DIET STARCH CONCENTRATION AND STARCH FERMENTABILITY AFFECT ENERGY INTAKE AND PRODUCTION OF DAIRY COWS DURING THE POSTPARTUM PERIOD

By

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ABSTRACT

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During the early postpartum (PP) period dairy cows experience suppressed appetite, negative energy balance and hypoglycemia; increasing the amount of starch in the diet can help to minimize these deficits. However, feeding diets with high starch concentration (SC) or highly fermentable starch can increase the production of propionic acid which can have hypophagic effects by stimulating the oxidation of fuels in the liver. We hypothesize that energy intake, energy balance and metabolic stress of cows in the early PP period can be improved by altering dietary SC and starch fermentability (SF). Our objective was to evaluate the effects of SC and SF of diets fed during the early PP period on DM and energy intake, energy balance, metabolic responses and production. Fifty-two multiparous Holstein cows were used in a completely randomized block design with a 2 X 2 factorial arrangement of treatments. Treatment diets were formulated to 22% (LS) or 28% (HS) starch with dry ground corn (DGC) or high moisture corn (HMC) as the primary starch source. Treatments were fed from 1 to 23 d PP and cows were switched to a common diet until 72 d PP to measure carryover (CO) effects. Treatment period (TP) diets were formulated for 22% forage NDF and 17% CP, and starch concentration was adjusted by substitution of corn grain for soyhulls. Throughout the experiment, DMI and MY were measured daily, and milk components, BCS and BW were measured weekly. During the TP, feeds, refusals, and fecal samples were collected and digestibility was determined weekly. Blood was collected weekly during the TP and every second week during the CO period. During the TP, HMC decreased intakes of DM and net energy of lactation (NE_L) more when included in

the HS (3.9 kg/d and 3.2 Mcal/d) than in the LS (0.9 kg/d and 0.6 Mcal/d) diets and HMC decreased yields of milk, fat, protein, 3.5% FCM and milk NE_L by 4.3, 0.19, 0.18, 4.8 kg/d and 2.8 Mcal/d, respectively. Treatments also interacted over time to decrease DMI and yields of milk and milk components more for HMC compared with DGC as time progressed during the TP. Over the TP, BCS loss was increased when HMC was fed in a HS diet (-0.38 vs. -0.17) and decreased when included in a LS diet (-0.21 vs. -0.29) compared with DGC with no effects on BW change. Energy balance was improved by HS compared with LS (-14.7 vs. -16.8 Mcal/d). Treatments interacted to affect plasma concentrations of TNFα, haptoglobin and lipopolysaccharide binding protein, with HMC increasing their concentrations for HS (9.29 vs. 8.42 pg/mL, 0.54 vs. 0.41 mg/mL and 5.85 vs. 4.67 μg/mL, respectively) and decreasing their concentrations for LS (5.88 vs. 11.3 pg/mL, 0.29 vs. 0.44 mg/mL and 4.41 vs. 6.02 μg/mL, respectively) compared with DGC. During the CO period, treatment effects on DMI diminished over time with no main effects of treatment for the entire period. Treatments interacted to affect yields of milk, milk fat and FCM during the CO period, which were greater for HS-DGC and LS-HMC (54.8 and 52.8, 1.76 and 1.81, and 51.3 and 52.2 kg/d, respectively) than for LS-DGC and HS-HMC (51.2 and 51.0, 1.68 and 1.64, and 48.4 and 48.6 kg/d, respectively). Treatments did not affect BCS change during the CO period but HS lost BW compared with LS (-5.7 vs. 7.0 kg). Concentrations of blood markers of inflammation were not affected by treatments during the CO period. Overall, our results are consistent with the Hepatic Oxidation Theory of control of feed intake and we were able to confirm that energy intake, production and metabolic stress during the early PP period can be improved by altering diet SC and SF. Our findings on production response during the CO period suggest a relationship with the inflammatory response elicited by treatments during the TP.

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KEY TO ABBREVIATIONS

AOP	Antioxidant potential
BCS	Body condition score
BFT	Backfat thickness
BHB	ß-hydroxybutyrate
BW	Body weight
CLA	Conjugated linoleic acid
CO	Carryover
CoA	Coenzyme A
CP	Crude protein
DE	Digestible energy
DGC	Dry ground corn
DM	Dry matter
DMI	Dry matter intake
EB	Energy balance
FA	Fatty acid
fNDF	Forage neutral-detergent fiber
GE	Gross energy
HMC	High moisture corn
HS	High starch
LBP	Lipopolysaccharide binding protein
LPS	Lipopolysaccharide
LS	Low starch

ME	Metabolizable energy
mRNA	Messenger ribonucleic acid
NDF	Neutral-detergent fiber
NE	Net energy
NEFA	Nonesterified fatty acids
NFC	Non fiber carbohydrate
OM	Organic matter
OSi	Oxidative stress index
PP	Postpartum
RONS	Reactive oxygen and nitrogen species
SC	Starch concentration
SCFA	Short-chain fatty acid
SEM	Standard error of the mean
SF	Starch fermentability
TCA	Tricarboxylic acid
TMR	Total-mixed ration
TNFα	Tumor necrosis factor alpha
TP	Treatment period

INTRODUCTION

Nutrient demand to support the last stage of gestation and onset of galactopoiesis are partially met by reducing nutrient uptake by peripheral tissues and from adipose tissue mobilization. Adipose and muscle tissue insulin resistance and increased sensitivity to lipolytic agents during late gestation drive this process (Bell, 1995), accompanied by a decrease in circulating insulin and increased metabolic stress after calving (Esposito et al., 2014; Sordillo and Mavangira, 2014). Suppression of appetite during the early postpartum (**PP**) period results in negative energy balance, exacerbating the metabolic stress and increasing risk for decreased health, productive and reproductive performance (Herdt, 2000; Butler, 2003; Ospina et al., 2010). Also, a review of studies revealed that inadequate nutrition during early lactation can cause a decrease in milk production of 22 to 63% between the following 3 to 12 weeks (Jørgensen et al., 2016), suggesting that early PP nutrition can have carryover effects later in lactation.

Increasing energy density of the diet by increasing starch concentration is a strategy commonly used to increase energy intake by dairy cows. Starch is an important source of fermentable energy for rumen microorganisms (Koenig et al., 2003) and supplies glucose and glucose precursors to the cow. However, there is no clear consensus among nutritionists and researchers regarding recommendations for the starch concentration (SC) and starch fermentability (SF) of diets fed to cows during the early PP period. Increasing diet starch concentration increased DMI in studies reported by Rabelo et al. (2003) and Andersen et al. (2003) but had no effect on DMI in studies reported by Nelson et al. (2011) and McCarthy et al. (2015). In addition, ruminal fermentability of starch varies greatly with grain type, processing

and conservation method (Allen, 2000), but increased starch fermentability decreased DMI in one study (Sadri et al., 2009) and had no effect in another (Rockwell and Allen, 2016). Previous research from our lab suggests that suppression of feed intake by cows receiving highly fermentable starch (e.g. high moisture corn) is likely from increased oxidation of fuels in the liver stimulated by propionate, the primary short-chain fatty acid derived from starch fermentation (Allen, 2000; Oba and Allen, 2003a; Oba and Allen, 2003b). However, the combined effects of SC and SF of rations fed during the early PP on energy intake and production have not been investigated, and its carryover effects are unknown.

Cows ability to metabolically adapt to the PP conditions is challenging and highly fermentable diets can exacerbate metabolic stress. Increasing diet SC (28% or more) have resulted in an increased inflammatory response in dairy cows (Gott et al., 2015; Emmanuel et al., 2008) and increased oxidative stress in lactating ewes (Sgorlon et al., 2008). In addition, a review from several studies concluded that feeding cows more than 44% of dietary concentrate containing highly fermentable starch sources (e.g. barley or wheat grain) linearly increases circulating markers of systemic inflammation (Zebeli et al., 2012). Increased inflammatory response to highly fermentable diets might be from absorption of bacterial lipopolysaccharide derived from lysis of gram-negative bacteria in the gastrointestinal tract (Khafipour et al., 2009) and this can also increase oxidative stress (Abaker et al., 2017). Yet, none of the studies mentioned above were performed in the critical postpartum period when cows are experiencing an innate metabolic stress, with carryover effects still unknown.

Considering the paucity of data previously mentioned, our objective is to evaluate effects of diet SC and SF of diets fed during the early PP period and its carryover effects on metabolic responses, energy intake and production. We hypothesize that energy intake, energy balance and

metabolic stress of cows in the early PP period can be improved by altering dietary SC and SF. We conducted a study to evaluate the effects of diet SC and SF of diets fed during the early PP period on 1) feed intake and yields of milk and milk components, as well as carryover effects on production, 2) diet digestibility, energy intake and balance, and milk fatty acid composition, and 3) inflammatory response and oxidative stress.

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CHAPTER 1. LITERATURE REVIEW

MEETING EARLY LACTATION COW ENERGY AND GLUCOSE DEMAND

After parturition, cow energy and glucose demand must increase to meet the needs for lactation, uterine involution, immune system, and increased splanchnic tissue. Bell (1995) estimated that demand by the mammary gland for glucose, amino acids, fatty acids and energy increases 2.7, 2, 4.5 and 3 fold, respectively, at 4 days postpartum compared to demand by the gravid uterus during late pregnancy. However, suppression of appetite during the early postpartum (**PP**) period along with increasing milk energy output results in negative energy balance which can decrease production, health and reproductive performance (Herdt, 2000; Butler, 2003; Ospina et al. 2010) and can lead to decreased production later in lactation (Jørgensen et al., 2016). The majority of health disorders in lactating dairy cows occur during the early PP period, likely exacerbating the negative energy balance. Supporting this notion, Kvidera et al. (2017) reported that a challenged immune system (with jugular infusion of lipopolysaccharide) increases glucose demand by up to 1 kg within 12 h post challenge in mid-lactation cows. During the early PP period, low insulin concentration and increased insulin resistance by adipose tissue reduces lipogenesis and stimulates lipolysis (Bell, 1995), increasing the oxidation of NEFA, likely suppressing feed intake (Allen et al., 2009) and decreasing supply of energy to the cow. Negative energy balance might be improved by increasing the energy density of the diet. Commercially available dietary fat supplements are often used to increase energy density of diets (Wang et al., 2010); however, their contribution of glucogenic precursors to dairy cows is negligible and they can depress DM and energy intake (Allen, 2000). Effects of individual fatty acids and their combinations on intakes of DM and energy, and their

physiological effects in dairy cows have not been fully elucidated (Piantoni et al., 2015; de Souza, 2018). Alternatively, starch from cereal grains can provide glucose and glucose precursors and energy, and diets with high starch concentrations are commonly fed to dairy cows for this purpose. However, recommendations for starch concentration in rations for lactating cows are highly variable, ranging from less than 20% to more than 28% DM, with recommendations being even more unclear for cows during the early PP period (Grummer, 2008). In addition, there is a large variability in ruminal starch fermentability among starch sources (Nocek and Tamminga, 1991; Firkins et al., 2001), and both amount and source of starch can affect ruminal pH and fiber digestibility (Krause and Oetzel, 2006; Firkins et al., 2001), as well as the type, amount, and temporal absorption of fuels derived from starch (e.g. propionate, acetate, lactate, glucose; Allen, 2000).

STARCH FOR DAIRY COWS

Starch is a polysaccharide comprised of glucose with α 1,4 (amylose) or α 1,6 (amylopectin) linkages that aggregate in semi-crystalline structures within granules which can vary in size, shape, and structure among cereal grains (Thomas and Atwell, 1999). Starch granules are found in the endosperm of cereal grains and typically contain about 25-28% amylose and 72-75% amylopectin (Colonna and Buléon, 1992). Granules are embedded within a protein matrix that acts as a barrier for starch degradation and is constituted of prolamins and other proteins (e.g. albumins, globulins, and glutelins; Shewry and Halford, 2002). The protein matrix surrounding the granule varies in solubility and resistance to digestion (Kotarski et al., 1992) and prolamin content in particular has been negatively correlated with ruminal starch digestibility (Correa et al., 2002). Prolamin content and distribution varies among grains, but within corn grain prolamin content is greater for vitreous endosperm compared with floury

endosperm. Accordingly, in vitro starch digestibility was decreased by 47% for corn grain with ~64% vitreous endosperm compared with corn grain with floury endosperm (0% vitreous endosperm; Lopes et al., 2009) and in vivo ruminal starch digestibility was decreased by 19% when corn grain with 66% vitreous endosperm was replaced with corn grain with 25% vitreous endosperm (Allen et al., 2008). However, different processing and conservation methods can solubilize or disrupt the protein matrix surrounding the starch granules increasing exposure to microbial enzymes and starch digestibility.

Ruminal Fermentability of Starch and Fuels Derived

Ruminal fermentability of starch is highly variable and is affected by particle size, starch gelatinization, and amount and solubility of endosperm proteins. Different grain types such as wheat, barley, corn, and sorghum have great differences in concentration and solubility of endosperm proteins, which can dramatically affect the rate of starch fermentation (Kotarski et al., 1992). Grain processing (e.g. grinding) and conservation method (e.g. ensiling) are also major factors that increase starch availability and digestion. Allen (2000) reviewed seven experiments with duodenally-cannulated lactating cows and reported that across different grains conserved and processed with different methods ruminal starch fermentability varied from less than 30% to more than 85%. More specifically for corn grain, the primary starch source fed to dairy cows in North America, ruminal fermentability ranged from less than 40% to more than 90% for various conservation and processing methods (Nocek and Tamminga, 1991). For example, in situ starch digestibility from semi-flint corn increased with grinding (0.6 mm) compared with coarse rolling (3.5 mm; 69.8% vs. 53.5%, respectively), and starch digestibility from dent corn was 19% greater with ground or medium rolling compared with coarse rolling (0.7-1.8 vs. 3.7 mm; Remond et al., 2004). Further, ruminal starch digestibility was greater for corn grain when

ensiled (89.9%) compared with steam-flaked (84.8%), steam-rolled (72.1%) or dry-rolled (76.2%; Huntington, 1997). In many areas of dairy production in North America, a common practice is to conserve corn grain as high moisture corn (**HMC**) to reduce field losses and drying costs. Ensiling solubilizes endosperm proteins, reducing prolamin content and increasing starch fermentability (Larson and Hoffman, 2008), and for HMC, starch fermentability was greater with increased moisture content at harvest and length of time ensiled (Allen et al., 2003).

Typical diets for lactating dairy cows in North America can contain up to 30% starch or more as percentage of diet DM, and its ruminal fermentability primarily determines the type and temporal absorption of fuels. Propionic acid is the primary short-chain fatty acid (**SCFA**) derived from ruminal starch fermentation, and its production increases from 15 to 45% of total SCFA as fermentable OM of the diet increases in diets with high vs. low forage to concentrate ratio (Davis, 1967). A review of studies revealed that from a wide range of diets fed to lactating dairy cows ruminal fermentation of OM ranged from 5.7 to 15.4 kg/d and production of SCFA ranged from 42 to 115 moles/d (Allen, 1997). Therefore, it can be inferred from Davis (1967) and Allen (1997) studies that ruminal production of propionic acid might range from ~ 6 to 52 moles/d. Ruminal starch fermentability and temporal supply of fuels to the cow are likely also influenced by other factors such as individual cow behavior (e.g. chewing rate and sorting), diet characteristics (e.g. fibrous forage content and particle size), management practices (e.g. grain supplementation vs. TMR) and facilities (e.g. feed bunk space). However, little research has been done in this regard and at a herd level, the starch source included in the ration is likely the most important factor influencing ruminal starch fermentability and fuels derived.

Intestinal Digestibility of Starch and Fuels Derived

Kinetics of ruminal starch digestion can be altered to shift starch site of digestion. Starch that escapes ruminal digestion can be degraded by pancreatic amylase in the duodenum, digested by enteric bacteria or excreted. In the small intestine, mucosal enzymes can further cleave bonds between saccharides (Harmon, 2009) with the available glucose being efficiently absorbed by intestinal epithelium. A meta-analysis by Moharrey et al. (2014) that covered a wide range of diets and production levels for lactating dairy cows reported that despite a larger proportion of the total starch intake being digested in the rumen, starch digested in the small intestine ranged from 114 to 901 g/kg of enteric starch. Also, in that study authors reported that starch sources with high total-tract digestibility increase starch digestibility in all digestive compartments (rumen, small intestine and hindgut), while sources with low ruminal starch digestibility have little compensatory starch digestion in the lower tract.

Glucose is the primary end product of starch digestion in the small intestine and after absorption by the intestinal epithelium it is partially metabolized to lactate (Reynolds et al., 2003), therefore little glucose appears in the portal circulation in ruminants (Huntington and Reynolds, 1986). Starch that is not digested in the small intestine can be partially digested by hind gut microorganisms into SCFA or excreted in feces. Therefore, diets with similar concentrations of starch but different ruminal starch digestibility can affect site and extent of starch digestion, affecting the type and temporal absorption of fuels.

EFFECT OF RUMINAL STARCH FERMENTABILITY ON FEED INTAKE

Replacing sources of starch with moderate fermentability for sources with highly fermentable starch decreased DMI of lactating cows by ~3 kg/d (~13%) in several studies (Allen,

2000). Consistent with this, Oba and Allen (2003a) reported a decrease in meal size (21%) and feed intake (7.6%) for cows during early lactation when DGC was replaced with the more fermentable HMC (2.3 vs.1.9 kg and 22.5 vs. 20.8 kg/d, respectively). Increasing starch fermentability shifted the primary site of starch digestion (rumen vs. post-rumen), increasing the amount and temporal supply of specific SCFA to the cow, with this last effect likely associated with the observed decrease in meal size.

Bradford and Allen (2007) reported an average decrease in feed intake of 2.0 kg/d for cows in mid- and late-lactation receiving HMC compared with DGC, but difference in DMI (more fermentable-less fermentable) ranged from +1.7 to -6.6 kg/d. In that study, hypophagic effects from HMC were exacerbated for cows with higher mean plasma insulin concentration and were quadratically related to insulin response to glucose infusion, indicative of an interaction between feed intake response and cow physiological state. Low insulin concentration and insulin sensitivity of tissues by cows during the early PP period induces a lipolytic state (Allen et al., 2009), but its interactions with starch concentration and ruminal starch fermentability are not known. One experiment reported by Dann et al. (1999) evaluated the effects of starch sources varying in ruminal fermentability (steam-flaked corn and cracked corn) for the first 9 weeks of lactation. The more fermentable starch source (steam-flaked corn) tended to decrease DMI over the 9-week experiment ($P = 0.13$) compared with the less fermentable starch source (cracked corn). However, responses were not reported for the early PP period separately, when the reduction in DMI by the more fermentable starch is expected to be greater. In accordance, Sadri et al. (2009) reported that increasing diet starch fermentability by substituting dry ground barley for DGC, decreased DMI 1.4 kg/d during the first 28 d PP despite a greater non fiber carbohydrate (NFC) concentration for the DGC diet (40.6%) compared with the ground barley

diet (38%). The hypophagic effects of highly fermentable starch sources are likely dependent upon the physiological state of the cow.

