhibited improvements in performance and metabolism in the prepartum period, with a possible synergistic effect of CS type and M supplementation on milk yield.

INTRODUCTION

The transition from pregnancy to lactation is a time of great metabolic adaptations for a dairy cow. Demands for glucose for milk production increased dramatically (Bell, 1995) concurrent decreased DMI, plunging the animal into negative energy balance (Grummer et al., 2004). To combat negative energy balance, adipose tissue is mobilized to provide energy to peripheral systems, as glucose is being spared for the mammary gland (Bauman and Currie, 1980). Excessive negative energy balance and mobilization of non-esterified fatty acids (NEFA) have been associated with metabolic dysfunction, poor production, and increased risk of disease postpartum (Grummer, 1993, Ospina et al., 2010a, b). Nutritional strategies that minimize the decrease in DMI and provide adequate glucose precursors through the transition period can improve metabolic status and ease energy balance drop postpartum (Overton and Waldron, 2004).

One such strategy is use of monensin, an ionophore that shifts the microbial population in the rumen to increase production of propionate (Armentano and Young,

1983, Kim et al., 2014), the primary precursor for hepatic gluconeogenesis (Reynolds et al., 2003). Effects of monensin are well documented in the literature across all production stages of dairy cows; increases in milk production, feed efficiency, and decreased blood BHB and risk of ketosis have been reported consistently (Duffield et al., 2008a, b, c). Increases in blood glucose and decreases in blood NEFA due to monensin supplementation have also been reported, though the response may be less consistent (Duffield et al., 2008a, Mullins et al., 2012, McCarthy et al., 2015b). Increasing propionate through monensin use would increase dietary glucose precursors without the need for change in DMI, which would be ideal in the transition dairy cow, as intake can be regulated by rumen fill (Dado and Allen, 1995), but also many metabolic and endocrine signals (Ingvartsen and Andersen, 2000) in the periparturient period.

Increased energy density is another nutritional strategy utilized to minimize negative energy balance in this period, though this energy can come from multiple sources. Increased concentrations of starch have been shown to increase production and energy balance status (Rabelo et al., 2003, McCarthy et al., 2015a, McCarthy et al., 2015b) however, it can also increase risk of subacute ruminal acidosis (SARA), which can lead to decreased DMI, milk production, and increased inflammation (Stone, 2004, Plaizier et al., 2008). Increases in fiber digestibility however, could increase dietary energy with less risk of SARA. Brown midrib corn silage (BMR) is a hybrid corn silage (CS) that has been shown to have lower lignin and cross-linking, increasing NDF digestibility (Oba and Allen, 1999, Ferraretto and Shaver, 2015, Raffrenato, et al., 2017). Feeding BMR has been shown to increase total tract NDF

digestibility, increase DMI, milk and milk protein yield (Ferraretto and Shaver, 2015). Specifically in the transition period, feeding BMR has been reported to increase DMI, milk production and yield of components (Stone et al., 2012), decreased BW loss and increased post-peak milk yield (Holt et al., 2013a), though few studies have evaluated energy metabolites.

There is a lack of published information investigating effects of BMR CS through the periparturient period on energy status and metabolites. While there are many studies investigating use of monensin alone in the periparturient period, studies investigating feeding CS with increased digestibility with or without monensin to investigate potential synergism and overall effects of the two different strategies are lacking. Our objective was to evaluate the effects of corn silage type and monensin supplementation throughout the transition period on DMI, milk yield, components and fatty acids (FA), blood metabolites, and health status. We hypothesized that feeding BMR and monensin would improve energy status, metabolic health, and performance of dairy cows during the transition period and early lactation.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

All animal protocols were approved by the Cornell University Institutional Animal Care and Use Committee (protocol # 2016-0079). Animals were enrolled in the experiment between December 2016 and March 2017. Multiparous Holstein cows (n = 85) were enrolled in a randomized design trial with a 2 × 2 factorial arrangement of treatments, with treatment assignment blocked to control for lactation number and previous 305-d mature equivalent milk production. A sample size calculation

determined 38 cows per main effect of treatment would detect a difference in milk yield of 3.5 kg per day with 95% confidence and 80% power based on data from previous studies in our lab (Chapter 3). Treatment variables were corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Rumensin, Elanco Animal Health, Indianapolis, IN]. Cows were moved to tie-stalls 28 d prior to expected parturition and fed the CON-NO diet for 7 d; from 21 d prior to expected calving through parturition cows were fed their respective treatment diets (CON vs. BMR and NO vs. M). On average, cows were fed prepartum treatment diets for 20 d, with a range from 9 d to 33 d. At parturition, cows were fed a fresh diet formulated to follow their assigned treatment (CON vs. BMR and NO vs. M) through 42 DIM. In the dataset 22 cows were in the conventional corn silage with monensin supplementation group (CON-M), and 21 cows were in each of the conventional corn silage, no monensin supplementation (CON-NO), BMR corn silage, no monensin supplementation (BMR-NO), BMR corn silage with monensin supplementation (BMR-M) treatment groups. Two cows were partially removed from the data set after suffering an injury (unrelated to treatment) and being unable to remain in the tie-stalls; one cow on CON-M treatment was removed after wk 2, and one cow on CON-NO treatment was removed after wk 4. Treatment diets were formulated to be the same except for the type of corn silage or monensin mix, diet ingredients, forage analysis, and analyzed composition of prepartum and postpartum diets are presented in Table 6-1, Table 6-2, Table 6-3, and Table 6-4 respectively.

Diet Formulation, Feeding Management, and Analysis

Diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS, version 6.55, Cornell University, Ithaca, NY; Van Amburgh et al., 2015). All diets were formulated to be similar to higher forage diets typically fed in the Northeastern United States. Diets were composed of a base TMR comprised of treatment corn silage (CON or BMR), hay crop silage, wheat straw and a grain mix that were shared across all treatments (prepartum and postpartum grain mix differed), as well as a small inclusion rate grain mix containing either NO or M. Corn silages were planted in spring 2015, harvested at a similar time and stored until the start of this study. Monensin comprised 0.29% on an as-fed basis of the small inclusion rate grain mixture for cows fed M, containing wheat middlings, canola meal, and soybean meal. The control small inclusion rate grain mixture for cows fed NO contained no monensin, but slightly higher amounts of wheat middlings, canola meal, and soybean meal on an as fed basis to compensate. The base TMR for each corn silage type was mixed for all cows receiving that treatment, and a small batch mixer was utilized to include small inclusion mixes prior to feeding. Formulated diet ingredients, forage analysis, and analyzed composition of prepartum and postpartum diets are presented in Table 6-1, Table 6-2, Table 6-3, and Table 6-4 respectively.

Cows were housed in tie-stalls and fed once daily, with lactating cows fed between 0600 and 0800 h and dry cows between 0830 and 1000 h each day. Refusals were removed and weight was recorded each day prior to feeding. Weight of feed delivered and refused along with weekly DM determinations were used to calculate

daily individual DMI for each cow and a refusal rate of 10% was targeted daily to ensure ad libitum intake.

Samples of all individual forages, grains, small inclusion mixes and TMR were collected weekly. A duplicate of each weekly TMR sample collected was sent to a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA) for DM determination at 135ºC (method 930.15, AOAC International, 2000) and NIR analysis to ensure diet targets were being achieved throughout the study. All other samples were dried at 40ºC for 96 h in a forced-air oven for on-farm DM determination and a portion of the sample was ground to 2-mm using a Wiley mill and retained. At the end of the experiment, all retained feed samples were composited by 4-wk intervals over the duration of the study and sent to a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA) for wet chemistry determination of DM at 135ºC (method 930.15, AOAC International, 2000), ADF (method 973.18, AOAC International, 2000), CP (method 990.03, AOAC International, 2000), aNDFom (Van Soest et al., 1991, Mertens, et al., 2002), starch (Hall, 2009), sugar (Dubois M. et al., 1952), ash (method 942.05, AOAC International, 2000), minerals (method 985.01, AOAC International, 2000), and NE_L (NRC 2001). *In vitro* NDF digestibility analysis (Goering and Van Soest, 1970, Raffrenato et al., 2019) for forages and canola meal were determined by a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA), whereas aNDFom and *in vitro* NDF digestibility at 240 h of fermentation for all TMR samples were performed as described previously by Raffrenato et al. (2019) in house. Analysis of all composited ingredients were inputted into diets in CNCPS (v 6.55 Cornell University,

Ithaca, NY; Van Amburgh et al., 2015), using chemical analysis of ingredients and actual average DMI for each diet and dietary MP supply was predicted.

Data Collection, Sampling Procedures, and Analysis

Body weight was measured and BCS was assessed once weekly throughout the experiment beginning 28 d prior to expected parturition and continuing through 42 DIM. Body condition score was assigned by two scorers weekly according to Edmonson et al. (1989) and scores averaged prior to analysis. Rumination time was recorded in 2-h intervals starting 28 d prior to expected calving through 42 d postpartum using rumination collars (HR tags; SCR Dairy, Netanya, Israel). Daily rumination time was calculated by adding all 2-h intervals in a 24-h period and daily rumination was averaged by week prior to statistical analysis. Cows were monitored daily for 14 days by research and farm personnel for presence of health disorders including; metritis, mastitis, displaced abomasum, retained placenta, hyperketonemia (whole blood BHB ≥1.2 mmol/L, blood samples only collected 2 x/ wk unless other symptoms were present), and respiratory disorders.

After calving, all cows were milked 3x daily at 0600, 1400, and 2200h and individual milk weights were recorded. Daily milk yield was determined as the sum of the three daily milkings and weekly means of daily production were calculated prior to analysis. Milk samples were collected from three consecutive milkings 2x/wk for the first 2 wk, and 1x/wk through 42 DIM and stored at 4ºC until transportation (within 48 h) to Cornell University for analysis.

Milk fat, true protein, and anhydrous lactose content were determined using a Fourier transform mid-infrared (**FTIR**) spectrophotometer (Lactoscope model FTA,

Delta Instruments, Drachten, The Netherlands). The prediction models used were the optimized basic model filter wavelengths and intercorrection factors described by Kaylegian et al. (2009). Calibration of the FTIR for measurement of fat, true protein, and anhydrous lactose was done using a 14 sample modified milk calibration set (Kaylegian et al., 2006) produced monthly. The reference chemistry values were an all lab mean produced by a network of 10 to 12 laboratories running all samples with the reference methods (Wojciechowski et al., 2016). The reference methods for fat, true protein, and anhydrous lactose measurement were determined in duplicate in each laboratory using the following validated methods (AOAC International, 2000): fat by modified Mojonnier ether extraction (method 989.05), true protein by Kjeldahl analysis (method 991.22), lactose by enzymatic analysis (method 2006.06) and total solids by atmospheric forced air oven drying (method 990.20). Milk somatic cell count was measured using a fluorometric flow cytometry method using DAPI (4′,6-diamidino-2-phenylindole) as the staining dye (Delta Instruments, SomaSmart, Drachten, The Netherlands). Calibration of the milk somatic cell counter was done using calibration samples from the USDA Federal Milk Markets with reference values established using the direct microscopic somatic cell count method (Fitts and Laird, 2004).

De novo, mixed origin and preformed milk fatty acids were measured directly as grams/100 g of milk by FTIR using the partial least squares prediction models described by Woolpert et al. (2016). The GLC reference chemistry for calibration of the milk fatty acid parameters was as described by Wojciechowski and Barbano (2016). The milk fatty acid calibration sample set was the same 14 sample set that was

used for calibration of the main milk components. The calibration concentration ranges for *de novo*, mixed origin and preformed milk fatty acids were: de novo 0.05 to 1.4 g/100 milk, mixed origin 0.08 to 2.2 g/100 g milk, and preformed 0.06 to 1.9 g/100 g milk. Milk urea N was measured by FTIR using a partial least squares (PLS) model developed by Delta Instruments (parameter number 502) and was calibrated using reference chemistry values from an enzymatic milk urea nitrogen assay (Megazyme, kit K-URAMR, Bray, Wicklow, Ireland).