Propionate Effects on Feed Intake

Propionate is the primary glucose precursor for ruminants and can alleviate glucose demand during the early PP period. However, hypophagic effects of propionate infusions (as propionic acid or sodium propionate) have been documented for dairy cows (Oba and Allen, 2003b; Gualdrón-Duarte and Allen 2018; Maldini and Allen, 2018). Anil and Forbes (1980) demonstrated that propionate is more hypophagic than butyrate or acetate when infused in the portal vein of sheep. In dairy cows during the early PP period, intra-ruminal infusions of propionate decreased DM and metabolizable energy (ME) intake by decreasing meal size when compared with isomolar infusions of acetate (Oba and Allen, 2003b). In addition, Gualdrón-Duarte and Allen (2018) conducted abomasal infusions of primary fuels derived from starch digestion in cows during the early PP period and reported that compared with control, propionic acid decreased intakes of DM and ME by 24% and 13%, respectively, while lactic acid infusion only affected DMI (14%) but not ME intake, with no effects of glucose infusion on either variable. These studies indicate that hypophagic effects of propionate are not related to energy intake but to specific mechanisms associated with the metabolism of propionate.

Piantoni et al. (2015) reported that DMI at 4 h post feeding by cows during the early PP period (~12 d PP) was negatively related to change (pre- vs. 4 h post-feeding) in hepatic acetyl CoA concentration and positively related to the reduction in plasma NEFA concentration within that same timeframe, with change in acetyl CoA concentration positively related to the reduction in NEFA concentration. In that study, the observed increase in plasma insulin likely reduced lipolysis and NEFA concentration, reducing the amount of acetyl CoA available for hepatic

oxidation and energy production in the liver and increasing DMI over the first 4 h post-feeding. In this regard, Anil and Forbes (1988) demonstrated that there is a relationship between the metabolism of propionate in the liver, the excitability of the vagus nerve and hunger or satiety signals. With a high rate of ruminal production, absorption and flux to the liver (Benson et al., 2002), propionate is metabolized by the hepatic enzyme propionyl CoA synthetase into propionyl CoA. Propionyl CoA can then be metabolized in the liver to produce glucose via gluconeogenesis or the carbon remains in the TCA cycle stimulating oxidation of acetyl CoA. In the TCA cycle, propionyl CoA promotes the complete oxidation of acetyl CoA, increasing liver energy charge and likely stimulating a satiety signal that decreases feed intake; this proposed mechanism of control of feed intake is known as the Hepatic Oxidation Theory (Allen et al., 2009). In accordance, Stocks and Allen (2012) demonstrated that intraruminal infusions of propionate in cows during the early PP period depressed DMI 20% compared with iso-osmotic infusions of acetate, and hypophagic effects of propionate were greater for cows with greater hepatic acetyl CoA concentration. Feeding different amounts of starch with different ruminal fermentability will likely yield differences in production of propionic acid in the rumen and the relative contribution of propionate to satiety.

DIET STARCH CONCENTRATION

Previous studies reported increases in DMI and milk yield during the early PP period when diet starch concentration was increased (Andersen et al., 2003; Rabelo et al., 2003). However, in those experiments the main starch sources were substituted for forage, decreasing the forage NDF (**fNDF**) concentration of the high starch diets. In a review of studies, Allen (2000) reported that fNDF is very filling compared with other dietary components, and likely greater fNDF concentration of the lower starch diets might have contributed to satiety by

increasing ruminal distention, especially as DMI over the lactation progressed and mobilization of body reserves diminished. Accordingly, in studies reported by Nelson et al. (2011) and McCarthy et al. (2015) increasing dietary starch concentration from ~21% to ~26% by replacing non-forage NDF sources with starch sources with moderate ruminal fermentability during the first 21 d PP did not affect DMI, likely because increasing dietary starch concentration did not reduce the filling effect of diets. These studies suggest that the isolated effect of dietary starch concentration cannot be evaluated in diets that differ in fNDF concentration.

A companion publication of the Rabelo et al. (2003) study in which dry corn grain was substituted for forage in diets fed to cows during the early PP period reported effects of treatments on estimated energy intake and energy balance (Rabelo et al., 2005). Increasing the amount of starch in the diet tended to increase DMI (7%) and estimated energy intake (10%), accompanied by a reduction in ruminal pH from 6.14 to 5.90. Dry ground corn has a moderate ruminal starch fermentability and different results might have occurred if the more fermentable HMC was included in the diet. In addition, the reduction in ruminal pH can decrease fiber digestibility and further decrease energy intake. Yet, the interaction between diet starch concentration and starch fermentability of diets fed during the early PP period has not been investigated.

STARCH EFFECTS IN THE RUMEN

Highly fermentable diets can decrease ruminal pH as fermentation acid production exceeds clearance and free hydrogen ions accumulate when the buffering capacity of digesta in the rumen is exceeded. Lower NDF intake in the early PP period compared with cows in early to mid-lactation, likely reduces rumen digesta mass and the buffering capacity of the rumen

contents (Allen and Piantoni, 2014). Decreased ruminal pH can reduce ruminal digestibility of fiber as demonstrated by a linear reduction in rate of digestion of potentially digestible NDF from over 4%/h to less than 1%/h as mean ruminal pH decreased from 6.5 to 5.7 in mid-lactation cows (Oba and Allen, 2003c), and this can affect energy intake and energy balance for cows in the early PP period. In addition, effects on fiber digestibility can impact production and in this regard Oba and Allen (1999) reported that for every unit increase in in vitro or in situ NDF digestibility, 4% fat corrected milk increased 0.25 kg/d.

Highly fermentable diets can also affect ruminal biohydrogenation pathways of polyunsaturated fatty acids and increase production of specific conjugated linoleic acid (**CLA**) isomers (e.g. trans-9, cis-12; trans-10, cis-12; cis-10, trans-12) that can affect energy partitioning and decrease milk fat yield (Bauman et al., 2008; Harvatine et al., 2009). Harvatine et al. (2007) summarized some of the effects associated with both diet- and CLA-induced milk fat depression in mammary tissue and reported decreases in mRNA abundance of genes involved in fatty acid transport, uptake, synthesis, and esterification (Harvatine et al., 2007). In contrast to reported changes in mammary tissue, gene expression for enzymes related to lipid synthesis in adipose tissue were increased by abomasal infusion of CLA trans-10, cis-12 (Harvatine et al., 2009). In accordance, Harvatine and Allen (2006) observed an increase in energy partitioned to body weight during milk fat depression for mid-lactation cows with increased ruminal passage of CLA trans-10, cis-12. Further, increasing supplementation (0, 200, 400 and 600 g/d) of a rumen-protected CLA supplement (containing a mix of CLA isomers including CLA trans-10, cis-12) during the peripartum period decreased milk fat content and yield in a dose-responsive manner during the early PP period, accompanied by a reduction in energy balance nadir by the two highest doses compared with control (0 g/d; Moore et al., 2004). Although there is a paucity of

evidence on the effects of highly fermentable diets fed during the early PP period and CLA isomers derived from altered biohydrogenation pathways, we hypothesize that increasing diet fermentability will improve energy balance by decreasing milk energy output and lipolysis in adipose tissue in cows during the early PP period.

METABOLIC STRESS DURING THE POSTPARTUM PERIOD

During the peripartum period, changes in hormonal and metabolic functions, paired with a negative energy balance, causes an increase in inflammation and metabolic stress. Insulin resistance has also been identified as a causative factor for low-grade inflammation in dairy cows (Sordillo and Mavangira, 2014). While physiological inflammation postpartum aids in the recovery of reproductive function in dairy cows (LeBlanc et al., 2012), it is also triggered to protect from harmful pathogens and tissue damage (Sordillo et al., 2009). Inflammation can induce changes in metabolism (e.g. increased lipolysis), which under acute inflammation can predispose the animal to disease. Oxidative stress occurs when increased metabolic activity generates an imbalance between reactive species (e.g. reactive oxygen species) and the availability of antioxidants. This imbalance favors immunosuppression and a more acute inflammatory response, increasing the risk for disease in ruminants (Sordillo and Aitken, 2009). When these effects are coupled with the suppression of appetite and the negative energy balance occurring during the early PP period, the risk for disease is increased. Evidence suggests that energy intake, inflammation and oxidative status are interrelated and can be modulated with dietary factors (Miller et al., 1993; Bertoni et al., 2015). Nutritional approaches can likely increase energy intake and minimize negative energy balance, reduce the severity and extent of the inflammatory response and oxidative stress, and improve herd health and reproductive performance during early lactation.

Effects of Diet Starch Concentration and Fermentability on Metabolic Stress

Research in ruminants has shown that nutrition can affect the extent and severity of the inflammatory response (Bertoni et al., 2015). Some studies have reported that ruminal acidosis can induce a severe inflammatory response in dairy cattle as well as oxidative stress (Khafipour et al., 2009; Guo et al., 2013). Whereas the methodologies implemented in these studies to cause an acidosis challenge do not represent common feeding conditions, high levels of starch in the diet (28% or more) have yielded an inflammatory response in dairy cows (Gott et al., 2015; Emmanuel et al., 2008) and increased oxidative stress in lactating ewes (Sgorlon et al., 2008). In addition, a review from several studies concluded that feeding cows more than 44% of dietary concentrate containing highly fermentable starch sources (e.g. barley or wheat grain) linearly increases circulating markers of systemic inflammation (Zebeli et al., 2012). Increased inflammatory response to highly fermentable diets might be from absorption of bacterial lipopolysaccharide (**LPS**) derived from lysis of gram-negative bacteria in the gastrointestinal tract (Khafipour et al., 2009). Even though there is a lack of research on the effects of diet starch fermentability on oxidative stress, a more acute inflammatory response when cows receive high quantities of rapidly fermentable starch could be accompanied by oxidative stress as inflammatory cells increase production of reactive species (Mier-Cabrera et al., 2011). Yet, none of the studies mentioned above were performed in the critical postpartum period when cows are experiencing an innate systemic inflammation, with carryover effects still unknown.

INTERACTION: DIET STARCH CONCENTRATION × STARCH FERMENTABILITY

No experiment has evaluated the combined effects of both concentration and ruminal fermentability of starch in diets fed to cows in the early PP period. While many commercial dairy farms feed a specific ration to cows in the PP period, the length of time this diet is fed

varies widely from less than 10 days to well past peak lactation at 60 days or more. Feeding the same diet for longer than 2-3 weeks makes it difficult to find an optimum diet because the potentially negative effects of highly fermentable diets in the early PP period are likely alleviated and possibly reversed once the lipolytic state and control of feed intake by hepatic oxidation diminishes several weeks into lactation and signals related to ruminal distention begin to dominate. Increasing the amount or the fermentability of starch in dairy cow diets results in increased ruminal SCFA production primarily because of greater propionate production (Bauman et al., 1971; Sutton et al., 2003). Depressed dry matter and energy intake in early lactation has detrimental effects on cow performance and immune system, and the depression in DMI can be exacerbated when highly fermentable starch sources are included in the diet. This suppression of appetite is likely controlled by the metabolism of propionic acid in the liver and increased when higher amounts of propionate reach the liver. Our objective was to determine the effects of concentration and ruminal fermentability of starch on production, energy intake and metabolic stress by cows during the early PP period. We hypothesize that feeding a highly fermentable starch source to cows during the critical early PP period will depress feed and energy intake, decreasing production and exacerbating metabolic stress. Findings from this study will allow us to provide dairy producers and nutritionists with guidance on ration formulation that will reduce risk of metabolic disorders and increase energy intake and energy balance by cows in the early PP period, improving cow health, production, and farm profitability.

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CHAPTER 2. HIGHLY FERMENTABLE STARCH AT DIFFERENT DIET STARCH CONCENTRATIONS DECREASED FEED INTAKE AND MILK YIELD OF COWS IN THE EARLY POSTPARTUM PERIOD

ABSTRACT

The objective of this study was to evaluate the effects of diet starch concentration and fermentability (SF) fed during the early postpartum (PP) period on DMI, yields of milk (MY) and milk components, body reserves and metabolism. Fifty-two multiparous Holstein cows were used in a randomized block design with a 2 X 2 factorial arrangement of treatments. Treatment diets were formulated to 22% (LS) or 28% (HS) starch with dry ground corn (DGC) or high moisture corn (HMC) as the primary starch source. Treatments were fed from 1 to 23 d PP and cows were switched to a common diet until 72 d PP to measure carryover (CO) effects. Treatment period (TP) diets were formulated for 22% forage NDF and 17% CP, and starch concentration was adjusted by substitution of corn grain for soyhulls. Throughout the experiment DMI and MY were measured daily, and milk components, BCS and BW were measured weekly. Blood was collected weekly during the TP and every second week during the CO period. During the TP, HMC decreased DMI more when included in the HS (3.9 kg/d) than in the LS (0.9 kg/d) diets and HMC decreased yields of milk, fat and FCM by 4.3, 0.19 and 4.8 kg/d, respectively. Treatments also interacted over time to decrease DMI and yields of milk and milk components more for HMC compared with DGC as time progressed during the TP. Loss of BCS was increased when HMC was fed in a HS diet (-0.38 vs. -0.17) and decreased when included in a LS diet (-0.21 vs. -0.29) with no effects on BW change during the TP. Treatments interacted with time to affect plasma concentrations of glucose and insulin with HS increasing concentrations early in the TP compared with LS but with similar effects by the end of the TP. During the CO

period, treatment effects on DMI diminished over time with no main effects of treatment for the entire period. Starch concentration and SF interacted to affect yields of milk, fat and FCM during the CO period which were greater for HS-DGC and LS-HMC (54.8 and 52.8, 1.76 and 1.81, and 51.3 and 52.2 kg/d, respectively) than for LS-DGC and HS-HMC (51.2 and 51.0, 1.68 and 1.64, and 48.4 and 48.6 kg/d, respectively). Treatments did not affect BCS change during the CO period but HS lost BW compared with LS (-5.7 vs. 7.0 kg). Blood glucose and insulin concentrations were not affected by treatments during the CO period. Feeding a highly fermentable starch source during the early PP period decreased DMI and yields of milk and milk components compared with a less fermentable starch source and the depression in DMI was greater when fed in the higher starch diet. However, diet starch concentration had no main effects on yield of milk or milk components.

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CHAPTER 3. DIET STARCH CONCENTRATION AND STARCH FERMENTABILITY AFFECT ENERGY INTAKE AND ENERGY BALANCE OF COWS IN THE EARLY POSTPARTUM PERIOD

ABSTRACT

Our objective was to evaluate effects of diet starch concentration and fermentability on energy intake and energy balance during the early postpartum (PP) period. Fifty-two multiparous Holstein cows were used in a randomized block design experiment with a 2 x 2 factorial arrangement of treatments. Treatment rations were formulated to 22% (LS) or 28% (HS) starch concentration with dry ground corn (DGC) or high moisture corn (HMC) as the primary starch source. Rations were formulated for 22% forage NDF and 17% CP and fed from 1 to 23 d PP. Starch concentration was adjusted by substitution of corn grain for soyhulls. DMI and milk yield were measured daily, and milk components, milk fatty acid composition, BCS, BW and back fat thickness (BFT) were measured weekly. Feeds, refusals, and fecal samples were collected and digestibility was determined weekly. HMC decreased DM and net energy (NE_L) intakes compared with DGC more when included in a HS diet (3.9 kg/d and 3.2 Mcal/d) than in a LS diet (0.9 kg/d and 0.6 Mcal/d). In addition, HS decreased total-tract NDF digestibility compared with LS, with a greater reduction (18%) for HMC compared with DGC (7%). Compared with DGC, HMC increased weekly BW and BFT loss when included in a HS diet (−34.7 vs. −8.4 kg/wk and −0.12 vs. −0.10 cm/wk) and decreased weekly BW loss but increased weekly BFT loss when included in a LS diet (−18.9 vs. −21.4 kg/wk and −0.11 vs. −0.02 cm/wk). Weekly BCS loss increased for HMC compared with DGC (−0.33 vs. −0.23 unit/wk). HMC also decreased milk NE_L compared with DGC (28.2 vs. 31 Mcal/d), but had little effect on energy balance, which was improved by HS compared with LS (−14.7 vs. −16.8 Mcal/d). Over time,

concentrations of milk de novo fatty acids (< 16 carbons) increased and concentration of milk preformed fatty acids (> 16 carbons) decreased for all treatments, but yields of both sources as well as yield of mixed fatty acids (C16:0 plus C16:1 cis-9) decreased over time with increased SF. Feeding HMC decreased energy intake and milk energy output, but had little effect on energy balance during the early PP period.

INTRODUCTION

The early postpartum (**PP**) period is characterized by a depressed feed intake and increased nutrient demand to sustain milk production and other body functions. Decreased energy intake and increased energy output results in negative energy balance, predisposing cows to health disorders and decreased performance. Increasing energy density of the diet by increasing the amount of starch, which provides glucose and glucose precursors, is a strategy commonly used to increase energy intake in dairy cows. However, diet starch concentration (SC) and starch fermentability (SF) affect cow production and metabolism differently depending on stage of lactation (Oba and Allen, 2003a; Albornoz and Allen, 2018), and these effects are likely associated with cow's blood insulin concentration and insulin response to glucose (Bradford and Allen, 2007; Allen et al., 2009), degree of lipolysis (Piantoni et al., 2015) and rate of propionate production and absorption (Oba and Allen, 2003b; Maldini and Allen, 2018). We demonstrated that feeding a highly fermentable starch source (e.g. high moisture corn) depressed DMI and milk production compared with a starch source of moderate ruminal fermentability (e.g. dry ground corn) during the early PP period, and effects were exacerbated when included in a high starch diet with the same forage NDF (**fNDF**) content as a low starch diet (Albornoz and Allen, 2018). Likely, the more fermentable starch source increased propionate production and

absorption, which can stimulate oxidation of fuels in the liver that triggers a satiety signal consistent with the hepatic oxidation theory (Allen et al., 2009).

Feeding high starch diets increased concentrations of blood plasma glucose and insulin (Oba and Allen, 2003a; McCarthy et al., 2015). However, the decrease in plasma insulin concentration and insulin sensitivity in adipose tissue during the peripartum period promotes lipolysis (Drackley, 1999), which can exacerbate hypophagic effects of propionate (Piantoni et al., 2015).

Increasing diet SC or SF can affect rumen microbial population and rumen pH. Reduced ruminal pH can decrease fiber digestibility and increase production of trans fatty acid intermediates that could increase the risk for diet-induced milk fat depression (Jenkins et al., 2003; Lascano et al., 2016). The combined effect of diet SC and SF on energy intake, energy balance and the risk for milk fat depression during the early PP period have not been investigated.

Our objective was to evaluate the combined effects of diet SC and SF for cows in the early PP period on energy intake, energy balance, glucose metabolism and milk fatty acid composition. The starch treatments were corn grain harvested as high-moisture (high ruminal fermentability) or dry (moderate ruminal fermentability). Starch concentration of diets were adjusted by substituting corn grain for soyhulls, keeping fNDF and the filling effect of diets constant. We hypothesized that rations with highly fermentable starch will decrease energy intake and affect metabolism involved in energy conservation by cows during the early PP period compared with rations with moderate starch fermentability, and effects will be greater for diets with greater starch concentration.

MATERIALS AND METHODS

Animal Care

This study was conducted from February 1st to November 15th, 2015, at the Dairy Cattle Research and Teaching Center at Michigan State University with all experimental procedures approved by the Michigan State University Institutional Animal Care and Use Committee (East Lansing, MI; AUF 11/13-254-00). Cows were housed individually in tie stalls, allowing for daily records of feed offered and refused, and fed once a day (0800 h) at 115% of expected intake and milked in a parlor twice a day (0400 h and 1430 h). All cows were in apparent good health at the beginning of the experiment and standard farm health and reproductive protocols were maintained during this study.

Experimental Design and Treatments

Fifty-two multiparous Holstein cows were used in a completely randomized block design experiment with 2 x 2 factorial arrangement of treatments with 13 cows per treatment. Blocking criteria included BCS observed within one week prior to expected calving date (up to 1 unit difference using a 5-point scale, where 1 = thin and 5 = fat; Wildman et al., 1982), previous lactation 305-d mature equivalent milk production (within 5,000 kg) and date of parturition (within 60 d). A common close up diet was fed from 21 d before expected parturition date until calving. This diet contained corn silage, mature grass hay, dry ground corn, soybean meal, SoyChlor (West Central Soy, Ralston, IA) and a mineral and vitamin mix, and was formulated to contain 42.5% NDF, 38.3% fNDF, 21.5% starch and 13.5% crude protein.

Treatments included diet starch concentration (**SC**; low starch = 22%, LS, or high starch = 28%, HS) and diet starch fermentability (**SF**; dry ground corn, **DGC**, or high moisture corn,

HMC). At calving, cows within each block were randomly assigned to 1 of 4 diet treatment combinations (LS-DGC, LS-HMC, HS-DGC, HS-HMC). Dry ground corn grain was stored in a covered gravity wagon and HMC was ground and ensiled in a bag (Ag-Bag Plastic, Cottage Grove, MN) for at least four months after harvest before utilization. Differences in SF were confirmed by 7-h in vitro starch digestibility analysis prior to and throughout the experiment (44.1% and 61.9% for DGC and HMC, respectively; Albornoz and Allen, 2018*)* according to Goering and Van Soest (1970). Starch concentration of treatment diets was adjusted by partially replacing the main starch source with soyhulls to maintain the same fNDF concentration across treatment diets. Treatment diets contained alfalfa silage, grass hay, corn grain treatments, soyhulls, soybean meal, minerals and vitamins and were formulated to 17% crude protein, and 22% fNDF. Cows received their respective diets beginning at day of calving if they calved before feeding time (0800 h) or at the following morning's feeding until 23 d PP. Dry matter concentration of fermented feeds was determined twice per week throughout the experiment and diets were adjusted accordingly. All rations were formulated to meet or exceed cows predicted requirements for protein, minerals, and vitamins according to NRC (2001) and ingredient and nutrient composition of treatment diets are reported in Table 3.1.

Data and Sample Collection

Feed offered, orts and milk yield were recorded on a daily basis throughout the experiment. Samples and measurements were collected or recorded on the same day of the week ± 3 d relative to expected calving date prepartum or relative to day of calving during the PP period. Backfat thickness (BFT), BCS, BW, feed ingredients, fecal samples and PM milk samples were collected on the same day of the week (5, 12, 19 d PP), with AM milk, fecal and

Table 3.1. Ingredient and nutrient composition of treatment diets

	LS[1]		HS[1]	
Item	DGC	HMC	DGC	HMC
Ingredient, % DM				
Corn silage	-	-	-	-
Alfalfa silage	37.0	37.1	37.7	37.0
Grass hay	8.25	8.35	8.35	8.21
DGC	27.5	-	35.4	-
HMC	-	28.1	-	36.2
Soyhulls	11.0	11.0	1.87	2.18
Soybean meal	11.7	11.1	12.2	12.4
Cottonseed	-	-	-	-
Wheat straw	-	-	-	-
Mineral-vitamin mix[2]	2.02	2.02	2.02	2.02
Limestone	0.55	0.55	0.55	0.55
Sodium bicarbonate	0.95	0.95	0.95	0.95
Dicalcium phosphate	0.95	0.95	0.95	0.95
Nutrient composition, % DM				
DM	58.4	55.2	59.2	53.1
OM	89.5	89.4	89.8	89.6
NDF	33.0	33.0	28.3	27.6
Forage NDF	22.4	22.8	22.6	22.2
Crude protein	17.2	16.7	17.3	16.9
Starch	21.4	21.9	27.1	27.8
Ash	10.5	10.5	10.2	10.3
Gross energy, Mcal/kg	4.21	4.21	4.25	4.25

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]Mineral-vitamin mix contained in a DM basis: 25.6% NaCl, 10.0% Ca, 2.0% Mg, 2.0% P, 30 ppm of Co, 506 ppm of Cu, 20 ppm of I, 2,220 ppm of Fe, 2,080 ppm of Mn, 15 ppm of Se, 2,030 ppm of Zn, 300 kIU/kg of vitamin A, 50 kIU/kg of vitamin D, and 1,500 kIU/kg of vitamin E.

orts samples collected the following morning. An additional milk sample was collected at each milking and stored at -20°C for fatty acid analysis. Also, an additional measurement of BFT was performed within a week prior to parturition and BCS and BW were also determined at calving to be used as a covariate for statistical analysis. A glucose tolerance test (GTT) was performed on day 14 PP according to Bradford and Allen (2007). Two days prior to the GTT, cows were fitted with an indwelling polypropylene jugular catheter (0.24 cm o.d. × 0.17 cm i.d. tubing,

MRE 095, Braintree Scientific, Braintree, MA) inserted through a 10-gauge needle until approximately 30 cm of tubing was inside the jugular vein. Patency of catheters was checked twice daily with 10 mL of heparinized saline (20 IU heparin/mL saline). On the day of the GTT, cows were blocked from feed at 0645 h and not allowed access until the procedure was completed. For the GTT, a sterile solution of 50% dextrose (wt/vol) was administered by intra-jugular bolus at a dose of 1.67 mmol glucose/kg of BW within 5 min. Catheters were flushed with sterile 4.2% sodium citrate after infusions (10 mL) and after blood collections (5 mL). Blood samples were collected in separate tubes containing potassium oxalate/sodium fluoride (for glucose analysis) and K2-EDTA (for insulin analysis) and centrifuged within 30 min (3,000 g × 15 minutes) to harvest plasma which was stored at -20°C.

Representative samples (0.5 kg) of feed ingredients and orts from each cow were collected weekly throughout the experiment and stored at -20°C for later analysis of DM and nutrient composition. Fecal grab samples (0.5 kg) were collected from the rectum every 8 h of a 24-h period to account for diurnal variation. Feces were stored in sealed plastic cups at -20°C until dried. Milk samples were collected weekly at each milking and stored with preservative (Bronopol, D&F Control Systems, San Ramos, CA) at 4°C for component and somatic cell count analysis (Universal Lab Services, East Lansing, MI). An additional milk sample was collected without preservative and stored at -20°C for milk fatty acid analysis. Body condition was scored by three trained investigators on a 5-point scale, as described by Wildman et al. (1982). Subcutaneous cross-section measurements of BFT were performed on the right side of the cow between the 12th and 13th rib by ultrasonography (Aloka SSD-500V monitor and UST-5044-3.5 MHz probe, Aloka Co., LTD, Japan). Back fat thickness was determined by performing an average of two measurements that were within 0.1 cm difference.

Sample Analysis

Feed ingredients, orts and fecal samples were dried in a 55°C forced-air oven for 72 h, analyzed for DM concentration, and ground with a Wiley mill (1-mm screen; Arthur H Thomas Co., Philadelphia, PA). Fecal samples were composited by cow by day on an equal DM basis before analysis. All feed ingredients, orts and fecal composites for each cow were analyzed by week for DM, ash, NDF, CP, starch and gross energy concentration. Nutrients were expressed as percentages of DM, determined by drying at 105°C in a forced-air oven for more than 8 h. Ash concentration was determined after 5 h of oxidation at 500°C in a muffle furnace. Concentration of NDF was determined according to Mertens (2002) and crude protein was determined according to Hach et al. (1987). The NDF residue after 240 h of in vitro fermentation (indigestible NDF; Goering and Van Soest, 1970) was used as an internal marker to estimate fecal output and nutrient digestibility (Cochran et al., 1986). Flasks for incubation contained rumen fluid from a nonpregnant dry cow fed dry hay only and were reinoculated at 120 h to ensure a viable microbial population. Gross energy was determined by bomb calorimetry according to manufacturers instructions (Parr Instrument Inc., Moline, IL). Starch in samples was measured by gelatinization with sodium hydroxide and subsequent hydrolysis to glucose using an enzymatic method (Karkalas, 1985). Glucose was then measured with a glucose oxidase method (PGO Enzyme Product No. P7119; Sigma Chemical Co., St. Louis, MO) and by determination of absorbance with a microplate reader (SpectraMax 190; Molecular Devices Corp., Sunnyvale, CA).

Intakes of DE and ME were calculated according to NRC (2001) as follow:

DE Intake = Gross energy intake (Mcal/d) x Gross energy digestibility

ME Intake = MEp (metabolizable energy at production levels of intake; Mcal/kg of DM) x DMI (kg/d)

Energy balance was determined according to NRC (2001) as follow:

NE_L balance (Mcal/d) = NE_L Intake (Mcal/d) − NE_L Maintenance (Mcal/d) − NE_L Lactation (Mcal/d)

Where NE_L Intake was calculated from DE through ME according to NRC (2001); NE_L Maintenance (Mcal/d) = 0.08 Mcal/kg x BW $(kg)^{0.75}$ (NRC, 2001); and NE_L Lactation (Mcal/d) = Milk yield (kg/d) x [(fat % x 0.0929) + (true protein % x 0.0563) + (lactose % x 0.0395)] (NRC, 2001).

Milk samples were analyzed for fat, true protein and lactose by mid-infrared spectroscopy (AOAC International, 1997) by the Michigan Herd Improvement Association (Universal Lab Services). Additional PM and AM milk samples for fatty acid analysis were composited by milk yield for each collection day for each cow. Fat cakes from composites were obtained by centrifugation at 1,300 × g for 20 min at 4°C before freezing. Fatty acid profile was determined as described by Rico and Harvatine (2013) with slight modifications. Briefly, lipid extraction was performed according to Hara and Radin (1978) using hexane: isopropanol. Fatty acid methyl esters were prepared by base-catalyzed transmethylation according to Chouinard et al. (1999). Fatty acid methyl esters were quantified by GC using an Agilent 6890A gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a fused-silica capillary column (SP-2560, 100 m × 0.25 mm i.d. with 0.2 μm film thickness; Supelco Inc., Bellefonte, PA) and a flame ionization detector with hydrogen as the carrier gas. Initial oven temperature was 80°C, which was increased by 2°C/min to 190°C and held for 15 min. Inlet and detector

temperatures were 250°C with a 100:1 split ratio. Constant gas flows were 1 mL/min for hydrogen carrier, 25 mL/min for detector hydrogen, 400 mL/min for detector airflow, and 40 mL/min for detector nitrogen plus carrier. Fatty acid peaks were identified using FAME standards (GLC 461, GLC 780, and pure CLA trans-10,cis-12 and CLA cis-9,trans-11, NuChek Prep Inc., Elysian, MN; Bacterial Acid Methyl Ester Mix, 47080-U, Sigma-Aldrich; and GLC 110 mixture, Matreya LLC., State College, PA). Recovery of individual FAs were determined using an equal weight reference standard (GLC 461; NuChek Prep Inc.). Correction factors for individual FAs and calculation of milk FA yield were carried out as described by Rico and Harvatine (2013).

Plasma samples from GTT were analyzed for glucose using a glucose oxidase method (PGO Enzyme Product No. P7119; Sigma Chemical Co., St. Louis, MO) and insulin with a commercial kit (Coat-A-Count RIA kit; Siemens Healthcare Diagnostics, Deerfield, IL). Area under the curve for glucose and insulin was calculated using the trapezoidal rule.

Statistical Analysis

All data were analyzed using the Fit Model procedure of JMP Pro (version 13, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijosf} = \mu + B_i + C(B_i)_j + J + O_o + S_S + F_f + S_sF_f + T + S_sT + F_fT + S_sF_fT + e_{ijosf}$$

where Y_{ijdsf} = response variable, μ = overall mean, B_i = random effect of block (i= 1 to 13), $C(B_i)_j$ = random effect of cow (j = 1 to 4) within block, J = random effect of Julian date, O_o = days offset from fixed weekly sampling day (o = -3 to +3), S_s = fixed effect of SC (s = 1 to 2), F_f = fixed effect of SF (f = 1 to 2), S_sF_f = interaction between SC and SF, T = fixed effect of

sampling day PP, S_sT = interaction between SC and day PP, F_fT = interaction between SF and day PP, S_sF_fT = interaction between SC, SF, and day PP, e_{ijosf} = residual error.

Day postpartum was included in the model as continuous measure and linear and quadratic interactions between main effects and sampling day PP were evaluated. Interactions with time were removed from the model for GTT data analysis and when non-significant and a reduced model was used to determine treatment effects. However, all interactions were included in the tables for informational purposes. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Goodness of normal fit was also tested with Shapiro-Wilk test and variables were transformed when necessary to fit a normal distribution. Due to storage issues with milk samples for fatty acid analysis for the first three blocks (12 cows) this analysis was performed on the subsequent ten blocks (40 cows). All cows were included for the analysis of all other variables. Treatment effects were declared significant at $P < 0.05$ and tendencies at $P < 0.10$. Interactions were declared significant at $P < 0.10$ and tendencies at $P < 0.15$.

RESULTS

Digestibility and Energy Balance

Dry matter, NDF, CP and starch intakes increased over time for both starch sources, but more for DGC compared with HMC throughout the treatment period ($P = 0.12$, $P = 0.02$, $P = 0.03$, $P = 0.04$, linear, respectively; Table 3.2.). The LS diets decreased starch intake (1.1 kg/d) and increased NDF intake (0.88 kg/d) compared with HS diets ($P < 0.01$). The HMC treatment decreased daily intake of DM, NDF, CP and starch compared with DGC ($P < 0.02$ for all) but the decrease was greater when included in the HS (3.9, 1.06, 0.65 and 0.89 kg/d, respectively) than

the LS diet (0.9, 0.20, 0.18 and 0.15 kg/d; interaction, $P = 0.07$, $P = 0.11$, $P = 0.12$ and $P = 0.10$, respectively).

The HS treatment increased total tract digestibility of DM, OM, and gross energy by 2.5, 2.3, and 2.4 percentage units compared with LS, respectively ($P < 0.01$; Table 3.2.). The HMC treatment decreased NDF digestibility 3.7 percentage units compared with DGC when included in the HS diet but had little effect when included in a LS diet (interaction $P = 0.10$). High moisture corn increased starch digestibility 2.0 percentage units compared with DGC ($P < 0.01$) but digestibility of crude protein was not affected by treatment.

Intakes of digestible, metabolizable and net energy of lactation all tended to increase over time but the rate of increase was less for LS-HMC than the other treatments (interactions; $P = 0.15$, $P = 0.12$ and $P = 0.12$, all linear, respectively; Figure 3.1.A). The HMC treatment decreased DE, ME and NE_L intakes compared with DGC ($P < 0.01$ for all) and the decrease was greater when included in the HS diet (8.7, 7.0 and 4.2 Mcal/d, respectively) than the LS diet (1.6, 1.0 and 0.6 Mcal/d, respectively; all interactions $P = 0.06$). The HMC treatment decreased the energy required for maintenance compared with DGC in the HS diet (10.7 vs. 11.1 Mcal/d), but did not differ between SF treatments when included in the LS diet (10.9 Mcal/d; interaction $P = 0.05$).

Table 3.2. Effects of diet starch concentration (SC) and starch fermentability (SF) on DMI, body reserves, total tract digestibility and energy balance

	LS[1]		HS[1]			*P*-value[2]					
Variable	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF	SC × Time	SF × Time	SC × SF × Time
Intake, kg/d											
DM	18.6	17.7	20.2	16.3	0.80	0.96	<0.01	0.07	0.97	0.12^{L}	0.84
NDF	5.98	5.78	5.53	4.48	0.28	<0.01	0.02	0.11	0.80	0.02^{L}	0.98
Crude protein	3.19	3.00	3.44	2.79	0.16	0.90	0.01	0.12	0.95	0.03^{L}	0.94
Starch	4.02	3.86	5.46	4.57	0.22	<0.01	0.02	0.10	0.18	0.04^{L}	0.90
Body reserves											
BCS change[3], unit/wk	-0.25	-0.30	-0.20	-0.36	0.06	0.91	0.08	0.33	0.88	0.06^{Q}	0.14^{Q}
BW change[3], kg/wk	-21.4	-18.9	-8.37	-34.7	4.80	0.77	0.02	<0.01	0.80	0.04^{LQ}	$<0.01^{LQ}$
BFT change[34],cm/wk	-0.02	-0.11	-0.10	-0.12	0.03	0.14	0.14	0.03	0.01^{Q}	0.51	0.49
Total tract digestibility, %											
DM	61.5	61.7	63.4	64.8	0.71	<0.01	0.27	0.43	0.79	0.91	0.61
Organic matter	63.0	64.0	65.2	66.4	0.73	<0.01	0.16	0.86	0.63	0.98	0.79
Gross energy	60.5	61.7	62.9	64.0	0.77	<0.01	0.12	0.96	0.73	0.89	0.93
NDF	38.9	39.6	36.2	32.5	1.33	<0.01	0.25	0.10	0.56	0.86	0.90
Crude protein	63.2	64.5	64.3	66.1	1.42	0.32	0.27	0.89	0.79	0.79	0.86
Starch	95.0	96.6	95.0	97.4	0.62	0.34	<0.01	0.35	0.35	0.82	0.75
Energy intake, Mcal/d											
DE	47.5	45.9	53.3	44.6	1.88	0.22	0.01	0.06	0.71	0.45	0.15^{L}
ME	39.4	38.4	44.7	37.7	1.57	0.14	0.01	0.06	0.67	0.52	0.12^{L}
NE_L	24.2	23.6	27.6	23.4	0.95	0.09	0.01	0.06	0.64	0.56	0.12^{L}
Production, Mcal/d											
Maintenance NE_L	10.9	10.9	11.1	10.7	0.09	0.97	0.01	0.05	0.89	0.08^{Q}	0.01^{LQ}
Milk NE_L	30.7	29.1	31.3	27.5	1.30	0.65	0.02	0.32	0.86	0.01^{L}	0.78
Energy balance, Mcal/d	-17.2	-16.4	-14.4	-15.1	1.03	0.05	0.93	0.44	0.56	0.01^{L}	1.00

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]Time = day postpartum. Polynomial interactions are identified with superscripts L = linear and Q = quadratic.

Table 3.2. (cont'd)
[3]Change represents the difference between final and initial measurement at each week postpartum.
[4]BFT = backfat thickness.

Over time, all treatments decreased energy required for maintenance, but HS-HMC had a more pronounced decrease during the second week PP when compared with the rest of the treatments ($P = 0.01$, quadratic). Milk energy output increased over time for DGC but decreased for HMC ($P = 0.01$, linear; Figure 3.1.B) and HMC decreased milk energy output 2.7 Mcal/d compared with DGC over the treatment period. Energy balance was negative for all treatments during the treatment period but HS improved energy balance compared with LS (-14.7 vs. -16.8, $P = 0.05$), and HMC decreased energy balance during the first week PP, and increase thereafter compared with DGC ($P = 0.01$, linear; Figure 3.1.C).