Prepartum and postpartum weekly energy balance (EBAL) was calculated according to NRC (2001) equations 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)], and

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 of NE_L/d) = weekly average DMI (kg/d) × diet NE_L (Mcal/kg of DM), maintenance requirement (Mcal of NE_L/d) = week metabolic BW ($kg^{0.75}$) × 0.08 (Mcal/ $kg^{0.75}$ per d); pregnancy requirement (Mcal) = (0.00318 × d of gestation - 0.0352) × (1/0.218); and lactation requirement (Mcal/d) = week average milk yield (kg/d) × [(0.0929 × fat percentage) + (0.0563 × true protein percentage) + (0.0395 × lactose percentage)].

Blood samples were collected via coccygeal vessels prior to fresh feed delivery between 0600 and 0730 h 1x/wk from d 28 prior to expected parturition through parturition, 2x/wk from parturition through 14 DIM, and 1x/wk from 15 through 42 DIM. Samples were collected using 10-mL sodium heparin evacuated tubes (158 USP,

Becton Dickinson and Company, Franklin Lakes NJ), using 20 ga vacutainer needles (Becton Dickinson and Company). Whole blood was harvested, and immediately used for analysis of BHB using the Nova Vet handheld meter (Nova Biomedical, Billerica, MA), the remainder was placed on ice and centrifuged for 20 min at 2,000 × *g* at 4ºC. Plasma was aliquoted into 1.7 mL microfuge tubes, snap frozen in liquid N_2, and stored at -20ºC until analysis. Samples were analyzed for NEFA using a commercial enzymatic kit [HR series NEFA HG(2) Wako Pure Chemical Industries, Osaka, Japan] and glucose by enzymatic analysis (glucose oxidase; protocol from kit 510-A Sigma Aldrich, St. Louis, MO) using commercial products (PGO Enzyme Preparation and o-dianisidine dihydrochloride, Sigma Aldrich). Spectrophotometric measurements were collected using a tunable microplate reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA). Inter- and intra-assay coefficients of variation were maintained <10% for all assays.

Statistical Analysis

Daily means for DMI, milk yield, and rumination were averaged by week prior to analysis. To standardize blood metabolite data, day of blood samples collected 2x/wk or 3x/wk were averaged to reference days prior to analysis. All statistical analyses were conducted using SAS (version 9.4, SAS Institute Inc., Cary, NC). Prepartum and postpartum data were analyzed separately. All data collected over time were analyzed as repeated measures ANOVA using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). Fixed effects of corn silage type, monensin treatment, time, all 2-way interactions, and a 3-way interaction of corn silage type, monensin treatment, and time were included in the model. Cow nested

within treatment was the random effect. Measurements collected during the week of enrollment were included in all models when available as covariates. Previous lactation 305-d mature-equivalent milk yield was used as a covariate for milk production. Denominator degrees of freedom were estimated using the Kenward Rogers method. Five covariance structures were tested for each model: first-order autoregressive, heterogeneous first-order autoregressive, compound symmetry, heterogeneous compound symmetry, and unstructured; and the covariance structure with the lowest Akaike's information criterion was used (Littell et al., 1996). In instances of a 2-way interaction without time where $P \leq 0.10$, the Tukey-Kramer adjustment was utilized to correct for multiple comparisons. In instances of 2-way interaction with time where $P \leq 0.10$, the slice option was added to the model to perform a partitioned analysis of the means of the interaction, producing an F-test for each level to determine at which levels treatments differed. In the instance of a 3-way interaction with time where $P \leq 0.05$, the slice option was used to determine at which time point treatment means differed and a Bonferroni correction (which incorporated the number of tests from the slice option, as well as the number of tests for each comparison within that time point) was utilized to correct for multiple comparisons. Residual analysis was performed to examine normality and homogeneity of residuals, when non-normality of residual variance was evident, data were transformed and analysis repeated. Body weight change and BCS change data were calculated as the change from wk 1 to 6, and as the only measurements not repeated over time, were analyzed using ANOVA in the MIXED procedure of SAS. Health disorders were analyzed using a Fisher's exact test to assess frequency of disorders for CS and M

treatment independently, and a stratified table analysis and Cochran-Mantel-Haenszel statistic was utilized to determine any interaction between CS and M on health disorder frequency in the FREQ procedure of SAS. All data presented are least squares means and standard error (SE), except in the case of transformed data (NEFA and BHB) where geometric means with back-transformed 95% confidence intervals (CI) are reported. Statistical significance was declared at $P \leq 0.05$, and trends at $0.05 < P \leq 0.10$.

RESULTS

Corn Silage and Diets

Corn silage composition is presented in Table 6-2. The conventional corn silage (CS) was slightly drier (35.4 vs 31.4% DM) and had higher NDF content (41.3 vs. 37.6 % of DM) than BMR CS. Fiber digestibility varied greatly between the two silages, with CON CS having lower 30 h NDFD (22.2 vs. 24.6 % of DM), and higher 240 h uNDF (14.2 vs. 9.3 % of DM) as compared to BMR CS. Despite differences in fiber characteristics, all other analyses were similar for both CS varieties.

Prepartum diet composition were similar between CS types (Table 6-3). Content of starch, sugar, and fat were similar, though fiber fractions were altered. The aNDFom of diets containing BMR CS was slightly lower (42.2 vs. 44.0 % of DM), and $uNDF_{240}$ as % DM were lower (13.2 vs 11.2 % DM) than those containing CON CS. Calculated MP supply was higher for diets containing BMR CS, likely due to the increase in fiber digestibility, as well as the increased intake for cows fed BMR diets. Postpartum diet composition followed the same trends and can be found in Table 6-4. Diets with BMR CS had slightly lower aNDFom and ~2 percentage units lower

$uNDF_{240}$ as % DM (representing a 15% decrease in $uNDF_{240}$) compared to diets with CON CS. Calculated MP supply was also higher for diets with BMR CS, despite no intake differences being observed in this period.

Prepartum

Results for prepartum DMI, rumination, energy balance, BW, BCS, and blood metabolites are reported in Table 6-5. Plasma NEFA and glucose, and whole blood BHB are shown over time in Figure 6-1. There were no interactions of corn silage type and monensin supplementation in the prepartum period; therefore, all results are discussed as main effect differences. Dry matter intake was lower for cows fed CON compared to cows fed BMR (14.0 vs. 14.7 kg/d; $P = 0.03$, Table 6-5), and for cows fed M compared to those fed NO (13.9 vs. 14.9 kg/d; $P < 0.01$). Supplementation of M resulted in lower starch intake (1.66 vs. 1.84 kg/d; $P < 0.01$), lower aNDFom intake (5.90 vs. 6.48 kg/d; $P < 0.01$) and lower $uNDF_{240}$ intake (1.66 vs. 1.84 kg/d; $P < 0.01$) when compared with cows not supplemented with M. Intake of $uNDF_{240}$ as percentage of BW also differed due to M supplementation, with cows fed M having lower $uNDF_{240}$ intake as a percentage of BW compared to cows fed NO (0.21 vs. 0.23% of BW; $P = 0.001$). Differences in these nutrient intakes due to M supplementation are likely related to the observed decrease in DMI for cows fed M. Intake of total aNDFom was not different due to CS type, however intake of $uNDF_{240}$ was lower for cows fed BMR compared to cows fed CON CS (1.65 vs. 1.85 kg/d; $P<0.01$), resulting in higher intake of potentially digestible NDF (data not shown, 4.55 vs. 4.31 kg/d; $P<0.03$). Intake of $uNDF_{240}$ as percentage of BW differed due to CS type such that cows fed BMR had lower $uNDF_{240}$ intake as percentage of BW when compared to

cows fed CON (0.20 vs. 0.23% of BW ; $P < 0.001$). A CS by M interaction was present ($P = 0.10$) for aNDFom intake such that cows fed BMR-M tended to have lower intake of aNDFom as compared to cows fed CON-NO, and cows fed BMR-NO had higher intake of aNDFom as compared to cows fed CON-M and BMR-M (Table 6-5). There were no differences in rumination due to CS type, but supplementation with M decreased rumination compared to cows fed NO (490.6 vs. 513.5 min/d ; $P = 0.02$). Predicted energy balance was also lower for cows fed CON compared to cows fed BMR (4.5 vs. 5.8 Mcal/d ; $P = 0.02$), and for cows fed M compared to those fed NO (4.4 vs. 5.9 kg/d ; $P = 0.01$). No differences in BW, BCS, or plasma glucose were observed in the prepartum period. A CS by time interaction observed for plasma NEFA such that cows fed CON had higher NEFA in wk -2 and wk -1 (Figure 6-1, $P = 0.05$) compared to cows fed BMR, there were no differences in plasma NEFA due to monensin supplementation. Cows fed M had lower BHB than cows fed NO (0.68 vs. 0.75 mmol/L ; $P = 0.04$), though no differences due to CS type were observed.

Postpartum

Results for postpartum DMI, intake of nutrients, rumination, energy balance, BW, BCS, and blood metabolites are reported in Table 6-6. Results for plasma NEFA and glucose, and whole blood BHB are shown over time in Figure 6-1. There were no observed interactions of corn silage type and monensin supplementation in the prepartum period; therefore all results are discussed as main effect differences. No differences in DMI were observed due to any treatment. Intake of $uNDF_{240}$ differed due to CS type such that cows fed BMR had lower $uNDF_{240}$ intake compared to cows fed CON both as kg/d (1.64 vs. 2.10 kg/d; $P < 0.01$) and as a percentage of BW (0.22

vs. 0.29% of BW; $P < 0.001$). Intake of $uNDF_{240}$ also differed due to M supplementation, cows fed M had lower $uNDF_{240}$ intake both as kg/d (1.81 vs. 1.94 kg/d; $P = 0.01$) and (0.25 vs. 0.26% of BW; $P = 0.04$) compared to cows fed NO. There were no differences in rumination due to CS type, but supplementation with MON did increase rumination compared to cows fed NO (574.2 vs. 542.2 min/d; $P < 0.01$). Energy balance was not different due to CS type; however, there was a treatment by time interaction for monensin supplementation, such that cows fed M tended ($P = 0.08$) to have lower predicted energy balance in wk 5 (-6.8 vs -5.2 Mcal/d) and wk 6 (-5.8 vs -4.1 Mcal/d) postpartum compared to cows fed NO. No differences in BW or BCS were observed in the postpartum period. Feed efficiency is shown over time in Figure 6-2. While there were no statistically significant main effect differences on feed efficiency, there was a trend for an interaction of M supplementation by time, and for a three way interaction of CS, M, and time ($P = 0.08$ and $P = 0.09$ respectively). No statistically significant differences in time points for the M by time or the CS by M by time interactions for feed efficiency were apparent upon further analysis, though numerically cows fed M had higher feed efficiency at wk 5 (2.17 vs. 2.27 ± 0.05), with the numerical difference being greater within cows fed BMR than within cows fed CON (0.16 and 0.03 units, respectively; Figure 6-2). Plasma glucose was lower for cows fed CON compared to cows fed BMR (47.9 vs. 50.5 mg/dL; $P = 0.03$), and tended ($P = 0.06$) to differ over time due to CS type as seen in Figure 6-1. Cows fed CON CS had higher plasma NEFA than cows fed BMR (473.2 vs. 379.9 mEq/L; $P < 0.01$). Cows fed M had lower plasma NEFA than cows fed NO (394.6 vs. 455.5 mEq/L; $P = 0.02$), and an interaction of M supplementation by time was

observed for plasma NEFA as depicted in Figure 6-1 ($P = 0.02$). Cows fed CON CS had higher whole blood BHB than cows fed BMR (1.20 vs. 1.00 mmol/L; $P = 0.01$), also a CS by time interaction observed for BHB such that cows fed BMR had lower BHB starting in wk 3 postpartum, as seen in Figure 6-1 ($P = 0.01$). Cows fed M also had lower whole blood BHB than cows fed NO (1.00 vs. 1.19 mmol/L; $P = 0.01$).

Results for milk yield, energy corrected milk (ECM), and milk components are reported in Table 6-7. A three way interaction of CS, M supplementation, and week relative to calving was apparent for fluid milk yield ($P = 0.02$), such that cows fed BMR-M tended to have higher milk yield compared to cows fed CON-NO during wk 6 postpartum (54.7 vs. 49.8 kg/d; $P = 0.10$; Table 6-7). Type of CS impacted milk yield such that cows fed BMR produced more milk than cows fed CON (48.3 vs. 45.8 kg/d; $P = 0.04$). No differences were seen in ECM between CS types, though cows fed M had significantly lower ECM at 4 DIM compared to cows fed NO (51.7 vs. 47.2 kg/d; $P < 0.01$), no other time points throughout the study were significantly different. Milk protein, fat, and lactose yields over time are shown in Figure 6-3. A CS by M by time interaction was present for milk protein yield ($P = 0.05$) such that cows fed BMR-NO had higher milk protein production than cows fed CON-NO on d 17 (1.63 vs. 1.44 kg/d; $P = 0.04$), and d 42 (1.59 vs. 1.40 kg/d; $P = 0.02$) postpartum, though no other treatments or time points were found to be different. A M by CS interaction trend for protein percentage was present such that cows fed BMR-NO had higher milk protein percentage than cows fed BMR-M (3.39 vs. 3.25%; $P = 0.02$), while cows fed CON did not differ due to M supplementation. A M by time interaction ($P = 0.02$) for milk fat (kg/d) was present such that cows fed M tended to have lower milk fat

production on d 4 compared to cows fed NO (1.84 vs. 2.04 kg/d; $P = 0.10$), with no other time points being significantly different. A trend for an interaction of M and CS interaction was present for milk fat yield and percentage ($P = 0.08$ and $P = 0.10$, respectively). Numerically, cows fed CON-NO and BMR-M had lower fat yield than cows fed CON-M or BMR-N, though in adjusting for multiple comparisons, no differences at specific timepoints could be elucidated. Milk fat percentage tended to be lower for cows fed BMR-M compared with those fed CON-M (4.28 vs. 4.63%; $P = 0.10$), whereas there were no differences for cows fed NO. An interaction of both CS type and time and M supplementation and time were apparent for lactose yield ($P = 0.01$ and $P = 0.04$, respectively). Cows fed BMR had higher milk lactose yield on d 14 (2.24 vs. 2.05 kg/d; $P < 0.05$), d 35 (2.50 vs. 2.30 kg/d; $P < 0.05$), and d 42 (2.54 vs 2.34 kg/d; $P < 0.05$) as compared to cows fed CON, which coincides with the increases in milk yield of cows fed BMR compared to cows fed CON, as shown in Figure 6-2. Cows fed M tended to have lower milk lactose yield (1.61 vs 1.74 kg/d; $P = 0.10$) at 4 DIM as compared to cows fed NO, and no differences in lactose yield appeared beyond 4 DIM. A CS by M supplementation interaction was apparent for total solids percentage ($P = 0.05$) such that, within cows supplemented with M, cows fed BMR tended to have lower total solids percentage than cows fed CON (13.2 vs 13.5 %; $P = 0.10$), whereas cows fed NO did not differ due to CS type. A CS by M supplementation by time interaction was evident for total solids yield ($P = 0.05$), though in correcting for multiple comparisons, no differences at specific timepoints were elucidated. There were no differences in SCS due to CS type or M supplementation. A CS by M interaction was present for MUN concentration ($P =$

0.01), while numerically BMR-M cows had lower MUN than BMR-NO cows (9.8 vs. 10.7) and CON-M cows (9.8 vs. 10.8), in correcting for multiple comparisons there were no significant differences.

Milk *de novo* fatty acids (FA) were not different due to CS type or M supplementation when expressed as grams per 100 g of FA or grams per day, though there tended to be a CS type by M supplementation interaction when expressed as grams per 100 g of milk (Table 6-7; $P = 0.10$). Numerically cows fed BMR-NO had higher *de novo* FA when expressed as grams per 100 g of milk than cows fed BMR-M, though no significant differences were found. Mixed fatty acids were not different when expressed as grams per 100 g of FA; however, a CS type by M supplementation interaction was present when expressed as grams per 100 g of milk and grams per day ($P = 0.05$ and $P = 0.03$, respectively). While numerically cows fed BMR-M had lower mixed FA expressed as grams per 100 g of milk compared to cows fed BMR-NO (Table 6-7) there were no significant differences. Presented as total grams per day of mixed FA, within cows not supplemented with M, cows fed BMR had higher grams per day of mixed FA compared with cows fed CON (462 vs. 587 g/d ; $P = 0.04$). An interaction of M supplementation with time occurred for mixed FA expressed as grams per day ($P = 0.04$) such that cows fed M on d 7 tended to have higher milk mixed FA than cows fed NO (606 vs. 572 g/d; $P = 0.10$), though no other differences were observed. A tendency for preformed FA was apparent where cows fed BMR had lower preformed FA as grams per 100 g of milk compared to cows fed CON (45.3 vs. 46.8; $P = 0.09$). There was an M by time interaction ($P = 0.06$) for preformed FA expressed as grams per day such that cows fed M had tended to have higher milk

preformed FA on d 35 (889 vs. 822 P = 0.06) compared to cows fed NO. No other differences in preformed FA were observed.

Incidence of farm-reported health disorders are reported in Table 6-8. There was no statistically significant difference ($P > 0.10$) of corn silage type or M supplementation alone on the incidence of hyperketonemia cases of cows < 10 DIM. There was an interaction of CS type and M supplementation, such that cows fed CON that were supplemented with M had lower incidence of hyperketonemia compared to cows fed CON with NO M supplementation (1 vs 6 cases; $P = 0.04$), whereas in cows fed BMR CS, there was no effect of M supplementation ($P = 0.61$). Similarly when stratified by M supplementation, within cows supplemented with M there was no difference due to CS ($P = 0.35$), while in cows fed NO feeding BMR CS decreased incidence of hyperketonemia (1 vs. 6 cases; $P = 0.05$).

Table 6-1. Formulated ingredient composition of diets for which ingredients besides corn silage type differed.

	Prepartum Diet		Postpartum Diet	
Ingredient, % of DM	NO[1]	MON[1]	NO	MON
Corn silage[1]	51.67	51.67	51.46	51.46
Hay crop silage	-	-	10.65	10.65
Wheat straw	23.33	23.33	2.66	2.66
Corn meal	-	-	15.08	15.08
Canola meal	4.57	4.57	6.45	6.45
Amino Plus[2]	4.00	4.00	5.32	5.32
Wheat middlings	1.93	1.92	1.40	1.39
Blood meal	1.67	1.67	1.77	1.77
Soybean meal	0.67	0.67	0.48	0.48
Citrus pulp	3.67	3.67	-	-
Rumensin[3], mg/d	0.00	0.01	0.00	0.01
Megalac-R[4]	-	-	1.42	1.42
Ca carbonate	2.67	2.67	1.33	1.33
Mg oxide	0.20	0.20	0.27	0.27
Sodium bicarbonate	-	-	0.71	0.71
Salt	-	-	0.53	0.53
Urea	-	-	0.18	0.18
Bio-chlor[5]	5.00	5.00	-	-
Smartamine M[6]	0.05	0.05	0.09	0.09
Vitamin and mineral mix[7]	1.03	1.03	0.18	0.18

[1] Treatment variables were corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 337 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN].
[2] Heat-treated soybean meal, Ag Processing Inc., Omaha, NE.
[3] Premix contained 26,400 g/t of monensin, Elanco Animal Health, Greenfield, IN. Total diet M content was 336.9 g/d prepartum and 449.7 g/d postpartum.
[4] Commercial fat product, Arm & Hammer Animal Nutrition, Princeton, NJ.
[5] Anionic mineral supplement, Arm & Hammer Animal Nutrition, Princeton, NJ.
[6] Rumen-protected methionine, Adisseo, Alpharetta, GA
[7] Prepartum mix contained 3,754 mg/kg Zn, 993 mg/kg Cu, 4,658 mg/kg Mn, 28.9 mg/kg Se, 82.8 mg/kg Co, 82.4 mg/kg I, 1,177 KIU/kg Vitamin A, 253 KIU/kg Vitamin D, and 55,784 IU/kg Vitamin E. Postpartum mix contained 25,560 mg/kg Zn, 7,154 mg/kg Cu, 21,958 mg/kg Mn, 214 mg/kg Se, 507 mg/kg Co, 331 mg/kg I, 3,704 KIU/kg Vitamin A, 922 KIU/kg Vitamin D, and 12,496 IU/kg Vitamin E (Central New York Feeds, Jordan, NY).

Table 6-2. Forage composition (DM basis except where noted) as analyzed by wet chemistry and *in-vitro* analysis by a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA) on two composite samples for each forage representative of the entire study period, presented as mean ± SD.

Item	Corn Silage		Hay crop silage	Wheat straw
	Conventional	Brown-Mid Rib		
DM, % of as fed[1]	35.4 ± 2.6	31.4 ± 1.7	33.6 ± 8.0	88.1 ± 3.8
CP, %	7.1 ± 0.2	8.0 ± 0.2	22.3 ± 0.2	3.0 ± 0.2
ADF, %	26.2 ± 0.5	22.6 ± 0.2	28.8 ± 0.2	54.5 ± 0.2
aNDFom, %	41.3 ± 2.0	37.6 ± 0.2	38.8 ± 0.2	77.4 ± 0.2
Starch, %	33.4 ± 3.2	32.4 ± 1.0	1.0 ± 0.2	0.7 ± 0.3
Sugar, %	1.2 ± 0.1	1.2 ± 0.1	2.7 ± 1.6	1.2 ± 0.4
Fat, %	3.1 ± 0.1	3.4 ± 0.1	4.0 ± 0.4	1.1 ± 0.2
In-vitro digestibility analysis				
30-h NDFD[2], %	22.2 ± 0.7	24.6 ± 2.4	22.6 ± 2.6	30.7 ± 0.1
30-h NDFD, % of NDF	53.8 ± 0.7	65.4 ± 2.4	58.2 ± 2.6	39.6 ± 0.1
$uNDF_{30}$[3], %	19.1 ± 0.7	13.0 ± 2.4	16.2 ± 2.6	46.8 ± 0.1
$uNDF_{30}$, % of NDF	46.2 ± 0.7	34.6 ± 2.4	41.8 ± 2.6	60.4 ± 0.1
$uNDF_{240}$[4], %	14.2 ± 0.4	9.3 ± 1.3	12.9 ± 1.3	32.7 ± 0.4
$uNDF_{240}$, % of NDF	34.6 ± 0.4	24.6 ± 0.7	33.2 ± 1.3	42.2 ± 0.4

[1]DM was analyzed (dried at 40º C in a forced air oven for 96 h) on fresh forages weekly throughout the study and further corrected for residual moisture using DM obtained from Cumberland Valley Analytical Services upon wet chemistry analysis of composite samples.
[2] NDF digestibility, determined via *in vitro* fermentation
[3] Undigested NDF at 30-h of *in vitro* fermentation
[4] Undigested NDF at 240-h of *in vitro* fermentation

Table 6-3. Nutrient profile of all prepartum diets (mean ± SD), obtained through wet chemistry analysis by Cumberland Valley Analytical Services (Waynesboro, PA), *in vitro* fermentation, or as predicted by Cornell Net Carbohydrate and Protein System (version 6.55, Cornell University, Ithaca, NY).