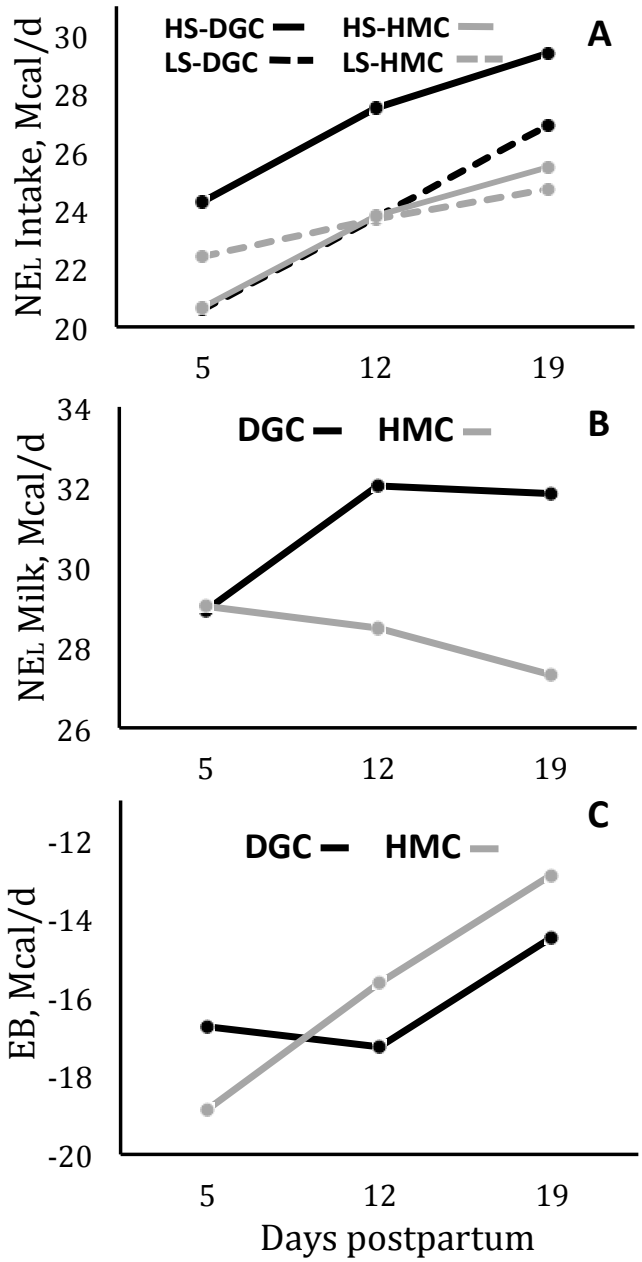

Figure 3.1. Effects of diet starch concentration (SC) and fermentability (SF) on A) NE_L intake and effects of diet SF on B) milk NE_L and C) energy balance (EB). For A) treatments are represented as 28% starch with dry ground corn (HS-DGC; black, solid line), 22% starch with dry ground corn (LS-DGC; black, broken line), 28% starch with high moisture corn (HS-HMC; grey, solid line) and 22% starch with high moisture corn (LS-HMC; grey, broken line). For B) and C) treatments are represented as dry ground corn (DGC; black line) and high moisture corn (HMC; grey line). Interactions among SC, SF and day postpartum are represented for A) NE_L intake ($P = 0.12$, quadratic) and interactions between SF and day postpartum for B) milk NE_L ($P = 0.01$, linear) and C) EB ($P = 0.01$, linear).

Body Reserves

Body condition score decreased over each week but the pattern of loss varied by treatment with a greater loss for the HS-HMC and less loss for the HS-DGC treatments compared with the other treatments until the second week PP and then less loss for the HS-HMC and greater loss for HS-DGC compared to the other treatments for the remainder of the treatment period ($P = 0.14$, quadratic, Table 3.2.). Overall, HMC tended to increase weekly BCS loss compared with DGC ($P = 0.08$), with both treatments reaching similar values by the third week PP ($P = 0.06$, quadratic). Body weight also decreased each week through the treatment period with patterns among treatments over time similar to BCS. The HS-HMC treatment combination increased and HS-DGC decreased BW loss compared with the other treatments until week two but HS-HMC decreased and HS-DGC increased BW loss compared with the other treatments for the remainder of the treatment period ($P < 0.01$, quadratic). Over the treatment period, HMC increased BW loss compared with DGC for HS (-34.7 vs. -8.4 kg/week) but decreased BW loss compared with DGC for LS (-18.9 vs. -21.4 kg/week; $P < 0.01$) with the greatest difference at the second week ($P = 0.04$, quadratic). Back fat thickness decreased for all treatments during the treatment period but HMC increased BFT loss more compared with DGC for LS (-0.11 vs. -0.02 cm/week) than HS (-0.12 vs. -0.10; $P = 0.03$; Table 3.2.) and HS increased loss more during the second week PP, with similar loss rate during the first and third week PP when compared with LS treatments (P = 0.01, quadratic).

Glucose Tolerance Test

Starch concentration and SF interacted to affect baseline (pre-glucose infusion) glucose concentration with HMC increasing glucose concentration compared with DGC for HS (47.7 vs. 42.1 mg/dL) and decreasing glucose concentration for LS (40.7 vs. 44.2 mg/dL, $P = 0.02$; Table

3.3.). However, treatment did not affect maximum glucose concentrations or time required to reach it. The HMC treatment tended to increase the rate of increase in glucose concentration from 10.2 to 11.0 mg/dL per min ($P = 0.09$) compared with DGC but the amount of time required to achieve baseline glucose concentrations post infusion and the area under the curve were not different among treatments.

Table 3.3. Effects of diet starch concentration (SC) and starch fermentability (SF) on response to glucose tolerance test

	LS[1]		HS[1]			*P*-value		
Variable	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF
Glucose								
Baseline, mg/dL	44.2	40.7	42.1	47.7	2.19	0.20	0.59	0.02
Maximum, mg/dL	151	151	148	158	4.40	0.64	0.28	0.25
Time to max., min	10.8	10.0	11.5	10.0	0.86	0.66	0.19	0.66
Rate, mg/dL x min	10.4	11.0	9.92	11.0	0.48	0.69	0.09	0.65
Time to baseline, min	91.5	83.1	78.5	74.6	6.64	0.12	0.36	0.73
AUC, mg/dL x min[2]	4388	4279	4225	3688	271	0.19	0.26	0.45
Insulin								
Baseline, mg/dL	2.34	2.10	3.73	3.45	0.59	0.01	0.61	0.98
Maximum, mg/dL	61.5	65.1	69.7	72.5	6.23	0.21	0.61	0.95
Time to max., min	13.8	12.5	13.1	15.4	1.36	0.46	0.73	0.21
Rate, mg/dL x min	4.72	5.52	5.68	4.87	0.64	0.82	1.00	0.23
Time to baseline, min	99.2	98.1	89.2	87.7	4.87	0.03	0.77	0.96
AUC, mg/dL x min[2]	2289	2407	2353	2435	235	0.84	0.66	0.94

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]AUC = area under the curve, calculated with the trapezoidal rule.

High starch diets increased baseline insulin concentration compared with LS diets (3.59 vs. 2.22 mg/dL, $P = 0.01$), but treatment did not affect maximum insulin concentration, time required to reach it or rate of increase in insulin concentration. The HS treatment reduced the amount of time required to reach baseline insulin concentrations post-infusion (88.5 vs. 98.7 min, $P = 0.03$), but area under the curve did not differ among treatments.

Milk Fatty Acids

Treatments had no main effects on concentrations of total de novo, mixed or preformed milk fatty acids (FA). The proportions of mixed FA were not affected by treatments, but proportions of de novo FA increased ($P = 0.02$, linear) and proportion of preformed FA decreased ($P = 0.10$, linear) over time for all treatments except that the shift in proportions of FA from both sources occurred after the second week PP for LS-HMC (Table 3.4.). Overall, SC and SF interacted to affect concentration of C14:1 cis-9 and C18:1 cis-11 with HMC decreasing their concentrations for LS (0.55% vs. 0.65% and 1.06% vs. 1.10%) but increasing their concentrations for HS (0.71% vs. 0.62% and 1.18% vs. 1.03%; $P = 0.03$ and $P = 0.08$, respectively). Over time, concentration of C14:1 cis-9 and C18:1 cis-11 were higher for LS-DGC and HS-HMC compared with LS-HMC and HS-DGC, with the greatest difference in concentration observed during week two PP with less difference among treatments by the third week PP ($P = 0.05$, quadratic and $P = 0.06$, linear, respectively). Treatments interacted to affect concentrations of iC16:0, C18:1 trans-11 and C18:0, with HMC increasing their concentrations for LS (0.21% vs. 0.18% , 0.76% vs. 0.65% and 13% vs. 11.9%) but decreasing their concentrations for HS (0.18% vs. 0.21%, 0.68% vs. 0.76% and 11.7% vs. 12.5%; $P = 0.10$, $P = 0.03$ and $P = 0.02$, respectively). Treatments also interacted with time to affect concentration of iC16:0 and C18:1 trans-11, which were higher for LS-DGC and HS-HMC compared with LS-HMC and HS-DGC, with less difference among treatments by the third week PP ($P = 0.06$, quadratic and $P = 0.10$, linear, respectively). Over time, concentration of C18:0 decreased for all treatments, with a greater reduction in concentration by week two PP for LS-DGC and HS-HMC compared with LS-HMC and HS-DGC, with all treatments reaching similar concentration values by the third week ($P = 0.03$, quadratic). Treatments did not affect total concentration of C18:1 trans FAs, but its concentration

increased for all treatments and decreased for LS-HMC after the second week PP ($P = 0.05$, quadratic).

Milk fat yield was decreased by HMC compared with DGC (190 g/d) and linearly increased over time for DGC and decreased for HMC (Albornoz and Allen, 2018). Accordingly, compared with DGC, HMC decreased yields of de novo FA (137 g/d; $P = 0.01$) and tended to decrease yields of mixed and preformed FA (86 and 158 g/d*;* $P = 0.06$ and $P = 0.07$, respectively), with yields of all sources being greater for DGC compared with HMC after the first week PP ($P = 0.09$, linear, $P = 0.08$, linear and $P = 0.10$, linear, respectively; Table 3.4.). Similarly, yields of individual FA and total C18:1 trans FA increased for DGC and decreased for HMC after the first week PP (all $P < 0.08$, linear). However, SC and SF interacted to decrease yield of C14:1 cis-9 for LS-HMC and HS-DGC (8.8 and 10.9 g/d) and increase for LS-DGC and HS-HMC (11.6 and 11.4 g/d, respectively; $P = 0.10$). Overall, treatments also interacted to affect yields of iC16:0, C18:1 trans-11 and C18:0, with HMC decreasing their yields for HS compared with DGC (2.67 vs. 3.78 g/d, 10.4 vs. 13.5 g/d and 178 vs. 224 g/d) and little difference between HMC and DGC for LS (3.25 vs.3.23 g/d, 11.9 vs. 11.4 g/d and 204 vs. 211 g/d; $P = 0.07$, $P = 0.06$ and $P = 0.14$, respectively). Additional milk fatty acids concentrations and yields are listed in appendix tables 3.5. and 3.6.

Table 3.4. Effects of diet starch concentration (SC) and starch fermentability (SF) on profile and yield of milk fatty acids (FA)

	LS[1]		HS[1]			*P*-value[2]					
Variable	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF	SC × Time	SF × Time	SC × SF × Time
Profile, %[3]											
De novo[4]	19.9	18.0	20.2	18.3	1.26	0.82	0.18	1.00	0.43	0.42	0.02^{L}
Mixed[4]	27.4	28.0	27.3	27.7	0.41	0.62	0.20	0.76	0.71	0.67	0.72
Preformed[4]	49.5	51.1	49.7	50.9	1.93	0.97	0.30	0.87	0.21	0.64	0.10^{L}
C14:1 cis-9	0.65	0.55	0.62	0.71	0.04	0.12	0.93	0.03	0.07^{L}	0.09^{L}	0.05^{Q}
iC16:0	0.18	0.21	0.21	0.18	0.01	1.00	0.94	0.10	0.41	0.01^{L}	0.06^{Q}
C18:1 trans-11	0.65	0.76	0.76	0.68	0.04	0.70	0.71	0.03	0.54	0.15^{Q}	0.10^{L}
C18:1 cis-11	1.10	1.06	1.03	1.18	0.05	0.63	0.33	0.08	0.45	0.42	0.06^{L}
C18:0	11.9	13.0	12.5	11.7	0.39	0.37	0.72	0.02	0.80	0.55	0.03^{Q}
C18:1 trans (total)	2.16	2.25	2.19	2.24	0.09	0.92	0.48	0.84	0.15^{L}	0.05^{L}	0.05^{LQ}
Yield, g/d[3]											
De novo[4]	341	296	373	282	28.0	0.77	0.01	0.35	0.32	0.04^{L}	0.09^{L}
Mixed[4]	462	437	487	426	27.2	0.83	0.06	0.42	0.15^{Q}	0.08^{L}	0.47
Preformed[4]	850	799	879	772	50.2	0.99	0.07	0.52	0.15^{Q}	0.10^{L}	0.65
C14:1 cis-9	11.6	8.8	10.9	11.4	1.22	0.35	0.24	0.10	0.74	<0.01^{L}	0.25
iC16:0	3.23	3.25	3.78	2.67	0.33	0.96	0.08	0.07	0.87	<0.01^{L}	0.24
C18:1 trans-11	11.4	11.9	13.5	10.4	1.06	0.73	0.18	0.06	0.67	0.07^{L}	0.75
C18:1 cis-11	19.5	16.8	18.1	18.1	1.41	0.97	0.36	0.36	0.78	0.02^{L}	0.71
C18:0	211	204	224	178	13.5	0.61	0.05	0.14	0.42	0.01^{L}	0.71
C18:1 trans (total)	38.2	35.4	38.9	33.2	3.44	0.83	0.21	0.67	0.89	0.08^{L}	0.70

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]Time = day postpartum. Polynomial interactions are identified with superscripts L = linear and Q = quadratic.
[3]A total of approximately 64 individual FA were quantified and used for calculations (summation by source).
[4]De novo FA originate from mammary de novo synthesis (< 16 carbons), preformed FA originate from extraction from plasma (> 16 carbons), and mixed FA originate from both sources (C16:0 plus C16:1 cis-9).

DISCUSSION

Feeding the highly fermentable starch source HMC depressed DMI when compared with DGC, and to a greater extent when included in the HS diet. Intakes of both NDF and starch followed DMI as well as diet composition for the SC treatment, whereas CP intake followed the same trend as DMI. Hypophagic effects of highly fermentable starch sources are likely related to the increased supply of propionate to the liver, which are greater for cows in a lipolytic state, and are consistent with the hepatic oxidation theory (Allen, 2000).

The HS treatment increased total-tract digestibilities of DM, OM and gross energy, but decreased NDF digestibility with a greater decrease for HS-HMC compared with HS-DGC. In contrast, Oba and Allen (2003c) reported no effects of treatment on total tract NDF digestibility when cows past peak lactation received HS (32%) or LS (21%) diets containing either HMC or DGC. Lower NDF intake in the early PP period compared with cows in early to mid-lactation, likely reduces rumen digesta mass and the buffering capacity of the rumen contents (Allen and Piantoni, 2014). Lower NDF intake and higher ruminal starch fermentability for HS-HMC compared with HS-DGC likely diminished ruminal buffering capacity and increased acid production, reducing ruminal pH and NDF digestibility.

Total-tract starch digestibility was similar for LS and HS treatments but greater for HMC compared with DGC. In contrast, in the study by Oba and Allen (2003c) total-tract starch digestibility was affected differently; SF had no effect whereas HS increased total-tract starch digestibility compared with LS. However, in that study, SC was adjusted by inclusion of fNDF while in the present study fNDF was constant across treatments. Interactions among

carbohydrates sources in the rumen likely alter ruminal starch digestibility and ultimately affect total-tract starch digestibility.

Intakes of digestible, metabolizable and net energy of lactation followed the same trends as DMI. Rabelo et al. (2003) reported that cows receiving a HS diet (47.2% NFC) increased DM and energy intake compared with cows receiving a LS diet (41.1% NFC) during the first 20 d PP. The starch source in that experiment was dry ground corn and the effect of SC on energy intake was similar to the DGC treatment but not the HMC treatment in the present experiment. Increasing SC from 22% to 28% did not increase intakes of DM or energy in our experiment despite the increase in DM digestibility by the HS compared with LS treatment. Greater BW loss by HS-HMC, particularly during the second week PP, was likely from a combination of decreased digesta mass from lower DMI and depletion of body reserves as assessed by BCS and BFT measurements, and resulted in lower energy required for maintenance when compared with the other treatments. These findings emphasize the importance of the contribution of DMI compared with DM digestibility to maximize energy intake during the early PP period.

The HMC treatments reduced yields of milk, 3.5% FCM and ECM (Albornoz and Allen, 2018) resulting in lower milk energy output compared with DGC, particularly during the second and third week PP which improved energy balance similarly over time. Although HMC decreased DMI compared with DGC, it did not affect energy balance because it also decreased milk energy output. The HS treatment improved energy balance compared with LS overall consistent with the study by McCarthy et al. (2015) who reported that a HS (25.5%) diet improved energy balance 5.1 Mcal/d compared with a LS (20.9%) diet during the early PP period with no effect on DMI, yields of milk or 3.5% FCM, or change in BW or BCS. These and our results suggest that when SC is increased, energy balance is likely improved, but when feed

intake is depressed by highly fermentable starch, decreased milk energy output is the primary mechanism involved in conserving energy.

Over time, HS diets increased glucose and insulin concentrations, with LS diets reaching similar values as HS treatments by the third week PP (Albornoz and Allen, 2018). Similarly, at the time of the GTT (second week PP), HS increased baseline (pre-glucose infusion) plasma insulin concentration compared with LS. In accordance with our findings for DMI, Bradford and Allen (2007) reported that hypophagic effects from HMC were exacerbated for cows with higher mean plasma insulin concentration, possibly because downregulated gluconeogenesis resulted in faster hepatic oxidation of fuels within meals. Whereas higher insulin concentration is expected to decrease lipolysis and increase lipogenesis in adipose tissue (Bauman, 2000), the opposite was observed for HS-HMC, which increased mobilization of body reserves. In addition, increased mean insulin concentration is expected to clear fuels from the blood faster, but at the time of the GTT, baseline (pre-glucose infusion) plasma glucose concentration increased for HMC compared with DGC when included in a HS diet and decreased for HMC compared to DGC when included in a LS diet. Reasons for this finding are not clear, but the opposite interaction was detected for BHB concentration (Albornoz and Allen, 2018) indicating differences in metabolism of fuels between treatments. Following the glucose infusion, HMC had higher rate of increase in glucose concentration compared with DGC that could relate to a reduced capacity for glucose uptake by tissues by cows that received HMC. Cows during the early PP period have low insulin sensitivity (Bell, 1995) and further research to elucidate how SC and SF affect insulin response to glucose and glucose precursors is needed.

Treatments had opposite effects on proportions of milk de novo and preformed FAs, but yields of both FA sources as well as mixed FA decreased with greater SF, following the same

trend as milk fat yield (Albornoz and Allen, 2018). Higher SF can affect FA biohydrogenation pathways in the rumen and increase synthesis of certain C18:1 isomers considered risk factors for milk fat depression (Mohammed et al., 2010). Whereas abomasal infusion of CLA trans-10, cis-12 causes milk fat depression, it also has been reported to increase abundance of genes related to FA synthesis in adipose tissue (Harvatine et al., 2009a). These effects could increase energy retention and improve energy balance. However, evidence for this is not present in our experiment; treatments did not affect concentration or yield of CLA trans-10, cis-12. Whereas the HS-HMC treatment decreased concentration and yield of C18:1 trans-11, a marker of normal biohydrogenation pathways (Harvatine et al., 2009b), the same shift in concentration was observed for LS-DGC. Further, the HS-HMC treatment decreased milk fat yield but not milk fat concentration compared with the other treatments (Albornoz and Allen, 2018), and cows receiving this treatment as well as those receiving the LS-DGC treatment increased loss of BW and BCS in a similar manner. This evidence indicates that milk fat depression via products of altered FA biohydrogenation pathway likely did not occur in our study and cannot explain the treatment differences observed for change in body reserves.

CONCLUSION

Feeding the highly fermentable starch source HMC decreased DM and energy intake during the early PP period. Negative effects from HMC compared with DGC were increased when included in a HS diet, despite HS diets increasing DM digestibility. However, over time, the decrease in milk energy output by HMC decreased the differences in energy balance between starch sources. Whereas the HS-HMC decreased digestibility of NDF compared with HS-DGC possibly a result of decreased rumen pH, there was no evidence of diet-induced milk fat

depression based upon a reduction in milk fat concentration, or concentration and yield of individual milk fatty acids associated with milk fat depression.

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APPENDIX

APPENDIX: MILK FATTY ACID CONCENTRATION AND YIELD

Table 3.5. Effects of diet starch concentration (SC) and starch fermentability (SF) on profile of milk fatty acids

	LS[1]		HS[1]			*P*-value[2]					
Variable	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF	SC × Time	SF × Time	SC × SF × Time
Profile, %											
4:0	5.44	5.43	5.51	5.23	0.22	0.79	0.57	0.57	0.08^{L}	0.44	0.28
6:0	1.97	1.75	2.04	1.83	0.16	0.64	0.20	1.00	0.94	0.42	0.03^{L}
8:0	0.86	0.71	0.90	0.78	0.08	0.59	0.15	0.91	0.50	0.26	0.04^{L}
10:0	1.52	1.25	1.60	1.34	0.17	0.65	0.15	0.98	0.22	0.24	0.09^{L}
11:0	0.02	0.02	0.02	0.02	0.01	0.62	0.94	0.63	0.05^{L}	0.35	0.46
12:0	1.57	1.31	1.63	1.38	0.17	0.73	0.17	0.95	0.25	0.32	0.05^{L}
13:0	0.05	0.04	0.05	0.05	0.01	0.80	0.96	0.69	0.08^{L}	0.96	0.65
14:0	6.57	5.82	6.58	5.94	0.45	0.90	0.16	0.91	0.20	0.46	0.01^{L}
15:0	0.69	0.63	0.66	0.61	0.05	0.64	0.28	0.87	0.10^{L}	0.71	0.53
16:0	24.7	25.4	24.8	25.0	0.40	0.65	0.23	0.61	0.64	0.74	0.99
16:1 cis-9	2.44	2.43	2.34	2.50	0.15	0.93	0.63	0.58	0.89	0.62	0.08^{L}
18:1 cis-9	28.0	29.2	28.0	29.6	1.08	0.85	0.25	0.85	0.09^{L}	0.39	0.04^{L}
18:1 trans-10	0.42	0.34	0.34	0.44	0.07	0.87	0.93	0.25	0.05^{L}	0.46	0.57

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]Time = day postpartum. Polynomial interactions are identified with superscripts L = linear and Q = quadratic.

Table 3.6 Effects of diet starch concentration (SC) and starch fermentability (SF) on yield of milk fatty acids

	LS[1]		HS[1]			*P*-value[2]					
Variable	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF	SC × Time	SF × Time	SC × SF × Time
Yield, g/d											
4:0	96.3	87.6	99.6	80.0	7.16	0.75	0.05	0.44	0.40	0.04^{L}	0.44
6:0	35.7	28.6	37.0	29.1	3.35	0.79	0.02	0.89	0.24	0.01^{L}	0.16
8:0	15.6	11.8	16.3	12.4	1.72	0.68	0.02	0.99	0.32	0.01^{L}	0.15
10:0	27.2	20.4	28.8	21.3	3.26	0.70	0.03	0.91	0.56	0.01^{L}	0.24
11:0	0.32	0.25	0.33	0.38	0.10	0.50	0.92	0.54	0.06^{L}	0.19	0.77
12:0	27.9	21.3	29.2	22.0	3.18	0.77	0.04	0.93	0.75	0.01^{L}	0.22
13:0	0.86	0.71	0.83	0.79	1.33	0.85	0.51	0.70	0.80	0.16	0.91
14:0	116	94.4	118	93.4	9.81	0.96	0.02	0.88	0.67	$<0.01^{L}$	0.19
15:0	12.2	10.0	11.8	9.68	0.96	0.70	0.03	0.99	0.83	0.01^{L}	0.76
16:0	435	399	440	384	24.5	0.83	0.06	0.69	0.30	$<0.01^{L}$	0.97
16:1 cis-9	43.8	38.1	40.8	39.0	3.62	0.76	0.30	0.58	0.10^{Q}	0.02^{L}	0.52
18:1 cis-9	492	459	494	444	33.2	0.86	0.22	0.80	0.19	0.02^{L}	0.60
18:1 trans-10	7.45	5.20	6.05	6.18	1.54	0.89	0.49	0.44	0.57	0.16	0.42

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]Time = day postpartum. Polynomial interactions are identified with superscripts L = linear and Q = quadratic.

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CHAPTER 4. DIET STARCH CONCENTRATION AND FERMENTABILITY AFFECT MARKERS OF INFLAMMATORY RESPONSE AND OXIDATIVE STRESS DURING EARLY LACTATION

ABSTRACT

Our objective was to evaluate the effects of diet starch concentration and starch fermentability on inflammatory response and oxidative stress markers during the early postpartum (PP) period and its carryover effects. Fifty-two multiparous Holstein cows were used in a completely randomized block design experiment with a 2 x 2 factorial arrangement of treatments. Treatments were starch concentration and starch fermentability of diets; diets were formulated to 22% (LS) or 28% (HS) starch with dry ground corn (DGC) or high moisture corn (HMC) as the primary starch source. Treatments were fed from 1 to 23 d PP and then switched to a common diet until 72 d PP to measure carryover (CO) effects. Treatment period (TP) diets were formulated to 22% forage NDF and 17% CP. The diet for the CO period was formulated to 20% forage NDF, 17% CP and 29% starch. Coccygeal blood was collected before feeding (0730 h) once a week during TP and every second week during CO. Liver and adipose tissue biopsies were performed at 0800 h within 2 d PP and at 20±3 d PP. Blood plasma was analyzed for concentrations of albumin, haptoglobin (HAP), reactive oxygen and nitrogen species (RONS) and antioxidant potential (AOP), with LPS-binding protein (LBP) and TNFα evaluated during the TP only. Oxidative Stress index (OSi) was calculated as RONS/AOP. Abundance of genes involved in glucose metabolism in liver and genes involved in lipogenesis were determined by real-time quantitative PCR. Data from blood markers and gene expression were analyzed in a Mixed Model, but separately for TP and CO periods including treatment interactions with time for blood markers, and including gene expression data from biopsies collected within 2 d PP as covariate in the

model. During the TP, treatments interacted to affect concentrations of TNFα, HAP and LBP, with HMC increasing their concentrations for HS (9.29 vs. 8.42 pg/mL, 0.54 vs. 0.41 mg/mL and 5.85 vs. 4.67 μg/mL, respectively) and decreasing their concentrations for LS (5.88 vs. 11.3 pg/mL, 0.29 vs. 0.44 mg/mL and 4.41 vs. 6.02 μg/mL, respectively) compared with DGC. Effects of treatments diminished over time for LBP and HAP with no differences by the end of the TP and no carryover effects of treatment on HAP. Opposite treatment interaction was observed for albumin, with HMC tending to decrease its concentration for HS (3.25 vs. 3.34 g/dL) and increase its concentration for LS (3.38 vs. 3.29 g/mL) compared with DGC, with no carryover effect. DGC increased the OSi during the first week of the TP compared with HMC, with this effect diminishing over time, but during CO HMC tended to increase the OSi for HS and decrease for LS compared with DGC, with this effect diminishing towards the end of CO. HMC increased hepatic abundance of genes associated with inflammation and gluconeogenesis for HS and decreased for LS compared with DGC. Abundance of genes associated with adipose tissue lipogenesis increased for HS compared with LS. Results during the TP suggest that feeding LS-DGC and HS-HMC elicited a more pronounced inflammatory response and induced an upregulation of genes associated with inflammation and gluconeogenesis in liver, without effects on OSi, but during the CO period effects on plasma markers of inflammation diminished.

INTRODUCTION

Cows failure to physiologically adapt to the challenging conditions during the peripartum period (e.g. increased nutrient demand and milk production) triggers metabolic stress (Sordillo and Mavangira, 2014), which is associated with excessive oxidative stress and inflammation (Abuelo et al., 2015). The postpartum period also involves a series of nutritional changes (e.g. new diet ingredients, diet fermentability, etc.) and environmental stressors (e.g. commingling,

movements to the parlor, etc.) that can further challenge cows metabolic adaptation. Exacerbated metabolic stress can have negative short- and long-term implications on cow well-being, production and reproduction (Sordillo and Aitken, 2009; Nightingale et al., 2015; Huzzey et al., 2015) and both oxidative stress and inflammation can be modulated with nutritional interventions (Miller et al., 1993; Bertoni et al., 2015).

Research in ruminants has shown that nutrition can affect the extent and severity of the inflammatory response (Bertoni et al., 2015). Some studies have reported that ruminal acidosis can induce a severe inflammatory response in dairy cattle as well as oxidative stress (Khafipour et al., 2009, Guo et al., 2013). Whereas the methodologies implemented in these studies to cause an acidosis challenge do not represent common feeding conditions, high levels of starch in the diet (28% or more) have yielded an inflammatory response in dairy cows (Gott et al., 2015; Emmanuel et al., 2008) and increased oxidative stress in lactating ewes (Sgorlon et al., 2008). In addition, a review from several studies concluded that feeding cows more than 44% of dietary concentrate containing highly fermentable starch sources (e.g. barley or wheat grain) linearly increases circulating markers of systemic inflammation (Zebeli et al., 2012). Increased inflammatory response to highly fermentable diets might be from absorption of bacterial lipopolysaccharide (**LPS**) derived from lysis of gram-negative bacteria in the gastrointestinal tract (Khafipour et al., 2009). Even though there is a lack of research on the effects of diet starch fermentability on oxidative stress, a more acute inflammatory response when cows receive high quantities of rapidly fermentable starch could be accompanied by oxidative stress as inflammatory cells increase production of reactive species (Mier-Cabrera et al., 2011). Yet, none of the studies mentioned above were performed in the critical early postpartum (**PP**) period when cows are experiencing an innate systemic inflammation, with carryover effects still unknown.

Cows that elicit a more pronounced inflammatory response compared with those that elicit a low-grade inflammatory response have different metabolic adaptations that along with other factors may determine the rate at which the inflammation is resolved, likely influencing the short- and long-term effects on health, productive and reproductive performance mentioned previously (Bradford et al., 2015). Further, the degree of inflammation and metabolic changes induced by the type and amount of starch in rations fed during the early PP period have not been investigated. Our objective was to evaluate effects of diet starch concentration (**SC**) and starch fermentability (**SF**) on the inflammatory response and oxidative stress of cows during the early PP period and its carryover effects. We hypothesize that cows fed a high starch ration with highly fermentable starch will elicit a more pronounced inflammatory response and oxidative stress during the early PP period.

MATERIALS AND METHODS

Animal Care

This study was conducted from February 1st to November 15th, 2015, at the Dairy Cattle Research and Teaching Center at Michigan State University with all experimental procedures approved by the Michigan State University Institutional Animal Care and Use Committee (East Lansing, MI; AUF 11/13-254-00). Cows were housed individually in tie stalls, allowing for daily records of feed offered and refused, and fed once a day (0800 h) at 115% of expected intake and milked at the parlor twice a day (0400 h and 1430 h). All cows were in apparent good health at the beginning of the experiment and standard farm health and reproductive protocols were maintained during this study.

Experimental Design and Treatments

Fifty-two multiparous Holstein cows were used in a completely randomized block design experiment with 2 x 2 factorial arrangement of treatments with 13 cows per treatment. Blocking criteria consisted of BCS observed within one week prior to expected calving date (up to 1 unit difference using a 5-point scale, where 1 = thin and 5 = fat; Wildman et al., 1982), previous lactation 305-d mature equivalent milk production (within 5,000 kg) and date of parturition (within 60 d). A common close up diet was fed from 21 d before expected parturition date until calving. This diet contained corn silage, mature grass hay, dry ground corn, soybean meal, SoyChlor (West Central Soy, Ralston, IA) and a mineral and vitamin mix, and was formulated to contain 42.5% NDF, 38.3% fNDF, 21.5% starch and 13.5% crude protein.

Treatments included diet starch concentration (**SC**; low starch = 22%, **LS**, or high starch = 28%, **HS**) and diet starch fermentability (**SF**; dry ground corn, **DGC**, or high moisture corn, **HMC**). At calving, cows were randomly assigned to 1 of the 4 diet treatment combinations (LS-DGC, LS-HMC, HS-DGC, HS-HMC). Dry ground corn grain was stored in a covered gravity wagon and HMC was ground and ensiled in a bag (Ag-Bag Plastic, Cottage Grove, MN) for at least four months after harvest before utilization. Differences in SF were confirmed by 7-h in vitro starch digestibility analysis prior to and throughout the experiment (Albornoz and Allen, 2018). Starch concentration of treatment diets was adjusted by partially replacing the main starch source with soyhulls to maintain the same fNDF concentration across treatment diets. Treatment diets contained alfalfa silage, grass hay, corn grain treatments, soyhulls, soybean meal, minerals and vitamins and were formulated to 17% crude protein, and 22% fNDF (Table 4.1.). Cows received their respective diets beginning at day of calving if they calved before feeding time (0800 h) or at the following morning's feeding until 23 d PP with this period identified as the

treatment period (**TP**). During the carryover period (**CO**) from 24 to 72 d PP, all cows received a common diet to evaluate possible residual effects of treatment diets (Table 4.1.). Dry matter concentration of fermented feeds was determined twice per week throughout the experiment and diets were adjusted accordingly. All rations were formulated to meet or exceed cows predicted requirements for protein, minerals, and vitamins according to NRC (2001) and ingredient and nutrient composition of treatment and CO diets are described in (Table 4.1.). Incidences of infectious and metabolic disorders during TP and CO period are reported on Table 4.2.

Table 4.1. Ingredient and nutrient composition of treatment and carryover diets

	LS[1]		HS[1]		
Item	DGC	HMC	DGC	HMC	Common diet
Ingredient, % DM					
Corn silage	-	-	-	-	25.6
Alfalfa silage	37.0	37.1	37.7	37.0	17.3
Grass hay	8.25	8.35	8.35	8.21	-
DGC	27.5	-	35.4	-	17.8
HMC	-	28.1	-	36.2	9.30
Soyhulls	11.0	11.0	1.87	2.18	-
Soybean meal	11.7	11.1	12.2	12.4	15.3
Cottonseed	-	-	-	-	7.2
Wheat straw	-	-	-	-	4.47
Mineral-vitamin mix[2]	2.02	2.02	2.02	2.02	2.15
Limestone	0.55	0.55	0.55	0.55	0.72
Sodium bicarbonate	0.95	0.95	0.95	0.95	0.75
Dicalcium phosphate	0.95	0.95	0.95	0.95	-
Nutrient composition, % DM					
DM	58.4	55.2	59.2	53.1	56.1
OM	89.5	89.4	89.8	89.6	91.8
NDF	33.0	33.0	28.3	27.6	28.1
Forage NDF	22.4	22.8	22.6	22.2	20.4
Crude protein	17.2	16.7	17.3	16.9	16.9
Starch	21.4	21.9	27.1	27.8	28.9
Ash	10.5	10.5	10.2	10.3	8.18
Gross energy, Mcal/kg	4.21	4.21	4.25	4.25	ND[3]

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]Mineral-vitamin mix contained in a DM basis: 25.6% NaCl, 10.0% Ca, 2.0% Mg, 2.0% P, 30 ppm of Co, 506 ppm of Cu, 20 ppm of I, 2,220 ppm of Fe, 2,080 ppm of Mn, 15 ppm of Se, 2,030 ppm of Zn, 300 kIU/kg of vitamin A, 50 kIU/kg of vitamin D, and 1,500 kIU/kg of vitamin E.
[3]Not determined.

Table 4.2. Health disorder events during treatment period (1 to 23 d postpartum) and carryover period (24 to 72 d postpartum)

	LS[1]		HS[1]	
Item	DGC	HMC	DGC	HMC
During the treatment period				
Ketosis	2	4	3	2
Displaced abomasum	1	2	3	0
Milk fever	0	2	0	2
Retained placenta	1	0	0	1
Metritis	1	0	0	0
Mastitis	0	1	0	1
During the carryover period				
Ketosis	1	0	0	0
Mastitis	1	0	0	2
Pneumonia	0	0	1	0
Udder eczema	0	0	1	0

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.

Data and Sample Collection

Blood samples were collected on the same day of the week ± 3 d relative to expected calving date prepartum or relative to day of calving during the PP period. Blood samples were collected via coccygeal venipuncture at 0730 h within a week prior to parturition and at 6, 13, 20, 27, 41, 55 and 69 d PP. The sample collected prepartum was used as a covariate for statistical analysis. Blood was collected in tubes containing K_2-EDTA and immediately centrifuged (3,000 g × 15 minutes) to harvest plasma which was flash frozen in liquid nitrogen and stored at -80°C. Liver and adipose tissue biopsies were performed at 0800 h within 2 d PP and at 20±3 d PP. Liver samples were collected by needle biopsy as described by Bradford and Allen (2005) and adipose tissue samples were collected from dorsal subcutaneous depot in the tailhead region as described by Harvatine et al. (2009). Both liver and adipose tissue samples were flash frozen in liquid nitrogen immediately after collection and stored on dry ice for transportation to finally be stored at -80°C until further analysis.

Markers of Inflammation and Oxidative Stress

Blood plasma samples were analyzed with commercial kits for the negative acute phase protein albumin (Albumin, Beckman Coulter Inc., Brea, CA) by colorimetric measurement on an autoanalyzer (Beckman Coulter AU680, Beckman Coulter Inc.) at the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI) and haptoglobin (Hp) was determined as positive acute phase protein with a commercial photometric colorimetric kit (Phase Haptoglobin Assay; Tridelta Development Ltd., Maynooth, Ireland; intra-assay CV: 3.7%, and inter-assay CV: 8.6%). Additional analyses were performed in blood plasma samples collected prepartum and during TP to determine concentration of lipopolysaccharide binding protein (**LBP**) and TNFα. Lipopolysaccharide binding protein was determined with a commercially available kit (LBP Elisa for various species, Hycult Biotech, Uden, the Netherlands; intra-assay CV: 5.1%, and inter-assay CV: 8.1%). Concentration of TNFα was determined by an ELISA method described by Farney et al. (2011; intra-assay CV: 5.1%, and inter-assay CV: 3.8%). Reactive oxygen and nitrogen species (RONS) were determined with a commercially available assay (ROS and RNS assay, Cell Biolabs, San Diego, CA; intra-assay CV: 6.3%, and inter-assay CV: 9.2%) as described by Abuelo et al. (2016) and antioxidant potential (AOP) using the trolox equivalents antioxidant capacity as described by Re et al. (1999; intra-assay CV: 1.6%, and inter-assay CV: 3.2%). Changes in oxidant status were assessed with the oxidative stress index (OSi) calculated from the ratio between pro-oxidant and antioxidant (RONS/AOP; Abuelo et al., 2013).

Hepatic Triglyceride Content

Liver samples were analyzed for triglyceride content as described by Zhou et al. (2016) and results expressed on a wet tissue basis.