	Prepartum Treatment Diets[1]			
Item	CON-NO	CON-M	BMR-NO	BMR-M
DM, % as fed[2]	45.7 ± 2.5	45.8 ± 2.4	43.4 ± 2.3	43.5 ± 2.3
ADF, % DM	27.1 ± 1.1	26.8 ± 0.8	26.1 ± 0.7	26.6 ± 0.3
aNDFom, % DM[3]	43.5	44.4	43.2	41.2
$uNDF_{240}$[4], % DM[3]	13.2	13.2	11.5	11.0
$uNDF_{240}$[4], % NDF[3]	30.3	29.8	26.5	26.7
CP, %	13.9 ± 0.4	14.0 ± 0.3	14.2 ± 0.4	14.0 ± 0.6
Starch, %	17.2 ± 1.0	17.8 ± 1.1	17.4 ± 0.6	17.1 ± 0.7
Sugar, %	3.6 ± 0.7	3.4 ± 0.9	3.2 ± 0.4	3.9 ± 0.6
Fat, %	2.1 ± 0.3	2.3 ± 0.4	2.4 ± 0.2	2.3 ± 0.2
MP[5], g/kg DM	94.8	94.4	99.2	98.5
NE_L, Mcal/kg	1.43 ± 0.02	1.45 ± 0.02	1.48 ± 0.01	1.48 ± 0.01
Ca, %	1.76 ± 0.25	1.85 ± 0.23	1.69 ± 0.33	1.58 ± 0.17
P, %	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.02
Mg, %	0.59 ± 0.05	0.58 ± 0.06	0.53 ± 0.06	0.52 ± 0.06
K, %	1.15 ± 0.04	1.13 ± 0.05	1.22 ± 0.05	1.24 ± 0.05
S, %	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.03	0.35 ± 0.04
Na, %	0.16 ± 0.02	0.16 ± 0.01	0.18 ± 0.02	0.18 ± 0.03
Cl, %	0.66 ± 0.05	0.66 ± 0.03	0.62 ± 0.05	0.62 ± 0.06

[1] Treatment variables were corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 337 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN].
[2] Determined via wet chemistry from fresh weekly samples sent to Cumberland Valley Analytical Services for NIR analysis.
[3] Obtained on a single composite sample which was representative of the entire study period via *in vitro* fermentation.
[4] Undigested NDF at 240-h of *in vitro* fermentation
[5] Formulated value given by Cornell Net Carbohydrate and Protein System v. 6.5 using analyzed forage values and mean DMI for each period and treatment.

Table 6-4. Nutrient profile of all postpartum diets (mean ± SD), obtained through wet chemistry analysis by Cumberland Valley Analytical Services (Waynesboro, PA), in vitro fermentation, or as predicted by Cornell Net Carbohydrate and Protein System (version 6.55, Cornell University, Ithaca, NY).

	Postpartum Treatment Diets[1]			
Item	CON-NO	CON-M	BMR-NO	BMR-M
DM, % as fed[2]	44.4 ± 2.4	44.6 ± 2.7	40.9 ± 1.7	41.0 ± 1.9
ADF, % DM	19.5 ± 0.6	19.1 ± 0.6	18.1 ± 0.3	18.2 ± 0.3
aNDFom, % DM[3]	33.1	32.3	31.3	30.8
$uNDF_{240}$[4], % DM[3]	9.4	8.9	7.2	6.9
$uNDF_{240}$[4], % NDF[3]	28.4	27.6	23.2	22.2
CP, %	15.6 ± 0.3	15.5 ± 0.4	16.3 ± 0.7	16.0 ± 0.8
Starch, %	28.0 ± 0.3	27.8 ± 0.8	27.2 ± 1.3	27.0 ± 1.2
Sugar, %	3.2 ± 0.9	3.0 ± 0.6	3.4 ± 0.4	3.4 ± 1.0
Fat, %	3.4 ± 0.3	3.2 ± 0.4	3.4 ± 0.2	3.6 ± 0.2
MP[5], g/kg DM	115.4	115.5	119.8	119.3
NE_L, Mcal/kg	1.65 ± 0.01	1.64 ± 0.01	1.69 ± 0.01	1.69 ± 0.01
Ca, %	1.26 ± 0.10	1.25 ± 0.10	1.23 ± 0.13	1.24 ± 0.20
P, %	0.36 ± 0.01	0.36 ± 0.01	0.36 ± 0.02	0.37 ± 0.01
Mg, %	0.39 ± 0.01	0.39 ± 0.02	0.38 ± 0.04	0.39 ± 0.04
K, %	1.27 ± 0.06	1.28 ± 0.05	1.36 ± 0.04	1.39 ± 0.07
S, %	0.26 ± 0.01	0.25 ± 0.01	0.28 ± 0.01	0.26 ± 0.02
Na, %	0.52 ± 0.05	0.52 ± 0.02	0.50 ± 0.07	0.53 ± 0.08
Cl, %	0.54 ± 0.04	0.55 ± 0.02	0.54 ± 0.06	0.55 ± 0.08

[1] Treatment variables were corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN].
[2] Determined via wet chemistry from fresh weekly samples sent to Cumberland Valley Analytical Services for NIR analysis.
[3] Obtained on a single composite sample which was representative of the entire study period via *in vitro* fermentation.
[4] Undigested NDF at 240-h of *in vitro* fermentation.
[5] Formulated value given by Cornell Net Carbohydrate and Protein System v. 6.5 using analyzed forage values and mean DMI for each period and treatment.

Table 6-5. Least squares means and SE of prepartum dry matter intake (DMI), intake of ash corrected NDF (aNDFom) and undigested fiber after 240 h in-vitro fermentation ($uNDF_{240}$), rumination, energy balance, body weight (BW), body condition score (BCS), and plasma glucose, and geometric means and back transformed 95% confidence intervals for confidence intervals for whole blood BHB and plasma NEFA (non-esterified fatty acids) for cows fed diets differing in corn silage type and monensin supplementation starting -21 d prior to expected calving through calving.

Item	Treatments[1]				SEM	P Value[2]					
	CON-NO	CON-M	BMR-NO	BMR-M		C	M	C×M	C×T	M×T	C×M×T
DMI, kg/d	14.4	13.7	15.3[b]	14.1	0.30	0.03	<0.01	0.44	0.30	0.47	0.85
Starch intake, kg/d	2.50	2.41	2.67	2.40	0.06	0.19	<0.01	0.18	0.33	0.47	0.89
aNDFom intake, kg/d	6.33[x]	6.00[a]	6.63[b]	5.79[a, y]	0.15	0.77	<0.01	0.10	0.31	0.44	0.90
$uNDF_{240}$ intake, kg/d	1.92	1.78	1.76	1.55	0.04	<0.01	<0.01	0.35	0.21	0.43	0.89
DMI, % of BW	1.82	1.69	1.87	1.77	0.06	0.22	0.05	0.84	0.39	0.97	0.34
$uNDF_{240}$ Intake, % of BW	0.24	0.22	0.22	0.19	0.01	<0.01	0.01	0.84	0.25	0.94	0.34
Rumination, min/d	519	497	508	484	9.75	0.23	0.02	0.94	0.94	0.32	0.32
Energy balance, Mcal/d[3]	5.28	3.73	6.56	5.14	0.58	0.02	0.01	0.91	0.19	0.79	0.23
BW, kg	821	812	831	804	19.7	0.96	0.35	0.63	0.61	0.71	0.64
BCS	3.47	3.45	3.43	3.46	0.02	0.50	0.94	0.27	0.61	0.39	0.12
Glucose, mg/dL	60.7	59.0	60.5	60.3	0.61	0.40	0.11	0.22	0.33	0.75	0.63
NEFA μEq/L	107 (94 - 122)	113 (100 - 129)	91.7 (80 - 104)	97.9 (85 - 112)	-	0.02	0.35	0.95	0.05	0.84	0.22
BHB, mg/dL	0.76 (0.7 – 0.8)	0.68 (0.6 – 0.7)	0.71 (0.7 – 0.8)	0.68 (0.6 – 0.7)	-	0.62	0.04	0.53	0.98	0.57	0.27

[1] Treatment variables were corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN].

[2] C = corn silage type, M = monensin supplementation, T = time

[3] Energy balance calculated using NRC (2001) equations.

[a, b] Values with different superscripts in the same row differ significantly ($P \leq 0.05$) due to CS by M interaction after Tukey correction for multiple comparisons.

[x, y] Values with different superscripts in the same row tend to differ ($0.05 < P \leq 0.10$) due to CS by M interaction after Tukey correction for multiple comparisons.

Table 6-6. Least squares means and SE of postpartum dry matter intake (DMI), intake of ash corrected NDF (aNDFom) and undigested fiber after 240 h in-vitro fermentation ($uNDF_{240}$), rumination, energy balance [calculated with NRC (2001) equations], body weight (BW), body condition score (BCS) , feed efficiency, and plasma glucose, and geometric means and back transformed 95% confidence intervals for confidence intervals for whole blood BHB and plasma NEFA (non-esterified fatty acids) for cows fed diets differing in corn silage type and monensin supplementation through 6 wk postpartum.

Item	Treatments[1]				SEM	P Value[2]					
	CON-NO	CON-M	BMR-NO	BMR-M		C	M	C×M	C×T	M×T	C×M×T
DMI, kg/d	22.7	23.2	23.9	22.7	0.55	0.57	0.55	0.14	0.50	0.67	0.44
Starch intake, kg/d	6.40	6.42	6.50	6.12	0.15	0.53	0.24	0.19	0.52	0.66	0.47
aNDFom intake, kg/d	7.56	7.46	7.48	6.99	0.18	0.13	0.10	0.28	0.58	0.66	0.43
$uNDF_{240}$ intake, kg/d	2.15	2.06	1.72	1.56	0.04	<0.01	<0.01	0.48	0.46	0.60	0.46
DMI, % of BW	3.12	3.14	3.19	3.10	0.08	0.86	0.70	0.53	0.26	0.82	0.28
$uNDF_{240}$ intake, % of BW	0.29	0.28	0.23	0.21	0.01	<0.01	0.04	0.77	0.16	0.59	0.24
Rumination, min/d	538	580	546	569	12.1	0.87	<0.01	0.44	0.16	0.96	0.52
Energy balance, Mcal/d	-8.0	-8.9	-7.9	-8.4	1.1	0.79	0.54	0.84	0.74	0.08	0.47
BW, kg	739	741	758	736	17.0	0.68	0.55	0.48	0.76	0.99	0.32
BW change, kg/d	-0.69	-1.02	-0.96	-0.88	0.22	0.74	0.36	0.56	-	-	-
BCS	3.23	3.12	3.19	3.19	0.03	0.54	0.56	0.45	0.18	0.86	0.84
BCS change[3]	-0.35	-0.36	-0.28	-0.31	0.05	0.26	0.70	0.82	-	-	-
Feed efficiency[4]	2.34	2.39	2.39	2.40	0.07	0.65	0.71	0.80	0.23	0.08	0.09
Glucose, mg/dL	47.5	48.3	49.7	51.4	0.61	0.03	0.31	0.73	0.06	0.94	0.21
NEFA μEq/L	487 (430 - 552)	460 (403 - 524)	426 (376 - 482)	339 (299 - 384)	-	<0.01	0.02	0.19	0.27	0.02	0.13
BHB, mg/dL	1.3 (1.1 – 1.5)	1.10 (1.0 – 1.3)	1.09 (0.9 – 1.2)	0.91 (0.8 – 1.0)	-	0.01	0.01	0.91	0.01	0.20	0.61

[1] Treatment variables were corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN].