RNA Extraction from Liver and Adipose Tissue

For RNA extraction ~30 mg of liver and ~50 mg of adipose tissue was homogenized in TRizol reagent (Thermofisher Scientific, Waltham, MA, USA) using 2.3 mm zirconia/silica beads (Biospec, Bartlesville, OK, USA) and bead mill homogenizer (Precellys, Bertin Instruments, Montigny-le-Bretonneu, France). Following homogenization, the TRizol homogenate was phase separated using chloroform. Total RNA was extracted from clear phase using the EZ gene™ Tissue RNA Miniprep Kit and genomic DNA is eliminated using DNase I Digestion Kit according to the manufacturer's instructions (Biomiga, San Diego, CA, USA). Purity, concentration, and integrity of total RNA was evaluated using a NanoDrop 1000 spectrophotometer (Thermofisher Scientific) and an Agilent Bioanalyzer 2100 system (Agilent Technologies). All samples had a 260:280 nm ratio between 1.96 and 2.05 and a RNA integrity number > 6. Reverse transcription was performed using the qScript cDNA SuperMix (Quantabio, Beverly, MA, USA) with 1 µg of total RNA.

Quantitative Real-Time PCR Analysis

Transcriptional studies were performed on the cDNA samples using high-throughput qPCR reactions on Wafergen SmartChip Real-time PCR system (Takara Bio Inc., Mountain View, CA, USA). In liver tissue, SYBR Green qPCR assays were performed based on custom designed primers with most of them previously reported by Gualdrón-Duarte and Allen (2018). Primers were designed either using web-based primer design software (Integrated DNA Technologies, Coralville, IO) or by Primer Express™ Software v3.0.1 (Appendix; Table 4.6.). These assays included SYBR-green select master mix (Thermofisher Scientific), 900 nM of primers (Appendix; Table 4.6.; Millipore-Sigma) and 1.5 ng/µL sample cDNA. Cycling conditions used were 50°C for 2 min and 95°C for 10 min holding; 45 cycles, 95˚C for 15 seconds denaturation

and 60˚C for 1 min amplification; final melting curve analysis was performed at 95°C for 15 seconds, 60˚C for 1 min and 95°C for 15 seconds. The quantitative PCR assays for adipose tissue were performed using TaqMan gene expression assays (Appendix; Table 4.7.; Applied Biosystems). A standard protocol provided by the manufacturer was used as previously described by Contreras et al. (2017a), and each 100 nL real-time PCR reaction contained 1X Applied Biosystems TaqMan Universal PCR Master mix (Thermofisher Scientific), 1X TaqMan gene expression assays and 1.5 ng/µL sample cDNA. The following real-time PCR cycling conditions were used for the chip, initial enzyme activation at 95°C for 10 min, 45 cycles of denaturation at 95°C for 10 seconds and annealing at 60°C for 53 seconds. All qPCR reactions were performed in duplicate and no template controls (NTC) were included in each chip for each TaqMan gene expression assay/custom designed primer. Finally, qPCR results were analyzed using SmartChip qPCR software (v 2.8.6.1), with amplification efficiency beyond the range (1.5–2.2) and a threshold cycle (Ct) above 40. Samples with multiple melting peaks were discarded. Threshold Cycle values (Ct values) from qPCR reactions were subsequently analyzed on qBase+ analysis software (Bio gazelle, Belgium). The software qBase+ calculates the stability of endogenous control genes and provides a value called M-value. The geometric mean of two endogenous control genes with low M-value were used to normalize the Ct values of target genes (Contreras et al., 2017a). The best endogenous control genes (PGK1 and RPS9 for liver and EIF3K and RPS9 for adipose) were identified according to Hellemans et al. (2007). Relative quantitation analysis was performed using ΔΔCt method as previously described by Livak and Schmittgen (2001).

Statistical Analysis

Data were analyzed separately for the TP (from 1 to 23 d postpartum) and for the CO period (from 24 to 72 d postpartum) as required to evaluate treatment effects during early PP and its residual effects. Variables were analyzed using the Fit Model procedure of JMP Pro (version 13, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijosf} = \mu + B_i + C(B_i)_j + J + O_o + S_s + F_f + S_sF_f + T + S_sT + F_fT + S_sF_fT + COV + e_{ijosf}$$

where Yijosf = response variable, μ = overall mean, Bi = random effect of block (i= 1 to 13), $C(B_i)_j$ = random effect of cow (j = 1 to 4) within block, J = random effect of Julian date, O_o = day offset from fixed weekly sampling day (o = -3 to +3), S_s = fixed effect of SC (s = 1 to 2), F_f = fixed effect of SF (f = 1 to 2), S_sF_f = interaction between SC and SF, T = fixed effect of sampling day PP, S_sT = interaction between SC and day PP, F_fT = interaction between SF and day PP, S_sF_fT = interaction between SC, SF, and day PP, COV = covariate variable corresponding to the response variable, e_{ijosf} = residual error.

Blood variables analyzed in samples collected prepartum and variables measured in tissues biopsied within 2 PP were included in the model as covariates. Day postpartum was included in the model as continuous measure for the analysis of linear and quadratic interactions between main effects and sampling day PP during TP and CO periods, but removed from the model for the analysis of hepatic triglyceride content and hepatic and adipose tissue gene expression data. Also, interactions with day PP were removed from the model when non-significant and a reduced model was used to determine treatment effects. However, all interactions were included in the tables for informational purposes. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of

residuals versus predicted values. Goodness of normal fit was also tested with Shapiro-Wilk test and variables were transformed when necessary to fit a normal distribution. Data from the last four weeks of the CO period from a cow receiving the HS-DGC treatment were removed because of abnormal recovery from an udder infection from eczema. Treatment effects were declared significant at $P < 0.05$ and tendencies at $P < 0.10$. Interactions were declared significant at $P < 0.10$ and tendencies at $P < 0.15$.

RESULTS

Markers of Inflammation

During the TP, haptoglobin and LBP concentrations decreased over time with all treatments reaching similar concentrations by the third week PP, but during the first and second week PP haptoglobin concentration was greatest for HMC when included in HS and lowest when included in LS compared with the DGC treatments that were intermediate and similar to each other ($P = 0.14$, quadratic), while LBP was greater for HS-HMC and LS-DGC compared with HS-DGC and LS-HMC ($P = 0.14$, quadratic; Table 4.3.; Figure 4.1.A and B). Treatments interacted to affect concentrations of haptoglobin, LBP and TNFα ($P < 0.07$), with HMC increasing their concentrations for HS (0.54 vs. 0.41 mg/mL, 5.85 vs. 4.67 μg/mL and 9.29 vs. 8.42 pg/mL, respectively) and decreasing their concentrations for LS (0.29 vs. 0.44 mg/mL, 4.41 vs. 6.02 μg/mL and 5.88 vs. 11.3 pg/mL, respectively) compared with DGC. The negative acute phase protein albumin tended to follow the opposite trend, with HMC decreasing its concentration for HS (3.25 vs. 3.34 g/dL) and increasing its concentration for LS (3.38 vs. 3.29 g/dL) compared with DGC (interaction $P = 0.13$). During the CO period, treatments did not affect concentrations of the acute phase proteins albumin and haptoglobin (Table 4.3.).

Table 4.3. Effects of diet starch concentration (SC) and starch fermentability (SF) on markers of inflammation and oxidative stress during treatment period (1 to 23 d postpartum) and carryover period (24 to 72 d postpartum)

	LS[1]		HS[1]			*P*-value[2]					
Variable	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF	SC × Time	SF × Time	SC × SF × Time
During treatment period											
Albumin, g/dL	3.29	3.38	3.34	3.25	0.06	0.51	1.00	0.13	0.22	0.56	0.16
Haptoglobin, mg/mL[3]	0.44	0.29	0.41	0.54		0.10	0.65	0.03	0.37	0.15^{L}	0.14^{LQ}
LBP, µg/mL[3]	6.02	4.41	4.67	5.85		0.92	0.77	0.07	0.55	0.96	0.14^{LQ}
TNFα, pg/mL[3]	11.3	5.88	8.42	9.29		0.68	0.17	0.07	0.68	0.71	0.70
RONS, RFU/µL[4]	52.8	58.9	51.2	56.2	4.47	0.47	0.07	0.84	0.72	0.23	0.98
AOP, TE/µL[4]	4.86	5.58	6.14	5.69	0.48	0.09	0.75	0.16	0.01^{Q}	0.02^{Q}	0.26
OSi[4]	11.3	11.3	7.90	10.1	0.20	0.09	0.37	0.25	0.26	0.02^{Q}	0.42
During carryover period											
Albumin, g/dL	3.48	3.52	3.51	3.46	0.05	0.81	0.93	0.41	0.39	0.80	0.97
Haptoglobin, mg/mL[3]	0.38	0.38	0.41	0.40		0.53	0.91	0.85	0.49	0.36	0.56
RONS, RFU/µL[4]	44.6	41.6	45.8	44.9	4.84	0.44	0.51	0.73	0.10^{L}	0.64	0.26
AOP, TE/µL[4]	7.13	7.11	6.92	6.80	1.09	0.39	0.86	0.53	0.02^{L}	0.02^{L}	0.21
OSi[4]	6.33	5.38	5.89	6.86	0.18	0.42	0.96	0.10	0.95	0.76	0.10^{L}

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]Time = day postpartum. Polynomial interactions are identified with superscripts L = linear and Q = quadratic.
[3]Data was log transformed to fit normal distribution.
[4]RONS = reactive oxygen and nitrogen species, AOP = antioxidant potential, OSi = oxidative stress index (RONS/AOP).

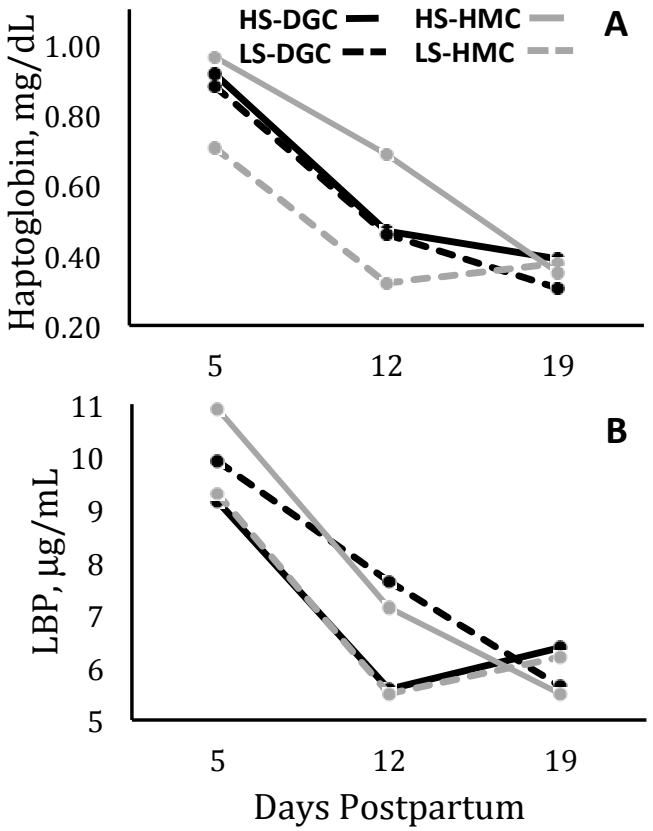

Figure 4.1. Effects of diet starch concentration (SC) and fermentability (SF) on blood plasma concentration of A) haptoglobin ($P = 0.14$, quadratic) and B) lipopolysaccharide binding protein (LBP; $P = 0.14$, quadratic) during treatment period (1 to 23 d postpartum). Treatments are represented as 28% starch with dry ground corn (HS-DGC; black, solid line), 22% starch with dry ground corn (LS-DGC; black, broken line), 28% starch with high moisture corn (HS-HMC; grey, solid line) and 22% starch with high moisture corn (LS-HMC; grey, broken line).

Markers of Oxidative Stress

During the TP, HMC tended to increase RONS compared with DGC (5.6 RFU/μL; P = 0.07; Table 4.3.). Despite a lack of interaction between treatments, AOP increased over time with all treatments reaching similar concentrations by the third week PP, but AOP increased for HS during the second week PP compared with LS (P = 0.01, quadratic) and increased for HMC during the first week PP compared with DGC (P = 0.02, quadratic). Over the TP, AOP tended to increase for HS compared with LS treatments (0.7 TE/μL; P = 0.09). The OSi decreased during the first week PP for HMC compared with DGC with this effect diminishing over time (P = 0.02, quadratic), but over the TP OSi tended to be increased by LS compared with HS (2.3; P = 0.09).

During the CO period, concentration of RONS decreased over time for HS compared with LS, with both treatments reaching similar concentrations by the end of the period P = 0.10, linear); however, there were no main effects of treatment overall (Table 4.3.). Despite a lack of interaction between treatments, AOP increased linearly over time but more for LS compared with HS (P = 0.02, linear), and more for DGC compared with HMC after the second week of the CO period (P = 0.02, linear). Treatments interacted over time to decrease OSi with all treatments reaching similar values by the end of the CO period, but LS-HMC and HS-DGC decreased OSi compared with LS-DGC and HS-HMC for most of the CO period (P = 0.10, linear). Accordingly, over the CO period, OSi increased for HMC when included in HS (0.97) and decreased when included in LS (0.95) compared with DGC (interaction, P = 0.10).

Hepatic Triglyceride Content and Gene Expression

Treatments did not affect triglyceride content in liver but we observed effects on hepatic gene expression (Table 4.4.). For genes associated with glucose metabolism in liver, HMC increased expression of NR2C2, FBP1, G6PC, PC and PCK1 compared with DGC when

included in a HS diet, and decreased their expression compared with DGC when included in a LS diet ($P = 0.12$, $P = 0.07$, $P = 0.08$ and $P = 0.08$, respectively). The HS treatment decreased expression of GCGR and LIPIN2 ($P = 0.04$ and $P = 0.09$, respectively; Table 4.4.) compared with LS. Treatments interacted to affect gene expression of AHSG, CD204, CD206 and SOCS1 ($P < 0.08$), with HMC increasing their expression for HS and decreasing their expression for LS compared with DGC.

Adipose Tissue Gene Expression

Related to expression of genes associated with lipogenesis, AGPAT2, GLUT4, GPAT-1, LPL, PGK1 and SRBEF1 increased or tended to increase ($P = 0.02$, $P = 0.10$, $P = 0.09$, $P = 0.09$ and $P <0.01$, respectively) for HS compared with LS, and DGC increased expression of ADIPOR2 and GLUT4 compared with HMC ($P = 0.03$ and $P = 0.04$, respectively; Table 4.5.). Abundance mRNA for LIPE decreased for HMC when included in HS and increased when included in LS compared with DGC ($P = 0.11$), with HS increasing expression of LPL compared with LS ($P = 0.06$). Related to fatty acid transport, expression of FATP3 tended to decrease for HMC with HS and increase with LS compared with DGC ($P = 0.13$). Finally, expression of the antigen CD44 increased for DGC compared with HMC ($P = 0.03$)

Table 4.4. Effects of diet starch concentration (SC) and starch fermentability (SF) on hepatic triglyceride content and gene expression at 20 d postpartum

	LS[1]		HS[1]			P-value		
Variable	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF
Triglyceride content								
mg/g wet wt	10.7	8.52	9.90	9.51	1.59	0.95	0.44	0.40
Carbohydrate metabolism[2]								
ADH1C	1.52	1.40	1.30	1.51	0.202	0.78	0.82	0.37
CS	1.02	1.06	2.05	1.18	0.597	0.33	0.49	0.43
FBP1	1.30	1.20	1.12	1.42	0.157	0.84	0.45	0.12
G6PC	1.59	1.32	1.25	1.57	0.203	0.78	0.88	0.07
GADPH	1.07	1.04	1.10	1.08	0.077	0.70	0.78	0.92
GCGR	1.13	1.10	0.97	0.98	0.072	0.04	0.91	0.76
GLUD1	1.58	1.39	1.32	1.42	0.115	0.31	0.71	0.20
GOT2	1.25	1.14	1.14	1.22	0.086	0.90	0.91	0.32
GPAM	1.22	1.24	1.28	1.06	0.081	0.56	0.28	0.19
HMGCS	0.20	0.22	0.04	0.09	0.206	0.33	0.81	0.93
HNF4A	1.21	1.14	1.14	1.21	0.169	0.99	0.99	0.67
MCEE	0.71	0.97	0.86	1.04	0.121	0.33	0.12	0.74
MUT	0.99	1.04	0.87	0.93	0.193	0.52	0.77	0.96
NR2C2	1.23	1.01	0.98	1.21	0.087	0.74	0.97	0.01
PC	1.00	0.81	0.73	0.95	0.121	0.61	0.92	0.08
PCCA	1.32	1.22	1.24	1.22	0.099	0.68	0.58	0.70
PCCB	1.15	1.13	1.13	1.25	0.140	0.76	0.73	0.63
PCK1	1.92	1.53	1.47	1.80	0.224	0.67	0.88	0.08
PCK2	1.02	1.10	0.94	1.16	0.095	0.93	0.35	0.43
PDK4	1.63	1.23	1.36	1.40	0.288	0.85	0.51	0.41
PPARA	1.21	1.15	1.14	1.07	0.082	0.40	0.51	0.98
SLC37A4	1.26	1.23	1.09	1.27	0.134	0.62	0.57	0.43
Fatty acid metabolism[2]								
CD36	1.40	1.21	2.34	1.46	0.623	0.36	0.43	0.59
FATP3	1.13	1.05	1.04	1.52	0.212	0.39	0.36	0.21
GPAT-1	0.63	0.61	0.66	0.54	0.240	0.89	0.65	0.72
LIPIN1	1.46	1.16	1.36	1.25	0.224	0.99	0.34	0.66
LIPIN2	1.42	1.40	1.15	1.18	0.142	0.09	0.97	0.83
LIPIN3	1.76	1.02	1.13	1.24	0.375	0.58	0.41	0.26
Inflammation[2]								
AHSG	1.99	1.80	1.49	2.05	0.210	0.53	0.37	0.07
CD204	1.27	0.88	0.82	1.11	0.101	0.24	0.59	<0.01
CD206	1.14	1.05	0.92	1.09	0.072	0.24	0.57	0.08
SOCS1	1.44	0.90	1.23	2.32	0.420	0.19	0.58	0.07

Table 4.4. (cont'd)

Oxylipids[2]								
GPR132	1.11	1.99	1.08	1.25	0.528	0.43	0.29	0.48

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.
[2]Values are least squares means of the relative expression of genes *ADH1C* = alcohol dehydrogenase 1C; *CS* = citrate synthase; *FBP1* = fructose-1,6-bisphosphatase 1; *G6PC* = glucose-6-phosphatase; *GADPH* = glyceraldehyde-3-phosphate dehydrogenase; *GCGR* = glucagon receptor; *GLUD1* = glutamate dehydrogenase 1; *GOT2* = aspartate aminotransferase 2; *GPAM* = glycerol-3-phosphate acyltransferase; *HMGCS2* = HMG-CoA synthase 2; *HNF4A* = hepatocyte nuclear factor 4 alpha; *MCEE* = methylmalonyl CoA epimerase; *MUT* = methylmalonyl CoA mutase; *NR2C2* = nuclear receptor subfamily 2 group C member; *PC* = pyruvate carboxylase; *PCCA* = propionyl-CoA carboxylase alpha; *PCCB* = propionyl-CoA carboxylase beta; *PCK1* = phosphoenolpyruvate carboxykinase 1; *PCK2* = phosphoenolpyruvate carboxykinase 2; *PDK4* = pyruvate dehydrogenase kinase; *PPARA* = peroxisome proliferator activated receptor alpha; *SLC37A4* = solute carrier family 37 A4; *CD36* = cluster of differentiation 36; FATP3 = fatty acid transport protein 3; *GPAT*-1 = glycerol-3-phosphate acyltransferase; *LPIN1* = lipin 1; *LPIN2* = lipin 2; *LPIN3* = lipin 3; *AHSG* = fetuin A; *CD204* = cluster of differentiation 204; *CD206* = cluster of differentiation 206; *SOCS1* = suppressor of cytokine signaling; GPR132 = G protein coupled receptor 132; *PGK1* = phosphoglycerate kinase 1; *RPS9* = ribosomal protein S9.

Table 4.5. Effects of diet starch concentration (SC) and starch fermentability (SF) on adipose gene expression at 20 d postpartum

	LS[1]		HS[1]			P-value		
Genes[2]	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF
Lipogenesis								
ACACA	1.26	1.22	1.39	0.94	0.176	0.65	0.19	0.23
ADIPOQ	1.50	1.31	1.56	1.11	0.275	0.78	0.26	0.61
ADIPOR1	1.07	1.11	1.22	0.93	0.132	0.93	0.35	0.21
ADIPOR2	1.07	0.84	1.40	0.87	0.175	0.29	0.03	0.36
AGPAT2	0.23	0.51	1.10	0.82	0.283	0.02	1.00	0.24
DGAT1	1.07	1.05	1.28	1.10	0.116	0.15	0.32	0.35
DGAT2	0.84	1.14	1.32	1.30	0.223	0.13	0.54	0.43
ELOVL6	1.17	1.37	1.73	1.31	0.275	0.42	0.70	0.29
FASN	1.03	1.37	1.37	1.56	0.287	0.28	0.31	0.75
GLUT4	1.14	0.76	1.43	1.07	0.190	0.10	0.04	0.94
GPAT-1	0.83	1.08	1.56	1.17	0.225	0.09	0.80	0.18
LIPIN-1	1.09	1.01	1.17	0.98	0.212	0.92	0.58	0.83
LIPIN-2	1.05	1.05	1.17	0.99	0.132	0.85	0.52	0.48
LIPIN-3	1.23	1.18	1.10	1.12	0.159	0.57	0.92	0.84
LPL	0.62	0.88	1.05	1.29	0.209	0.06	0.25	0.97
PGK1	0.82	0.86	0.95	0.92	0.061	0.09	0.90	0.56
PPARG	0.81	0.60	0.93	0.81	0.165	0.30	0.31	0.77
PRKAA1	0.78	0.89	1.20	0.91	0.182	0.28	0.66	0.33
SCD1	0.21	0.93	0.38	0.40	0.299	0.56	0.26	0.26
SREBF1	0.51	0.75	1.35	1.11	0.180	0.00	0.98	0.22
THRSP	0.79	1.04	0.76	0.91	0.495	0.88	0.71	0.92

Table 4.5. (cont'd)

Lipolysis								
ABDH5	1.05	1.51	1.45	1.13	0.279	0.96	0.82	0.20
LIPE	1.07	1.12	1.33	0.93	0.143	0.80	0.23	0.11
PNPLA2	0.59	0.76	1.01	0.79	0.195	0.22	0.90	0.28
Fatty acid transport								
FABP4	1.03	1.16	1.24	1.16	0.142	0.56	0.91	0.56
FATP1	0.40	0.56	0.74	1.10	0.677	0.77	0.94	0.96
FATP3	0.96	1.16	1.45	1.10	0.169	0.23	0.70	0.13
Adipose tissue energy status								
LEP	0.89	1.53	1.13	1.00	0.359	0.62	0.48	0.24
Inflammation								
CCL2	0.79	1.59	0.95	0.96	0.401	0.54	0.31	0.30
CD44	0.71	1.11	0.85	1.25	0.177	0.40	0.03	0.97
AHSG	1.64	1.40	3.00	1.37	0.570	0.28	0.13	0.24
IL10	1.26	0.47	0.49	0.73	0.458	0.61	0.56	0.31
IL6	0.73	0.57	1.38	1.02	0.329	0.24	0.55	0.82
SIRPA	0.40	0.37	0.55	0.49	0.157	0.22	0.70	0.88
SPP1	2.67	2.65	2.97	1.52	1.416	0.77	0.62	0.60
TNFα	2.29	0.36	0.24	0.81	0.957	0.48	0.50	0.35
Oxylipids								
5LOX	1.06	0.89	1.09	1.05	0.187	0.60	0.59	0.73
COX2	1.74	1.17	2.30	1.26	0.524	0.67	0.27	0.75
CYP3A4	0.40	0.99	2.50	0.46	0.917	0.45	0.48	0.19
EPHX2	1.12	1.10	1.13	1.05	0.142	0.89	0.73	0.83
GPR132	2.17	1.94	3.01	1.09	0.592	1.00	0.11	0.21
NR2C2	1.10	1.06	1.56	1.09	0.161	0.14	0.14	0.19
PTGS1	0.91	0.83	0.69	1.12	0.224	0.87	0.50	0.31

[1]LS = 22% starch, HS = 28% starch, DGC = dry ground corn, HMC = high moisture corn.

[2]Values are least squares means of the relative expression of genes *ACACA* = acetyl-CoA carboxylase 1; *ADIPOQ* = adiponectin; *ADIPOR1* = adiponectin receptor 1; *ADIPOR2* = adiponectin receptor 2; *AGAPAT2* =1-acylglycerol-3-phosphate O-acyltransferase 2; *DGAT1* = diacylglycerol acyltransferase-1; *DGAT2*= diacylglycerol acyltransferase-2; *ELOVL6* = fatty acid elongase 6; *FASN* = fatty acid synthase; *GPAT*-1 = glycerol-3-phosphate acyltransferase*; GLUT4* = glucose transporter type 4; *LPIN1* = lipin 1; *LPIN2* = lipin 2; *LPIN3* = lipin 3; *LPL* = lipoprotein lipase; *PGK1* = phosphoglycerate kinase 1; *PPARG* = peroxisome proliferator-activated receptor gamma; *PRKAA1* = 5'-AMP-activated protein kinase catalytic subunit alpha-1; *SCD1*= stearoyl-Coenzyme A desaturase-1; *SERBF1* = sterol regulatory element-binding transcription factor 1; *THRSP* = thyroid hormone responsive; *ABDH5* = abhydrolase domain containing 5; *LIPE* = lipase E; *PNPLA2* = patatin like phospholipase domain containing 2; *FABP4* = fatty acid binding protein 4; *FATP1* = fatty acid transport protein 1; *FATP3* = fatty acid transport protein 3; *LEP* = leptin; *CCL2* = C-C motif chemokine Ligand 2; *CD44* = CD44 antigen; *AHSG* = fetuin A; *IL10* = interleukin 10; *IL6* = interleukin 6; *SIRPA* = signal regulatory protein α; *SPP1* = secreted phosphoprotein 1; *TNF* = tumor necrosis factor α; *5LOX* = arachidonate 5-lipoxygenase; *COX2* = cyclooxygenase-2; *CYP3A4* = cytochrome P450 3A4*; EPHX2* = epoxide hydrolase 2; *GPR132* = G protein coupled receptor 132*; NR2C2* = nuclear receptor subfamily 2 group C member*; PTGS1* = prostaglandin-endoperoxide synthase 1; *RPS9* = ribosomal protein S9; *EIF3K* = eukaryotic translation initiation factor 3 subunit K.

DISCUSSION

Treatment Period

The objective of our study was to evaluate the interaction between the innate metabolic stress occurring during the early PP period and the amount and source of starch fed to dairy cows. During the TP, LS-DGC and HS-HMC elicited a more pronounced inflammatory response identified by the increase in plasma concentration of the positive acute phase proteins haptoglobin and LBP and the pro-inflammatory cytokine TNFα, and decrease in plasma concentration of the negative acute phase protein albumin compared with LS-HMC and HS-DGC. Evidence suggests that increasing the amount of grain in the diet (from 15 to 30% of diet DM) with a highly fermentable starch source (e.g. rolled barley) increases blood concentration of markers of inflammation (Emmanuel et al., 2008), but this response did not occur with a moderate increase in the amount of grain (from 9.9 to 19.9% of diet DM) using a starch source with moderate ruminal fermentability (e.g. dry ground corn; Gott et al., 2015). The increase in inflammatory response in the Emmanuel et al. (2008) study was attributed to an increased absorption of lipopolysaccharides (LPS) derived from lysis of gram-negative bacteria in the gastrointestinal tract as indicated by an increased concentration of serum LBP. In accordance, we observed a more pronounced inflammatory response and increase in LBP in cows fed HS-HMC; however, cows that received LS-DGC had similar concentration of plasma LBP and elicited a similar inflammatory response, but reasons for this are not clear. Inflammation has implications on energy requirements and partitioning, and it has been estimated that a challenged immune system (with jugular infusion of LPS) increases glucose demand by up to 1 kg within 12 h post challenge in mid-lactation cows (Kvidera et al., 2017). Treatments that increased inflammation also decreased plasma BHB and increased lactate concentrations during the TP and the opposite

was observed for treatments that decreased inflammation (Albornoz and Allen, 2018; Figure 4.2.A).

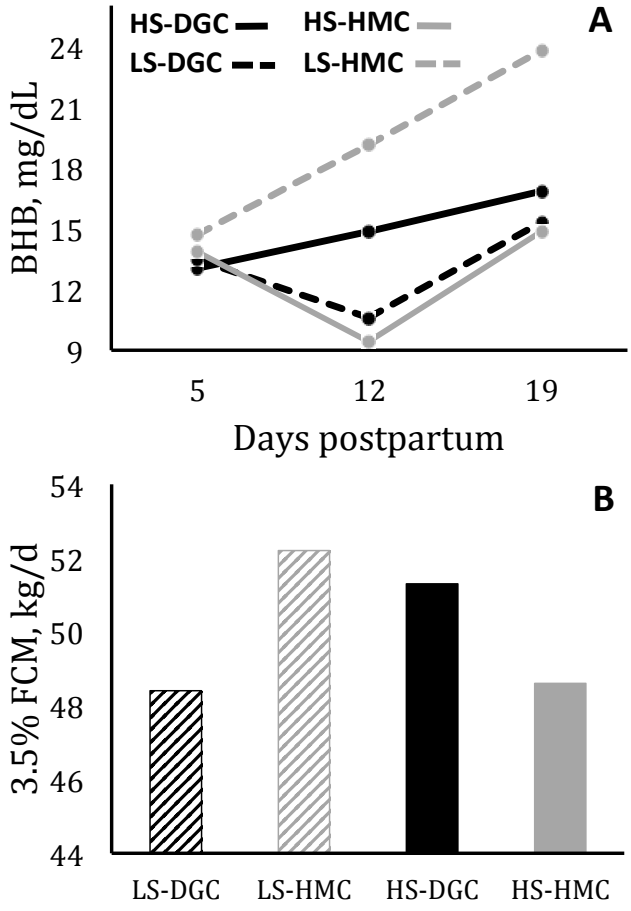

Figure 4.2. Effects of diet starch concentration (SC) and fermentability (SF) on blood plasma concentration of A) ß-hydroxybutyrate (BHB; $P = 0.03$, quadratic) during treatment period (1 to 23 d postpartum) and B) 3.5% fat corrected milk yield (3.5%FCM; interaction $P = 0.06$) during carryover period (24 to 72 d postpartum). Treatments are represented as 28% starch with dry ground corn (HS-DGC; black, solid line or column), 22% starch with dry ground corn (LS-DGC; black, broken line or column), 28% starch with high moisture corn (HS-HMC; grey, solid line or column) and 22% starch with high moisture corn (LS-HMC; grey, broken line or column).

Inflammatory challenges increase muscle catabolism (Doyle et al., 2011; Duan et al., 2016) and likely the export of lactate in order to satisfy the increased demand for energy and glucose precursors. Further, low-grade inflammation can increase insulin resistance during the early PP period (Farney et al., 2013a) and this effect may have spared glucose for the immune system and mammary gland at the expense of increased use of BHB as an energy source by other tissues. However, increased insulin resistance normally promotes lipolysis and we observed inconsistent results between the mobilization of body reserves (BCS, BW, backfat thickness) and plasma NEFA concentration among treatments that increased inflammation (Albornoz and Allen, 2018; Chapter 3*).* Alternatively, BHB may be involved in modulating the inflammatory response during the early PP period. Previous studies that performed intramammary (Waldron et al., 2006; Zarrin et al., 2014) or intravenous (Werling et al., 1996) LPS challenges in bovines reported decreases in circulating BHB concentration, but mechanisms involved in reducing BHB and its role during an inflammatory response are not fully understood. Evidence suggests that BHB can act as an anti-inflammatory metabolite by inhibiting inflammation activators or signaling pathways (Youm et al., 2015; Grinberg et al., 2008; Fu et al., 2014) and therefore the increase in BHB by LS-HMC and HS-DGC might have been involved in modulating the inflammatory response. We are unable to discern if the differences in BHB concentrations induced by treatments were from differences in liver metabolism and energy partitioning or from differences in short-chain fatty acid (e.g. butyric acid) production or absorption from the gastrointestinal tract.

Inflammation has been linked to decreased DMI, milk production (Bertoni et al., 2008; Trevisi et al., 2015) and energy balance (Esposito et al., 2014) in cows during the early PP period. However, in our study, LS-DGC increased DMI and milk production (Albornoz and

Allen, 2018) and decreased energy balance (Chapter 3) compared with HS-HMC, with both treatments increasing inflammation. Similarly, HS-DGC increased DMI, milk production and energy balance compared with LS-HMC (Albornoz and Allen, 2018; Chapter 3), with both treatments decreasing inflammation. The discrepancy between results from previous studies and our results are likely associated with the mechanisms involved in controlling feed intake and affecting production and energy balance. During the early PP period different signals interact to control feed intake (e.g. metabolism of fuels, ruminal distention, etc.) and the relative contribution of each signal is dependent on several factors (e.g. degree of lipolysis, diet composition, etc.). In our study the observed depression in feed intake by the highly fermentable HMC was likely related to hypophagic effects from propionate related to hepatic oxidation (Allen et al., 2009), while in studies associating the feed intake depression with increased inflammation a different mechanism may have been involved (e.g. signals from pro-inflammatory cytokines; Johnson and Finck, 2001; Dantzer and Kelley, 2007). It is possible that the degree of inflammation observed in cows in our study was not sufficient to depress feed intake and the hepatic oxidation of fuels was the dominant mechanism controlling feed intake.

In agreement with the study reported by Yuan et al. (2013) in which a low-grade inflammation was induced during the early PP period, we did not observe effects of treatments in liver triglyceride content. Consistent with our findings on plasma inflammatory markers, treatments that increased inflammation (LS-DGC and HS-HMC), increased hepatic abundance of suppressor of cytokine signaling (SOCS-1), macrophage scavenger receptors (CD204 and CD206) and the acute phase protein Fetuin-A (AHSG) compared with treatments that decreased inflammation (LS-HMC and HS-DGC). Circulating LPS bound to LBP is first recognized by TLR4 and LPS-induced inflammation in mice revealed that SOCS-1 negatively regulates LPS

responses (Kinjyo et al., 2002) and enhances expression of CD204 (Hashimoto et al., 2017), a suppressor of TLR-4 mediated inflammation (Ohnishi et al., 2011). This evidence further supports the notion that cows that elicited a more pronounced inflammatory response in our study (LS-DGC and HS-HMC) were exposed to higher concentration of circulating LPS. Fetuin-A is normally characterized as a negative acute phase protein with anti-inflammatory properties under acute inflammation (Wang and Sama, 2012); however, under chronic inflammation Fetuin-A can be upregulated and stimulate pro-inflammatory cytokines (Dasgupta et al., 2010). It is possible that the long-term (~first 3 weeks PP) increase in plasma LPS concentration by cows fed LS-DGC and HS-HMC may have induced a prolonged inflammatory response and upregulated the expression of Fetuin-A.

Oxidative stress represents an imbalance between pro- and anti-oxidants and the OSi is a reliable indicator of the shift between pro- and anti-oxidants during early lactation (Abuelo et al., 2013). There is an interrelation between oxidative stress and inflammatory response (Sordillo and Aitken, 2009), and feeding a high-starch diet (25.3% vs. 32.8%) was associated with increased ruminal LPS translocation and hepatic oxidative stress in mid-lactating dairy cows (Abaker et al., 2017). However, we did not observe an increase in OSi by cows that received HS or a relationship in OSi between treatments that increased inflammation (LS-DGC and HS-HMC). The effects observed by Abaker et al. (2017) likely were not observed in our study because the HS diet used in that study caused sub-acute ruminal acidosis (ruminal pH < 5.8 for 270 min/d) that lead to milk fat depression. Sub-acute ruminal acidosis increases ruminal LPS production and translocation into the bloodstream (Emmanuel et al., 2008; Khafipour et al., 2009) and LPS can predispose dairy cows to oxidative stress (Shi et al., 2016; Bromfield and Iacovides, 2017). In our study we did not measure ruminal pH, but indications of severe ruminal

pH disturbances (e.g. milk fat depression or fatty acids indicative of altered ruminal biohydrogenation) by treatments were not observed (Albornoz and Allen, 2018; Chapter 3). Further, a previous study from our group did not report indications of sub-acute ruminal acidosis by cows fed diets with 21 or 32% starch containing either HMC or DGC as the primary starch source during early lactation (~55 d PP; Oba and Allen, 2003). Likely, in our study, cows experienced a low-grade inflammatory response compared with cows experiencing sub-acute ruminal acidosis and the risk for LPS-induced oxidative stress was diminished.

Treatments had different effects on the expression of genes associated with energy metabolism in liver and adipose tissue. In liver, abundance of key regulators of gluconeogenesis (NR2C2, FBP1, G6PC, PC and PCK1) were increased by treatments that elicited a more pronounced inflammatory response (LS-DGC and HS-HMC). However, effects on plasma glucose concentration did not accompany those results (Albornoz and Allen, 2018). Previous studies have reported conflicting results in hepatic expression of genes associated with glucose metabolism during an inflammatory event. Bradford et al. (2009) simulated a low-grade inflammation in late lactation cows with daily subcutaneous injections of rbTNFα (2 μg/kg of BW) for 7 d and reported a decrease in abundance of PCK1 and G6PC compared with control, while Yuan et al. (2013) using the same treatment regime at lower and higher doses (1.5 and 3 μg/kg of BW) in cows during the early PP period did not observe differences in abundance of those same genes. Further, Garcia et al. (2015) reported an increase in PC abundance when liver slices were incubated with LPS (0.2 μg of LPS/mL of total culture medium), and the abundance was greater for slices collected from cows in the early PP period than mid-lactation cows. These studies and our data suggest that adaptation of hepatic gluconeogenesis to an inflammatory state depends on the physiological state of cows and likely on the severity of the inflammatory

response. Opposite to the effects observed in liver, it is apparent that abundance of genes associated with lipogenesis in adipose tissue was primarily related to the supply of glucogenic precursors to the cow rather than inflammation. During the TP, cows fed HS increased plasma concentration of glucose and insulin over time (Albornoz and Allen, 2018) and the abundance of key regulators of lipogenesis in adipose tissue (AGPAT2, GLUT4, GPAT-1, PGK1, SREBF1 and LPL). Feeding HS likely increased energy partitioned towards lipogenesis, but this effect did not accompany the observed shift in mobilization of body reserves (BW, BCS and backfat thickness; Albornoz and Allen, 2018), likely because lipolysis prevails over lipogenesis during the early PP period (Contreras et al., 2017b) and energy intake was different among treatments (Chapter 3). These effects suggest that a low-grade systemic inflammation might modulate hepatic metabolism of glucose but has little impact on energy partitioning in adipose tissue during the early PP period.