[2] C = Corn silage type, M = Monensin supplementation, T = Time

[3] Calculated as the difference in BCS over the course of the experiment; BCS Change= BCS at wk 6 - BCS at wk 1

[4] Feed efficiency = energy corrected milk /DMI

Table 6-7. Least squares means and SE milk production, energy corrected milk (ECM), and milk composition data for cows fed diets differing in corn silage type and with our without monensin supplementation starting -21 d prior to expected calving through 6 wk postpartum.

Item	Treatments[1]				SEM	*P* Value[2]					
	CON-NO	CON-M	BMR-NO	BMR-M		C	M	C×M	C×T	M×T	C×M×T
Milk yield, kg/d[3]	45.5	46.2	48.0	48.5	1.22	0.04	0.61	0.95	0.14	0.92	0.02
ECM, kg/d	52.3	54.0	55.9	53.6	1.50	0.28	0.84	0.17	0.56	<0.01	0.13
Fat, %	4.52	4.63[x]	4.52	4.28[y]	0.11	0.10	0.52	0.10	0.99	0.58	0.59
Fat, kg/d	2.02	2.12	2.16	2.02	0.07	0.75	0.78	0.08	0.75	0.02	0.14
True protein, %	3.32	3.34	3.39[x]	3.25[y]	0.04	0.83	0.09	0.02	0.11	0.12	0.15
True protein, kg/d	1.48	1.52	1.61	1.53	0.04	0.04	0.68	0.09	0.54	0.04	0.04
Lactose, %	4.51	4.50	4.52	4.55	0.03	0.34	0.68	0.62	0.65	0.80	0.94
Lactose, kg/d	2.05	2.09	2.19	2.20	0.06	0.03	0.67	0.76	0.01	0.04	0.28
Total solids, %	13.4	13.5[c]	13.5	13.2[d]	0.12	0.21	0.30	0.05	0.99	0.52	0.36
Total solids, kg/d	6.04	6.23	6.50	6.27	0.16	0.13	0.93	0.20	0.16	0.01	0.05
MUN, mg/dL	10.0	10.9	10.7	9.80	0.35	0.56	0.94	0.01	0.34	0.56	0.86
SCS	1.65	1.54	2.20	1.82	0.31	0.18	0.43	0.65	0.68	0.66	0.52
De novo FA											
g/100 g of milk	0.88	0.92	0.97	0.88	0.04	0.44	0.44	0.10	0.74	0.42	0.50
g/100 g of FA	20.9	21.2	22.8	21.4	0.76	0.16	0.46	0.30	0.66	0.60	0.28
g/d	389	411	448	408	26.10	0.24	0.71	0.19	0.74	0.55	0.48
Mixed FA											
g/100 g of milk	1.35	1.38	1.38	1.30	0.03	0.40	0.39	0.05	0.53	0.42	0.82
g/100 g of FA	32.1	32.2	32.6	32.7	0.42	0.21	0.78	0.91	0.45	0.82	0.90
g/d	589[a]	627	642[b]	618	14.3	0.11	0.58	0.03	0.14	0.03	0.09
Preformed FA											
g/100 g of milk	1.96	1.98	1.90	1.84	0.06	0.09	0.76	0.49	0.66	0.86	0.91
g/100 g of FA	46.9	46.6	44.6	45.9	1.05	0.15	0.63	0.43	0.47	0.57	0.81
g/d	876	916	896	871	38.5	0.74	0.85	0.39	0.68	0.06	0.44

[1] Treatment variables were corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN].

Table 6-7. Continued

[2] C = Corn silage type, M = Monensin supplementation, T = Time

[3] Analyzed as weekly means of daily milk yield, all other data in table analyzed as reference day of milk sample (days 4, 7, 10, 14, 21, 28, 35, 42).

[a, b] Values with subscripts in the same row differ significantly ($P \leq 0.05$) due to CS by M interaction after Tukey correction for multiple comparisons.

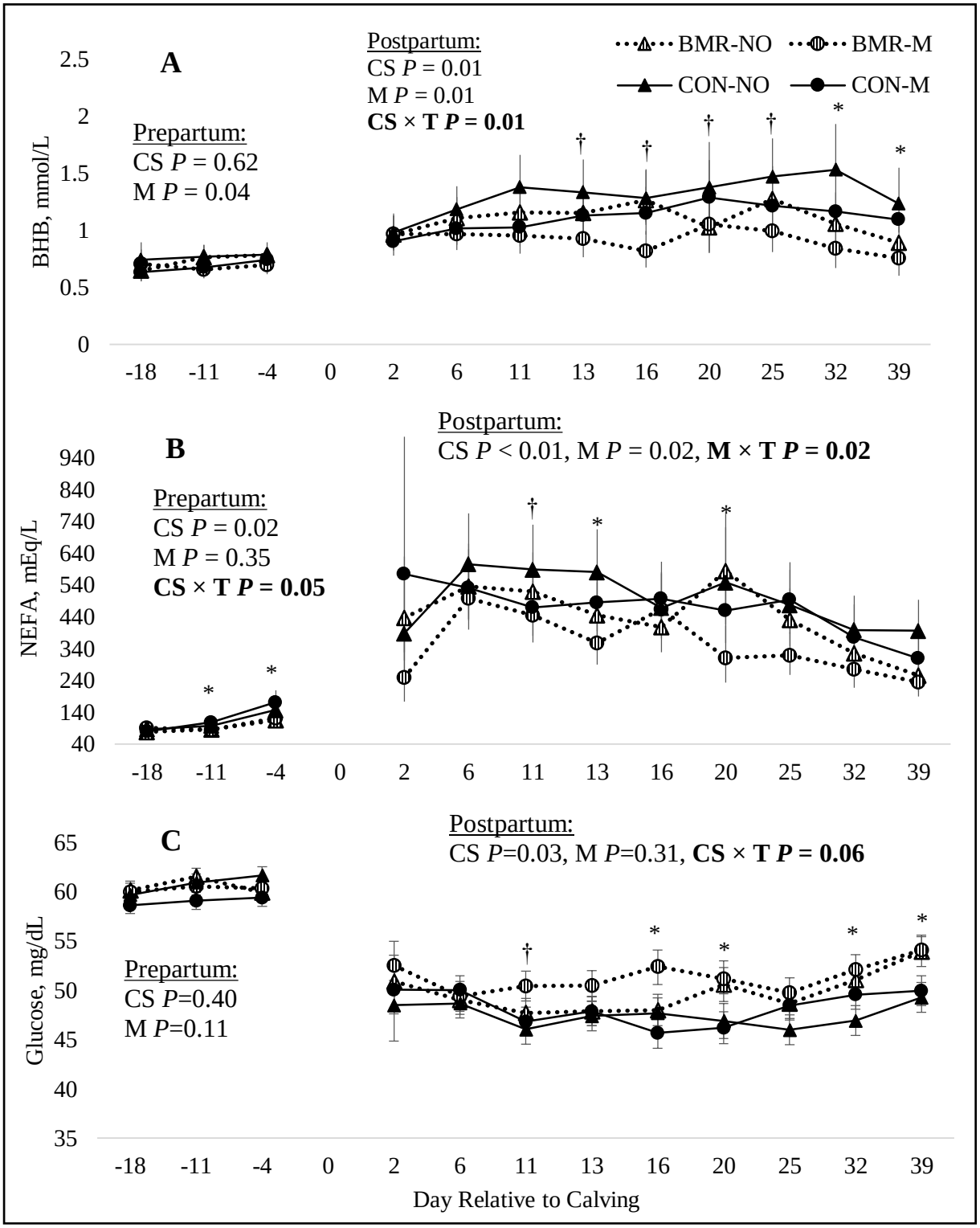
[x, y] Values with subscripts in the same row tend to differ ($0.05 < P \leq 0.10$) due to CS by M interaction after Tukey correction for multiple comparisons

Table 6-8. Incidence of health disorders during the postpartum period for cows fed diets differing in corn silage type and with or without monensin supplementation starting -21 d prior to expected calving through 6 wk postpartum.

	Dietary Treatment [1]				*P*-Value[2]		
Disorder	CON-NO	CON-M	BMR-NO	BMR-M	C	M	C×M
Hyperketonemia[3]	6[a]	1[b]	1[b]	3	0.27	0.25	0.04
Clinical Mastitis	0	1	0	2	NS	NS	NS
Metritis	0	1	0	0	NS	NS	NS
Retained placenta	0	4	3	2	NS	NS	NS
Pneumonia	2	0	1	2	NS	NS	NS
Displaced abomasum	1	0	0	1	NS	NS	NS

[1] Treatment variables were corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN].
[2] Determined using Fisher's exact test. C = Corn silage type, M = Monensin supplementation
[3] Defined as BHB ≥ 1.2 mmol/L in cows <10 DIM.
[a,b] Values with subscripts in the same row differ significantly ($P \leq 0.05$) in incidence due to the interaction of corn silage type by monensin supplementation.

Figure 6-1. Geometric means and back transformed 95% confidence intervals of (A) whole blood BHB, (B) plasma non-esterified fatty acids (NEFA), and least squares means (± SEM) of plasma glucose (C) by day relative to calving for cows fed diets differing in corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN], starting from 21 d prior to expected parturition through 42 DIM. Bold *P* values indicate the interaction with time for which the asterisks (*) and crosses (†) indicate significance, asterisks indicate significant differences of $P \geq 0.05$, crosses indicate trends $0.10 \leq P < 0.05$.


A
Prepartum:
CS P = 0.62
M P = 0.04
Postpartum:
CS P = 0.01
M P = 0.01
CS × T P = 0.01
BMR-NO
BMR-M
CON-NO
CON-M
BHB, mmol/L
2.5
2
1.5
1
0.5
0
-18 -11 -4 0 2 6 11 13 16 20 25 32 39
B
Prepartum:
CS P = 0.02
M P = 0.35
CS × T P = 0.05
Postpartum:
CS P < 0.01, M P = 0.02, M × T P = 0.02
NEFA, mEq/L
940
840
740
640
540
440
340
240
140
40
-18 -11 -4 0 2 6 11 13 16 20 25 32 39
C
Prepartum:
CS P=0.40
M P=0.11
Postpartum:
CS P=0.03, M P=0.31, CS × T P = 0.06
Glucose, mg/dL
65
60
55
50
45
40
35
-18 -11 -4 0 2 6 11 13 16 20 25 32 39
Day Relative to Calving