Carryover Effects

During the CO period, cows received a common diet and effects of treatment on markers of inflammation diminished. However, we observed positive carryover effects on oxidative stress and production by treatments that decreased inflammation (LS-HMC and HS-DGC) compared with those that increased inflammation (LS-DGC and HS-HMC) during the TP. Both LS-HMC and HS-DGC, decreased the OSi and increased yields of milk, fat and 3.5% FCM (2.7, 0.125 and 3.25 kg/d, respectively) compared with LS-DGC and HS-HMC, without differences in DMI across all treatments (Albornoz and Allen, 2018). Previous studies also reported carryover effects on production, as well as health and reproductive performance, by cows with decreased or increased inflammation during the early PP period (Farney et al., 2013b; Nightingale et al., 2015; Huzzey et al. 2015; McCarthy et al., 2016). For example, Huzzey et al. (2015) reported that for

every increase of 1 mg/mL in plasma haptoglobin during the first 3-10 d PP cows decreased 305 d mature equivalent milk production by 464 ± 136 kg, and cows on third or greater parity administered the non-steroidal anti-inflammatory drug sodium salicylate (123.3 ± 5.5 g/d) in water bowls during the first 7 d PP increased yields of milk and milk fat (2,469 ± 646 kg and 130 ± 29 kg, respectively) over the lactation compared with control cows of the same parity (Farney et al., 2013b). However, mechanisms to explain the relationship between inflammation and carryover effects on production have not yet been elucidated.

We observed that treatments that increased BHB concentration during the early PP period, LS-HMC and HS-DGC, also had positive effects on production during the CO period, increasing yield of 3.5% fat corrected milk (52.2 and 51.3 kg/d, respectively) compared with treatments that decreased BHB concentration during the early PP period (LS-DGC and HS-HMC; 48.4 and 48.6 kg/d, respectively; Figure 4.2.B). A similar relationship was present in previous studies with cows receiving either non-steroidal anti-inflammatory drugs (Farney et al., 2013a; Farney et al., 2013b; Carpenter et al., 2016) or dietary treatments (Piantoni et al., 2015; de Souza, 2018) within the first 4 weeks PP, and these effects were not consistent with DMI, production or blood concentration of hormones and other metabolites during the treatment period. As suggested previously, BHB can act as an anti-inflammatory metabolite. Supporting this notion, ruminal infusion with sodium butyrate increases levels of circulating BHB in the bovine (Herrick et al., 2018), and supplementation with sodium butyrate reduces inflammation of the rumen epithelium and translocation of LPS into the bloodstream, contributing to a reduction in mammary gland cell apoptosis in goats (Dai et al., 2017; Chang et al., 2018). Reduction of cell apoptosis and necrosis for several cell types such as fibroblasts, neurons, myocytes, glial and epithelial cells when BHB is increased has been summarized by Grinberg et al. (2008), and the

balance between mammary cell proliferation and apoptosis rates are deemed important factors in determining persistency of lactation in bovines (Capuco et al., 2001). The importance of BHB and butyrate as signaling molecules and inhibitors of histone deacetylases, and the effects of some inhibitors of histone deacetylases as anti-inflammatory agents and their role on epigenetics have been extensively reviewed in human literature (Adcock, 2007; Berni Canani et al., 2012; Newman and Verdin, 2014; Hull et al., 2016; Woolf et al., 2016; Cavaleri and Bashar, 2018). Further, Ylioja et al. (2018) recently demonstrated that administering sodium salicylate to dairy cows during the first week PP increased global DNA methylation in mammary tissue, supporting the idea that modulating the inflammatory response during the early PP period might have epigenetic effects and alter long-term production responses. Future work investigating the mechanisms by which circulating BHB concentrations affect the inflammatory response and epigenetic effects in dairy cows could help elucidate some of the carryover effects observed in our study.

CONCLUSION

Consistent with our hypothesis, HMC elicited a more pronounced inflammatory response when included in HS, but failed to increase oxidative stress during the TP. However, LS-DGC also increased the inflammatory response. Further, treatments that increased inflammation during TP also increased abundance of genes associated with gluconeogenesis, but did not affect triglyceride accumulation in liver. The reduction in inflammation by LS-HMC and HS-DGC during the TP could be associated with the positive effects observed in production (Albornoz and Allen, 2018) and oxidative stress during the CO period. Signaling effects from circulating BHB could potentially modulate the inflammatory response during the early PP period and long-term effects on production, but further research to elucidate the mechanisms involved in mediating

this response in dairy cows is required.

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APPENDIX

APPENDIX: PRIMERS

Table 4.6. Primer sequences and accession numbers for genes analyzed in liver tissue

Gene[1]	NCBI Accession Number	Forward Sequence (5'-3') Reverse Sequence (5'-3')
Carbohydrate metabolism[2]		
ADH1C	NM_001206387.1	CAGGACTACGAGAAACCCATC TCCTACAATGACGCTTACACC
CS	NM_001044721	ACTAATGCATGTAGTGTGGGTTAGGT AAGAGCCAGATTCCCACTCTGA
FBP1	XM_019966535.1	GGCACGGGCGAGATGA CTTTGACCGCGGTGCAA
G6PC	NM_183364	CCATATCCGAAACCAATCAAGAG GAACAGGCAAGGAGGAGGAGTT
GAPDH	XM_019960295.1	TGCCGCCTGGAGAAACC CGCCTGCTTCACCACCTT
GCGR	XM_019982395.1	GAGCCCACCAGATGCGTTAT GATGAGGGTCAGTGTGGACTTG
GLUD1	NM_182652.2	CGCTCTGCCAGGCAAATC GGTCCAGCCCCAGGTTATACTT
GOT2	XM_019979412.1	GGCTGTGCGCCACTTCAT TTGGCATAGGACTGGCAGAGA
GPAM	NM_001012282	GAAGTCATGAGGGTGACGAGAAA TGCCACAACCTGAGCTTACACT
HMGCS2	AA112667	CCTGCTGCAATCACTGTCATG TCTGTCCCGCCACCTCTTC
HNF4A	NM_001015557	GGCGTCCCGCCAGACT GCACAGGACCCGATGACATC
MCEE	NM_001045995	GGGCTGGCCCTGTATGG GCACTGCGACTGCAACATG
MUT	NM_173939	CTCGCTGCTGGGCACAA AAGGGCGTTGAGTTCCTTGA
NR2C2	NM_001192061.1	CAGAGTGAACGGAAACCCTTTG CGCAGCACAATTGCTTGGT
PC	NM_177946	AGGCAAGACGCTGCACATC GCCCGCCCGGTTGA
PCCA	NM_001083509	GGTCAGGAAATCTGTGTGATTGAA GTCTTCCCGGCCGTCATA
PCCB	BC109784	CGAATCTGCTGTGACCTGGAT TCCTCCAAGGGCGCTGTAC
PCK1	NM_174737	CAGCCAAGCTGCCCAAGA CCGGCCTTGTCCTTTCG

Table 4.6. (cont'd)

PCK2	XM_019968310.1	CCCCCACCCCTACTCCAA TGCTCATAGTTTCTGCAAGCCTAGT
PDK4	NM_001101883	TGCTCATAGTTTCTGCAAGCCTAGT GAGGTGGTGTTCCCCTGAGA
PPARA	NM_001034036	CAGCGCCGAGGAGTCATC TGTCCCCGCAGATCCTACAC
SLC374A	NM_001193045	TTGTCATGCCGTCGTTGGT GGTGATGAGCCCCAAGTCA
Fatty acid metabolism[3]		
CD36	NM_001278621.1	TGTGCATGCAGATCAAAGAGAGT AAGGTCCTGACATAGCTGGTTCA
FATP3	XM_015462580.1	CAGAGACCTTCAAGCAGCAGAA ACAGTGTGCTTGGGTCAAAGC
GPAT-1	NM_001278621.1	TGAGTTTGGTGGTGCTGTAGGT TCCCGCTCCCTTCACTTG
LPIN1	NM_001206156.1	CCCCTCCTGACATCCTGTGA CACGTCCCCAAACCTATCCTT
LPIN2	XM_025273164.1	ACCAGGGTATCGCGAAGCT GGCCGAGCAGTACAGGAACTT
LPIN3	NM_001192540.1	GGGCATCACCAGTCTCTACCA GCCGAGCAGTACAGGAACTTG
Inflammation[3]		
AHSG	NM_173984.3	GCGCTGGCTACCTTCAATG CGAGAAATTTCCACCAGCTGTA
CD204	NM_001113240.1	TGCCCATCATTGGCATAGTG CCGTGCAATTCTTCGTTTCC
CD206	XM_003586772.4	GGTTATCCAAATGCCTTCATCTG CGGGACAACTGTGGCATTG
SOCS1	XM_002697964.4	GCTCCTTCCCCTTCCAGATC CCCACATGGTCCAGGAAAAT
Oxylipids[3]		
GPR132	XM_002696852.3	CAGGGTGGCCGGGTACTACT ATGAACAGCGGGACAGCAA
Reference genes		
GADPH[3]	NM_001034034	ATCTCGCTCCTGGAAGATG TCGGAGTGAACGGATTCG
HPRT1[3]	NM_001034035[2]	TGGCGTCCCAGTGAAATCA CAGCTGGCCACAGAACAAGA
PGK1[2]	NM_001034299.1	CAAGGATGTTTTGTTCTTGAAGA GGGTCAGCACAAGCCTTCTC
RPS9[2]	XM_019978604.1	TCCCGCCTCGACCAAGA TTTGTTCCGGAGCCCATACT

Table 4.6. (cont'd)

[1]*ADH1C* = alcohol dehydrogenase 1C; *CS* = citrate synthase; *FBP1* = fructose-1,6-bisphosphatase 1; *G6PC* = glucose-6-phosphatase; *GADPH* = glyceraldehyde-3-phosphate dehydrogenase; *GCGR* = glucagon receptor; *GLUD1* = glutamate dehydrogenase 1; *GOT2* = aspartate aminotransferase 2; *GPAM* = glycerol-3-phosphate acyltransferase; *HMGCS2* = HMG-CoA synthase 2; *HNF4A* = hepatocyte nuclear factor 4 alpha; *MCEE* = methylmalonyl CoA epimerase; *MUT* = methylmalonyl CoA mutase; *NR2C2* = nuclear receptor subfamily 2 group C member; *PC* = pyruvate carboxylase; *PCCA* = propionyl-CoA carboxylase alpha; *PCCB* = propionyl-CoA carboxylase beta; *PCK1* = phosphoenolpyruvate carboxykinase 1; *PCK2* = phosphoenolpyruvate carboxykinase 2; *PDK4* = pyruvate dehydrogenase kinase; *PPARA* = peroxisome proliferator activated receptor alpha; *SLC37A4* = solute carrier family 37 A4; *CD36* = cluster of differentiation 36; FATP3 = fatty acid transport protein 3; *GPAT*-1 = glycerol-3-phosphate acyltransferase; *LPIN1* = lipin 1; *LPIN2* = lipin 2; *LPIN3* = lipin 3; *AHSG* = fetuin A; *CD204* = cluster of differentiation 204; *CD206* = cluster of differentiation 206; *SOCS1* = suppressor of cytokine signaling; GPR132 = G protein coupled receptor 132; *GADPH* = glyceraldehyde 3-phosphate dehydrogenase; *HPRT1* = hypoxanthine phosphoribosyltransferase 1; *PGK1* = phosphoglycerate kinase 1; *RPS9* = ribosomal protein S9.
[2]Reported by Gualdrón-Duarte and Allen (2018).
[3]Custom designed

Table 4.7. Taqman assay information for analyzes of gene expression in adipose tissue

Gene[1]	NCBI Accession Number	Product
Lipogenesis		
ACACA	NM_174224.2	Bt03213371_m1
ADIPOQ	NM_174742.2	Bt03292341_s1
ADIPOR1	NM_001034055.1	Bt03225429_m1
ADIPOR2	NM_001040499.2	Bt03233609_m1
AGPAT2	NM_001012727.1	Bt03244182_m1
DGAT1	NM_174693.2	Bt03251718_g1
DGAT2	NM_205793.2	Bt03259837_m1
ELOVL6	NM_001102155.1	Bt00907566_m1
FASN	NM_001012669.1	Bt03210481_m1
GLUT4	BC114082.1	Bt03215316_m1
GPAT-1	custom designed	APU63EN
LPIN1	NM_001206156.1	Bt04290056_m1
LPIN2	custom designed	
LPIN3	NM_001192540.1	Bt04292449_m1
LPL	NM_001075120.1	Bt03240493_m1
PGK1	NM_001034299.1	Bt03225857_m1
PPARG	NM_181024.2	Bt03217547_m1
PRKAA1	NM_001109802.2	Bt01562310_m1
SCD1	NM_173959.4	Bt04307476_m1
SREBF1	NM_001113302.1	Bt03276364_m1
THRSP	NM_001040533.1	Bt03211511_m1

Table 4.7. (cont'd)

Lipolysis		
ABDH5	NM_001076063.2	Bt03248391_m1
LIPE	NM_001080220.1	Bt03253697_m1
PNPLA2	NM_001046005.2	Bt03234128_m1
Fatty acid transport		
FABP4	NM_174314.2	Bt03213820_m1
FATP1	NM_198580.2	Hs01587911_m1
FATP3	custom designed	APTZ9UR
Adipose energy status		
LEP	NM_173928.2	Bt03211909_m1
Inflammation		
CCL2	NM_174006.2	Bt03212321_m1
CD44	NM_174013.3	Bt03212355_m1
AHSG	NM_173984.2	Bt23250
IL10	NM_174088.1	Bt03212727_m1
IL6	NM_173923.2	Bt03211905_m1
SIRPA	NMz_175788.1	Bt03224468_m1
SPP1	NM_174187.2	Bt03213107_m1
TNF	NM_173966.3	Bt03259154_m1
Oxylipids		
5LOX	NM_001192792.1	Bt00386520_m1
COX2	NM_174445.2	Bt03214492_m1
CYP3A4	NM_001099367.1	Bt03293209_sH
EPHX2	NM_001075534.1	Bt03241449_m1
GPR132	custom designed	APKA3X4
NR2C2	NM_001192061.	Bt00991823_m1
PTGS1	NM_001105323.1	Bt03817775_m1
Reference genes		
ACTB	NM_173979.3	Bt03279174_g1
B2M	NM_173893.3	Bt03251628_m1
EIF3K	NM_001034489.2	Bt03226565_m1
GAPDH	NM_001034034.2	Bt03210913_g1
PPIA	NM_178320.2	Bt03224615_g1
RPLP0	NM_001012682.1	Bt03218086_m1
RPS9	NM_001101152.2	Bt03272016_m1

[1] *ACACA* = acetyl-CoA carboxylase 1; *ADIPOQ* = adiponectin; *ADIPOR1* = adiponectin receptor 1; *ADIPOR2* = adiponectin receptor 2; *AGAPAT2* =1-acylglycerol-3-phosphate O-acyltransferase 2; *DGAT1* = diacylglycerol acyltransferase-1; *DGAT2*= diacylglycerol acyltransferase-2; *ELOVL6* = fatty acid elongase 6; *FASN* = fatty acid synthase; *GPAT*-1 = glycerol-3-phosphate acyltransferase*; GLUT4* = glucose transporter type 4; *LPIN1* = lipin 1; *LPIN2* = lipin 2; *LPIN3* = lipin 3; *LPL* = lipoprotein lipase; *PGK1* = phosphoglycerate kinase 1; *PPARG* = peroxisome proliferator-activated receptor gamma; *PRKAA1* = 5'-AMP-activated protein kinase catalytic subunit alpha-1; *SCD1*= stearoyl-Coenzyme A desaturase-1; *SERBF1* = sterol regulatory element-binding transcription factor 1;

Table 4.7. (cont'd)

THRSP = thyroid hormone responsive; *ABDH5* = abhydrolase domain containing 5; *LIPE* = lipase E; *PNPLA2* = patatin like phospholipase domain containing 2; *FABP4* = fatty acid binding protein 4; *FATP1* = fatty acid transport protein 1; *FATP3* = fatty acid transport protein 3; *LEP* = leptin; *CCL2* = C-C motif chemokine Ligand 2; *CD44* = CD44 antigen; *AHSG* = fetuin A; *IL10* = interleukin 10; *IL6* = interleukin 6; *SIRPA* = signal regulatory protein α; *SPP1* = secreted phosphoprotein 1; *TNF* = tumor necrosis factor α; *5LOX* = arachidonate 5-lipoxygenase; *COX2* = cyclooxygenase-2; *CYP3A4* = cytochrome P450 3A4*; EPHX2* = epoxide hydrolase 2; *GPR132* = G protein coupled receptor 132*; NR2C2* = nuclear receptor subfamily 2 group C member*; PTGS1* = prostaglandin-endoperoxide synthase 1; *ACTB* = actin beta*; B2M* = beta-2-microglobulin *; EIF3K* = eukaryotic translation initiation factor 3 subunit K; *GADPH* = glyceraldehyde 3-phosphate dehydrogenase*; PPIA* = peptidylprolyl isomerase A*;* RPLP0 = ribosomal protein lateral stalk subunit P0; *RPS9* = ribosomal protein S9.

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CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

Consistent with the Hepatic Oxidation Theory of control of feed intake, our study demonstrated that feeding the highly fermentable starch source high moisture corn (HMC) decreased DM and energy intake during the early PP period compared with the less fermentable dry ground corn (DGC). The effects were increased when diet starch concentration (SC) increased despite high starch (HS) diets increasing DM digestibility. The HMC also decreased yields of milk and milk components, but SC had no main effects on any of those variables. Over the early PP period, the decrease in milk energy output resulting from feeding a diet with HMC diminished differences in energy balance of cows fed different starch sources. Whereas HS-HMC resulted in reduced digestibility of NDF compared with HS-DGC likely as a result of decreased ruminal pH, there was no evidence of diet-induced milk fat depression based upon a reduction in milk fat concentration, or concentration and yield of individual milk fatty acids associated with milk fat depression. Further, the observed shift in mobilization of body reserves and abundance of genes associated with lipogenesis and lipolysis did not indicate an effect of CLA isomers from altered ruminal biohydrogenation pathways on gene expression and energy retention in adipose tissue. We did not observe an increase in oxidative stress from feeding HMC, but HMC diet elicited a more pronounced inflammatory response when included in a HS diet, and this last effect was similar for cows that received DGC in a low starch (LS) diet. In addition, LS-DGC and HS-HMC treatments increased the abundance of genes associated with gluconeogenesis compared with LS-HMC and HS-DGC, and there were no differences on triglyceride accumulation in liver among treatments.

During the carryover period, yields of milk and fat were increased by feeding LS-HMC and HS-DGC diets, without differences in DMI across all treatments. Reasons for this effect are

uncertain, but it is possible that the decrease in inflammation by those treatments during the early PP period may have programming effects on production later in lactation. Signaling effects from circulating BHB during the early PP period could have potentially modulated the inflammatory response and induced long-term effects on production, but further research to elucidate the mechanisms involved in mediating this response is required. In vivo work performing intravenous infusions of LPS and BHB in cows during the early PP period could provide evidence of anti-inflammatory and epigenetic effects of BHB.

Overall, our study suggests that feeding a highly fermentable starch source during the early PP period should be minimized or avoided. Also, feeding a HS diet containing a starch source of moderate ruminal fermentability (e.g. DGC) can improve energy intake and production, and reduce the metabolic stress during the early PP period, as well as improve production later in lactation. However, evidence suggests that interactions among SC, SF and forage NDF content of diets fed during the early PP period need to be considered. In addition, a large-scale study should be conducted to determine if the positive effects observed on DM and energy intake by cows receiving HS-DGC during the early PP period would also improve cow health and reproductive performance. Lastly, interactions with diets fed during the close-up period and after the early PP period should be investigated in order to guarantee a successful transition into the rest of the lactation and maximize cow well-being, reproduction, production and farm profitability.