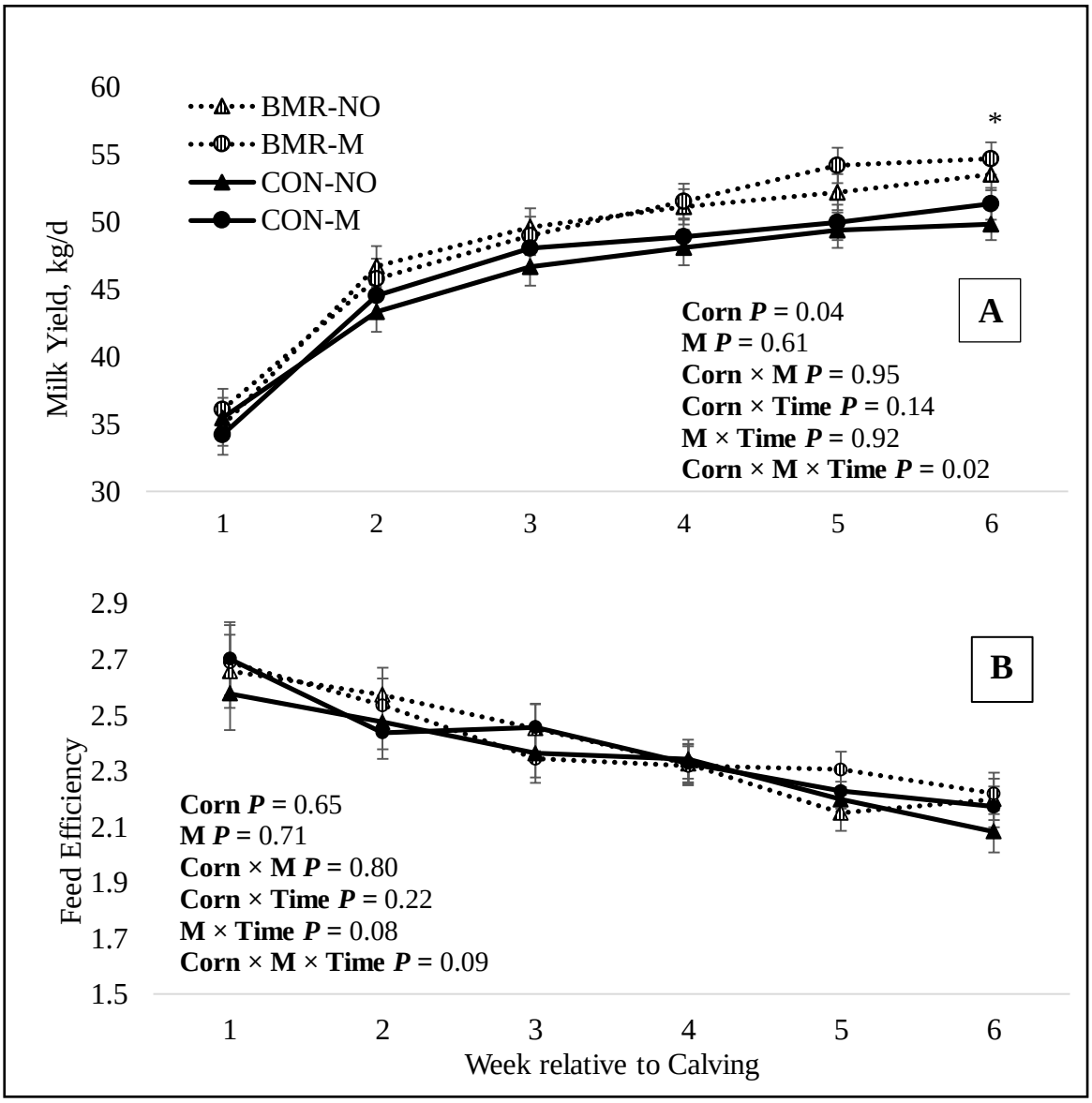


Figure 6-2. Least squares means and standard error of milk yield by week relative to calving (A) and feed efficiency (calculated as feed efficiency = Energy Corrected Milk /DMI; B) for cows fed diets differing in corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN], starting from 21 d prior to expected parturition through 42 DIM. Asterisk (*) indicates a significant differences of $P < 0.10$ in the three way interaction of corn silage by monensin supplementation by time after adjustment for multiple comparisons using the Bonferroni correction.

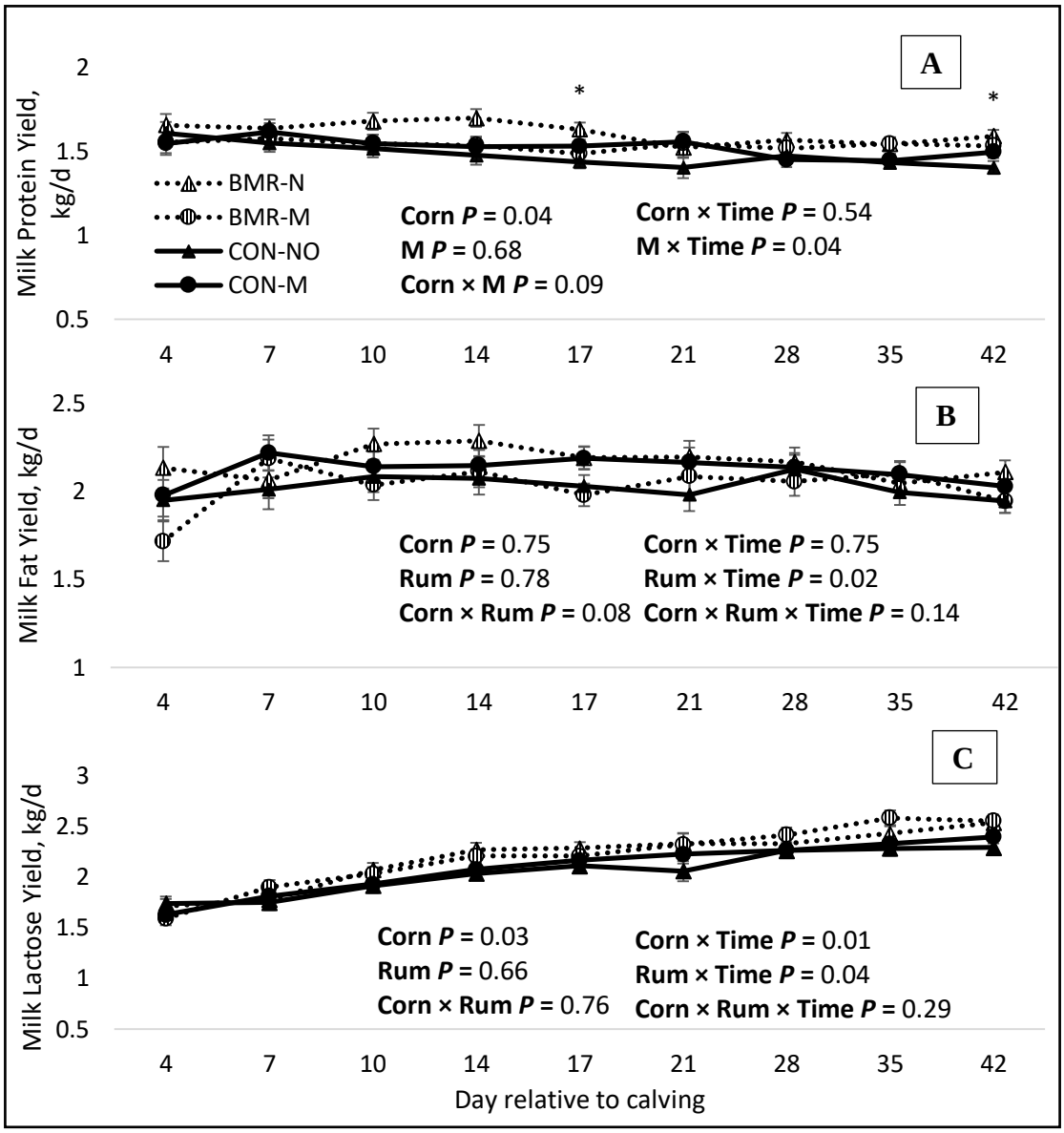


Figure 6-3. Least squares means and standard error of milk component yield, milk protein (A), milk fat (B), and milk lactose (C), by week relative to calving for cows fed diets differing in corn silage type [conventional corn silage, TMF hybrid (CON) vs. brown mid-rib, BM3 hybrid (BMR), Mycogen Seeds, Indianapolis, IN] and monensin supplementation [0 mg/d prepartum and postpartum (NO) vs. 330 mg/d prepartum and 450 mg/d postpartum (M), Elanco Animal Health, Indianapolis, IN], starting from 21 d prior to expected parturition through 42 DIM. Asterisks (*) indicate significant differences of $P < 0.05$ in the three way interaction of corn silage by M supplementation by time after adjustment for multiple comparisons using a Bonferroni correction.

DISCUSSION

The majority of effects observed in this study were for main effects of treatment, with few interactions related to milk yield and efficiency; thus, the majority of discussion will focus on these main effect differences.

Diets and Intake

Brown midrib corn silage has been well documented to have higher *in-vitro* digestibility of NDF and lower lignin content (Oba and Allen, 1999, 2000c, Stone et al., 2012, Holt et al., 2013a). Recently, $uNDF_{240}$ has been investigated as a better measure of the indigestible NDF of a feedstuff as compared to lignin, and likely plays a role in intake and rumen dynamics (Harper and McNeill, 2015, Williams et al., 2016, Grant et al., 2018, Raffrenato et al., 2019). In our experiment, BMR CS had higher *in-vitro* digestibility (11.6 unit increase in $NDFD_{30}$ as % NDF) and lower $uNDF_{240}$ (10 unit decrease as % of NDF) content compared to CON CS, indicating more potentially digestible NDF for the cow. Diet NDF content is linked to DMI (Hayirli et al., 2002) through the physical constraints caused by rumen fill of high NDF diets (Allen, 1996); therefore, increased digestibility of NDF in BMR CS should ease the restriction of physical fill and allow for increased DMI.

Increased DMI when feeding BMR CS-based diets have been observed in several studies (Oba and Allen, 1999, Tine et al., 2001, Ferraretto and Shaver, 2015) , but not others (Oba and Allen, 2000a, Greenfield et al., 2001, Kung et al., 2008). In the prepartum period of our study, cows fed BMR had increased DMI compared to cows fed CON; however, no differences were observed in the postpartum period, despite lower $uNDF_{240}$ intake and higher *in-vitro* fiber digestibility. Several studies have

reported increases in *in-vitro* digestibility, without seeing similar effects on in-vivo NDF digestibility (Oba and Allen, 2000c, Holt et al., 2010), while some have reported accompanying increases in NDF digestibility in vivo (Oba and Allen, 1999, Greenfield et al., 2001, Ferraretto and Shaver, 2015). Overall, the in-vivo rate of digestibility could be dependent on a number of cow factors, including energy balance and demand for nutrients, which can alter passage rate and rumen retention time (Oba and Allen, 2000c).

Reports of increased DMI with BMR CS specifically in the transition period are also mixed. Stone et al. (2012) reported increased DMI for cows fed BMR prepartum and immediately postpartum, whereas Holt et al. (2013a) did not report any differences in DMI until cows were greater than 60 DIM, at which point an increase in DMI was sustained through 180 DIM. While there is evidence that cows in negative energy balance can be limited by gut fill (Allen, 2000), there are many other metabolic, endocrine, and hormonal factors that can be playing a role as well (Ingvartsen and Andersen, 2000), indicating that gut fill may not be the only factor regulating intake at this time.

Despite differences in fiber digestibility and differences in intake prepartum, rumination was not different due to CS type at any time during the study. While rumination has been associated with aspects of NDF previously (Beauchemin et al., 1994, Dado and Allen, 1995, Beauchemin et al., 2003), and differences in rumination with feeding of BMR have been observed (Taylor and Allen, 2005a), several others report no differences in rumination due to BMR feeding (Oba and Allen, 2000b, Holt et al., 2010, Zontini et al., 2015). In recent studies examining rumination time due to

differences in $uNDF_{240}$ and peNDF in forage based diets several researchers have reported no differences in rumination (Zontini et al., 2015, Williams et al., 2016, Grant et al., 2018); however, Zontini et al. (2015) reported feeding high $uNDF_{240}$ in a low forage diet with high inclusion of byproducts resulted in lower rumination compared forage based diets formulated for both high and low $uNDF_{240}$. The lack of differences within forage based diets with different NDF digestibility may indicate NDF digestibility or physical characteristics may not be the primary driver of rumination when adequate fiber fractions are provided in the diet, even if intake is limited, though further investigation is warranted.

Monensin supplementation has been shown to decrease DMI by 0.3 kg in a meta-analysis which includes all stages of production (Duffield et al., 2008b). Cows fed M in the prepartum period had 1 kg/d lower DMI compared to cows not fed M. This decrease in DMI is significantly lower than reported in the meta-analysis, and other recent studies reporting no difference in prepartum DMI with M supplementation (Mullins et al., 2012, McCarthy et al., 2015a); though, tendencies for decreased DMI in the prepartum period have been reported (Petersson-Wolfe et al., 2007), and decreases in DMI of 1.2 kg/d over the periparturient period have been observed with monensin administration (Sauer et al., 1989). It is possible that the increases in propionate provided by monensin resulted in a hypophagic effect due to increased hepatic oxidation in this positive energy balance state (Allen et al., 2009). No differences in DMI due to M supplementation were observed postpartum, similar to findings of others (Mutsvangwa et al., 2002, Zahra et al., 2006, Mullins et al., 2012), indicating

intake regulation in the immediate postpartum period is likely not affected by the same mechanisms as in the prepartum period.

Rumination was altered in both periods due to M supplementation, although in opposite directions between the prepartum and postpartum periods. Prepartum M supplementation decreased rumination time by 23 min/d compared to cows not fed M, which corresponds with the decreased overall DMI, aNDFom intake, and $uNDF_{240}$ intake observed for cows supplemented with M in this period. This follows with findings previously reported in the literature, as rumination has been reported to be related to dietary NDF content (Beauchemin et al., 1994, Dado and Allen, 1995). However, in the postpartum period cows supplemented with M had increased rumination of 32 min/d compared to cows not fed M, with no accompanying change in DMI. To the authors' knowledge, increases in rumination due to M supplementation without changes in DMI have not been previously reported. This could potentially be due to different metabolic status of animals fed M. Monensin supplementation has been demonstrated to decrease plasma BHB and decrease risk of ketosis (Duffield et al., 2008a, c). Decreased rumination has been reported for cows with ketosis (-25 min/d) with further reductions for cows with ketosis accompanying any other health disorder (-44 min/d; Kaufman et al., 2016). Decreased rumination has also been reported for cows at higher risk of subacute rumen acidosis in the transition period (DeVries et al., 2009), while M supplementation has been well documented to increase ruminal pH in beef cows (Nagaraja et al., 1985, Burrin and Britton, 1986), the data supporting a role for it in dairy cows is less clear. Some researchers have reported no effect of M on rumen pH (Mutsvangwa et al., 2002, Mathew et al., 2011), others report

increases in rumen pH with M supplementation (Green et al., 1999), or smaller standard deviation of rumen pH (Mullins et al., 2012) in cows supplemented with M possibly creating a more stable rumen environment. It could be possible that rather than a direct effect on rumination, M supplementation decreases metabolic disorders, thus resulting in increased rumination compared to cows not supplemented with M that are at higher risk of metabolic disease that can decrease rumination.

Energy Status, plasma metabolites, and health outcomes

Preventing excessive negative energy balance is critical for a smooth transition to lactation. To compensate for the inability of dietary energy and nutrients to meet milk production demands, NEFA are mobilized from body tissue (Herdt, 2000). However excessive mobilization of body tissues both prepartum and postpartum can result in metabolic dysfunction and disease (Grummer, 1993, Ospina et al., 2010b). Energy balance of cows in this experiment did not differ due to CS type postpartum, though cows fed BMR maintained higher energy balance and had lower plasma NEFA approaching calving compared to cows fed CON in the prepartum period. Stone et al. (2012) also reported a 1.6 Mcal/d increase in energy balance for cows fed BMR prepartum with no difference in energy balance postpartum. In both studies, the increased energy balance was likely caused by increased DMI also observed during the prepartum period.

In the postpartum period, despite no differences in calculated energy balance due to CS type, cows fed BMR exhibited lower circulating NEFA, BHB, and higher circulating glucose concentrations. While impacts of BMR on nutrient utilization, feeding behavior, production, and efficiency are well documented (Oba and Allen,

2000a, b, c, Ferraretto and Shaver, 2015) effects on energy metabolites are not well-documented. Stone et al. (2012) observed no difference in plasma NEFA or BHB of cows fed BMR prepartum or postpartum; however, samples were collected less frequently than in the current study and perhaps failed to capture any transient effects. There were no differences in DMI postpartum for cows fed BMR, however the intake of NDF fractions (calculated via measured *in-vitro* digestibility) were different due to CS type. Though cows fed BMR ingested similar amounts of total NDF, they consumed less $uNDF_{240}$ in the postpartum period compared to cows fed CON which would result in higher intake of more potentially digestible NDF. The more favorable energy metabolites observed for cows fed BMR are likely due to increased intake of more digestible material for cows fed BMR compared to CON CS. Though evidence of *in-vivo* digestibility differences following *in-vitro* digestibility are mixed, generally studies with no postpartum differences in DMI, similar to this study, have reported increased digestibility of NDF and OM (Greenfield et al., 2001, Lim et al., 2015), indicating increased nutrients could be available to meet the increased energy demands in the early lactation period. Several studies have reported either a decrease in the ruminal acetate:propionate ratio, or increases in propionate alone for cows fed BMR compared to cows fed CON CS (Greenfield et al., 2001, Mutsvangwa et al., 2002, Taylor and Allen, 2005b). As propionate is the primary substrate for gluconeogenesis (Reynolds et al., 2003), it would follow that cows fed BMR would have increased plasma glucose concentrations. Increasing propionate supply to the liver eases glucose demands, and thus decreases the incomplete oxidation of FA for ketone production

(Drackley et al., 2001), while increases in energy balance status associated with increased blood glucose would subsequently decrease plasma NEFA (Herdt, 2000).

Cows fed M tended to have lower predicted energy balance prepartum, likely caused by decreased DMI, but also exhibited lower whole blood BHB when compared to cows not supplemented M. Several studies have found decreased blood BHB overall, including prepartum and postpartum values (Arieli et al., 2008, Mullins et al., 2012) and a meta-analysis reported 13% decrease in BHB due to M supplementation across all production stages (Duffield et al., 2008a). Postpartum energy balance tended to differ over time, with lower energy balance for cows fed M in wk 5 and 6 postpartum, though plasma NEFA and blood BHB were also lower. It is interesting that cows fed M had lower DMI and energy balance in the prepartum period, yet had more favorable energy metabolites throughout the periparturient period. Recently, decreasing the magnitude of DMI change from the dry period through parturition to protect against excessive negative energy balance (Grummer et al., 2004) has been examined through both restriction of intake and controlled energy diets; while these approaches seem to have positive impacts on postpartum metabolism and performance, energy metabolites are often increased in the prepartum period (Douglas et al., 2006, Janovick et al., 2011, Mann et al., 2015). In this experiment, however, cows fed M prepartum had lower overall intake and energy status, but showed favorable effects on blood metabolites that carried through to the postpartum period as well. Monensin effects on blood metabolites are well-documented throughout the literature, with decreases of BHB (Mullins et al., 2012, McCarthy et al., 2015b) and NEFA (Duffield et al., 2008a) being common postpartum. Monensin increases glucose precursors via

shifts of microbial populations in the rumen, which increases propionate production (Bergen and Bates, 1984). In negative energy balance, ketone bodies are formed from partially oxidized fatty acids as fuel for peripheral body systems, as glucose is being spared for the mammary gland (Bauman and Currie, 1980, Drackley et al., 2001). Increases in propionate supply to the liver for gluconeogenesis, such as during M supplementation, decreases ketone production (Drackley et al., 2001), as acetyl-CoA would likely be redirected to the TCA cycle for complete oxidation (Allen et al., 2009).

Monensin has been well-documented to decrease risk of postpartum health disorders, including ketosis, DA, and mastitis (Duffield et al., 2008c), though research on impacts of CS type on disorder incidence is lacking. In this study within cows fed CON CS, feeding M decreased incidence of hyperketonemia in cows <10 DIM compared to those fed NO, and when not supplementing M, cows fed BMR had decreased incidence of hyperketonemia in cows <10 DIM compared to cows fed CON CS. Decreased incidence of hyperketonemia due to BMR CS in cows fed NO is likely due to increases in digestibility of nutrients for cows fed BMR (Lim et al., 2015), causing increased blood glucose and subsequent decreased lipolysis and ketone production as evidenced by decreased NEFA and BHB. Decreased incidence of hyperketonemia due to M supplementation within cows fed CON CS were also likely due to changes in energy metabolism and increased propionate availability with M treatment. While an increase in plasma glucose due to M supplementation was not apparent in this study it has been reported previously (Duffield et al., 2008a), the characteristic decreases in circulating NEFA and BHB would indicate overall better energy status in cows fed M. Duffield et al. (1998) reported decreases in incidence of

subclinical ketosis (BHB <1.2 mmol/L) of 50% with M supplementation, which is lower than what we observed in this study for cows supplemented with M and fed CON CS. The lack of effects of M supplementation for cows fed BMR, or of BMR within cows fed M would suggest that when cows are in a state of increased metabolic health and energy balance due to one dietary factor, a second may not further improve incidence of disease.

Milk production and milk composition

Though evidence of increased production with feeding of BMR CS is mixed from study to study, a meta-analysis across multiple trials and stages of lactation report increased production of 1.5 kg/d, accompanied by a decrease (0.09%) in milk fat percentage, and an increase (0.05 kg/d) in milk protein yield (Ferraretto and Shaver, 2015). This is similar to results for this study, though increases in milk yield (4.9 kg/d) and the tendency for decreased milk fat percentage (0.17%) were higher than reported, higher yields of protein (0.07 kg/d) were similar to reported. Higher milk lactose yield was also observed (0.08 kg/d), which has also been previously reported (Oba and Allen, 2000a, Stone et al., 2012, Ferraretto et al., 2015), likely attributed to increases in milk yield. Higher overall milk yield is often attributed to accompanying increases in DMI, however with no differences in DMI postpartum increases in milk production and changes in milk composition are attributed to changes in rumen fermentation and overall digestibility (Lim et al., 2015). Lower milk fat percentage for cows fed BMR has been well-documented in the literature (Oba and Allen, 2000a, Holt et al., 2010, Lim et al., 2015), though not in all studies (Stone et al., 2012). Decreases in milk fat percentage can likely be attributed to shifts in rumen fermentation, as diets containing

BMR often shift content and ratios of VFA, and can lower rumen pH (Oba and Allen, 2000a, Taylor and Allen, 2005b), which ultimately affect the biohydrogenation pathway (Bauman and Griinari, 2003). Cows fed BMR tended to have lower preformed FA as a percentage of total FA compared to cows fed CON, and when not supplemented with M cows fed BMR tended to have higher grams per day of mixed FA. Previous associations at the herd level would suggest lower preformed FA are associated with higher milk fat percentage (Woolpert et al., 2016, 2017, McCarthy et al., 2018); however we observed the opposite with feeding BMR CS, as cows tended to have lower preformed FA and also lower milk fat percent as compared to cows fed CON CS. As milk preformed FA can be contributed by intestinal FA or FA mobilized from adipose tissue (Woolpert et al., 2017), it is likely the increased preformed FA for cows fed CON were contributed from the increase in body tissue mobilization evidenced by higher circulating NEFA and BHB. Increases in milk protein yield are likely influenced by increases N efficiency and increased microbial protein synthesis and efficiency often reported with BMR CS (Oba and Allen, 2000c, Holt et al., 2013b) likely due to increased ruminal digestibility or increased passage rate.

Similarly, impacts of M supplementation on milk yield and composition have also been well-documented throughout the literature. The meta-analysis across all production stages by Duffield et al. (2008b) reported increased milk yield of 0.7 kg/d, with decreased milk fat and protein percentage, and increased in milk protein yield; however evidence specifically in the transition period is mixed. Mullins et al. (2012) reported no changes in yield or any components, Arieli et al. (2008) reported no change in components as a percentage, but increased milk yield and yield of fat,

protein, and lactose, while McCarthy et al. (2015a) reported increased milk yield, tendencies for decreased milk fat and lactose percentage. In the current study M supplementation decreased milk fat and total solids yield and tended to have lower milk lactose yield on d 4 postpartum, which likely caused the decrease in ECM on d 4 as well, though no other time points were impacted. The reason for this transient decrease in milk components is unknown, as most other effects are reported as means for the entire period measured while in this experiment, no differences were evident after 4 DIM. Monensin supplementation has been thought to stabilize rumen environment through more constant rumen pH immediately after calving (Mullins et al., 2012) rather than causing shifts that might alter microbial fermentation towards a state which results in decreased nutrient utilization. Lower milk fat yield could be due to decreased circulating FA (as NEFA and BHB) available for incorporation into milk fat which were reported for M supplemented cows, though further research in this area is warranted.

As effects of BMR CS and M supplementation have both been well-documented in the literature to increase production and energy availability through increased efficiency, increased intake, or a mixture of the two as mentioned previously, this study was designed to examine any interactions between the two nutritional strategies. Given that sample size of this study was determined to detect a difference between main effect means however, it is likely that we lacked the sample size within individual treatment groups to detect statistical differences of interactions, indicating the type II error could be high. Interactions of CS by M, or three-way interactions of CS by M by time were apparent for milk production and components. A three way

interaction was present for milk yield, while a slice effect of time revealed differences in wk 5 and 6 postpartum, controlling for multiple comparisons revealed only a tendency for a 4.8 kg/d increase of wk 6 milk yield for cows fed BMR-M compared to CON-NO. Numerically, in wk 5 and 6 postpartum, cows fed CON-NO had the lowest and BMR-M had the highest milk production, while cows fed BMR-NO and CON-R were similar. As both BMR and M supplementation have been reported to increase milk yield in meta-analyses (Duffield et al., 2008b, Ferraretto and Shaver, 2015), this result is not surprising; however, this interaction suggests a possible synergistic effect of feeding BMR and M for milk production.

A CS by M interaction was apparent for milk fat, protein, and total solids percentages, as well as milk MUN such that cows fed BMR-M had the lowest values when compared to either cows fed CON-M (milk fat and total solids percent), or BMR-NO (true protein percent). Decreases in milk fat and total solids percentages for cows fed BMR-M as compared to CON-M may would suggest that previously reported and discussed decreases of milk fat for cows fed BMR (Oba and Allen, 2000a, Holt et al., 2010, Lim et al., 2015), and cows fed M (Duffield et al., 2008b, McCarthy et al., 2015a) may have also been synergistic, resulting in the lowest milk fat and total solids percent for cows fed both BMR and M. McCarthy et al. (2018) examined impacts of several dietary factors on milk fat in from 79 herds across the Northeast and upper Midwest. Though no single dietary characteristic accounted for >11% of variation in herd level milk fat, several factors including particle size, monensin dose, starch, and TMR PUFA and MUFA together accounted for 32% of the variation in milk fat depression. While BMR and M act through separate mechanisms to alter milk fat,

when combined in the same diet they may both contribute to further increase the magnitude milk fat depression.

Milk fatty acid composition was also altered when feeding BMR and M. An interaction of CS by M trend was present such that numerically within cows fed BMR, cows fed M had the lowest and NO had the highest grams per 100 g of milk *de novo* fatty acids and mixed FA. Altered rumen environments such as those created with excess PUFA or high dietary carbohydrate fermentation can lead to low *de novo* FA synthesis (Harvatine and Bauman, 2011), and lower *de novo* FA has been associated with cows at higher risk for metabolic disease (Bach et al., 2019). Feeding BMR CS has been reported to alter rumen VFA concentrations and lower rumen pH (Oba and Allen, 2000c, Taylor and Allen, 2005b), and M supplementation can also impact rumen environments and alter rates of biohydrogenation (Fellner et al., 1997, Duffield et al., 2008b). While alone these impacts may not have been enough to negatively alter *de novo* FA synthesis, it is possible that the combination altered the rumen environment enough to impact de novo milk FA synthesis.

Feeding BMR CS has been reported to increase milk protein, likely due to increased microbial efficiency and supply to the duodenum (Oba and Allen, 2000c, Holt et al., 2013a). A tendency for decreased milk protein percentage in cows fed BMR-M compared to cows fed BMR-NO would suggest M supplementation may have a blunting effect of the increased microbial efficiency and supply to the small intestine due to BMR CS. Monensin supplementation has been reported to decrease milk protein percentage, the effects are hypothesized to be due to M altering rumen microbial population, possibly decreasing peptide availability (Duffield et al., 2008b). Though

not significant after correcting for multiple comparisons, cows fed BMR-M had a numerical decrease of 1.1 mg/dL of MUN compared to cows fed CON-M. In the literature, cows fed BMR have lower MUN as compared to cows fed CON (Stone et al., 2012, Ferraretto and Shaver, 2015), whereas cows supplemented with M have been reported to have higher MUN values (McCarthy et al., 2015a). The interaction would suggest cows fed BMR-M are still able to increase efficiency of utilization of N (Oba and Allen, 2000c), despite increases due to M supplementation.

CONCLUSIONS

Increasing energy availability in the periparturient period, through CS type or monensin supplementation, had positive impacts on energy status and production. In the prepartum period feeding BMR increased, while feeding M decreased, DMI and no differences were observed in the postpartum period. Feeding BMR or M resulted in more favorable blood metabolites both pre and postpartum, and decreased incidence of hyperketonemia. Interactions of CS and M were observed for milk yield and components, while a positive effect of BMR and M supplementation was evident for milk yield, milk fat percentage was decreased, fatty acid makeup was altered, and milk MUN was lower for cows fed both BMR and M. While some interactions were unclear, CS type and M supplementation did interact to impact milk production and composition, but not energy metabolites or disease incidence in the postpartum period, more research is warranted to examine interactions further.

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CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTIONS

The transition period is one of opportunity, as successful transitions from pregnancy to lactation often result in a healthier animal and ultimately higher milk production. Nutritional and management strategies that provide adequate energy and promote healthier animals are critical to ensuring success. Dietary energy density can alter metabolism and health status of the animal both pre- and postpartum. Prepartum energy density that meets or is slightly below animal requirements has been found to increase postpartum metabolism and limit metabolic dysfunction. Energy density in postpartum diets however, does not provide a clear consensus due to rumen impacts of energy source. Opportunities to increase energy density in the postpartum period while considering rumen health may increase overall energy balance while also resulting in a more consistent response.

CONCLUSIONS

The first objective of our research was to investigate the effects of varying fiber fractions in high starch postpartum dairy cow diets on performance, hepatic metabolism, and energy balance. High levels of $uNDF_{240}$ and peNDF in the postpartum period can limit DMI and milk production and further increase energy deficit. Intake of total aNDFom was similar between treatments, though with increases in $uNDF_{240}$ of the high fiber treatment less potentially digestible fiber was available for metabolism. Limitations in $uNDF_{240}$ intake occured above approximately 0.32 percent of BW, with an optimum range proposed from 0.29 to 0.32 percent of BW. In cows that were limited by intake, energy metabolites reflected a more negative energy balance and those cows were in a more altered state of metabolism. Altered metabolism was evidenced by both changes in hepatic gene expression and metabolic

rates of fatty acid metabolism, indicating increased hepatic fatty acid load and increased liver triglyceride accumulation.

The second objective of our research was to investigate interactions of inflammation, hepatic metabolism, and metabolic hormones related to energy balance. Though dietary fiber levels impacted metabolism and energy balance via limitations in DMI, overall few effects were observed for dietary treatment on inflammatory markers or metabolic hormones. Plasma IGF-I production was higher for cows fed a diet with lower fiber diet, which accompanied more positive energy balance in wk 3 postpartum, though all other timepoints were similar. Feeding lower fiber diets actually subtly increased markers of inflammation as compared to cows fed higher fiber, which was possibly indicative of inflammation associated with SARA in the lower fiber diet. Correlation analysis revealed associations of increased gene expression of GHR and IGF-I and signs of increased energy balance and improved hepatic health via associations with acute phase proteins and metabolic enzymes. Conversely, gene expression of TNFα and its receptor were associated with markers of decreased energy balance, production, and decreased hepatic health via associations with acute phase proteins and metabolic enzymes. Taken together metabolic shifts, energy balance, liver health, and inflammation are all invariably linked in the transition period.

The final objective of our research was to investigate nutritional strategies to increase dietary energy through multiple mechanisms in the periparturient period. Increasing dietary energy through use of a higher digestibility corn silage or through monensin supplementation positively impacted energy metabolites, health status, and

production. Feeding BMR with monensin had a combined impact to increase milk production and may improve nitrogen utilization, though milk components were decreased and milk fatty acid composition were altered indicating possible impacts on rumen dynamics.

FUTURE DIRECTIONS

Throughout the process of conducting the research presented in this dissertation, I have discovered several findings of interest that may warrant further investigation. First the level of $uNDF_{240}$ in postpartum diets, the study described in Chapter 3, and previous case study research mentioned previously would suggest an optimal range of $uNDF_{240}$ of approximately 0.29 to 0.32 percent of BW; lower than this could induce metabolic dysfunction, and higher can limit DMI. However, given observations from the study discussed in Chapter 5, the lower limit of $uNDF_{240}$ as a percent of BW may be even lower, as cows displayed no negative impacts on intake, plasma metabolites, or milk yield or composition as low as 0.23 percent of BW when fed BMR corn silage. It is likely that the energy density, source, and overall fiber pools, play a role in the ability of the rumen to function optimally with different levels of $uNDF_{240}$ intake. Rather than intake of $uNDF_{240}$ and its impacts being reported generally, further research should examine effects of $uNDF_{240}$ in diets with forages of differing fiber digestibility and varying fermentable carbohydrate levels as these interactions may help quantify impacts of $uNDF_{240}$ across different common dietary scenarios.

Future research on fiber digestibility and energy density of diets in the transition period should also include parameters of rumen health. In Chapter 5, several

alterations were observed with feeding both BMR and monensin that would indicate alterations in rumen fermentation, though without rumen pH and VFA information, we cannot for certain make claims that that was the case. Additionally, for future studies investigating $uNDF_{240}$ interaction with fermentable carbohydrates, this could be particularly useful in understanding performance and metabolic parameter effects. Overall, research investigating rumen pH and dynamics when feeding typical diets in the transition period is limited. Most research on SARA is done through challenge models on mid-lactation or dry cows, which might not accurately reflect the severity and consequences of SARA experienced by cows going through the transition period; thus increasing the number of studies which report such measures in transition cows would likely further our understanding greatly.

Management factors can also greatly impact feeding behavior and rumen dynamics, which could alter impacts observed. For example, all of the research trials reported in this dissertation were conducted with cows that were in tie-stalls and had individual and ad-libitum access to feed, which removes social pressures associated with feeding that can induce SARA. It is possible that when feeding higher risk diets, such as those with lower fiber and higher starch or monensin, in a more applied setting with social pressures surrounding feeding, that rumen dynamics and overall results may have differed greatly. Again, this would imply that research conducted to explain mechanisms related to rumen dynamics might not be representative of cows in a group housed environment that make up the majority of housing the dairy industry today. While removing impacts of social pressures and management factors that might influence metabolism is essential for studies that establish a new idea or investigate

underlying mechanisms, larger studies that are more representative of current management practices are likely of great importance when conducting applied research on feeding strategies.

Research into nutritional strategies and management considerations which might promote optimal metabolism and health for transition cows will continue to be an exciting and evolving field. Further research on nutritional strategies that increase energy availability and metabolism while incorporating rumen fermentation dynamics in the postpartum period will only continue to improve our understanding of how best to navigate the transition period. I look forward to being able to share the knowledge I have gained throughout my PhD to promote successful transitions of cows from pregnancy to lactation as I continue with a career in the dairy industry.